Plant Pathology

Third Edition

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Third Edition

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Dedicated to

Professor S. B. Saksena
For his inspiration as a teacher
(On his centenary birth anniversary)

Preface to the Third Edition

Overview

Anyone who ever grows a plant or crop not only enjoys the rewards of beautiful flowers, fruits, seeds, vegetables, etc., but also faces the disappointment when the plants or crops become diseased. Plant diseases cause great losses annually throughout the world and reduce the yield even with the use of improved varieties and technologies. The prospects of increasing production of various crops by increasing land under cultivation are limited. Because of dependence of humans for food, fiber and other resources, understanding plant diseases and their control is vital for our survival, and there is a need to increase productivity of various crops to meet the future demand and to achieve the goal of sustainable agriculture development.

We all know that newer researches and findings in the field of sciences regularly enhance the knowledge-base and dimensions of the subject. Nature has empowered us with the intellect and curiosity to think, to question, and to go beyond the limits of general perceptions. Science gives us a direction to discover, experiment and reveal the hidden truths of the Universe for further studying and exploring them. A theory or a notion without queries and research becomes out of date very soon. In the field of Plant Pathology also, knowledge of techniques and newer researches are always necessary to remain up to date.

Aim of the Book

Keeping in view these facts, this new edition of *Plant Pathology* is written to provide recent information and advances in the field, mainly for the students of plant pathology, agriculture and plant sciences. The present book is a joint effort made after years of teaching and research and months of focused thinking so that it may help students to develop a deep understanding of the subject.

We have presented the latest information, leavened the lessons learned from our own experiences as well as colleagues, students and others, on precise and well-defined topics in a concise manner.

Salient Features

- The textbook covers approximately 132 diseases of crop plants, providing details of symptoms, latest nomenclature of causal organisms and their control measures.

- It attempts to provide postgraduate and professional readers, already familiar with the subject, with a means to acquire latest and deep knowledge on various aspects of plant pathology.
- Profusely illustrated with more than 191 figures in the form of diagrams and photographs. Some diagrams are redrawn so as to improve the quality of the book.
- Most of the chapters have been revised with recent information like Chapter 2 (Food safety, Biological warfare, Bioterrorism); Chapter 4 (Cercosporin toxin), Chapter 5 (Effect of polysaccharides and growth regulators on pathogen/ diseases); Chapter 6 (Defence through genes); Chapter 7 (Genetics of virulence in pathogens and resistance in host plants); Chapter 8 (Effect of silicon on nutrition and disease development); Chapter 9 (Overwintering of pathogens and their role in epidemics); Chapter 10 (Host physiology/Factors based prediction, role of molecular tools, GIS, GPS and remote sensing based prediction); Chapter 11 (Control of diseases through use of transgenic plants); Chapter 12 (recent classification of fungi by Kirk *et al*., 2008); Chapter 13 (Collar rot of apple); Chapter 17 (Fusarium Head Blight of wheat, Bakanae or foot rot disease of rice); Chapter 20 (Post harvest decay of grains and legumes, different mycotoxins, methods of preservations); Chapter 26 (Recent classification of nematodes); Chapter 29 (role of innovative tools like monoclonal, recombinant antibodies, flow cytometry, FISH, PCR, DNA microarray, gene pyramiding in the field of plant pathology); and Chapter 30 (maintenance and preservation of microbial cultures).

Chapter Organization

The book is unique as it contains information on general concepts as well as information on specific diseases. From chapters 1 to 10, the authors have discussed the host pathogen interactions in detail, and in the later chapters, have given a detailed account of diseases caused by fungi, bacteria, viruses, phytoplasmas and nematodes.

The book adopts multidisciplinary approach in addressing both basic and applied aspects of plant diseases management. Latest information regarding disease management has been added from journals, review articles and books to make the subject up to date in this edition.

R. S. Mehrotra
Ashok Aggarwal

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Piracy-related issues may also be reported.

Preface to the First Edition

Plant pathology is intimately related to the health of plant and the production of crop yields. It has special relevance in agricultural countries like India which are fast developing, and where much still needs to be done in the modernization of control measures against plant diseases. Rough estimates have suggested that in India, on an average, there is a loss of about 10 per cent in crop yield due to plant diseases. This loss would certainly amount to hundreds of crores of rupees annually, taking into account the output of all the cultivated crops. Hence, a book on plant pathology is always welcome.

During the last quarter of the century, there have been enormous developments in the science of plant pathology. Many new techniques and chemicals have been evolved to fight plant diseases. Efforts have been made to understand the nature of diseases at molecular levels and the relationship between the host and parasite has been put, in many cases, on a sound biochemical footing. It is a stupendous task to present this knowledge in a volume of suitable size to a young post graduate or research student.

The book has several important features, one of which is the "all-in-one" approach which will be useful to postgraduate students. To the best of my knowledge there is no book with both generalized and specialized topics discussed in parallel which caters to the needs of undergraduate, postgraduate and research students at the same time. Most of the important aspects of plant pathology, such as physiology of diseased plants, root diseases, seed-borne diseases, post harvest diseases of fruits and vegetables, diseases caused by fungi, bacteria, viruses, mycoplasmas, nematodes and non-parasitic diseases have all been dealt with suitable detail for the benefit of students. Besides, the book should also serve as a good reference for research workers.

It is hoped that this work will enrich the background of those who wish to study plant pathology intensively. Suggestions for improving the book will be gratefully received and acknowledged.

R. S. Mehrotra

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R. S. Mehrotra
Ashok Aggarwal

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1 Introduction

Nature and Concept of Plant Diseases

It is imperative to define and discuss the meanings of certain terms commonly used in phytopathology (*phyton*- plant; *pathos*- suffering; *logos*- knowledge) before we discuss the basic principles and concept of plant pathology. It is essential to include these definitions and terms in an introductory chapter, since an understanding of these terms is required before the reader can be made aware of the details of the processes involved in plant diseases.

Phytopathology is the study of the diseases of plants and covers the entire field of biological and scientific activity that is concerned with the understanding of this complex phenomenon. Phytopathology is thus the study of the nature, development, and control of plant diseases.

Disease, being a complex phenomenon, is difficult to define in a few words. We have to keep in mind what Locke said in *Human Understanding*: "Though definitions will serve to explain the names of substances as they stand for our ideas, yet they leave them not without great imperfection as they stand for things." According to modern conception, disease is an interaction among the host, parasite, and environment. A simple dictionary meaning of disease is: any departure from health, presenting marked symptoms, malady, illness, disorder. Diseased plants are distinguished by changes in their morphological structures or physiological processes, which are brought about by unfavourable environment, or by parasitic agencies. Several definitions of diseases in plants have been proposed. Some examples are: "a series of harmful physiological processes caused by continuous irritation of the plant by a primary agent"; "a harmful deviation from the normal functioning of physiological processes"; "a continuous impairment of metabolism". Stakman and Harrar (1957) defined plant disease as "a physiological disorder or structural abnormality that is harmful to the plant or to any of its part or products that reduces the economic value".

Agrios (1997) has defined disease in plants as a series of invisible and visible responses of plant cells and tissues to a pathogenic microorganism or environmental factor that results in adverse changes in the form, function, or integrity of the plant and may lead to partial impairment or death of the plant or its parts. However, *A Guide to the Use of the Terms in Plant Pathology* (1973) prepared by the Federation of British Plant Pathologists recommends that the use of the term should be restricted to malfunctions caused by plant pathogenic organisms or viruses and those caused by other factors (the so called non-infectious diseases) should be termed as "disorders". According to Horsfall and Cowling (1977a),

disease is a malfunctioning process that is caused by continuous irritation. Of course this process may result in some suffering, and this produces symptoms. This conception of the disease is accepted by the Committee of Terminology of the American Phytopathological Society and by their counterpart, Committee of the British Mycological Society.

Simple Interest Disease

This denotes a disease, the increase of which is mathematically analogous to simple interest in money. There is only one generation of disease in the course of one epidemic.

A simple interest disease develops from a common source of inoculum, that is, the capital is constant, and often there is one generation of infection in a season. In the increase of simple interest disease, the $\log_e [1/(1-x)]$ is plotted against time (where x = the proportion of diseased tissue), thus allowing for the fact that with increasing infection the number of plants susceptible to infection decreases (Fig. 1.1). Soil-borne diseases such as vascular wilts and soil-borne smuts, which infect seedlings and subsequently sporulate in the inflorescence of the mature plants, provided that the pathogen does not spread from the smutted heads, are examples of simple interest diseases.

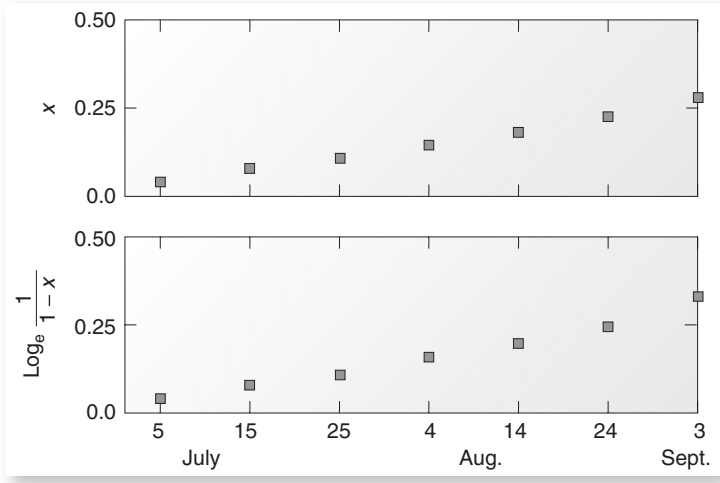


Figure 1.1 The progress of an epidemic of wilt of cotton caused by *Fusarium oxysporum* f. *vasinfectum*. In the upper graph x is plotted against time; the lower part plots $\text{Log}_e [1(/1-x)]$ against time. (Redrawn from JE Van der Plank. *Plant Disease Epidemics and Control*, Academic Press, New York and London, 1963, p.34.)

Compound Interest Disease

This denotes a disease, the increase of which is mathematically analogous to compound interest money. There are several or many generations of the pathogen in the life of the crop, that is, the capital is increased by the amount of interest. In plotting the amount of disease against time, the transformation $\log_e (x/1-x)$ is used, where x represents the proportion of infected susceptible tissue or of infected plants if the pathogen develops in a systemic manner (Fig.1.2). Late blight of potato, stem rust of wheat, or powdery mildew of wheat are examples of compound interest diseases.

If we know that a particular disease is of the simple interest type, suitable control measures can be applied. For example, sanitation affects the simple interest disease more than the compound interest type.

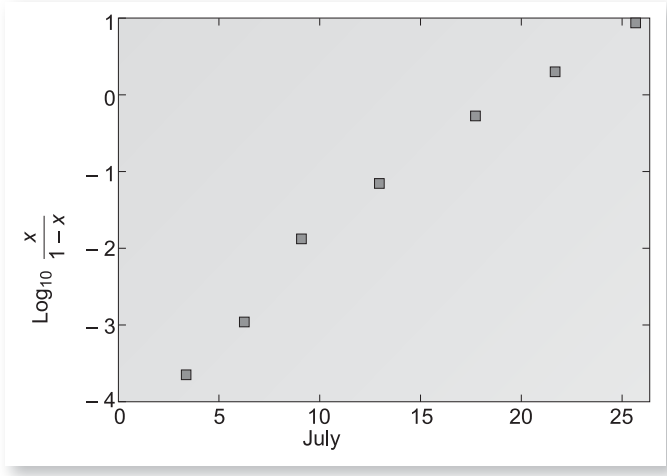


Figure 1.2 Progress of stem rust of wheat caused by *Puccinia graminis tritici*. (Redrawn from JE Van der Plank. *Plant Disease Epidemics and Control*, Academic Press, New York and London, 1963, p.32.)

If the disease is of the former type, sanitation, including the eradication of the inoculum of the pathogen from planting material and sites, should be insisted upon. If it is of the compound interest type, and the epidemic spreads rapidly and lasts long, the infection rate should be reduced by a timely application of fungicides. The stress should shift from sanitation to reducing the infection rate in proportion to the increase in the rate of spread and duration of the epidemic.

Cause of Diseases

When a parasitic microorganism enters into the causal complex of a disease, it is commonly considered to be the cause of the disease. However, we know that sometimes a variation in the environment also makes a plant liable to infection. The microorganism is thus not the sole causal factor. After a microorganism has successfully infected a susceptible plant, the subsequent interaction between the plant and the microorganism (which results in a disease) is also subject to environmental influences. Thus, strictly speaking, it is not correct to refer to a microorganism as the cause of a given disease since this implies that it is the sole cause. The term 'causal organism' is proper since it implies that the organism is a part of the causal complex. Most causal organisms are parasites. A parasite is not synonymous with the pathogen. In some cases, organisms are parasitic without becoming causal factors in the disease. Many parasitic establishments do not become pathogenic if the parasite compensates for the damage done by its presence. The mycorrhizal fungus is certainly parasitic on the roots of trees but it is not pathogenic. The root nodule bacterium (*Rhizobium leguminosarum*) is another example. In other cases, the byproducts of a strict saprophyte are an important part of the causal complex. Leaf tip injury to oats may be due to the absorption of Griseofulvin from soil. Milo root rot is caused by a powerful toxin produced by the fungus (*Periconia circinata*) growing in the root zone.

Thrower (1996) modified a definition offered by the British Mycological Society in 1950 and defined a parasite as "an organism or virus existing in an intimate association with another living organism from which it derives an essential part of the materials for its existence". In a literal sense, a pathogen is an

agent that causes damage. According to *A Guide to the Use of Terms in Plant Pathology* (1973) prepared by the Federation of British Plant Pathologists, a pathogen is an organism or virus capable of causing disease in a particular host or range of hosts. Most, but not all pathogens, are also parasites in that they derive the materials they need for existence from a living plant (the host or the suscept) as distinct from saprophytes, which derive these materials from dead organic matter.

Pathotype

This is a subdivision of a species distinguished by the common characters of pathogenicity, particularly in relation to the range of hosts.

Serotype

A serotype is the population of a pathogen (usually a bacterium or virus) in which all individuals possess a given character of serology in common (Robinson, 1969). On the basis of serological tests, differences between apparently similar organisms or viruses may be found and the subdivisions thus formed are called serotypes or strains. For example, only one serotype was designated amongst 45 isolates of *Xanthomonas oryzae* pv. *oryzae* on the basis of agglutination reactions and gel diffusion tests (Addy and Dhal, 1977).

Physiotype

It is a population of pathogens in which all individuals have a particular character of physiology (but not pathogenicity) in common (Robinson, 1969).

Pathogenicity

It is the quality or characteristic of a pathogen capable of causing disease.

Pathogenesis

It is the sequence of progress in disease development from the initial contact between a pathogen and its host to the completion of the syndrome. Horsfall and Dimond (1960) liken the relationship between the microorganisms and higher plants to a situation in which your mother-in-law lives with you. If she contributes to the harmonious running of the household, your relation is symbiotic; if you support her and she does not do her share of work, she is a parasite and you are the host; if she stirs up trouble between you and your wife, she is a pathogen. Her actions, your reactions, and her reactions constitute pathogenesis.

Host

An organism that harbours or supports the activities of a parasite is known as the host.

Whetzel (1929) termed the diseased plant suscept, but suscept may actually mean the plant that is susceptible or prone to disease.

Inoculum

Inoculum is the infectious material that can cause disease, and it is that portion of individual pathogens that is brought into contact with the host.

Inoculum Potential The term inoculum potential describes the degree of infectivity. Garrett (1956) defined inoculum potential as "the energy of a fungal parasite available for infection of a host at the surface of the host organ to be affected".

Primary Inoculum The overwintering or oversummering stage may be in or on the seed of the host, in the perennial wild host, in debris of infected plant of the previous season, in dormant spores, sclerotia or other organs in the soil, or in the form of an inhabitant of the soil flora. Whatever the source, it causes primary infection. It may serve as an infective agent and thus becomes the primary inoculum, or it may multiply as a saprophyte (or as a parasite on the perennial host) to produce spores which become the primary inoculum. The rust fungus oversummers in India in the uredinial (uredial) stage on summer wheat in the hills, from where the primary inoculum is carried by air currents to the next crop grown at the foot of the hills and then to the plains. When the host becomes diseased, the organism commonly produces another crop of spores or infective bodies which are known as the secondary inoculum and cause secondary infection. This last cycle may be repeated several times. The uredinial (uredial) pustules formed in the wheat crop as a result of primary infection become a source of secondary infection to other plants as the urediospores are blown down to these leaves and plants.

Penetration This is the first step in the contact of the inoculum with the host. Penetration may take place in one of a number of ways characteristic of the organism and are sometimes influenced by the environment. The methods of penetration have been discussed in Chapter 3. There is a clear distinction between penetration and infection. Penetration refers to the initial invasion of the host by an organism. We may have many cases of penetration without infection, for example, in the case of apple scab fungus (*Venturia inaequalis*) conidia, the infection hyphae penetrate readily whether or not the host variety and fungal strain are compatible; but in the resistant host variety, the fungus is not able to develop further and cause infection due to the influence of the vital activities of the cell.

Infection It implies the establishment of the pathogen inside the host following penetration in which a parasitic relationship between the two organisms is established.

Incubation Period There may be a long period between penetration, infection, and the appearance of the disease, but ordinarily one follows the other. This interval of time between the penetration of the host by the pathogen and the first appearance of the symptoms on the host is known as the incubation period. In the case of black stem rust of wheat, the pathogen *Puccinia graminis tritici* enters the host (wheat) a day or so after the urediniospores (urediospores) have germinated, but the actual symptoms of the disease in the form of rust pustules develops only after a week or so at 20°C. In the case of *Sphacelotheca sorghi* (covered smut of *Sorghum*), the very young seedling is infected from soil-borne or seed-borne spores, but no visible symptoms appear until the infected plant produce smutted flowers. In such cases, the incubation period is technically several months, although increased respiration of the diseased plant may occur much sooner. In case of the pathogen *Mycosphaerella musicola*, the incubation period is about three or four weeks.

Disease Development It refers to a series of events which occur between the time of infection and complete development of the disease.

Epidemiology Epidemiology of disease is a study of the factors affecting the outbreak of an infectious disease. Van der Plank (1963) defined epidemiology as the science of disease in a population. The founders of epidemiology have kept the identity that epidemiology deals with population of the host and pathogens under varying environment.

Symptoms Disease development is a long drawn process, which involves the production of secondary inoculum and progression of external or internal evidences of the disturbance of morphological and physiological processes of the host plant. These external or internal reaction or alterations as a result of a disease are referred to as symptoms.

Syndrome Diseased plants show several valuable symptoms by which a disease is recognized. These symptoms are collectively known as a syndrome.

Disease Cycle A series of events involved in disease development, including the stages of development of the pathogen and the effect of the disease on the host is called the disease cycle. When a pathogen is involved, the disease cycle is intimately associated with the organism. It is distinct from the life cycle of the organism.

Infectious Organism An infectious organism is one that may be transmitted from a diseased host plant to a healthy plant, and which is capable of inciting the disease in a favourable environment.

Infectious Disease An infectious disease is one in which a transmissible causal organism is part of the causal complex. In other words, on infection the disease can spread from a diseased to a healthy plant.

Various Levels of Parasitism

Symbiosis

Two living organisms may live in association with one another and in a sense; each may be parasitic upon the other. However, they are not only non-pathogenic to one another but are also essential to each other's development. This phenomenon is known as symbiosis. Lichens are classical examples of symbiosis in which a fungus and alga live in close association. The alga through its photosynthetic activity, converts the kinetic energy of sun's rays into the potential energy of carbohydrates which is useful for its own development and essential to the life of fungus. The later through its property of producing hydrolytic enzymes breaks down the material in the substrate, and thus makes energy available and provides minerals and organic nutrients essential to itself and the alga. Another case of symbiosis is that of *Rhizobium* species forming root nodules in leguminous plants.

Obligate Parasites

There are various categories of parasitism. Parasites which are restricted to living tissues (also known as biotrophs) are called obligate parasites. Obligate is also used to denote parasite which has not been grown in axenic cultures, that is, free from any other organism.

Facultative Saprophytes

The term facultative is both cumbersome and confusing. Comprehension of the phrase *facultatively saprophytic* requires a mental pause for translation to *strongly parasitic.* Facultative saprophytes ordinarily

have a life cycle similar to that of obligate parasites in that they pass most of the cycle in association with the host and do not subsist as saprophytes. They may, however, subsist as saprophytes for part of their life cycle but do not complete their life cycle away from the host. Many smut fungi are good examples of this.

Facultative Parasites

This term implies that they are ordinarily saprophytic but under certain conditions they may become parasitic. Many damping-off fungi, such as *Pythium* and *Rhizoctonia* are good examples of facultative parasites.

Saprophytes

Saprophytes are those organisms which have no relation with the living cell and secure their nutrients from dead organic tissues or from available inorganic materials.

In fact, a number of fungi once considered to be obligate parasites, have been cultured away from their hosts on artificial media. This makes the term obligate inappropriate. Luttrell (1974) recognizes three broad categories of parasitism which are as follows:

1. Biotrophs These are organisms which, regardless of the ease with which they can be cultured in the laboratory or not, in nature (*in vivo*) obtain their food from the living tissues on which they complete their life cycles. Some typical examples are rusts, smuts and mildews.

2. Hemibiotrophs These are organisms which attack living tissues in the same way as biotrophs but continue to develop and sporulate after the tissue is dead. Typical examples of these are leaf-spotting fungi.

3. Perthotrophs These are organisms which kill host tissues in advance of penetration and then live saprophytically. *Sclerotium rolfsii* is a typical fungus included in this category.

Virulence

Aggressiveness and virulence are ambiguous terms. Many pathologists equate virulence with pathogenicity; nevertheless, some shade of difference in usage is usually apparent. Thus, the statement that an organism is pathogenic merely implies that it can cause a disease. But a virulent organism is considered to have a high capacity to do so. Aggressiveness is equally ambiguous and has been used by Gäumann (1950) to describe the capacity of a parasite to invade and grow in its host plant and to reproduce on or in it. High aggressiveness can be combined with low pathogenicity as in the case of some obligate parasites which invade plants efficiently but cause minimal damage to it, at least in the early stages of attack. Aggressiveness is also used as a measure of pathogenicity.

Predisposition

It may be defined as the effect of one or more environmental factors which makes a plant vulnerable to attack by a pathogen. It is a process which antedates penetration and infection.

Susceptibility

It is the inability of a plant to resist the effect of a pathogen or any other damaging factor. Susceptibility of a given individual may be increased or decreased by environmental factors. It may also vary between individuals, varieties or species because of differences in the inherited characteristics which affect susceptibility.

Resistance

Resistance can be described as the inherent ability of a plant to prevent or restrict the establishment and subsequent activities of a potential pathogen. It may be regarded as the opposite of susceptibility and may also be influenced by environmental factors. It is the ability of the plant to withstand, oppose, or overcome the attack of a pathogen completely or to some degree. The new concepts of vertical resistance (*VR*) and horizontal resistance (*HR*) were put forward by JE Van der Plank (1968). Resistance may be defined as horizontal (uniform) when it is evenly spread against all races of a pathogen, or as vertical (differential) when it is completely effective against some races of a pathogen but not against others. It may be *polygenic* (determined by many genes), *oligogenic* (several genes) or *monogenic* (one gene). The genes involved may be major or minor depending on their effectiveness in conferring resistance.

Figure 1.3 shows the foliage of two potato varieties to 16 races of *Phytophthora infestans*. Both varieties show vertical (complete) resistance to races (0), (2), (3), (4), (2, 3), (2, 4), (3, 4), and (2, 3, 4), while resistance to other eight races is horizontal and greater in Maritta than in Kennebec.

Vertical resistance is complete but impermanent, while horizontal resistance, though incomplete, is of a permanent nature.

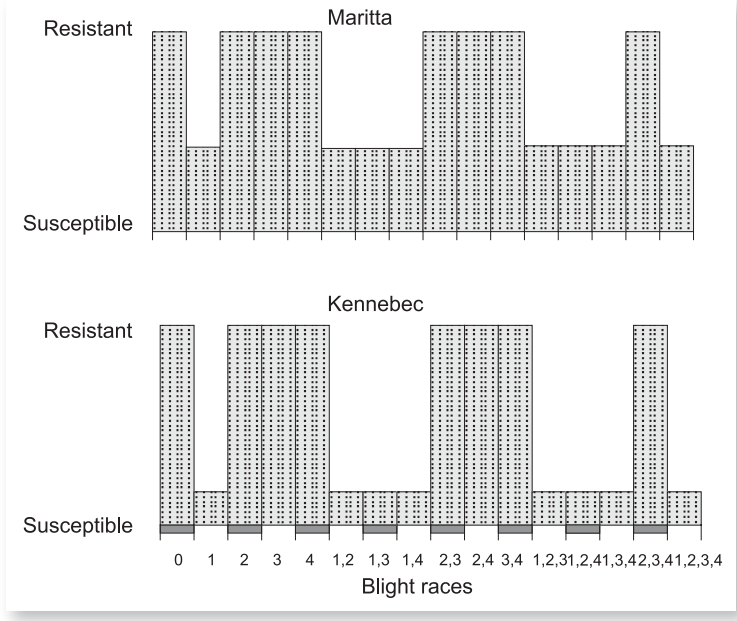


Figure 1.3 Vertical and horizontal resistance in two varieties of Potato (Redrawn from JE Van der Plank. *Plant Diseases Epidemics and Control*, Academic Press, New York and London,1963, p. 175.)

Hypersensitivity

This results in violent local reaction to attack by a pathogen and the prompt death of tissue around the points of entry prevents further spread of infection. Hypersensitivity thus confers high resistance and is sometime used in the sense of immunity.

Symptomless Carriers

Symptomless carriers are those plants in which the agent inciting disease is present but shows no symptoms. This is an extreme case of tolerance in some plants.

Active Resistance

Active (or dynamic) resistance is "due to reactions incited by the attack", while passive (or static) resistance is "due to qualities innate in the host prior to the attack". These terms describe resistance mechanisms and do not necessarily connote any epidemiological significance.

Pathodeme

Pathodeme is that population of a host in which all individuals have a particular character of resistance in common (Robinson, 1969).

Biotype

A biotype is a population of individuals which are genetically identical (literally any species subdivision which is based on criteria other than morphological).

Immunity

This implies exemption from infection by pathogen and in this sense is an absolute quality.

Aetiology

Aetiology is the science of the cause of disease. When used in a broader sense it includes the study of causal factors, their nature, and their relation with the host.

Fungitoxic, Fungicidal, Fungistatic

Fungitoxic substances are harmful to fungi and include fungicides. The word fungicide has originated from two Latin words, *fungus* and *caedo*. The word caedo means "to kill". Thus, literally speaking, a fungicide is any agency which has the ability to kill a fungus. According to this meaning, physical agencies like heat and ultraviolet light should also be called fungicides. However, this is not so. In common usage the meaning of the word fungicide is restricted to chemicals. Hence, fungicide means a chemical capable of killing fungi. Some chemicals do not kill fungi but inhibit fungus growth temporarily. Such a chemical is called *fungistat* and the phenomenon of temporarily inhibiting growth is called *fungistasis*.

Integrated Disease/Pest Management

Keeping in mind environmental and health concerns, a determined effort was made in the last few years to reduce and rationalize the use of chemicals (pesticides, fungicides and nematicides etc.) worldwide in order to manage pests/pathogens more effectively. This approach led to the emergence of a new discipline called IDM/IPM (Integrated disease management/Integrated pest management). In the IDM/IPM, a fungicide/pesticide is applied only when the pathogen population reaches a level inducing economic crop losses. In other words, IDM/IPM concentrates on reducing the number of noxious agents with agrochemicals. So, IDM/IPM can be defined as: "A disease management system that in the context of the associated environment and the population dynamics of the pest/pathogen species utilizes all suitable techniques and methods in a manner as compatible as possible and maintains the pest/pathogen population at levels below this causing economic injury." IPM/IDM in the broad sense has been further defined as: "the optimization of pests/pathogens control in an economically and ecologically sound manner, accomplished by coordinated use of multiple tactics to assure stable crop production to maintain pathogen/pest damage below the economic injury level while minimizing hazards to humans, animals, plants and the environment." Health of plants depends upon their interaction with pathogen/pests and a further complex cohort of other organisms, as well as with the physical and chemical environment in which the plant is growing. Moreover, the basic concern of IPM/IDM is with designing and implementing pest/disease management, practices that meet the goals of farmers, consumers, and government in reducing pest/disease losses while at the same time safeguarding against the longer term risks of environmental pollution, hazards to human health, and reduced agricultural sustainability.

Importance of Plant Diseases

Man is directly dependent upon plants for his survival because plants are his prime source of food, fiber, and drugs. The late Professor James G Horsfall, formerly Director of the Connecticut Agricultural Experiment Station, said that 30 million mouths were added every year. When we cannot feed the present population of the world, which is around four thousand million, how can we expect to feed it in 2000, by which year the world population will have doubled? This cannot be brushed aside with the quip that man should not be as prolific as plant pathogens. The role of plant pathology is important for alleviating losses to our food, fodder, fiber, and other plant products. Plants are also important to man because they utilize CO_2 in photosynthesis and release O_2. According to Agrios (2005) around 14% of the crops are lost to plant diseases alone: the total annual loss worldwide for plant diseases is about $220 billion. To these should be added 6–12% losses of the crops after harvest which is particularly higher in the developing countries.

Effect of Plant Diseases on Human Affairs

An example of the tremendous impact that plant diseases can have on the course of human history is given by the late blight of potatoes, caused by the fungus *Phytophthora infestans*. Between 1840 and 1847 it resulted in a famine among the working class population of Ireland who had come to depend on potato as their major source of food. In 1845 the potato blight devastated millions of acres in Europe, the USA, and Canada. So sudden and so complete was the catastrophe that in only a few days, fields

which had promised abundant harvests, were transformed into blackened wastes of vegetation overlying foul and putrefying masses of rotten tubers. As a result of this total failure of potato crop in Ireland there was a great famine and about a million people died, and almost the same number of people migrated to other continents. In the early 1870s another fungal disease, the coffee rust (*Hemileia vastatrix*), wiped out the coffee plantations of Sri Lanka (Ceylon). The eminent English plant pathologist, H Marshal Ward, made a thorough investigation of the disease but did not succeed in devising ways of checking its ravages. Coffee growing was therefore abandoned, at least for many decades, and much of the world's coffee production was shifted to the western hemisphere where the disease did not occur. The tea and rubber industry replaced coffee in Sri Lanka and other eastern areas. In the 1930s the entire banana industry in Central and South America was threatened with extinction by Sigatoka (*Mycosphaerella musicola*) and the industry could be saved only by 1940 when Bordeaux mixture was used on a massive scale (Wardlaw, 1961).

The eating habits of people are very difficult to change and plant diseases appear to be responsible for many deeply implanted eating habits. While in northern and southern Europe, wheat bread is more common; in central Europe people eat rye bread. In the southern parts of the USA people eat corn bread while in the north, wheat bread is more common. Horsfall (1956) had suggested that this situation resulted due to the ravages of wheat rust. In France, between 1878 and 1882, the wine industry was threatened due to the introduction of downy mildew (*Plasmopara viticola*) from the USA. In India, the 1942 Bengal famine was perhaps largely due to the *Helminthosporium* disease of rice caused by *Helminthosporium oryzae* Breda de Haan. About two million people died of starvation. According to Padmanabhan (1973), nothing is as devastating as the Bengal epiphytotic of 1942 has been recorded in plant pathological literature. In 1946–47, the wheat rust epidemic was responsible for food shortage as well as seeds in Madhya Pradesh. The bacterial blight of rice, thought to be confined to Japan, was reported in 1951 in Bombay (Srinivasan *et al*., 1959) and in a short time became very serious and widely distributed, attacking the commonly cultivated varieties. The disease broke out in an epidemic form in the Shahbad district of Bihar in 1963. With the introduction of Taichung Native-1, the disease appeared in a severe form in 1966 throughout the country wherever the crop was grown. Thus, with the introduction of one susceptible variety, a disease which was unknown in all but two states earlier became pandemic within two years. At present, bacterial blight is a major hurdle in stepping up rice yields (Srivastava *et al*., 1966; Srivastava, 1967). Epidemics of ergot disease of bajra caused by *Claviceps fusiformis* (Syn.C. *microcephala*) have occurred throughout the whole country. In the USA (between 1969–70), there occurred one of the most devastating epidemics in the recent history of plant pathology: the southern corn leaf blight caused by *Helminthosporium maydis*. In 1970, over 70 percent of the North American maize crop carried Texas male sterile cytoplasm because a single type of cytoplasmic male sterility (Tms) was used in the production of hybrid seed. A virulent race of *Helminthosporium maydis*, known as race T, was present and the weather conditions favoured the development of the epidemic. The epidemic led to a great crisis in North America at that time. Coffee rust (*Hemileia vastatrix*) was recently discovered in Brazil where the pathogen had spread rapidly over the entire area. Coffee rust epiphytotic caused colossal losses in Brazil during 1974–1975. Thus, the disease had crossed the Atlantic Ocean to Brazil and Columbia where it was spreading.

Chestnut blight caused by the fungus *Cryphonectria* (*Endothia*) *parasitica* nearly wiped out the American chestnut. It is believed the causal organism was introduced from eastern Asia at the beginning of this century. The first report of the disease came from the New York Zoological Park in 1904. A year later, the disease assumed serious proportions and the Forestry Division of the Department of

Agriculture became alarmed. Although the causative agent of the disease was soon discovered, no suitable control measure could be found to control the disease. The result that about 30 billion board feet of wood, used for furniture, flooring, poles, railroad tiers (sleepers) and coffins, was lost. The tanning industry, which was dependent on this tree, collapsed and this forced the leather makers of the USA to obtain this item from other source. The loss of one species created far reaching effects that have not been completely overcome till now. The dramatic biological and human events of this epiphytotic and the attempts to stop it have been documented in an excellent paper by Hepting (1974).

Another instance of serious loss by a fungus disease is due to red rot of sugarcane (*Glomerella tucumanesis*) which reached its peak in 1938–39 in the white sugar belt of northern India: Bihar, Punjab and Uttar Pradesh. In the badly affected areas, most of the mills could crush only 33 percent of their normal quantity (Woodhead *et al*., 1945). The farmers suffered tremendously.

Catastrophic plant diseases are ably summarized by Klinkowski (1970) in which he mentions diseases like peach yellow, phony peach, sugarcane mosaic, pear decline, swollen shoot of cacao, plum pox, sugarbeet yellow, tobacco veinal necrosis, late blight of potatoes, blue mould of tobacco, hop downy mildew, Dutch elm disease, St. Anthony's fire, cereal rusts, white pine blister rust, brown spot of rice, red rot of sugarcane, and certain catastrophic nematode diseases which had disastrous consequences for man and made a dramatic impact on his affairs.

Information on internationally important plant diseases exists (Holliday, 1971; Klinkowski, 1970; Meredith, 1973; Padwick, 1956; Riker, 1964; Mukhopadhyay and associates, 1992). Some of the plant diseases which can assume serious proportions have been ably discussed by Thurston (1973). Some common epidemics have been discussed by Horsfall and Cowling (1978).

A large number of plant diseases and the destruction they caused can be quoted and the above few examples amply prove that diseases can entirely change the course of history and the economy of a country, and they have been and still are a limiting factor in crop production. The advances made in food production in developing countries due to the green revolution could be lost if proper attention is not given to plant diseases and other pests. The higher and increased yields of green revolution are based on practices which are generally favourable to attacks by plant pathogens. Today, there is an increasing awareness on the part of the scientific agricultural community that only through a deeper and more fundamental understanding of all the interacting components of agriculture can we expect to improve our capabilities of feeding the expanding world population. Therefore, the role of plant pathologists is assuming greater importance in the present day world. The role of plant pathology in the scientific and social development of the world had been discussed by Stakman (1958) who had also dissertated on the opportunities and social obligations in plant pathology (1964). The future and the sociology of plant pathology have been discussed by Walker (1963) and Horsfall and Cowling (1977b), respectively. The scope and contribution of plant pathology have been dealt with by Houten (1959). Zentmyer (1994) has given a 55-year retrospective of plant pathology. Keen (2000) has reviewed "A century of plant pathology: A retrospective view on the understanding of host—parasite interaction".

In our country it is believed that the following are the key diseases of national and international importance (Pathak *et al*., 1996) (Table 1.1).

Agrios (2005) has given examples of severe losses caused by plant diseases and additional diseases likely to cause severe losses in future throughout the word.

Table 1.1

Crop	Disease	Pathogen
Rice	Tungro	Viral
	Bacterial blight/wilt	*Xanthomonas oryzae* pv. *oryzae*
	Blast	*Pyricularia grisea* (*P. oryzae*)
	Brown leaf spot	*Helminthosporium oryzae*
	Sheath blight	*Rhizoctonia solani* (Perfect state-*Thanatephorus cucumeris*)
	False smut	*Ustilaginoidea virens* (Tanaka *et al*.,2008 have renamed as *Villosiclava virens* for the teleomorph stage) (Perfect state-*Claviceps oryzae sativae*)
	Nematodes pest	Nematodes
Wheat	Rusts	*Puccinia graminis* f. sp. *tritici*, *P. striiformis* f. sp. *tritici*, *P. recondita*
	Smut of wheat	*Ustilago segetum* var. *tritici* (*U. tritici*)
	Karnal bunt	*Tilletia* (*Neovossia*) *indica*
	Foliar blights	*Helminthosporium sativum* and *Alternaria triticina*
	Nematode pests	*Heterodera avenae*
	Molya	*Corynebacterium*
	Ear cockle	*michiganense* var. *tritici* and *Anguina tritici*
Pearl Millet	Downy mildew	*Sclerospora graminicola*
	Ergot	*Claviceps fusiformis*
	Smut	*Moesziomyces* (*Tolyposporium*) *penicillariae*
Sorghum	Grain mould	
Corn or maize	Stalk rot	Fungal and bacterial
Pigeon pea	Sterility mosaic	Viral
	Phytophthora blight	*Phytophthora drechsleri* f. sp. *cajani*
	Fusarium wilt	*Fusarium udum* (perfect state *Gibberella indica*)
Chick pea	Ascochyta blight	*Ascochyta rabie*
Soybean	Yellow mosaic	Viral
	Bud blight	Viral
Pea	Powdery mildew	*Erysiphe polygoni*
Potato	Viral diseases	Viral
	Bacterial wilt	*Ralstonia*(*Pseudomonas*) *solanacearum*
	Late blight	*Phytophthora infestans*
	Nematode pests	*Globoderma rostochiensis* (*Heterodera rostochiensis*) and several species of *Meloidogyne.*
Ground nut	Aspergillus root rot	*Aspergillus* spp.
	Rust	*Puccinia arachidis*
	Tikka disease	*Cercospora arachidicola* and *Cercosporidium personatum* (Perfect stage *Mycosphaerella berkeleyi*)

(Contd.)

Crop	Disease	Pathogen
Mustard	White rust Alternaria blight	*Albugo candida* *Alternaria* spp.
Mango	Mango malformation Powdery mildew Diplodia stem and other rots of fruits	A complex caused by *Fusarium moniliforme* var. *subglutinans* and mites *Oidium mangiferae* *Diplodia* sp.
Banana	Bunchy top Bacterial wilt Nematode pests	Mycoplasmal *Ralstonia*(*Pseudomonas*) *solanacearum* Nematodes
Guava	Wilt	*Fusarium oxysporum* f. sp. *psidi*
Apple	Scab	*Venturia inaequalis*
Tea	Blister blight	*Exobasidium vexans*
Coconut	Root wilt *Phytophthora* bud rot Ganoderma wilt	Mycoplasmal *Phytophthora palmivora* *Ganoderma lucidum*
Areca- Nut	Fruit drop	*Colletotrichum* sp.
Sugarcane	Red rot Smut	*Colletotrichum falcatum (Glomerella tucumanesis)* *Ustilago scitaminea*
Cotton	New cotton wilt	*Fusarium oxysporum* f. sp. *vasinfectum*
Tobacco	Leaf curl Phytophthora black leg Bacterial wilt	Viral *Phytophthora nicotianae* var. *nicotianae* Bacterial
Tapioca	Mosaic	Viral
Ginger	Rhizome rot	*Pythium aphanidermatum* and *Pythium myriotylum*
Cardamom	Katte disease	Viral
Coffee	Die back Rust	*Colletotrichum coffeanum* *Hemileia vastatrix*
Rubber	Phytophthora leaf fall Pink disease	*Phytophthora palmivora* *Pellicularia salmonicolor*
Jute	Macrophomina stalk rot Bacterial wilt	*Macrophomina phaseoli* *Ralstonia (Pseudomonas) solanacearum* var. *asiaticum* along with *Rhizoctonia bataticola* and *Fusarium solani*.
Black Pepper	Phytophthora fruit fall and Phytophthora wilt Nematode pests	*Phytophthora capsici*
Brinjal	Little leaf	Mycoplasmal
Okra	Yellow vein mosaic	Viral

(Contd.)

Crop	Disease	Pathogen
Cauliflower and cabbage	Sclerotinia rot Curd rot of cauliflower	*Sclerotinia sclerotiorum* Bacterial
Tomato	Leaf curl complex Bacterial wilt	Viral *Ralstonia* (*Pseudomonas*) *solanacearum*

According to Mukhopadhyay and associates (1992), the following are the diseases of international importance (Table 1.2).

Table 1.2

Fungal diseases	
Karnal bunt of wheat	Bean anthracnose
Blast of rice	Potato late blight
Wheat rusts	Downy mildew of lettuce
Brown spot of rice	Phomopsis blight and fruit rot of egg plant
Sheath blight of rice	Rust of groundnut
Downy mildew of maize	Tikka disease of groundnut
Downy mildew of pearl millet	Apple scab
Ergot of pearl millet	Sunflower rust
Anthracnose of sorghum	Powdery mildew of apple
Eye spot of cereals	Downy mildew of grapes
Pigeon pea blight	Phytophthora disease of citrus
Wilt of pigeon pea	Fusarium wilt of banana
Chickpea wilt	Phytophthora root rot of papaya
Ascochyta blight of chickpea	Mango malformation
Grey mildew of lettuce	Phytophthora root rot of avocado
Red rot of sugarcane	Blue mould of tobacco
Phytophthora root rot of avocado	Leaf rust of coffee
Red rot of sugarcane	Coffee berry disease
Smut of sugarcane	Armillaria root disease of trees
Blight and wilt of cotton	Phytophthora root rot and dieback of forest trees
Bacterial Diseases	
Bacterial blight of rice	Black rot of crucifers
Fire blight of apples and pears	Black leg of crucifers
Moko disease of bananas	Bacterial blight of soybean
Bacterial canker of stone fruits	Citrus canker
Common bacterial blight of bean	Ratoon stunting of sugarcane

(Contd.)

Viral / Mycoplasmal/ Viroid Diseases	
Barley yellow dwarf	Citrus tristeza
Rice tungro	Sandal spike
Maize dwarf mosaic, Maize chlorotic dwarf	Cadang-cadang of coconut palm
Sterility mosaic of pea	Lethal yellowing of coconut palm
Bean mosaics	Bunchy top of banana
Pea seed borne mosaic virus disease	Curly top of sugarbeet
Potato mosaics	
Nematode diseases	
Cereal cyst nematodes	Root knot of vegetables
Root knot of vegetables	Soybean cyst nematode
Cereal cyst nematodes	

Classification of Plant Diseases

Diseases may be classified in various ways on the basis of:

1. Host plant affected, such as cereals, millets, fruits, trees, vegetables, etc.
2. Part of the plant affected, such as root, stem, leaf, flower, fruit, etc.
3. Symptoms produced in the host plants, such as wilt, blight, soft rot, anthracnose, rust, smut, mildew, damping off, etc.
4. The mode of spread and severity of infection, such as epiphytotic, endemic, sporadic, and pandemic.
5. Causal organism/ factor.

Classification of Plant Diseases on the Basis of Spread and Severity of Infection

Endemic Diseases A disease is classified as endemic when it is constantly present in a moderate or severe form and is confined to a particular country or district, for example, the wart disease of potato caused by *Synchytrium endobioticum* is endemic in Darjeeling. In plant pathology, this term is generally applied to simple interest diseases which are either indigenous or of ancient introduction.

Epidemic or Epiphytotic Diseases An epidemic or epiphytotic disease usually occurs widely but periodically in a destructive form. The pathogen may be present, as in endemic diseases, but the environmental factors responsible for the development of the disease occurs only periodically, for example, cereal rusts, powdery mildews, etc.

Sporadic Diseases Sporadic diseases, in reality, belong to the epidemic group. The term is applied to those diseases which occur at very irregular intervals and locations and in relatively fewer instances. Angular leaf spot and blotch disease of cucumber are sporadic diseases (Pathak, 1972).

Pandemic Diseases Pandemic outbreak is an epidemic which occurs over vast and extensive areas causing severe losses. These occur all over the world and result in mass mortality, for example, late blight of potato.

Diseases Classified According to Major Causal Factors

Walker (1969) has classified plant diseases into three main types:

1. Non parasitic diseases
2. Parasitic diseases
3. Mycoplasmal and viral diseases based on the major causal factors

These are further subdivided on the basis of the causal agency or organism involved. These are as follows:

Sr. no.	Non-parasitic diseases (1)	Parasitic diseases (2)	Mycoplasmal, Spiroplasmal, Viral and Viroidal diseases (3)
1.	Low temperature, i.e. freezing injury	Bacteria, i.e. bacterial blight of paddy	Such as little leaf of brinjal, citrus stubborn, tobacco mosaic, and potato spindle tuber.
2.	High temperature, i.e. heat canker of flax	Plasmodiophoromycota, i.e. club root of crucifers	
3.	Unfavourable oxygen, i.e. black heart of potato	Chytridiomycota, i.e. wart of potato	
4.	Unfavourable soil moisture, i.e. flooding injury	Oomycota, i.e. late blight of potato	
5.	Injurious atmosphere, i.e. apple scald or black tip of mango.	Ascomycota, i.e. ergot of pearl millet	
6.	Lightning injury, i.e. damage to potato plants due to lightning	Basidiomycota, i.e. pink disease of citrus	
7.	Mineral excess, i.e. internal bark necrosis of delicious apple	Deuteromycota, i.e. blast of rice	
8.	Mineral deficiency, i.e. red leaf of cotton or khaira disease of rice	Phanerogams, i.e. diseases due to giant mistletoe. Animal parasites (Nematodes, i.e. ear cockle of wheat; insect, i.e. insect galls of various trees; Protozoans, i.e. Hart rot of coconut palms, phloem necrosis of Coffee).	

Trends in Teaching and Research in Plant Pathology Worldwide

Agrios (1997) has very briefly summarized the trends in teaching and training in plant pathology. Trends in plant pathology-significance for the future have been critically discussed by Weinhold (1988).

Sequeira (1986) had critically evaluated the issues facing the development of the subject of plant pathology. Phytopathology has existed barley for the last 150 years when it was separated from the basic sciences, of botany and medicine. As the date of its origin the publication of the work of Julius Kuhn *Die Krankheiten der Kulturgewachse* (Diseases of the cultivated plants) in 1858 may be considered. To obtain a clear understanding of the modern trends in phytopathological research it may be necessary to briefly recapitulate the earlier developmental trends of the subject. From the time of Theophrastus (Father of Botany), a transcendental origin was attributed to plant diseases and epidemics. This view dominated until the end of the middle ages. This era is called the predispositional era in plant pathology. The etiological view developed essentially in the 19th century and culminated in the activities of Prevost, Tillet and de Bary. The mycological trends of de Bary and Brefeld were followed by the bacteriological and virological directions of research.

The requirement of industry for agricultural raw materials rose considerably in the 20th century. Efficient field-plant protection machines such as aircraft, helicopters, and new means of mechanical and thermo-mechanical aerosol treatment, use of potent fungicides and so on spread considerably in the modern control of plant diseases and epidemics. According to Ubrizsy (1964), phytopathology has been characterized in the 20th century by prophylaxis and breeding for resistance and more particularly pathophysiological trends of research started in the 1950s. The next 50 years saw the development of research in the areas of the physiological-biochemical processes of diseased plants, the nature of parasitism as well as disease resistance. The modern trends of research tend to disclose the true causes of cell proliferation, elucidate the nature of biosynthesis of viruses, possibilities of inhibition of virus multiplication, the mechanism of fungal toxicity, the manner of action of toxins, the role of hormone-like growth substances (regulators) in pathogenesis and the importance of extracellular enzymes in the etiology of the diseases. Chemotherapy and chemically-induced resistance are areas where a lot of attention is being directed. Some of the frontline areas of research in plant pathology are the molecular biology of diseases resistance, plant diseases epidemic modeling, forecasting and modern methods of diseases control including biocontrol of plant pathogens. According to Sequeira (2000) the 21st century will present our profession with dramatic challenges to meet the demands of increased food, fiber and feed production for a declining agricultural base. This could be accomplished only if plant pathology retains its integrity as a profession and remains abreast of advancements in the fields of biotechnology and communications.

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Questions

1. Define the term 'disease'.
2. Distinguish the following pairs of terms :
   i. Simple interest disease and compound interest disease
   ii. Parasite and pathogen
   iii. Pathotype and serotype
   iv. Pathogenicity and pathogenesis
   v. Host and suscept
   vi. Primary inoculum and secondary inoculum
   vii. Penetration and infection
   viii. Disease cycle and life cycle
   ix. Symptoms and Syndrome
   x. Obligate and facultative parasites and saprophytes
   xi. Biotrophs and Perthotrophs
   xii. Vertical resistance and horizontal resistance
   xiii. Pathodeme and Pathotype
   xiv. Epidemiology and Aetiology
   xv. Fungicidal and fungistat
3. Name some plant disease that resulted in grave consequences for many nations.
4. Give definition and examples of the following types of disease:
   i. Epidemic or Epiphytotic disease
   ii. Sporadic disease
   iii. Endemic disease
   iv. Pandemic disease
   v. Integrated disease/pest management
5. What are the recent trends in Phytopathological research and training worldwide?

History of Plant Pathology 2

It may be asked as to why is it necessary to have an historical account of plant pathology? A study of the history of any science helps to make the subject clearer. By studying the history of a science, we get a better perspective of the subject; we come to know the contributions made in that field; the problems that were encountered; and the manner in which they were tackled. We can follow the guidelines set by previous scientists while studying any particular branch of science.

Whetzel (1918) has given a useful account in his book, *Outline of the History of Plant Pathology*. Large (1940) had dealt with the subject in his remarkable book, *The Advance of the Fungi.* The historical development of plant pathology had been ably presented by Keith (1959). Parris (1968) had discussed the chronology of plant pathology. The history of plant pathology in Australia had been given by Fish (1970); and in India, Burma, and Sri Lanka by Das Gupta (1958), and Raychaudhuri *et al.,* (1972). The history of plant pathology in the middle ages had been discussed by Orlob (1971). Factors important for the growth of the science of plant pathology had been considered by Bennett (1973). Thurston (1973) gave an interesting account of some threatening plant diseases. There are several other reviews, such as *Plant Pathology: Changing Agricultural Methods and Human Society* by Ten Houten (1974). In Japan, a historical perspective of the science of plant pathology had been given by S. Akai (1974). Some highlights in plant pathology had been given by J C Walker (1975) in the USA. The history of plant pathology in South America, Central America, and Mexico had been reviewed by Nolla, Manuel and Valiela (1976). Fuchs in 1976 had written the history of physiological plant pathology. Modern trends in plant pathological research had been discussed by Ubrizsy (1964). *Landmarks in Plant Virology: Genesis of Concepts* had been discussed by Markham (1977). Zentmyer (1994) had reviewed "Plant Pathology—A 55-year Retrospective". "Legacy for the Millennium: A Century of Progress in Plant Pathology" has been reviewed by Sequeira (2000).

It is interesting to note that the largest number of botanists specialize in mycology and plant pathology. This is primarily due to the fact the fungi, bacteria, and actinomycetes yield antibiotics; they have a role in the decomposition of organic matter, fermentation, nitrogen fixation, they are sources of food (mushroom culture); and lastly, some of these organisms are responsible for plant diseases which cause great annual losses throughout the world. It is estimated that in India there is an annual loss of five thousand crores of rupees as a result of plant diseases. In the USA the annual loss is estimated to be 9.1 billion dollars.

The earliest known writings make references to the ravages of plant diseases. There can be little doubt that plant diseases preceded man on earth and that ever since he has practised agriculture, they have taken their toll of crops. We must agree with the late Professor Whetzel (1918) of Cornell University, that phytopathology, like all natural sciences, had its beginnings in the dawn of man's civilization. There are references in the Vedas (1500 BC) to plant diseases and the methods of control. The first book in which plant diseases have been discussed is *Vraksha Ayurveda,* written by Surapal in ancient India. There are references in the Old Testament of blasting, mildew and insect pests. The Romans used to celebrate a festival, Robigalia, to ward off rust. The annual festival of Robigalia is reported to have been initiated by king Numa Pompilius, in probably about 700 BC, and was continued with modifications in the Christian era. Theophrastus, who lived from 370 BC to about 286 BC, was the first botanist to study and write about the diseases of trees, cereals and legumes, although his approach was observational and speculative rather than experimental. He noticed that some kinds of plants are more liable to certain diseases than are others. He was not aware that diseases are caused by microorganisms, and it was generally accepted that fungi and various other organisms observed in association with diseased or decaying plants, arose spontaneously from plants or from the environment. Comparatively, little was added to the knowledge of botany or plant pathology for nearly two thousand years after the time of Theophrastus. From the 5th century AD until the Renaissance, there was lack of intellectual activity and little was written on botany and plant pathology. In his writings on the plant pathology of that period, Whetzel (1918) stated that one bright spot in that dark period was the work, in the 10th century AD, of an Arabian landed proprietor, Ibn-al-Awam, who was said to have described accurately the symptoms of many diseases of trees and vines and to have given extensive consideration to their control. The period between 300 AD and 1300 AD is said to be the middle or 'dark ages' in history. There were petty wars, religious dogmas, and superstitions and there was practically no increase in the knowledge of plant pathology during that period. Then came the Renaissance from the 14th to the 16th century AD. It is during this period that printing (1440) and the microscope (1685) were invented. The compound microscope was invented by Anton van Leeuwenhoek, a linen draper, surveyor and wine gauger of Delft, Holland; and Robert Hooke, an English scientist, observed the cell for the first time under the microscope in 1660.

The Herbalists

The beginning of the revival of interest in botany is reflected in the writings of the herbalists and these are more important from the mycological standpoint.

1. *Pinax Theatri Botanici* (1623) written by Gaspard Bauhin, a Swiss, who attempted to compile all the plants known to him or his predecessors.
2. *Nova Plantarum Genera* (1729) written by Pier Antonio Micheli, an Italian, who cultured fungi on pieces of water melon.
3. *Species Plantarum* (1753) by C. Linnaeus (1707–78), a Swedish, who is credited with giving the binomial nomenclature. He is often called the 'father of botany'.

The Systematists

C H Persoon's *Synopsis Methodica Fungorum* (1793) is the chief starting point for the nomenclature of the Uredinales, Ustilaginales, and Gasteromycetes. Persoon, a native of South Africa; was educated in

Germany, and Holland; and did most of his work in France. Although a doctor of medicine, he devoted most of his time to the study of fungi.

Elias Magnus Fries (1794–1878) was Swedish and is regarded as the 'Linnaeus' of mycology. His *Systema Mycologicum* (1821–32) is the chief starting point for the nomenclature of fungi. Fries was the son of a Swedish clergyman and was a Professor of Botany, first at the University of Lund, Sweden, and then later at Uppsala, where he succeeded Linnaeus. Fries regarded the rust and smut fungi as products of diseased plants. *Systema Mycologicum* had put the taxonomy of most fungi on a more scientific footing than any other earlier work. He was one of the most respected mycologists of his time.

Matheiu Tillet (1714–91), was director of the Mint at Troyes, France, and an amateur botanist and agriculturist; he had published an important paper on wheat bunt. In 1755 he tested the effect of dusting seed with the black mass contained in the diseased kernels. Thus, he demonstrated experimentally that the bunt of wheat was contagious and thought that the spores contained a poisonous entity. Tillet did not understand the full implication of his experiments. He, however, suggested the control of disease by treating seeds with salt and lime. It can be said that he was an experimenter who lived ahead of his times. Felice Fontana (1730–1805), an Italian physicist and naturalist, published a paper in the year 1767 in which he expressed the view that the grain rust was a distinct parasitic entity. He distinguished the red and black stages of wheat rust and made microscopic drawings.

Benedict Prevost (1755–1819) who was a Swiss Professor of Philosophy at the Academy of France in 1807 observed the germination of the spores of the wheat bunt organism and conceived the idea that this organism penetrated the young wheat plant and was the actual cause of the disease. Prevost's experiments provided the first proof and interpretation of the role of a microorganism in the causation of disease. Prevost also demonstrated the control of smut by steeping seeds in a solution of copper sulphate. His contemporaries did not accept his views until the devastating potato blight in 1845 which attracted the attention of mycologists and plant pathologists to plant diseases. Thus, Matheiu Tillet, Felice Fontana, and Benedict Prevost were the three experimenters who were definitely ahead of their times in their thinking with regard to the causation of plant diseases.

The Beginning of Modern Period

In 1845, the devastating epidemic of the potato blight in North Europe and particularly in Ireland focused attention on plant diseases and gave a great fillip and stimulated interest in the subject of plant pathology. Much of the early literature on this disease is found in the *Gardener's Chronicle*.

Scientists at that time were unable to agree on the cause of the disease or to suggest a successful remedy. The autogenetic theory of diseases was still dominant, although opposition to this was rapidly increasing. Dr John Lindley, editor of the *Gardener's Chronicle* and Professor of Botany at University College, London, was the leader of the autogenetists. MJ Berkeley (1803–89) who was then the most prominent British mycologist, was at first somewhat reserved in supporting the parasitic theory, but advocated it strongly in 1846. Montagne in France, in 1845, described the fungus as *Botrytis infestans*. Morreu in Belgium experimented with disease in 1845. He described the disease and fungus and regarded the fungus as the major cause of the disease, although he lacked convincing evidence. That the fungus was the real cause of the disease and not the result, was proven experimentally by Speerschneider in 1857 and de Bary in 1861 and 1863 (Jones *et. al.*, 1912).

Following the publication of the *Origin of Species* in 1859, evolution became accepted as a fact and the systems of classifications of both plants and animals were given a phylogenetic slant. Anton de Bary

(1831–88) became an outstanding figure in the botanical field. Much of his research was on fungi and the relation of parasitic fungi to their hosts. De Bary was born in 1831 in Frankfurt-am-Main, Germany. His mycological interests were more biological and physiological than systematic. From his investigations on life histories, parasites and saprophytes, the nature of lichens, etc. he made new and important discoveries. Heteroecism in the Uredinales was made clear by his experiments and he gave accounts of the development and sex in a number of Phycomycetes and Ascomycetes. He was the first to indicate the nature of obligate and facultative forms. His well-known text book, *Morphologie and Physiologie der Pilze, Flechten, und Myxomyceten* in 1866 and its second edition in 1884, records the broad classification of fungi. He is truly regarded as the 'founder of modern mycology'. A large number of students from different parts of the world came to his laboratory. More than sixty of them became prominent in their fields and carried his techniques and influences to many parts of the world. Famous among them were MS Woronin of Russia, O Brefeld of Germany, PA Millardet of France, HM Ward of England, WG Farlow of the USA and A Fischer of Switzerland.

In his studies on the *Sclerotinia* disease of carrots and other vegetables, de Bary noted that the host cells are killed in advance of the pathogen and that the juice from the rotted tissue can break down healthy tissues of the host. On the other hand, if the juice is boiled it has no effect on the healthy tissues. De Bary concluded that the pathogen produces enzymes that bring about the degradation and killing of plant cells. De Bary's noteworthy contribution to plant pathology were his studies on the Peronosporaceae and the diseases they incite, especially the late blight of potato, the discovery of heteroecism in the rusts, and his work on *Sclerotinia sclerotiorum* (1886) which opened up an entirely new field of investigation, that is the physiology of parasitism.

Confirmation of Prevost's Work

In the decade of 1840s, the Tulasne brothers (Louis Rene Tulasne and Charles Tulasne) who were botanists at the Museum of Natural History in Paris began extensive morphological studies on fungi. They made illustrated drawings of rusts, smuts and Ascomycetes which are regarded as classics in mycology. L R Tulasne (1815–85) is often called the 'Reconstructor of Mycology'. In 1847, the two brothers published studies on wheat fungus and confirmed the observations of Benedict Prevost with regard to the causal organism of the bunt disease of wheat. Julius Gotthelf Kuhn (1825–1910) was a contemporary of de Bary. He was initially a farm manager. He published his famous textbook for farmers in 1858 entitled, *The Diseases of Cultivated Crops: Their Causes and Their Control*. This was the first textbook to be published in which fungi were regarded as causal factors in plant disease. The diseases were grouped according to causes: unfavourable climatic and soil conditions; influence of animals; and parasitic plants. Nematodes were also included as they were known as plant parasites since the time of Needham (1774). Their economic importance at the end of the 19th century promoted nematological studies as a part of plant pathology (Raski, 1959).

Doctrine of Spontaneous Generation

This theory held that the living organisms could arise spontaneously from inanimate or non-living matter. In 1748, Needham, an English clergyman and a naturalist, supported the theory of spontaneous generation. To prove his point, he boiled meat in a flask which was corked. He demonstrated that the

contents had spoiled due to microorganisms. We now know that, while he killed the vegetative bacteria, the bacterial spores were not killed at the temperatures used. In 1775, Spallanzani, an Italian investigator challenged Needham and proved that the decay of meat by bacteria and other lower forms of life could be prevented by heating the material in a flask which was sealed in such a way as to exclude contamination from the air. His experiments supported his contention. Others failed to get similar results consistently. Nevertheless, the views put forward by Spallanzani at that time were not accepted. Antoine Laurent Lavosier (1743–94) discovered the presence of oxygen in the air and its indispensability to sustain macroscopic forms of life. In 1775, he argued that this essential gas is vital for life, and therefore, sealed flasks that did not permit the entrance of oxygen were incapable of allowing spontaneous generation to occur.

Schwann (1837) and Cagniard Latour (1838) discovered the yeast cell and maintained that it caused fermentation. On the other hand, in 1839, Berselius, a Swedish chemist, strongly defended the mechanistic explanation of fermentation and he was supported by Liebig, a German agricultural chemist. It was in 1860 that Pasteur furnished irrefutable evidence that microorganisms arise from pre-existing living entities and that fermentation is a biological phenomenon rather than a purely chemical one. It may be mentioned that the autogenetic theory of disease continued to be dominant throughout the first half of 19th century. The most influential proponents were Unger, Meyen and Liebig. Unger was an Austrian Physician and Professor of Botany. He thought that fungi, associated with plant diseases, arose from the diseased plant because of abnormalities in plant juices and were thought to be products rather than the causes of diseases. His best known work on plant pathology was *Die Exantheme de Pflanzen* (1833). Thus, Needham, Lavosier, Liebig, and several others could not interpret the results of their experiments correctly because of their reluctance to give importance to new scientific ideas. Pasteur was born in 1822 to humble parents in the village of Dole in France. He began his life as a chemist. He worked as a teacher at Strassburg and then at the Ecole des Beaux Arts in Paris. He never studied medicine, though his discoveries about the protozoan nature of the silkworm and the confirmation of the bacterial origin of anthrax buried forever the controversy over the theory of spontaneous generation. The germ theory in relation to diseases of man and animals was established in 1876 by Robert Koch. Koch was a Germen physician and a co-worker of Pasteur. The first final proof of the causal relation of the anthrax bacillus to anthrax disease was published by Robert Koch in Germany in 1876. Koch (1843–1910) was trained as a physician; however he turned his attention to the new science of bacteriology. He began his work by establishing laboratory procedures and techniques that would yield reproducible results. Koch established the principles of pure culture technique although it was Lister, the English surgeon, who pioneered work in the field of aseptic surgery. He was impressed by Pasteur's work and kept it as a basis of his own research. He was the first man to culture a bacterium capable of souring milk. In the medical field, Robert Koch discovered the bacterium that causes tuberculosis. He enunciated certain rules or criteria that should be satisfied before the identity of the disease-producing organism in a particular disease can be established. These rules are known as **Koch's postulates** and can be briefly summarized as follows:

1. A specific organism must always be associated with a disease.
2. The organism has to be isolated in pure culture.
3. The organism must be identified.
4. The organism when inoculated must produce disease in a healthy susceptible host.
5. The organism must be isolated from the experimental host again in pure culture and its identity established.

Brefeld (1875, 1881, and 1883) in Germany worked on fungi and was mainly responsible for the early development of modern techniques of growing microorganisms in a pure culture. Along with the improvement made by Koch, Petri *et al.,* his techniques are the basis for the pure culture methods currently employed. He was the pioneer in tracing the life cycles of the cereal smut fungi and their role in the causation of diseases. He made important contribution both to pure culture of smut fungi and to the methods of inoculation.

Forest Pathology

Robert Hartig was born in Braunschweig in Germany in 1839. He was an enthusiastic and successful teacher. He is well known for his contributions to many aspects of forestry, botany and entomology. He is aptly called the 'Father of Forest Pathology'. His foremost contributions are W*ichtige Krankheiten der Waldaume* in 1874 and *Lehrbuch der Baumkrankheiten* in 1882. The latter publication was an authoritative work for many years.

Discovery of Bordeaux Mixture

The discovery of this fungicide has an interesting story. It was discovered by Pierre Marie Alexis Millardet in 1882. Millardet was born in Montmery-la Ville in France in 1838. He studied medicine but was more interested in botany. He learnt botany from the great botanist, Hoftmeister at Heidelberg and de Bary at Freiburg. He returned to France and took his doctorate both in medicine and science. In 1876, he joined the University of Bordeaux, France, and served there till his retirement in 1899. Millardet was a man of broad interests. His contributions fall into the following three categories:

1. Studies on the morphology, physiology, and systematic relationships of plants.
2. Investigation on the root aphid *Phylloxera* of the grape vines and hybridization experiments in order to have resistant plants.
3. Research on the downy mildew of grapes caused by *Plasmopara viticola* and its cure.

The downy mildew of the vine was first reported in Europe in 1878. Millardet and Planchon discovered it at about the same time in France, where it had evidently been introduced from the USA somehow or the other during the importation of resistant root stocks of grape vines for root aphid *Phylloxera*. The disease spread rapidly and threatened the vineyards of Europe. Millardet began a thorough study of the disease and the methods of control. In 1882, he noticed that the vines that had been treated with a mixture of copper sulphate and slaked lime to prevent the grapes from being stolen retained their leaves, whereas the untreated vines were defoliated. This fact suggested to Millardet a means of curing the disease. In 1883 and 1884, he performed extensive spraying experiments with many preparations of copper, calcium and iron salts, used singly and in various mixtures and also arranged tests by viticulturists. Both the seasons were dry and little mildew developed. Being a conservative scientist, Millardet preferred to delay the publication until he could recommend a thoroughly tested spraying programme. However, the news of his work spread and others began to publish the effects of copper preparation on mildew. It May 1885, Millardet published his works and gave details of spraying with a mixture of copper sulphate and slaked lime, which was later known as the Bordeaux mixture. The downy mildew appeared in a severe form in 1885 and when people followed Millardet's recommendation, spectacular results were obtained in controlling the disease. He, as well as others, rapidly improved spraying methods and studied

numerous copper and other fungicidal preparations. The Bordeaux mixture emerged and for many years continued to be the most important and successful fungicide.

Plant Pathology in The Twentieth Century

The major trends initiated in the latter half of the 19th century progressed in several directions. The rediscovery of Mendel's laws in the beginning of the century had its impact and the genetics of the host as well as the pathogen received the attention of plant pathologists. Some of the major trends were in the following areas:

1. Physiological plant pathology
2. Genetics of the host and the pathogen
3. Fungicidal research
4. Environment and its relation to plant disease
5. Nature of disease resistance in plants
6. Biochemistry and physiology of the diseased state
7. Molecular biology of pathogenesis and induced systemic resistance
8. Tissue culture in plant pathology
9. Biotechnological approach towards disease control
10. Ecological studies of plant pathogens
11. Bacteriology
12. Virology (Viruses, Viroids, and Prions)
13. Mycoplasma group of pathogens, etc., Mollicutes (Phytoplasmas)
14. Nematology
15. Protozoans as plant pathogens

These major trends are now discussed in brief.

Physiological Plant Pathology

The physiological trend in plant pathology can be divided into three aspects:

1. Studies on physiogenic diseases.
2. Work on the indirect or secondary causal relationship of the environment to infectious diseases.
3. Investigations into the physiology of parasitism.

Sorauer, born in Germany in 1839, initiated modern studies in physiogenic diseases. His best known work is *Handbuch der Pflanzenkrankheiten* which was written in 1909. Sorauer laid tremendous emphasis on physiogenic diseases.

Prevost (1807) demonstrated experimentally and interpreted clearly the indirect or secondary causal role of the environment in the etiology of parasitic disease. H M Ward (1880) emphasized the role of the environment in the epidemiology of coffee rust. He may be reckoned as the father of tropical plant pathology. He was the first to point out that the exclusive cultivation of a crop over unbroken areas is one of the most important prerequisites for an epidemic. The effect of copper fungicides against endophytic fungal pathogen was also demonstrated by him first; and L R Jones (1926) at Wisconsin initiated a new field of research on the effects of soil temperature and moisture and other environmental factors upon the epidemiology of the disease.

De Bary (1886) in his last work pioneered research on the physiology of parasitism. De Bary worked on *Sclerotinia sclerotiorum* and the disease it causes to carrots and other plants. He demonstrated that the pathogen killed the cells of the host in advance of the invading hyphae of the pathogen. He found that juice from rotted tissue could rapidly break down healthy host tissues. The capacity to break down tissues was completely lost after boiling. L R Jones did pioneering work on pectic enzymes secreted by soft rot bacteria. William Brown's papers on the physiology of parasitism form the foundation of further researches in the role of enzymes in plant pathogenesis. After the Second World War, mainly on the initiative of the American, Japanese, Soviet, English and Indian Researchers, the so-called physiological era commenced. The outstanding names of this era are J C Walker, K O Muller, J G Horsfall, A E Dimond, L P Miller, R G Ovens, T Akazawa, I Uritani, W H Fuchs, E Gaumann, R K S Wood, T S Sadasivasn, H E Wheeler, J M Duniway, J A Kuc, F Fric, G F Pegg, R Heitefuss, R P Scheffer, K Rudolph, D F Bateman, R N Goodman and I A M Cruickshank.

Genetics of The Host and The Pathogen

In England, in as early as 1905, R H Biffen began breeding cereals resistant to yellow rust. The genetics of the host plant and pathogen were studied extensively with regard to the inheritance of resistance and virulence, when Biffen's rust resistant varieties succumbed to infection in Australia, and the fact of genetically bound disease resistance was doubted. E C Stakman, in as early as 1914, contributed valuable information on delineating and cataloguing physiological races of the pathogen. It has been demonstrated by crossing formae specialis (Stakman *et al.*, 1930) and pathotypes (Newton *et al*., 1930) of *Puccinia graminis* that the virulence of the pathogen is also inherited, according to Mendel's laws. The same is true for various other pathogens (Person and Sidhu, 1971). The demonstration by Biffen (1905) of the Mendelian nature of the inheritance became the starting point for the systematic genetic analysis of resistance. An increase in the knowledge of the sexuality of fungi by Blakeslee (1904), Craigie and Dodge (1927) enabled a genetic study of pathogenic fungi. Gaumann (1946) proposed that in many host–parasite combinations plants remain resistant due to hypersensitivity. Flor (1955) explained host parasite interaction in flax rust caused by *Melampsora lini.* He gave a gene for gene hypothesis to explain this reaction. Van der Plank (1963, 1968) gave the concept of horizontal and vertical resistance in plants. Other pioneers in the field of breeding disease resistant varieties are W A Orton and LR Jones of the USA, and B P Pal and K C Mehta of India.

Fungicidal Research

The earliest known fungicide is sulphur. The history of the development of fungicides is fascinating. In the 20th century, several biochemists and organic chemists along with plant pathologists started investigations on this subject. Fungicides in the 20th century, especially in the 1930 period, were mercury, or Bordeaux mixture. From the Bordeaux mixture developed the inorganic insoluble copper fungicides, such as cuprous oxides or oxychlorides. The emphasis shifted from the oxides of copper to the organic fungicides, the organo-mercurial, the dithiocarbamates and bisdithiocarbamates. W H Tisdale and I Williams (1934) revolutionized the study of organic fungicides by discovering alkyldithiocarbamates. Alkylene bisdithiocarbamates were discovered by Dimond, Heuberger and Horsfall in 1943. Interest in the chemotherapy of plants has been generated and works on an interesting group of compounds, such

as chloranil and systemic fungicides and antibiotics, is of current interest to workers in this field. The mechanism of fungicidal action has been explored by Horsfall (1945, 1956), Sisler and Cox (1960), Sisler (1969), Rich (1960), Owen (1963) and others. In 1956 J G Horsfall published an authoritative book entitled *Principles of Fungicidal Action*. An informative book on systematic fungicides was edited by R W Marsh in 1972. Fry (1982) published a very useful book *Principles of Plant Disease Management*. Knight *et al.* (1997) have reviewed the "Rational and Perspectives on the Development of Fungicides." McManus *et al.,* (2002) have discussed "Antibiotic Use in Plant Agriculture". Staub (1991) has critically discussed all aspects of "Fungicide Resistance: Practical Experience with Anti- Resistance Strategies and the Role of Integrated Use" .

Environment in Relation To Plant Disease

L R Jones pioneered the study of the effect of soil temperature on cabbage yellows at Wisconsin. He developed soil temperature tanks to see the effect of soil temperature on the development of disease. For the type of experimental approach, a thermostatically controlled water bath was devised into which water tight vessels containing plants growing in soil or other substrate could be inserted. This apparatus is known as the Wisconsin soil temperature tank.

Before the acceptance of the germ theory in relation to plant disease, the environment was considered to be the major factor. It is now well known that in cases where a parasitic micro-organism is part of the causal complex of a disease, the environment plays an important part. Experimental studies in disease development in relation to various environmental factors have now become quite extensive and the predisposing factors are considered to play an important role in the development of any disease.

Nature of Disease Resistance

The fact that many plants show a natural resistance to disease has long been used by plant geneticists in their breeding programmes. Rapid advances in modern biochemistry have now made it possible for plant pathologists to investigate the biochemical basis of this resistance. Valuable contributions in this field had been made by G L Farkas and Z Kiralay (1962), J Kuc' (1963), I Uritani (1963) and other who investigated the role of phenolic compounds in natural chemical resistance.

The biochemical nature of induced resistance is still shrouded in obscurity. It is an interesting and important phenomenon in both virus and bacterial diseases. A significant discovery by Loebenstein showed that as a consequence of viral infection, interferon-like substances are produced in the host plant. In infections by animal viruses, similar antiviral substances appear in the host plant. Müller (1956) and later Cruickshank (1963) proposed that in the reaction to infections caused by microbes, a mechanism, leading to the formation of antibody-like materials, operates in plants. This recognition led to a demonstration of the substances called *phytoalexins* which are currently known to be synthesized post-infectionally in the host plant and have a role in the tissue resistance phenomenon. An ever increasing body of research on this class of compounds that has been accumulating during the last few decades (Cruickshank, 1963; Kuc', 1972, 1995) but more convincing evidence as to how phytoalexins play a deciding role in host pathogen interaction, leading to resistance, is still lacking.

Biochemistry and Physiology of The Diseased Host Plant

In recent years, much of the earlier symptomatological approach to problems of plant disease has given way to an integrated study of cause and effect. It is now becoming increasingly clear that a sufficient knowledge of plant physiology and biochemistry is essential for interpreting the metabolic changes in plants during pathogenesis. As a matter of fact, the biochemical and pathological trends of research started about 55 years ago and were founded on the physiological research of host–parasite relations. These modern trends of research intend to unravel the physio-biochemical processes of the disease plant (such as photosynthesis, respiration, nitrogen metabolism and growth). They wish to determine the true causes of cell-proliferation, the possibilities for the inhibition of virus multiplication, the mechanism of fungal toxicity; the mode of action of toxins; the role of hormones or hormone-like substances in pathogenesis, as well as to elucidate the virus biosynthesis. The importance of extracellular enzymes in the etiology of the disease is emphasized by workers currently interested in this area of investigation.

Molecular Biology of Pathogenesis and Induced Systemic Resistance

The present era of plant pathology has seen much progress in molecular biology of pathogenesis and induced systemic resistance (Vidhyasekaran, 1998). Recent advances in molecular plant pathology have paved the way for development of innovative techniques to manage crop diseases. Crop plants are endowed with numerous defense genes which encode various proteins responsible for synthesis and accumulation of defense arsenals in plants. The defense genes are involved in the production of antimicrobial compounds like phenolics, phytoalexins, pathogenesis related proteins (PR Proteins) and active oxygen species are involved in reinforcement of cell wall by accumulating hydroxyproline rich glycoproteins, callose, and lignin and wall phenolics. Many of the proteins produced by defense genes are enzymes but some of them are not enzymes. Some of them show antifungal activity, some show elicitor-releasing activity, some increase mechanical strength of the host wall and some others are involved in release of second messengers and systemic signals (molecules). It is now believed that signals are required to activate defense genes which are commonly present in both susceptible and resistant plants. Signal molecules have been detected in fungi (Schaffrath *et al.*, 1995; Vidhyasekaran, 1988a,b, 1993, 1997 and 1998), bacteria (He *et al.*, 1993) and viruses (Culver and Dawson, 1991). These are produced by pathogens as well as non-pathogens and are released by the host enzymes when these pathogens come into contact with plants. Signal transduction system plays a key role in disease resistance. Thus the use of molecular genetic techniques has provided new insights into how plants defend themselves against attack. In turn this new understanding is suggesting novel ways in which plant diseases may be controlled.

Tissue Culture in Plant Pathology

Tissue culture has had the widest impact on the study of "crown-gall". It has enabled plant pathologists to grow organs, tissues and free cells alive *in vitro* for almost an indefinite period. In the words of White (1968): "Tissue culture is a method of simplifying organisms in order that they may be handled more effectively". In the early 1920s an off-shoot of conventional tissue culture and organ culture made its modest appearance and has since established itself as a method of choice in many areas of plant physiology

and plant pathology, biological and medical research, biochemistry of drugs, antibiotics, hormones, virus research, etc.

Biotechnological Approach Towards Disease Control

One of the frontier areas of plant pathology is the biotechnological approach towards plant disease control. The genetic modification of organisms and application of modern techniques to study cellular and molecular processes are now expanding our knowledge of interactions between organisms and allowing a greater degree of control over biological processes. Specific genes may now be transferred between plants of different cultivars, lines, and species, from microbes to plants and from plants to microbes using suitable vectors such as plasmids, to integrate new characters into the plant genome (Issac, 1992). Transgenic plants are current interests of both plant pathologists and agriculturists throughout the world.

Ecological Studies of Soil-Borne Fungal Plant Pathogens

Initially it was thought that knowledge of the life cycle of a plant parasite was sufficient to ensure its speedy control. Pathologists have now realized that a wider outlook is required which embraces entire dynamics of microbial ecology, and that knowledge of life cycles is only a prerequisite to the proper understanding of a soil-borne fungus pathogen in relation to its environment. The surroundings of an organism in the soil are biologically complex and the interactions of the organisms themselves produce balance. The most important worker in the field of root disease investigations was Dr. S D Garrett. To paraphrase Garrett (1970), his subject has opened for him, and for others too, a window upon a world wider than that of the diseased plant. This world is that of the living soil, which is the source of all terrestrial plant life, and therefore, of direct concern to every human being and of interest to every biologist. Inside the soil, many kinds of living organisms are interacting continuously with one another and with the root systems of green plants. Thus, diseases of the root system cannot be studied in isolation as a simple interaction between the root system and the pathogen; hence, the root-disease investigator should be concerned with everything in the soil, such as changes in the soil texture, temperature, moisture, pH and other biological effects.

Bacteriology

Thomas J Burrill (1839–1916) of the University of Illinois, USA, was the first to describe a bacterial disease of plant in 1878 and he showed that the fire blight of apples and pears is caused by the bacterium known as *Erwinia amylovora.* Several other plant diseases were described shortly afterwards. Wakker (1883) showed that the yellow disease of hyacinth is caused by a bacterium. Savastano (1887) worked on the olive knot disease and EF Smith worked on the bacterial wilt of cucurbits. Smith is regarded as the most outstanding man who used the best available methods and standards in the study of bacterial diseases of plants and in establishing the modern bacteriological trend in plant pathology. Smith's name is still remembered as he resolved the controversy with the German bacteriologist, Alfred Fisher (1897, 1899), who did not think that bacteria were the primary cause of disease in plants. The subject of bacteriology has now developed considerably and has its own techniques and specialists in the field.

Landmarks in the development of Phytobacteriology have been discussed in 1984 by Starr and emerging problems in plant bacteriology have been critically discussed by Verma (1992). Verma *et al.,* (1994) have reviewed work on "Plant Diseases Caused by Bacteria in India" in book entitled *Botany in India—History and Progress*. In India, in 1948 a school of bacteriology was established at Pune by M K Patel. Chand (1989) had discussed "Phytobacteriology in India—Past, Present and Future". Gopal Krishanan and Verma (2006) have reviewed the significant achievements and current status in bacteriology in India.

Virology (Viruses, Viroids and Prions)

The German born Adolph Mayer (1886) worked in Holland on a serious disease of tobacco (tobacco mosaic) and this work can be said to be the alpha of virology. He showed that the juice from infected plants, if applied to healthy plants, could reproduce the disease. In the same way, EF Smith (1891) showed that the peach yellows was a contagious disease and could be bud-transmitted. In 1862, Dimitri Ivanowski, a Russian, showed that the causal agent of tobacco mosaic could even go through a chamberland filter that withholds bacteria. It was Beijerinck (1898), a distinguished Dutch microbiologist, and Professor of Microbiology at the Technical University of Delft, Holland, who demonstrated that it could pass through porcelain filter and diffuse through an agar agar membrane and concluded that the tobacco mosaic was caused by a non-corpuscular *contagium vivum fluidum* which he called virus. In 1935, the first major contribution was made regarding the nature of viruses when Stanley obtained a crystalline protein which he thought to be gamma globuline. It was in 1938 that Bawden and Pirie discovered the real nature of the tobacco mosaic virus. In 1956, Gierrer and Schramm proved that the nucleic acid fraction of the virus is actually the infectious agent. Within the attacked cell, the virus somehow mobilizes nitrogenous and other compounds of the plant and replicates itself. The relationship between an insect and a virus was discovered by a Japanese farmer, Hashimoto, who worked in 1894 with the rice dwarf disease and the leaf hopper *Nephotettix apicalis* var. *cinticeps*. This information was however not available to the western world for quite some time (Smith, 1974). About (1907), the three American workers—Ball, Adams, and Shaw—proved the connection between the curly top of sugar beet and the leaf hopper *Eutettix tenella*.

During the last few decades, much more information of the relationship of plant viruses with their vectors has accumulated. It is now clear that in addition to the insects which transmit plant viruses, other organisms such as mites, nematodes, worms, and fungal spores have all been associated as vectors. Other advances in the study of plant viruses include an increased knowledge of incomplete viruses and the discovery of satellite viruses (Kassanis, 1966) and 'viroids' (Diener and Raymer, 1967). Potato spindle tuber virus (Diener and Raymer, 1967) and the rice dwarf virus (Hiura *et al.*, 1966) are shown to contain double stranded RNA. There are termed as viroids. Viroids differ from viruses by the absence of a dormant phase (Virions) and by genomes that are much smaller than those of viruses. The algal viruses which form a very distinct group of DNA viruses are another important and interesting field of research.

The rod-shaped tobacco mosaic virus was first reconstituted from its protein and RNA components (Fraenkel-Conrat and Williams, 1955). Since then, several spherical viruses have been reconstituted. "The tide of molecular biology has now reached the shores of plant virology", says Dr. Kenneth M. Smith; and more precise knowledge of the ultra structure of viruses has been obtained by electron microscopy and X-ray diffraction, techniques. A photographic rotation technique has been developed to increase the details of high resolution electron micrographs. The mechanism of replication of the viral

nucleic acid and other constituents within the cells is being looked into critically, and in the case of the tobacco mosaic virus, the work of K K Reddi (1964) stands out prominently. By modifying the purine bases of RNA, Mundry (1959) succeeded in inducing TMV mutations; thus, he was the first to elucidate the biochemical mechanism of mutation. Varma and Ramachandran (1994) have given a historical perspective on the "Plant Viruses" research work done in India and discussed histopathology, ultra structural changes in virus infected tissues, physiology of the diseased plant, vector transmission, transmission through seeds, and electron microscopic diagnosis of viral diseases, serodiagnosis, epidemiology and management of plant viral diseases.

Prions

Prions were first proposed in 1972 by Prusiner who was later awarded the Nobel Prize in physiology and medicine in 1997. Prions are at first normal small protein molecules which are produced in nerve cells and other cells of the brain. Prions are infectious proteins which cause diseases in animals and humans. Prions have not been observed in plants or other organisms.

Mollicutes

Before 1967, only fungi, bacteria, nematodes and viruses were considered as main incitants of plant diseases. However, in as early as 1898, Nocard and Roux had discovered another agent of diseases caused in animals which was similar to viruses in size but which could be cultured on artificial media. This group was known as mycoplasma. *Mycoplasma mycoides*, the causal agent of infectious pneumonia, had been cultured by that time. In 1967, Japanese scientists Doi and colleagues found that mycoplasma-like organisms (MLOs) could be responsible for most of the disease of the yellows type which were earlier thought to be caused by viruses in plants.

Mycoplasma-like organisms found in certain leaf hopper-transmitted diseases are morphologically similar to animal mycoplasma, but Ploaie and Maramorosch (1969) were unable to grow the leaf hopper organisms in culture. The cells of mycoplasma lack a cell wall and hence are pleomorphic. They do have a cell membrane (10 nm unit membrane). They are very small in size (from 100 nm to 1 mm in diameter) and because of the lack of cell walls, can easily pass through bacteria proof filters. They contain ribosomes and thin stranded DNA and are sensitive to antibiotics of the tetracycline group. The reader may refer to an excellent review of *Mycoplasmas as Plant Pathogens: Perspectives and Principles* by Hampton (1972). There are some other reviews, such as by Ghosh and Raychaudhuri (1972), Whitcomb and Davis (1970), Whitcomb and Tuly (1989) and Paul, Khurana *et al.,* (1988). Plant Mollicutes and bacteria like organisms with a historical perspective have been reviewed by Varma and Ahlawat (1994). Some of the yellows types of diseases (YTDs) are caused by Spiroplasmas which have a helical structure (Bove *et al.,* 1973) which resemble Mycoplasmas and belong to the same class of Mollicutes. Most however are round to elongate but are not spiral and are now called as Phytoplasmas. Mycoplasmas are saprophytic or cause diseases in animals. Execpt for the genus *Spiroplasma*, mollicutes that infect and cannot be grown in culture are called as Phytoplasmas. So far no YTD causing Mycoplasma has been cultured on synthetic medium making their identity tentative, hence the name MLO. Similarly, bacteria like organisms (BLOs) which cause important diseases like citrus greening, cannot be cultured on synthetic medium.

Nematology

The first report of nematode association with a plant disease was made in England by T Needham in the year 1743 AD with some microscopical observations on the farina of red lilly, and of worms discovered in smutty corn. He described the wheat gall nematode now known as *Angunia tritici*. C Devaine almost 100 years after Needham described the life cycle of *Angunia tritici*. There are some important landmarks in the development of phytonematology. For example the root nematode was the second phytonematode to be discovered and reported. In England, M J Berkeley (1855) noticed galls on the roots of Cucumber plants growing in the green house. However, the credit goes to Cornu who in 1879 for the first time made a specific mention of a root knot nematode which he described under the name *Anguillula mationi*. *Ditylenchus dispaci* a stem nematode was first reported by Kuhn in 1857 while beet cyst nematode was first noticed in Germany by H Schacht in 1859. N A Cobb studied the structure of many nematodes and classified them and coined the term nematology.

The association of nematodes with diseases caused by other organisms had been noted as early as 1892 when Atkinson reported that Fusarium wilt of cotton was more severe in the presence of root nematodes. Similarly the bacterial wilt of tomato was facilitated by root knot nematodes. According to Singh (2008) an outstanding discovery was made by Hewitt in 1858 that the grapevine fan leaf virus was transmitted by a nematode. Several plant viruses are transmitted through soil by means of nematodes.

In India, Butler (1906) reported for the first time root knot nematode on black pepper in Kerala and also reported *Ditylenchus angustus* on rice in Bengal. Hutchinson (1970) also reported for the first time Tundu disease of wheat caused by *Angunia tritici*. Dastur (1936) for the first time has discovered the rice leaf nematode (*Aphelenchoides besseyi*). In India, research work on nematode parasites of insects was started by Basir in 1940 and he has done a lot during 1940–70, which was followed by Siddique in 1960 onwards, Parveen and Jairajpuri (1980–90), Rijvi and Jairajpuri 1995, Ganguly 2000, Ali 2000 onwards mainly in North India. Professor Abrar Khan at Aligarh Muslim University laid the first foundation for nematode diseases of vegetable crops and their control. In South India, significant contributions and series of reports were made by Narayan Rao (1962–95) followed by Meena Kumari (1965–75), Hussaini 1995 onwards and others. Gopal Swaroop (1990) discussed critically "Nematode–microorganism interaction—An Appraisal." P C Trivedi at Rajasthan University, Jaipur, has contributed significantly on various aspects of plant parasitic nematode diseases and biocontrol measures. The centres working on entomopathogenic nematodes in India are AMU, Aligarh; Allahabad University, Allahabad; Osmania University, Hyderabad.

Protozoans as Plant Pathogens

Lafont in1909 reported for the first time trypanosomatid protozoa in the latex bearing cells of the family Euphorbiaceae which were thought to parasitize the plant latex without causing disease on the host plants. In 1931, Stahel reported for the first time that the flagellates were found to be infecting the phloem of coffee trees, causing abnormal phloem formation and wilting of trees in Surinam, Guyana, Brazil, Salvadore and Colombia. There are now large numbers of diseases caused by trypanosomatids such as Emply root of Cassava, wilt and decay of red ginger (*Alpinia purpurata*) an ornamental plant.

Some of the important plant pathologists of the present era are G A Zentmyer of the University of California, Riverside, USA (Phytophthora diseases); C E Yarwood of the University of California,

Berkeley, USA (powdery mildews); David Gottlieb (antibiotics); R S Ludwig of the Canada Department of Agriculture (toxins and plant diseases); N E Borlaug (composite varieties); J W Eckert (chemical control of post harvest diseases); K Maramorosch of the Waksman Institute of Microbiology, Rutgers University, New Brunswick, Canada (viruses and mycoplasmas); C S Holton of the US Department of Agriculture (smut fungi); G C Kent (cereal pathology); T Mizukami and S Wakimoto (epidemiology and control of leaf blight of rice); Vander Plank of Plant Protection Research Institute, Pretoria, South Africa (plant disease epidemics and control and principles of plant infection—theoretical aspects mainly); S D Garrett of Cambridge University, England (soil-borne root diseases and their control); HS Sisler of the University Maryland, USA (fungicides); N F Robertson of the University of Hull, England (growth processes in fungi); C J Hickman of the University of Western Ontario, Canada (biology of Phytophthora zoospores); M E Gallegly of West Virginia University, Morgantown, USA (genetics of host pathogen interaction); Ralph Baker of Colorado State University, Fort Collins, USA (mechanism of biological control of soil-borne pathogens); R N Goodman of the University of Missouri, USA (bacterial pathogens and physiology and biochemistry of plant diseases); Charles E Bracker of the University of California, Davis, USA (ultra structure of fungi); R P Scheffer of Michigan State University, East Lansing, USA (host specific toxin); John L Lockwood of Michigan State University, East Lansing, USA (soil fungistasis); P E Waggoner, Director Connecticut Agriculture Research Station, New Haven (microclimate and plant disease); J C Walker of the University of Wisconsin (bacterial diseases and physiological plant pathology); D F Bateman of Cornell University, USA (Enzymes and tissue degradation); J M Daly of the University of Nebraska, Lincoln, USA (the physiology of diseased plant); H E Wheeler of the University of Kentucky, USA (permeability alterations in diseased plants); I Uritani of Nagoya University, Japan (protein metabolism); G F Pegg of Wye College, University of London (growth regulatory metabolism of the diseased plant); J A Kuc' of the University of Ketucky, USA (phytoalexins); A H Ellingboe of Michigan State University, USA (genetics of host parasite interaction); R Heitefuss of the Institute fur pflanzenpathologie und Pflanzenschutz der Universitat, Gottingen, West Germany (nucleic acid metabolism of the diseased plant); R K S Wood of Imperial College, London (toxins and enzymes); F Grossman of the University of Hohenheim, West Germany (systematic fungicides); I A M Cruickshank of the Division of Plant Industry, CSIRO, Canberra, Australia (phytoalexins); Michael Shaw of the Department of Biology, University of Vancouver, Canada (host–parasite relationships in rusts); G Ovens of the Boyce Thompson Institute for Plant Research, New York, USA (chemistry and physiology of fungicidal action); J Dekker of the Agricultural University, Binnenhaven, Netherlands (acquired resistance to fungicides); GA Strobel of Montana State University, Bozeman, USA (toxins); S Akai of Kyoto University, Kyoto, Japan (host–parasite relationships); etc.

History of The Development of Plant Pathology in India

Bagchee (1939) was the first to review the work on Indian forest mycology and plant pathology in India. Das Gupta (1958) gave the history of plant pathology and mycology in India till that period. History of plant pathology in India has been reviewed by Raychaudhuri *et al.,* (1972) and Raychaudhuri (1991). Reminiscences and observations on the growth of plant pathology in India had been given by Saksena (1977). Mehta (1963) in his presidential address had discussed plant pathology in India—past, present and prospects. Role of plant pathology in India in crop production had been critically discussed by Raychaudhury (1968). Grover (1975) had critically discussed plant pathology in India—introspection

and prospects. Plant pathology, past, present and future in India has been discussed by Mehrotra and Aggarwal (1994). Nagarajan (2000) has discussed "Plant Pathology and Indian Agriculture—Past, Present and Future". Chahal *et al.,* (2006) have given an overview of "One hundred Years of Plant Pathology in India". Chahal (2010) has reviewed in brief the historical development of plant pathology in India and a road map to meet future needs.

Patel and Kulkarni (1958) had reviewed the work of bacterial plant pathology in India in 1958. Phytobacteriology in India—past, present and future prospects were reviewed by Chand (1989). Verma (1992) has analysed the emerging problems in plant bacteriology. Gopalkrishnan and Verma (2006) have reviewed the significant achievements and current status in Bacteriology in India till 2006.

Chenulu (1984) had discussed in his presidential address to the members of Indian Phytopathological Society the status of plant virology in India, while Reddy (1990) had discussed the development virology in developing countries of the world.

Some of the important events in the development of plant of pathology in India are as follows:

1. Barclay (1886) Uredinales of Simla.
2. Publications of two journals "Memoirs of the Department of Agriculture" and Agriculture Journal of India.
3. Appointment of EJ Butler in 1901 as a Cryptogamic Botanist to the Government of India and then later transferred to the then Imperial Agricultural Research Institute established in Pusa in 1905 as the first Imperial Mycologist. Butlers work on Phytophthora blight of potato from Assam, Bengal and Eastern Himalyas and Nilgiris in 1903 and fusarial wilt of pigeon pea caused by a new species of *Fusarium* in 1906 and 1910 are pioneering contributions in phytopathology in India. Along with Hayman he published a note in 1906 in the Memoirs of the Department of Agriculture on wheat rust problem. Elsewhere with H P Sydow he commenced a series of articles which ultimately led to the Cryptogamae Indiae Orientalis (National herbarium) in 1905 and the publication of Fungi of India with G B Bisby in 1931. This is being constantly updated by Vasudeva (1960, 1962), Bilgrami *et al.,* (1979, 1981, and 1991), Tandon and Chandra (1964), Sarbhoy *et al.,* (1974, 1994) and Tilak and Ramchandran (1970). His (E.J. Butler's) book *Fungi and Diseases in Plants* became a classic for Indian phytopathologists.
4. Among Butler's contemporaries, F J F Shaw's contribution in 1912 along with S.L Ajrekar on the genus *Rhizoctonia* with special reference to its morphology and parasitism are one of the landmarks in plant pathological research in India (Raychaudhri, 1991).

The Following are important plant pathologists of past and present era in plant pathology in India.

D D Cunningham, (1889) identified the causal organism of red rust of tea in Assam caused by *Cephaleuros virescens.* W McRae, (Bud rot of palm) made detailed study of Phytophthora diseases of rubber in South India. S Sundaraman (1922) reported *Pyricularia oryzae* on rice for the first time from India; he described this disease for the first time from India. B B Mundkur (1948) laid the foundations of India Phytopathological society and later published a book in 1949 entitled *Fungi and Plant Disease*. Other significant contributions of Mundkur include the monograph on *Ustilaginales of India* and the *Supplements to Fungi of India* and *Genera of Rusts* in collaboration with Dr. M J Thirumalachar. K C Mehta (1940) published the Monograph entitled *Further Studies on Cereal Rusts in India* and did pioneering work on the wheat rust problem in our country. R Prasad (his contributions include the cereal rusts, linseed rusts, Alternaria blight of wheat caused by *A. triticina*). J F Dastur (studies on the genus Phytophthora, cotton wilt, foot rot of betel, ripe rot of bananas, stem breaking of cotton, pink disease of citrus, anthracnose and *Nematospora* on cotton, black thread of *Hevea* are some of his important contributions).

Some of the other important plant pathologists of India, past and present, are as follows: S N Das Gupta (black tip of mango), B L Chona (sugarcane diseases), R S Vasudeva (root rot of cotton, and virus diseases of potato), J C Luthra and A Sattar (gram blight and wilt, solar heat treatment against loose smut of wheat), B N Uppal (cereal smuts, downy mildews of millets and maize), M Mitra (Helminthosporium diseases, discovered the new bunt of wheat caused by *Neovossia indica*), K P V Menon (coconut diseases), S Y Padmanabhan (diseases of rice), S B Chattopadhyay (diseases of rice and revised the book entitled *Fungi and Plant Diseases* originally written by B B Mundkur), K Bagchee (Forest Pathology), B K Bakshi (Forest Pathology), M J Thirumalachar (Monograph on "Ustilaginales of India", Uredinales of the World, Cercospora, *Physoderma, Cephalosporium* etc. At Hindustan Antibiotics Ltd., Pimpri, Pune discovered a number of antibiotics such as Haymycin, Aureofungin, Antibiotics 326, Antiamoebin etc.). M J Narasimhan (Phytophthora diseases, sandal-spike, downy mildews of grasses and cereals, heteroecism in rusts, entomogenous fungi and biocontrol of insect pests).

T S Sadasivan (established the school of Mycology and Plant Pathology at Madras University, Madras [now Chennai] and it was here that the foundations of research on soil borne pathogens was laid and the physiology of hydromycotic wilting specially cotton wilt was investigated and the control of wilt diseases by trace element amendment was tried), R Kalyansundram (Wilt diseases, Fusaric acid as a wilt toxin), C S Venkata Ram (Tea diseases), R N Tandon (Physiology of fungal pathogens and post harvest diseases of fruits and vegetables), V Agnihothrudu (Tea diseases), R P Ashthana (Control of smuts of millets), C V Subramanian (Wilt of cotton), N Prasad (Fusarium diseases and Phytophthora blight), D Suryanayana (Downy mildew diseases), T S Ramakrishnan (contributions on genera *Pythium, Phytophthora, Colletotrichum* and the rusts and his monograph on "Diseases of millets" published by I.C.A.R., M K Patel (Bacterial diseases, advocated a new family Phytobacteriaceae to include all phytopathogenic bacteria), A P Misra (Helminthosporium diseases), S P Raychaudhuri (Virus diseases of crop plants), P R Mehta (Diseases of cereals and millets and plant protection), H K Saksena (Rhizoctonia diseases and gram rust), M K Hingorani (Bacterial diseases), L M Joshi (Wheat rusts), M N Kamat (Ascomycologist, control of plant diseases and author of Introductory plant pathology), R K Saksena (Cytology of Pythiaceae and ecology of soil fungi), S B Saksena (Mycologist of eminence, discovered *Saksenea* which is a human pathogen, root diseases and biocontrol), D N Srivastava (Bacterial blight of paddy and diseases of fruits and vegetables), S Sinha (Rusts), Y L Nene (Khaira disease of rice and authored *Fungicides in Plant Disease Control*), M M Payak (Maize diseases), S B Mathur (Seed Pathology), V P Bhide (Bacterial diseases), S P Kapoor (Virus diseases), Pushkarnath (Potato diseases), G Rangaswami (bacterial diseases, authored *Diseases of Crop Plants in India, Bacterial Plant Diseases in India*), A Mahadevan (Physiological plant pathology), Y S Kulkarni (Bacterial diseases of plants), H C Arya (Downy mildew of pearl millet, axenic culturing of *Sclerospora graminicola),* BP Chakravarty (Bacterial diseases), D Ganguly (Helminthosporium disease of rice), B B Nagaich (Virus diseases), R P Purkayastha (Phytoalexins and induced resistance), V P Agnihotri (Pythium, Sugarcane diseases), V Muniyappa (Virus diseases), Anupam Varma (Virus diseases), T K Narianai (Virus diseases), J P Verma (Bacterial diseases specially bacterial blight of cotton), R P Thakur (Diseases of millets), H S Shetty (Induced resistance in pearl millet), Chtreshwar Sen (Biocontrol of plant diseases), Hari Om Agrawal (Virus diseases), S Mukhopadhyay (Virus diseases), A N Mukhopadhyay (Biocontrol of plant pathogens), K S Bhargava (Virus diseases), R K Hegde (Phytophthora diseases of arecanut, coconut and *Piper nigrum*), Kishan Singh (Sugarcane diseases), Abrar Khan (Nematode diseases of vegetables and control), M V Pagvi (Indian Ustilaginales, *Physoderma, Tapharina maculans* and rusts), V R Reddy (Virus diseases), KS Bilgrami (Mycotoxins), J N Chand (Bacterial diseases), H S Sohi (Diseases of ornamentals and fruit

crops and mushroom culture), S K Saxena (Nematode diseases), K M Safeeulla (Diseases of pearlmillet), R S Singh (Organic amendments and control of soil borne pathogens. Author of the books *Plant Diseases* and *Diseases of Vegetable Crops*), M V Naydu (Virus and bacterial diseases), C D Mayee (Groundnut diseases, Sunflower diseases and Plant disease forecasting), Dharam Vir (Fungicides), M S Chatrath (Fungicides), S J Singh, (Virus diseases), R J Singh, (Rice diseases), Y S Ahlawat (Virus diseases), K C Alexander (Sugarcane diseases), R S Dwivedi (Soil mycologist and root diseases), M W Khan (Powdery mildews), S Maiti (Betel vine diseases), S M Paul Khurana (Virus diseases of potato), G S Shekhawat (Bacterial diseases of potato), Uma Kant (Insect galls and other teratomas), A Narian (Minor diseases of rice and toxins), R Jeyarajan (Biocontrol and Virus diseases), V N Pathak (Post harvest diseases of fruits and vegetables), A K Sarbhoy (Fungal taxonomist and *Rhizoctonia* diseases), B L Jalali (Mycorrhizae and integrated disease management), D V Singh (Bunts of wheat), J S Grewal (Diseases of Pulse Crops), K G Mukerji (Mycorrhiza and biocontrol), D J Bagyaraj (Mycorrhiza and biocontrol), C Manoharachary (Post harvest diseases and mycorrhiza), Sudhir Chandra (Post harvest pathology, mycorrhiza and biocontrol), J S Chauhan (Diseases of oil seed crops), Y R Sarma (Diseases of plantation crops), P K Koshy (Nematode diseases in plantation crops), Bharat Rai (Wilt diseases of pulse crops), B P Singh (Virus diseases), S S Sokhi (Vegetable diseases), V K Gupta (Diseases of apple), RK Grover (fungicides) ,P Ramarao (soil-borne diseases), P Vidhyasekaran (Physiological plant pathlogy), R A Singh (Rice diseases), J S Jhooty (powdery mildews), B M Singh (Plant disease management), U P Singh (diseases of pulse crops), V Muniyappa (viral diseases), S S Chahal (cereal and pearl millet diseases), S S Aujla (wheat diseases), T N Lakhanpal (tree diseases and ectomycorrhiza), B P Singh (potato diseases), R D Parasher (bacterial diseases), Gopal Swarup (nematology), Amerika Singh (Wheat diseases), K Jindal (bacterial diseases), J L Kaul (diseases or fruits), BN Johri (mycology and mycorrhiza), W M Khan (powdery mildew), Satyavir (sugarcane diseases), Y S Ahlawat (viral diseases), D Kumar (mycorrhiza), S B Sharma (nematode diseases), D Lalitha Kumari (fungal biotechnology of antagonistic organisms), M N Khare (pulse diseases), B Chandra Mouli (tea diseases), V Mariappan (rice diseases), V R Mali (viral disease), B L Chopra (disease of cotton), S P Kapoor (viral disease), K M Vyas (soil borne diseases), D K Arora (soil borne diseases and biocontrol), R K Jain (viral diseases), T S Thind (fungicides and Phytophthora diseases), RK Khetrapal (plant quarantines and viral diseases), O P Mall (wilt and mycotoxins), Sudha Mall (potato diseases and biological control), D P Tiwari (betel vine disease), R K S Chauhan (mycotoxins and phytoalexins), Sashi Chauhan (mycotoxins), Geeta Sumbali (post harvest diseases and mycotoxins), Arun Arya (post harvest diseases and mycorrhiza), N S Atri (mushroom cultivation), P J Singh (disease of wheat and gram), P C Trivedi (nematode diseases) M.P. Srivastava (fungicides and plant disease clinics) and A Dixit (control by botanicals).

Trends in Teaching and Training in Plant Pathology in India

Plant pathology in India began with the establishment of the Agricultural Research Institute in 1905 at Pusa, Bihar, now called the Indian Agricultural Research Institute and located in Delhi; and the appointment of E J Butler as the first Indian mycologist. The first Indian Universities that were established in 1857 at Calcutta, Madras, and Bombay emphasized on the taxonomy of fungi. Plant pathology as a university science became established at Lucknow, Allahabad, and Madras Universities (founded in 1921, 1887, and 1857 respectively), only in the 1930s. There are now 29 agricultural universities and 4 deemed universities in our country. Work on plant pathology is carried out at various agricultural research institutes in the country, agricultural universities, and botany departments of traditional universities.

The need to bring about a rapid increase in food production in the post-independence period necessitated re-examination of the prevailing system of agricultural research and education. Under the existing system agricultural research and education was managed by the state departments of agriculture. For purposes of examination and award of degrees, the colleges of agriculture were affiliated to conventional or traditional universities which also controlled the syllabi and courses of reading. It was felt that these institutions and procedures oriented to the needs of the past could not meet the requirements of the present day. It was around this time that the agricultural universities came into existence, which could integrate research and education extension with education. The Indian Agricultural Research Institute, New Delhi, was the first to adopt the new system when the present post graduate school was set up in 1958. It was in Uttar Pradesh that the first agricultural university was set up in 1960. The establishment of the agricultural universities was an important landmark in the development of agricultural education and research in our country and which revolutionized the process of education of agricultural sciences in our country including the subject of plant pathology. In the beginning, for a master's degree in plant pathology, the course work-content emphasized on mycology and major diseases caused by fungi, bacteria and viruses. Now in addition to these courses, well-planned courses are offered in specialized areas such as epidemiology, forecasting and loss assessment, physiology and biochemistry of plant infection, soil plant disease relationships, phytopathological techniques, seed pathology, nematology, chemistry of fungicides. A student doing his master's programme or Ph.D. in plant pathology is now up to date not only in his area of specialization but also in supporting minor subjects. A young plant pathologist is now better equipped to deal with problems in the field. In these agricultural universities extension in education became an important part which brought farmers and scientists in the agricultural universities closer and made it possible to transmit practical knowledge to the farmers which led to awareness among the public of the role of plant pathological science in ameliorating problems related its plant diseases. Extension is a very important part of pathological science. It puts the scientific worker to test. With feedback from the community, the plant pathologist learns his mistakes and can rectify them. Extension plant pathology in Indian agriculture was critically evaluated by Upadhyay and Bharat Rai (1983). Extension activities in plant pathology are better organized today than they were 40 years ago. The whole concept of extension has undergone a change. Many agricultural universities hold farmers fairs and there is no substitute for developing closer links between farmers and plant pathologists. Extension pathologists are doing very meaningful and valuable work.

Trends in research and teaching in plant pathology in India have been discussed by Saksena (1977). From 1977 there has been a remarkable shift in the areas of research in plant pathology. In the beginning the emphasis was on taxonomic aspects of plant pathology. Nene (1987) has critically suggested various facets of plant pathology in India and plant pathology in 2000 AD. Chahal *et al.,* (2006) and Chahal (2010) briefly discussed "Plant Pathology in India—A Road Map to Meet the Future Needs."

Holistic Approaches For Plant Health Management

Plant diseases cause substantial reduction (nearly 40%) in yield worldwide (Oerle *et al*., 1994). According to Agrios (2005) it is conservatively estimated that diseases, insects, and weeds together annually interfere with the production of or destroy between 31–42% of all crops produced worldwide. Man in his endeavour to provide protection to plants and ensure food security or availability to ever growing population has greatly depended on pesticides, which have been used since the 19th century. It is

earnestly believed that the pesticides would still continue to be used as the most important arsenals for controlling plant diseases. However, their use has to be made more judicious, which unfortunately is not, and their reckless and indiscriminate use has created more problems. It would be worthwhile to recapitulate the events as to how pesticides became suspect and how the concept of holistic approaches to plant diseases developed. Pesticides came under serious criticism with the publication of Silent Spring in 1962 by Rachel Carson. Then in 1970 Frank-Graham too expressed similar views on pesticides in his book *Since Silent Spring*. Another publication, *Silent Spring Revisited*, by American Chemical Society in 1987 also corroborated the concerns of Rachel Carson. In order to minimize increasing hazards due to pesticides and to prevent ecological disruption and in making pest control cost effective, ecologically sound and socially acceptable, a balanced approach can be adopted by integrating pesticide with other measures of control: the IPM approach of what is common parlance is called as holistic approach. This has been suitably projected by van Embden and Peakall (1996) in their book, *Beyond Silent Spring*. The last chapter of *Silent Spring* by Rachel Carson (1962) modestly hints towards IPM, although in fact the concept of IPM was first proposed by Stern *et al.,* (1959) in a paper published in the journal *Hilgardia* much earlier. But IPM caught worldwide attention only after UNCED (United Nations Conference on Environmental Development) at Rio de Janeirio in June, 1992.

Plant Disease Clinics

According to Barnes (1994) the evolution of the plant disease clinic arose from a perceived need for an organized systematic, professional effort to assist in the identification of plant disease problems. In the United States of America for many years, most states operated a plant disease clinic through their departments of plant pathology (Agrios, 1997). Growers, country extension agents, and homeowners would send diseased plants, soil from areas infested with diseased plants, and sometimes insects, to the plant disease clinic and the pathogen or insect would be identified and some suitable control measures suggested by extension plant pathologists. Barnes (1994) has reviewed the role of plant diseases clinics in disease diagnosis and education in the North American perspective.

The plant disease clinic frequently represents “the tie that binds”, bridging various aspects of basic and applied features of plant pathology into a meaningful, functional, responsive, accountable component of agriculture (Barnes, 1994).

Earlier plant disease clinics were manned mainly by students of M.Sc. of Agricultural or Ph.D. students who would identify the diseases with the help of some basic facilities such as dissecting microscope, culture dishes, test tubes, surface sterilizing agents, and culture media. Viral disease identification was made by symptomatology; but after 1970, greater attention was paid to these plant disease clinics which have now facilities for viral disease diagnosis, host range tests, serological tests, cell inclusion identification, electron microscopy, and dot–blot assays of radioactive or color production probes. In the most agriculturally advanced countries such as the USA, modern computers with data bases, videodisc facilities, and e-mail for transmitting of the results of the diagnosis and recommendations for the management of the disease, now exist.

In our country also the plant disease clinics have now come up in almost all the agricultural universities when a modest beginning was made by Punjab Agricultural University, Ludhiana, and sometime back. Srivastava (2009, 2013) has discussed and highlighted the importance of plant health security through integrated pest management/ eco-friendly pesticides and plant health clinic to prevent huge losses of about 40 percent occurring from field to fork.

Some private plant disease clinics have come up in the United States of America. We do not have such a system so far in our country. It may be emphasized that the plant disease clinics have a significant contribution to make in the education of students of plant pathology and perform a critical function by providing diagnostic capabilities that are essentially unavailable elsewhere as well as unique and varied educational opportunities. These have an important role in the transfer of technology as far disease management is concerned, from lab to land.

According to Agrios (1991, 1997), plant pathology, unlike its sister sciences of medicine and veterinary sciences, has yet to develop a teaching and training programme that will produce practitioners, physicians, and veterinaries who can be professional enough to identify all types of cause of disease and injury to plants and of making recommendations to control or manage these disease. In some of our agricultural universities, plant disease clinics have been established based on the concept that there can be doctors in plant pathology similar to human or animal pathology.

Education and Information Technology in Plant Pathology

According to Agrios (1997) numerous advances have been made in developing, collecting, organizing and transmitting information for use by researchers, extension pathologists, teachers, and students of plant pathology. Ariena and Bruggen (1991) have discussed the importance of the computer-based diagnosis of plant diseases. Computers have provided the capacity to simulate epidemics and their modeling. Some of the facilities available now are electronic mail (e-mail), videodiscs, and use of videotapes and access to satellites to uplink and downlink television. In the western countries of the world computers are being used more and more for database management and are being made use of in diagnostic clinics. However, in our country we have to develop these areas more so that computer based diagnosis of plant disease is available not only for diagnosing a disease but also suggesting a suitable control strategy.

Innovations in Teaching Plant Pathology

The teaching environment for plant pathology is changing in both positive and negative ways. Teaching expectations are increasing and resources are decreasing but recent educational research and instructional technology offer new approaches to meet these challenges. Plant pathologists are teaching courses that may attract new students to the discipline or at least improve agricultural awareness.

Many faculty members are now using modern modes of teaching including powerpoint presentation, web pages, simulations or other software. Major constrain to using instructional technology include technical support, classroom equipment and time. Recently Schumann (2003) has reviewed this topic. Teachers are looking for more forms of active learning to engage student's interest and to encourage class participation through discussion. Active learning and application to real world problems not only give students satisfaction in their ability to use what they have learned but may also increase the likelihood of their retaining the information. Inquiry based learning, group learning and collaborative learning are strategies frequently discussed by teachers seeking to engage students more fully.

Some teachers have adapted television game shows to review sessions in plant pathology and students look forward to them. Plant pathologists have provided educational services through extension education programmes to farmers, gardeners and others. Apart from this, new digitized images are available

for teaching purposes. A image resource also is helpful for review of materials from past classes and for use when a class is missed.

The internet allows rapid access to unlimited current information related to plant pathology. Students can access this information from a computer at any location. The ease with which information can be obtained from the internet may limit the use of older books and journals because older publications are not available in digital form.

Finally it can be said that there is no one right way to teach, just as there is no one right way to learn. Highly motivated students will learn under any circumstances. An alert and dedicated teacher continually assesses the level of learning in a classroom by eliciting questions from students. No computer programme can replace these qualities of a good teacher.

Some Aspects of Applied Plant Pathology

Plant Biotechnology

It can be defined as the use of tissue culture and genetic engineering techniques to produce genetically modified plants that show new or improved desirable traits. The desirable traits or characteristics are better yield and quality, resistance to disease, pests and environmental factors like drought and salinity etc. As a result of plant biotechnology, improved crops, ornamental and forest plants have been modified genetically by scientists all over the world in the large number and continues to grow. Transformed plants become resistant by coding for enzymes that mobilize other enzymes that carry out numerous defensive functions, such as breaking down of structural compounds of the pathogen, some enzymes produced in the plants that are toxic to the growth and spread of the pathogen. These crop plants have been tested for resistance in the field with excellent results. Examples of genetic engineering of disease resistant plants include tomato, melon, tobacco, squash, and papaya that are protected from viral diseases. For example, engineering tobacco with a chimeric transgene containing sequences from two different viruses (turnip mosaic and tomato spotted wilt) resulted in new plants resistant to both viruses. Molecular biology and biotechnology have made possible the development and use of non toxic chemical substances that when applied to plant, stimulate the plant and elicit the activation of their natural defence mechanism. One such chemical Actigard where one application increases the plants resistance against fungal and bacterial diseases and second one Messenger-derived from the fire blight bacterium gene coding for the protein harpin which elicit a hypersensitive response and systemic-acquired resistance in plants.

In transforming plants, it is necessary to modify their nucleic acid by adding genetic material from another plant or a pathogen. In most cases, these nucleic acids become active, producing in the plant compounds that may be toxic to pathogen or pests. At times, these nucleic acids may find their way into other plants, making these plants also resistant to the pathogen or pest. Several kinds of plants have been engineered to produce toxins against certain pathogens; to produce vaccines against human pathogens that can be used to treat diseases of humans and animals. The fear by some people that these products will enter into human food chain and can cause some adverse effects has resulted in non-acceptability of biotechnology. That type of products has led many buyers to refuse to buy and use products produced by genetically modified organisms (GMO). Apart from this there are many more arguments against biotechnology like:

1. It would replace local varieties with GM crops.
2. It will force farmers to buy seeds from big companies.

3. It can develop pests and pathogens that can resist transformed resistant crops.
4. It threatens to upset the plant balance and through it the entire biotic balance of the environment.

Food Safety

Some of the food-borne microorganisms/pathogenic to humans like *Salmonella*, *Listeria*, *E.coli*, the protozoa *Cyclospora*, *Giardia* and *Hepatitis* A Virus reach and contaminate our food in variety of ways like:

1. Fruit juices, fruit and vegetable salads carry large number of food-spoiling bacteria and mycotoxin producing fungi.
2. Inadequate food processing procedures in processed products.
3. Long storage, unhygienic harvesting, and packaging
4. The presence of pets and animals which carry human pathogens on their bodies.

When these products are transported from one place to another, these pathogenic microorganisms multiply and expand the effects of a local contamination of food products. One of the main reasons of fears about food safety is economic. Not only have these increased the cost of control measures but at times there is also the fear of rejection of whole food product due to contamination at the point of destination because of refusal of buyers to such products. In addition to the above mentioned type of contamination of food with pathogens, there are other contaminations with pathogenic microorganisms that are resistant to antibiotics used in plants, genetically engineered plants that carry genes of chemical toxic to plants, such as *Bacillus thuringiensis* toxin, genes of activating defensive mechanism of plants, genes of edible or otherwise delivered human vaccines and antibodies (plantibodies) against human pathogen etc.

People are afraid of especially in developed countries that some of these genes are introduced in the inedible plants like tobacco. Plants with such genes may accidentally find their way into food and feed and will affect adversely the health of humans and animals. There is another form of having food and water contaminated with bacterium causing the disease anthrax or with toxic substances by terrorists.

Biological Warfare, Bioterrorism

It is defined as the use of pathogenic microorganisms that could infect people and cause disease in a large population. Biological warfare is used against enemy armies whereas in bioterrorism the purpose is to frighten and terrorize civilian population.

The best example of bioterrorism occurred in 2001 when political leaders and television news media in USA received letters through the mail containing spores of the bacterium *Bacillus anthracis*, the cause of deadly anthrax disease. In biological warfare, several countries have been experimenting with and stockpiling microorganisms that can infect and destroy important staple food crop for certain countries, e.g., rice, potatoes, wheat which could affect the availability of food and thereby survival of the people. This type of agricultural biological warfare has revolved around some important pathogens of such crops, e.g., *Phytophthora infestans* causing late blight of potato, *Puccinia graminis tritici* causing black rust of wheat, and *Magnaporthe grisea* causing blast disease of rice. If a particular crop is grown in a particular area year after year, it becomes evident that such areas become extremely vulnerable to agroterrorism. Agrios (2005) has discussed in details the applied aspects of plant pathology including food safety and biological warfare and bioterrorism.

Plant Pathology Today and Future Directions: Molecular Approaches

Recently great emphasis has been laid regarding the role of molecular approaches in disease development (Agrios, 2005). More molecular techniques have been used in viruses in comparison to fungi and bacteria because most or all genes of many viruses have been worked in detail. These genes have been isolated and excised and finally have been incorporated either to host plant for resistance or into bacteria for protein they code for are isolated and studied. Similar studies have been made in few bacterial and fungal genes coding for certain pathogenesis related (PR) proteins.

The beginning of era of molecular plant pathology was started by W Stanley in 1935 by isolating TMV as a crystalline protein which he thought to be infectious in nature. Later in 1956, Gierer and Schramm reported that the RNA was responsible for the infection and reproduction of complete virus particle and not the protein coat of TMV. In the meantime, Beadle and Tatum in 1941 proposed one gene one enzyme hypothesis. In 1942, Flor showed that in flax rust fungus (*Melampsora lini*), a single gene is responsible for pathogenicity and that the rust fungus gene corresponds to a single gene for resistance in flax plant. Later in 1953, Watson and Crick showed that DNA exists in a double helix. In the mid-1960s, full information of the genetic code of TMV according to which specific base triplets of DNA and RNA code for an amino acid was studied. Further in mid-1970s, T-DNA of its Ti plasmid of *Agrobacterium tumefaciens* revealed the presence of several genes, of which two were coding for the formation of galls or tumors by the infected plants. It was further seen that the two genes of Ti plasmid can be replaced with one or more genes from other organism. This discovery started the era of genetic engineering where foreign genes can be transferred using viruses as vector.

With this technology, few bacterial and fungal genes coding for certain enzymes that breakdown the cell wall of the pathogens have been engineered into plants and have given the plant with resistance to these pathogens.

P Albersheim and his associates in the University of Georgia (USA) in 1984 identified the molecule in the cell wall of the Oomycete *Phytophthora megasperma* that acts as the elicitor of defence response in its soybean host. It was further found that the elicitor does this by interacting with a receptor molecule on the plant cells. B J Staskawicz and his coworkers from the University of California, Berkeley, isolated first avirulence gene from *Pseudomonas syringae* pv. *glycinea*. Bacterial hrp (hypersensitive response protein) genes were discovered in 1986 which were thought earlier required for bacterial pathogenicity and production of the hypersensitive response. But now it is known that they affect the transport of proteins in pathogenic bacteria and also the transport of bacteria into plant cells (Agrios, 2005). The first authentic report of molecular plant pathology in improving disease resistance came in 1986 when R Beachy and associates obtained tobacco plant resistant to TMV by transforming them. Similarly in 1989, M B Dickman and P E Kolattukudi, in the Iowa University, USA, a fungus that normally could enter host plant only through wounds, with a cloned gene coding for the enzyme cutinase. The enzyme cutinase helped the fungus to enter host plants directly through cuticle, thereby proving the role of enzyme cutinases in penetration of some plants by fungi. In 1991, R Broglie and colleagues in the Cornell University, Geneva, USA, that plants transformed with the gene that codes for chitinase exhibit enhanced resistance to disease caused by fungi that contain chitin in their cell walls. In 1990, R Cheim and co-workers reported that transgenic tobacco plants have the gene for stilbene synthetase, the enzyme that synthesizes a phytoalexin that accounts for resistance. DF Klessig and colleagues and J Ryals and associates in 1990s reported that systemic acquired resistance (SAR) is due to salicylic acid, a relative of aspirin. P J G M De Wit in the University of Wageningen, Netherlands, isolated the first avirulence gene

(avr 9) from *Cladosporium fulvum* while the first resistance gene (Hm-1) was isolated from corn by S P Briggs and J D Walton. The latter further reported that gene Hm-1 operates by producing a protein that detoxifies the host specific toxin of the pathogen *Cochliobolus carbonum*. Later on, many plant disease resistance genes were isolated from many plants.

Another important defence mechanism against plant pathogens are of "RNA Silencing", i.e., of regulating genes based on targeting and degrading sequence-specific RNAs. In plants, RNA silencing has been shown to serve as a defence against virus infections. Many plant viruses carry genes that encode proteins that suppress the silencing of their RNA by the plant. Further new tools and techniques are used to detect and identify pathogens when they are present in very small number or in mixtures with other closely related pathogens. Such tools include use of monoclonal antibodies, isozymes, or fatty acid analysis of pathogens, analysis of fragments of their nucleic acids produced by specific enzymes and determination of nucleotide sequences of the nucleic acids of the pathogens. The present era in molecular plant pathology is the use of genomics, i.e., sequencing of the entire genomes of plants and their pathogens. Genomes of *Arabidopsis thaliana*, some plant viruses, viroids and of the plant pathogenic bacteria *Ralstonia* and *Xylella*, fungus like *Phanerochaete* and nematode *Caenorhabditis* have been sequenced. Some of the destructive plant pathogenic fungi like *Magnaporthe grisea* (rice blast), *Ustilago maydis* (corn smut), *Botrytis cinerea* (gray mold of fruits and vegetables), *Fusarium graminearum* (head scab of wheat), *Phytophthora infestans* (late blight of potato) and many other pathogens, genomes have been sequenced. It is earlier to locate, identify, compare, isolate and manipulate the genes for pathogenicity in the pathogens and of resistance in their host plants so that it can be introduced into the plant genome where they would be effective. The practical implications of such developments cannot be overestimated, as they are likely to revolutionize the control of plant diseases by providing us the cultivars than can resist disease in the presence of the pathogen.

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Questions

1. Mention five important milestones in the development of the subject of plant pathology.
2. What have been the three great controversies of mycology and plant pathology?
3. Name any three workers who "lived ahead of their times".
4. List five major breakthroughs in phytopathology during the 20th century and the scientists responsible for them.
5. Name five contemporary scientists of plant pathology.
6. Name the person who introduced plant pathology in India. Mention his important contributions.
7. Mention the most important contribution of the following:

i. L. Spallanzani	ii. A. Leeuwenhoek
iii. P.A. Micheli	iv. M. Tillet
v. B. Prevost	vi. A. de Bary
vii. T.J. Burrill	viii. M.S. Woronin
ix. O. Brefeld	x. L. Pasteur
xi. P.A. Millardet	xii. H.M. Ward
xiii. J.G. Kühn	xiv. W.G. Farlow
xv. R.Koch	xvi. R. Hartig
xvii. A. Mayer	xviii. D.Ivanowski
xix. V. M. Biejerinck	xx. E.Gäumann
xxi. W.Brown	xxii. K.O. Müller
xxiii. S.A. Waksman	xiv. E.J. Butler
xxv. R.H. Biffen	xxvi. E.C. Stakman
xxvii. J.E. Vander Plank	xxviii. J.C. Walker
xxix. J.G. Horsfall	xxx. S.R. Bose
xxxi. T.S. Sadasivan	xxxii. K.C. Metha
xxxiii. J.F. Dastur	xxxiv. M.J. Thirumalachar
xxxv. R.N. Tandon	xxxvi. G.A. Zentmyer
xxxvii. T.O. Diener	xxxviii. S.P. Raychaudhuri
xxxix. Anupam Varma	xl. S.B. Saksena

8. What are the recent trends in Phytopathological research and training worldwide?
9. What are Prions? Who discovered them for the first time? Do they cause any plant disease?
10. Name at least four important Virologists of the country.
11. Name at least four Bacteriologists of the country.
12. Name at least four Nematologists of the country.
13. What is bio-terrorism?
14. What are some important innovations in teaching of plant pathology?

Pathogenesis

3

Penetration and Entry by Plant Pathogens

Contact

Pathogenesis, caused by an infectious agent, begins as soon as the pathogen comes into contact with any portion of a susceptible plant. Most of the bacterial and fungal pathogens come into contact with their hosts accidently in the form of wind-borne spores or propagules. Some fungi, many bacteria, and most of the viruses are carried to their host plants by insects, nematodes, and other vectors. Motile propagules of fungal pathogens are attracted to root exudates such as the zoospores of many plant pathogens (Hickman and Ho, 1966). Attraction and accumulation of zoospores to root in the soil just behind the root tip has been demonstrated by Mehrotra (1970) [Fig. 3.2 (a, b)], and the evidence of the attraction of nematodes to root has been ably summarized by Webster (1969).

Interesting research by Kerr and Flentje (1957) demonstrated that the hyphae of the fungus *Rhizoctonia solani* aggregate on the outside of cellophane bags in contact with the roots indicating that root exudates induce the formation of infection cushions by the fungus. Surface attachment and pre-penetration stage development by plant pathogenic fungi has been reviewed by Tucker and Talbot (2001) wherein they have reviewed the processes implicated in surface attachment, germ tube elongation and development of appressoria.

Spore Attachment Mechanism

Adhesion of fungal spores to the plant surface is the first step in initiating a pathogen to the establishment of disease. Primarily adhesion serves anchor to the propagules to the plant surface, but it may also be required for host recognition and subsequent development. Fungal adhesives are basically water-insoluble glycoproteins, and labeling with lectins has been used to predict both their composition and distribution. Lipids and polysaccharides have also been detected in adhesive material. In the case of the rice blast fungus, *Magnaporthe grisea*, moist air or dew is required for hydration and extrusion of a spore tip mucilage, which serves to attach the conidium, by its apex, to the hydrophobic plant surface. The adhesive material contains α-linked-mannosyl and glucosyl residues in addition to protein and lipid component and is immediately released upon hydration. Adhesion of *M. grisea* is therefore a passive process involving the release of a preformed adhesive. Also, apical attachment of the pyriform conidia produced by *M. grisea* allows them to resist the flow of water and as water is essential for later stages of

development in this fungus. Similar mechanisms of spore attachment are likely to be widespread among pathogenic fungi. Conidium attachment by the barley powdery mildew pathogen *Blumeria graminis* f.sp *hordei* and many other powdery mildews differ markedly from the situation in *M. grisea* largely because free water is not absolutely required for pre-penetration phase development.

Attachment and appressorium formation in *B. graminis* are accompanied by release of cutinase from the spore. It is believed that this enzyme not only cements the conidium to the leaf surface but also to make the surface of both the attachment zone and conidium more hydrophilic, favouring further attachment and development of primary and appressorial germ tubes. It has been reported for a number of fungi, a thigmotropic response is also essential before release of spore adhesives. In contrast to the passive release of preformed adhesive from conidia of *M. grisea*, spore attachment in other species can involve energy-requiring metabolic processes. In the corn anthracnose pathogen *Colletotrichum graminicola*, for example, spores do not attain maximum adhesion to the surface until 30 minutes after landing indicating that *de novo* glycoprotein synthesis is involved in the adhesion of ungerminated conidia in this fungus. Similar is the case in the pea wilt fungus, *Nectria haematococca.*

How exactly spores adhere to plant surface is not known but it seems to either involve a very specific interaction of the spore with the host plant surface via lectins, ionic interaction or hydrophobic contact with the plant cuticle, or involve stimulation of the spore by physical rather than chemical signals.

Spore Germination and Perception of the Host Surface

It is not clear that what exactly triggers spore germination but stimulation by the contact with the host surface, hydration and absorption of low molecular weight ionic material from the host surface and availability of nutrient play a role, conversely nutrient deprivation may be important in some biotrophic and hemibiotrophic species. Although, the stimuli may be diverse, the process of germination in all fungal spores requires mobilization of storage reserves—lipids, polyols, and carbohydrates such as trehalose—accompanied by polarization and rapid membrane and cell wall biosynthesis during germ tube extension. The germ tube is a specialized structure and is distinct from vegetative fungal hyphae, growing sometimes for only a very short distance before differentiating into an appressorium. Germ tube extension and differentiation can occur in response to a number of signals including surface hardness, hydrophobicity, plant signals and surface topography.

According to Tucker and Talbot (2001) and Agrios (2005), the perception of signals from plant surfaces by pathogenic fungi seems to be the result of signaling pathways mediated by cyclic adenosine monophosphate (cAMP) and mitogen-activated protein kinase (MAPK) which have been implicated in regulating the development of infection-related phenomena in many fungi (Fig. 3.1)

Recognition between Host and Pathogen

It is still not clear as to how pathogens recognize their hosts and vice versa. Recognition plays a central role in interactions between plants and their pathogens.

Pathogens must be able to recognize the presence of their host plants in their environment and often must recognize specific surface features of their host in order to effect successful penetration and infection. Successful pathogens must also be able to recognize and overcome plant defence responses. Since plants are sessile, they must, in return, have evolved sophisticated mechanisms to detect the multitude of potential pathogens in their environment and to activate various diverse defence mechanisms. The mutual signalling between plant hosts and potential pathogens has been the focus of extensive researches during the last few decades. The emphasis has been to identify and characterize the molecules involved in recognition between the two organisms.

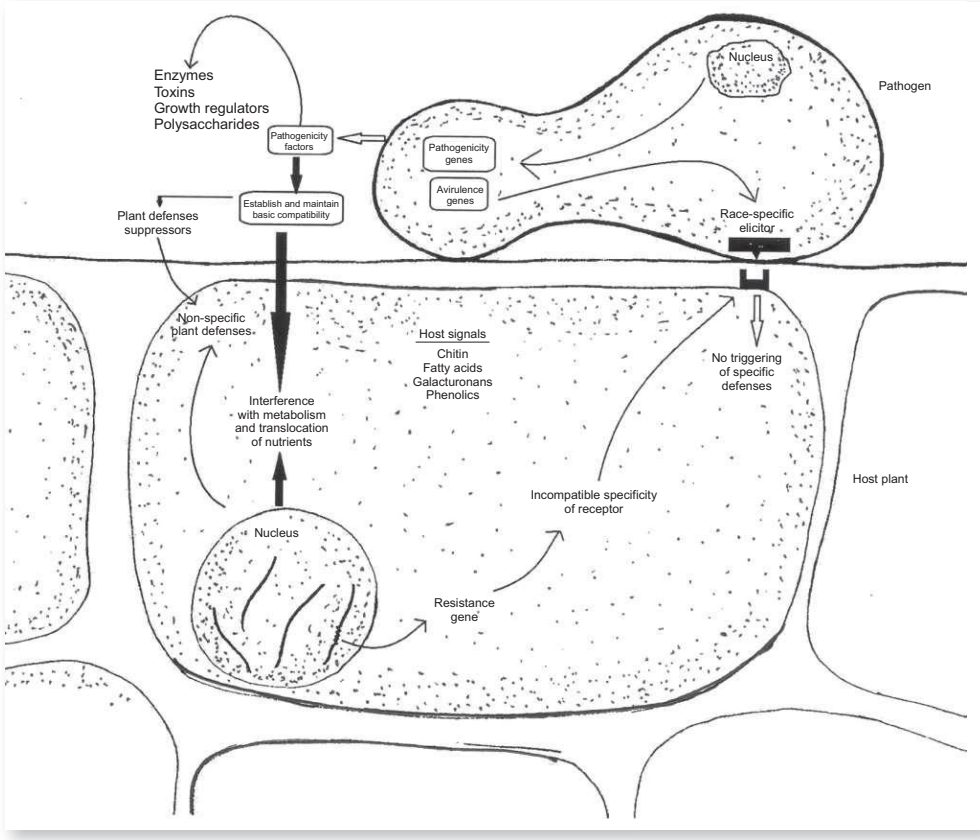


Figure 3.1 Establishment of infection between a pathogen and its host (Redrawn from G.N. Agrios (2005), *Plant Pathology*, 5th edition, Academic Press)

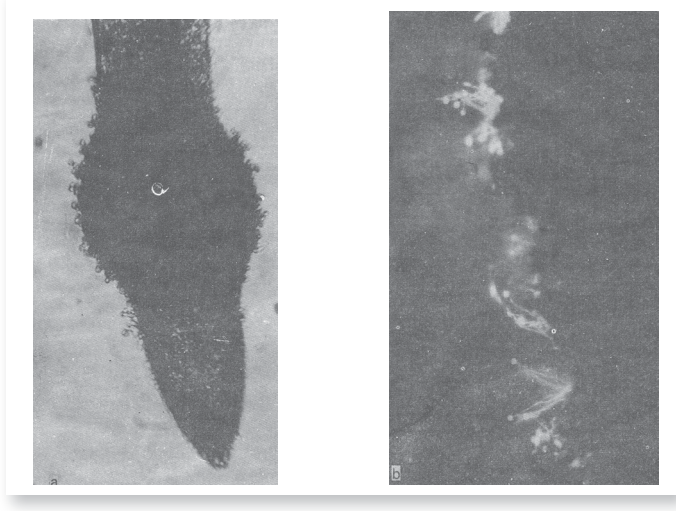


Figure 3.2 Accumulation of zoospores on root tip: **(a)** in petridish **(b)** in soil-encysted zoospores with their germ tubes fluorescing under the fluorescent microscope

Host components acting as signals for activation of pathogens are numerous. These may include fatty acids of the plant host cuticle that activate production of cutinase enzymes which break down cutin of the host galacturonan molecules of pectin which stimulate the production of pectin lyase enzymes by the fungal or bacterial pathogen, phenolic compounds, such as which stimulate the activation and germination of propagules of some pathogens, and phenolics and sugars released from plant wounds that activate a series of genes in certain pathogens leading to infection.

The term elicitor was originally used to refer to the molecules and other stimuli that induce the synthesis and accumulation of antimicrobial compounds (phytoalexins) in plant cells, but is now commonly used for molecules that stimulate any plant defence mechanism. Examples of plant defence mechanisms induced by elicitors include the synthesis and accumulation of antimicrobial phytoalexins, the production of glycosyl hydrolases capable of attacking surface polymers of pathogens, the synthesis of proteins that inhibit degradative enzymes produced by pathogens, the production of activated oxygen species (oxidative burst), and the modification of plant cell walls by the deposition of callose, hydroxy proline-rich glycoproteins and /or lignin.

Pathogen components that act as elicitors of recongnition by the host plant and subsequent mobilization of plant defences are still poorly known? The induction of these plant defence reactions or otherwise is mediated by an initial recognition process between plants and pathogen, which involves detection of certain unique structural features of incompatible or compatible pathogens by recognition molecules in plants thereby setting of a cascade of biochemical events leading to resistance or susceptible reaction. When the initial signal by the pathogen favours growth and development of the pathogens, disease may be induced, if the signal suppresses the growth and development of the pathogens disease does not occur. On the other hand, if the initial recongition elicitor received by the host triggers a defence reaction, the growth of the pathogens and its activity is slowed down or completely inhibited. If the elicitor either suppresses or bypasses the defence reactions of the host, disease may develop.

Daly (1984) had reviewed the role of recognition in plant disease. Plant–microbe interactions—molecular and genetic perspectives had been brought out in an edited volume by Kosuge and Nester (1984). Microbial elicitors and their receptors in plants have recently been reviewed by Hahn (1996). Molecular mechanism of fungal pathogenicity to plants has been reviewed by Schaffer (1994), while molecular biology of pathogenesis and induced systemic resistance has been reviewed by Vidhyasekaran (1998).

Host Penetration and Infection

When a pathogen comes in contract with a host plant, a complex of interacting factors, including temperature, moisture, susceptibility of plant tissues, effects of other microorganisms present, aggressiveness of the pathogen, etc. determine whether infection will occur or not. Pathogenicity is the property of a microorganism whereby it becomes a part of the causal complex. Pathogenesis is a process or the chain of events by which disease development takes place. The events of pathogenesis will be discussed under the following heads:

1. Prepenetration
2. Penetration
3. Post penetration or further colonization of plant by the pathogen

Prepenetration

The propagules of bacteria and fungi have on their surface or at their tip mucilaginous substances consisting of mixtures of water-insoluble polysaccharides, lipids etc., which when moistened become sticky and help the pathogen adhere to the plant. These propagules have some mechanisms that prevent their germination until they get some stimulus. Once the stimulation for germination has been received by the propagules, the latter mobilizes its stored food and directs them towards the germ tube formation. The germ tube also perceives stimulus from the host and if it does not receive the right stimulus, the germ tube remains undifferentiated and it stops growing. When appropriate signals are present, the germ tube differentiation i.e., formation of appressorium takes place. The perception of signal by the pathogenic fungi seems to be controlled by the cyclic adenosine monophosphate (cAMP) and mitogen-activated protein kinase (MAPK). In response to a signal from the host plant, the fungus perceives the extracellular signal and its transmission via the plasma membrane and it accumulates intracellular signaling molecules and induces a phosphorylation cascade. In some fungi the receptor of the signal is a protein in the plasma membrane of the fungal spores. Transmission of the cAMP signal moves via the cAMP-dependent activity of protein kinase A (PKA) and subsequent phosphorylation of target proteins. The main activity of PKA is the mobilization of lipids and carbohydrates to the appressorium site and is therefore important in the production of functional appressoria. In some fungi, cAMP signaling is required for the initiation of appressorium development at which time intracellular cAMP concentration rises during differentiation of conidia and emergence of the appressorium germ tube. Signaling pathways for infection-related development are also achieved through mitogen-activated protein kinases. All of these comprise a functional unit that ultimately transmits input signals from the cell to cell nucleus to elicit the expression of appropriate genes. After attachment of the propagule to the host surface, germ tubes also produce mucilaginous materials that allow them to adhere to the cuticular surface of the host. Once appressoria are formed, they stick tightly to the host surface. After that appressoria secrete some extracellular enzymes and generate physical pressure to bring about penetration of the cuticle. The turgour pressure inside an appressorium is quite high and it is due to the accumulation of glycerol in the appressorium, which due to its high osmotic pressure draws water into the cell and generates hydrostatic pressure that pushes appressorial penetration peg further. Tucker and Talbot (2001) have discussed critically the surface attachment and the prepenetration stage development by plant pathogenic fungi.

In fungal pathogens, this includes spore germination and growth of the resulting tube on the surface of the host plant. Germination is essentially the change from a low metabolic rate to a high metabolic rate and involves a change from near-dormancy to intense activity; for this, an energy source is needed such as a carbohydrate or fat reserve in the propagule. Fungal invasion is chiefly by germ tubes or structures derived from them. There are two well-known situations involving hyphae acting in a concerted way to achieve host penetration. Hyphae of *Rhizoctonia solani* often aggregate to form an infection cushion from which multiple penetration occurs by means of appressoria and penetration pegs. This is shown in Fig. 3.3 (a, b). The other example of penetration is exemplified by the penetration of intact root periderm by the rhizomorphs of *Armillaria mellea,* which is shown in Fig. 3.4 in the ectotrophic infection habit among the specialized pathogens such as *Gaeumannomyces (Ophiobolus) graminis*, the fungus progresses epiphytically over the root system as a sparse network of dark hyphae known as runner hyphae from which hyaline branches quickly penetration and infect the underlying cortex [Fig. 3.5 (a, b)]. The entry of bacteria and viruses is achieved in various ways. According to the nature of the spore and the environmental conditions, germination of fungal spores occurs in various ways.

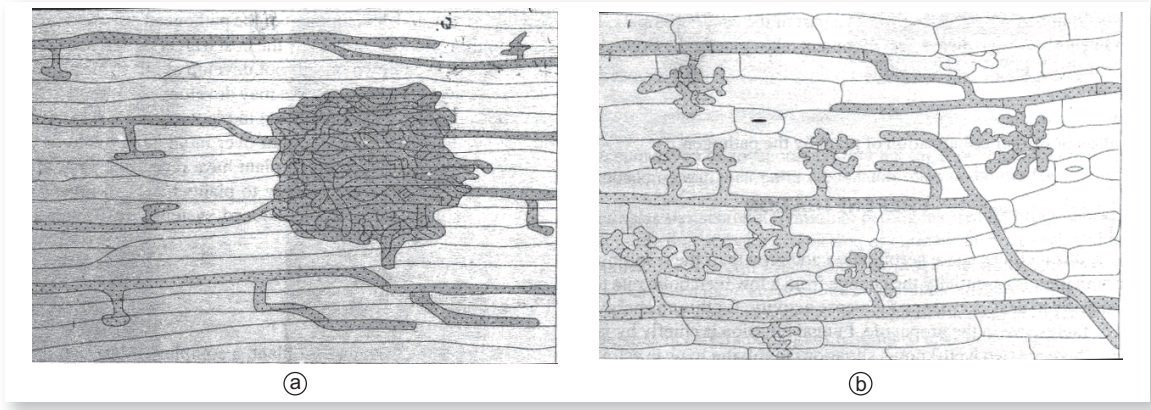


Figure 3.3 **(a)** Formation of infection cushion by crucifer isolate of *R. solani* on radish hypocotyl. **(b)** Production of lobate appressoria by solanaceous isolate of *R. solani* on tomato hypocotyl

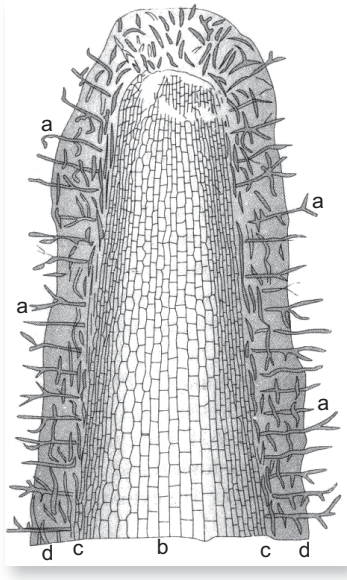


Figure 3.4 Apical region of rhizomorph of *Armillaria mellea* in longitudinal section **(a)** mantle of filamentous hyphae **(b)** central conducting cells **(c)** cells of the rind **(d)** boundary of enveloping mucilaginous layer

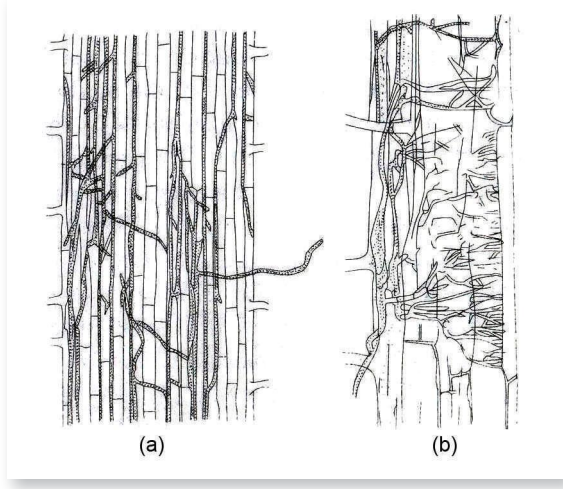


Figure 3.5 Ectotrophic infection of *Gaeumannomyces* (*Ophiobolus*) *graminis* **(a)** in surface view **(b)** in longitudinal section

Sporangia of Chytridiomycetes, Oomycetes, and parasitic slime molds produce zoospores in wet conditions and germ tubes in dry conditions, while those of some others appear always to germinate by zoospores (e.g. *Plasmopara viticola)* or always by germ tubes (e.g. *Peronospora* sp.). Teliospores of Uredinales and Ustilaginales normally germinate by producing a promycelium on which basidiospores, (sporidia) are borne. Budding and fission are characteristics of some yeasts and bacteria, although conidia, ascospores (e.g. *Taphrina* sp.) and basidiospores (Ustilaginales) may bud under certain conditions.

Some spores germinate easily on release and sometimes even before release, while resting spores have a dormancy period during which they do not normally germinate. There can be chemical and biological factors inhibiting or stimulating germination. The physiology of spore germination will not be discussed here nor the requirements for spore germination (such as moisture, temperature, light, *p*H, oxygen, carbon dioxide, and biological factors). The behaviour of germ tubes growing on a plant surface depends on many factors. In order to procure food, plant pathogens have to establish an intimate connection with their hosts; this means that they have to enter into tissues or send haustoria into surface cells. Entry is mostly affected by the germ tube from spores, but can also often be accomplished by mycelial hyphae. The pathogen invades plants through natural openings (stomata, lenticels, nectarthodes, hydathodes), through wounds (natural or due to insects or nematodes), or by direct penetration. While some enter in one way only, others enter in more than one way. In Uredinales, the basidiospores penetrate directly while uredospores generally, but not invariably, invade through stomata. *Botrytis cinerea* may invade directly or through wounds and some fungi which normally grow through stomata may penetrate directly under certain conditions. Gäumann (1950) deals in an exhaustive manner with the penetration and infection processes in various host parasite combination. The infection of plants by fungi is reviewed by Eide (1955) and Wood (1967). Cytology of virus infection and virus transport had been discussed by De Zoeten (1977). Physiological and cytological aspects of the bacterial infection process had been reviewed by Goodman (1977). The cytology of penetration and infection by fungi had been reviewed by Aist (1976).

Entry Through Natural Openings

The process by which fungi enters through stoma will be illustrated by the stem rust fungus *Puccinia graminis tritici* (Fig. 3.6a). Urediniospores of pathogen will germinate in water on a leaf surface in an hour under optimum condition. The germ tube from the uredospore grows over the leaf and approaches the stoma. The protoplasm of the germ tube accumulates at the tip to leave the older parts empty. On reaching stoma, the end of the germ tube swells to form an appressorium in the stomatal aperture. The empty germ tube is cut off by the formation of a cross wall. From the appressorium a blade-like wedge grows through the stomatal slits and swells inside to form a substomatal vesicle into which the contents of the appressourin pass, leaving the latter empty. One or more hyphae grow from the vesicle towards the cell lining the substomatal cavity and after making contact proceed to penetrate the cell wall. After penetration, a haustorium is produced in the cell. In diseases caused by obligate parasites and facultative saprophytes and in a few caused by facultative parasites, there is no massive penetration of the cell cavities of the host by ordinary hyphae of the pasasite. Instead, the cell wall is penetrated by short branches which usually grow to a limited extent in the lumen of the cell before their ends are transformed into structures called haustoria, the forms of which may be simple or relatively complex depending on different parasites. Haustoria are presumed to have an absorptive function and are the main source of metabolic exchange between the host and parasite. A detailed account of haustoria will be given later.

There are variations in the behaviour of the germ tube at the time of penetration through the stomata, as in *Peronospora destructor* infecting onion leaves the germ tube continues to grow after the formation of the first appressorium (Fig. 3.6b).

On the other hand, zoopores of a species of *Phytophthora* infecting fennel (*Foeniculum vulgare*), almost always enter through stomata while *Phytophthora infestans* almost always enter plants directly, but at times through stomata after the formation of an appressorium. In *Pseudoperonospora cubensis* (Fig. 3.6c), the zoospores swim along the boundary lines of the epidermal cells towards the stomata; and

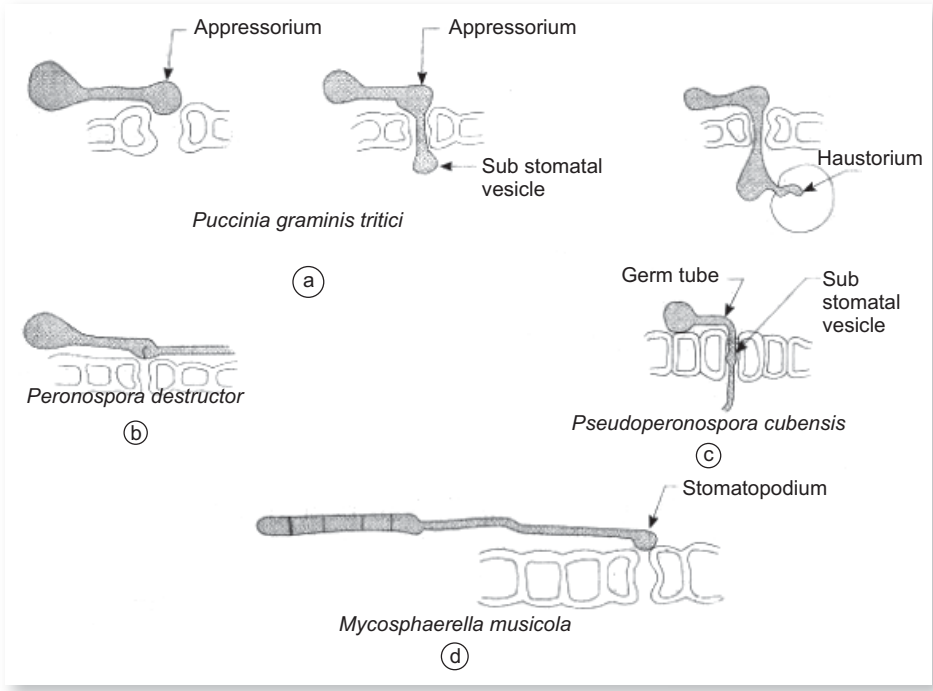


Figure 3.6 Penetration through natural openings

encyst, above the lines separating the guard and epidermal cells. Hyphae penetrate the stomatal aperture and swell to form a substomatal vesicle from which in turn other hyphae may grow to form haustoria in the adjacent cells of the leaves. *Mycosphaerella musicola* (Fig. 3.6d), which is responsible for Sigatoka disease of bananas, enters leaves in a similar manner as do the rusts. The germ tube from a conidium grows slowly over the leaves for a few days before its penetration. This indicates that it can withstand period of desiccation. A small structure called a stomatopodium is formed over the pore of a stoma. A hypha then arises from it which grows into the substomatal chamber and swells to form a vesicle, which in turn gives rise to hyphae which invade palisade tissues.

Leaf infecting fungi-imperfecti generally enters through stomata. Some of the examples are *Cladosporium fulvum* infecting tomato, *Cladosporium cucumerinum* infecting cucumber, and *Stemphylium solani* infecting tomato. Members of Sphaeropsidales entering through stomata are *Phoma trifolii* and *Phoma herbarum* var. *medicaginis* on *Trifolium pratense* and *Medicago sativa* respectively. These fungi also enter by direct penetration under certain conditions.

The mode of entry into plants by *Sclerotinia cinerea,* which causes stone fruits to rot dependes on the host species infected. It enters plums through the stomata, apricots through the stomata or cuticle, peaches through hair sockets and cherries and nectarines through the cuticle.

Stomata are the most important or the only pathways of entry for many parasitic bacteria such as *Pseudomonas tabaci* on tobacco, *Erwinia amylovora* infecting apples and pears, *Xanthomonas campestris pv. phaseoli* infecting *Phaseolus* spp., *Xanthomonas campestris pv. malvacearum* infecting cotton, and *Pseudomonas phaseolicola* infecting *Phaseolus* spp. Bacteria enter stomata only through films of water that extend from outside through the stomatal aperture into the inside of the shoots. Because of this the water content of shoots is of great importance in determining whether or not they will get

infected. The stomatal morphology may in fact determine whether successful infection will take place or not. In case of citrus fruits, the conformation of the cuticle around the stoma either prevents or allows the passage of water droplet containing the bacterial pathogen *Xanthomonas campestris pv.citri.*

Lenticels

In many dicotyledonous plants, a protective layer develops as the epidermis becomes redundant. This is called periderm. Periderm also develops on surfaces exposed by abscission of plant parts or on surfaces produced by the wounding of tissues. It also develops on the surface of storage tissues, such as potato tubers.

Streptomyces scabies, the cause of the common scab of potato, enters young developing tubers through stomata or lenticels. *Oospora pustulans,* which causes the skin scab of potato tubers, also enters through lenticels. *Penicillium expansum,* which is responsible for storage rot of apples and *Gloeosporium perennans*, the cause of another storage rot, also enters through stomata or lenticels. *Nectria galligena*, the cause of apple canker, can enter twigs through lenticels. Lenticular wound penetrations are somewhat similar; cork being involved in both and many lenticular invaders can also enter through wounds, particularly soil-borne pathogens, such as *S.scabies*, *Armillaria mellea*, *Spongospora subterranea*, *Phymatotrichum omnivorum*, and *Pectobacterium carotovorum.*

Nectarthodes, Hydoathodes and Trichomes

Erwinia amylovora enters through nectaries of apple and pear flowers. In pears, nectaries occur in saucer-shaped tissues between the point of emergence of the style and stamens. Droplets of nectar are exuded through stoma-like nectarthodes at the bottom of depresions on the surface of the nectaries. Within 24–28 hours of adding suspensions to open pear blossoms, the bacteria are found locally in great number beneath the nectarthodes.

Hydathodes are structures through which water is discharged from the interior of leaves, usually more or less directly from bundles of terminal tracheids which end in thin walled parenchyma known as epithem. Hydathodes are the only means of entry for certain bacteria. Bacteria, which enter through hydathodes, are *Erwinia amylovora*, and *Xanthomonas campestris.*

In high humidity, bacteria accumulates in water from hydathodes and are drawn into epithem and tracheids as the water recedes when conditions reduce water congestion, for example, in rice leaves and *Xanthomonas campestris pv. oryzae*. Baker *et al*. (1995) have reported that *Botrytis cinerea* colonizes necrotic tisue around the hydathodes of *Methiola incana*, this necrosis (tip-burn) perhaps resulting from high salt concentration due to the evaporation of the hydathode exudate. *Clavibacter* (*Corynebacterium*) *michiganense*, which causes the bacterial canker of tomato plans, often enters through trichomes.

Direct Penetration

Direct penetration of plants has been known for many years. de Bary (1886) was the first to study critically the nature of direct penetration by *Sclerotinia sclerotiorum*. He laid emphasis on the relationship of nutrients in the infection drop. The physicochemical characteristics of cuticles and cell walls are important factors in direct penetration.

Structure of Cell Walls and Cuticles

According to Dickinson (1960), the external layers lining the intercellular spaces and between the adjacent cells (middle lamella) consist largely of pectic substances, particularly calcium and magnesium pectates and can be disintegrated by the fungel and bacterial pathogens which produce pectolytic enzymes. Proteins have also been reported to occur in the middle lamella. Within this is a primary cell wall consisting largely of cellulose embedded in a matrix of pectic substance and hemicelluloses. The cellulose may be present as micellae. Other substances, such as proteins, lignin, suberin, and cutin have been reported in the primary cell wall. In some tissues, a secondary cell wall consisting chiefly of cellulose is laid down within the primary wall. The cuticle is a non-cellular membrane which covers most of the aerial parts of the higher plants. The outer waxy layer is hydrophobic and largely impermeable to water but the inner layer can absorb moisture, and swell and force the wax aggregates apart. The innermost layer of the cuticle passes gradually into a predominantly pectic layer which in turn merges into the largely cellulose outer walls of the epidermal cells.

The intact or uninjured plant surface or epidermis which is covered by a well defined layer of cuticle is penetrated directly by a mechanical process. In most cases, penetration is preceded by the formation of a special structure, namely, penetration hypha or appressorium which exerts a mechanical pressure on the intact wall of the host. Infection peg, arising from the appressorium, pierces the host wall to cause infection. The penetration of the cuticle is regarded by many as purely mechanical although there is evidence of the chemical degradation of the cuticle. Many root pathogens accumulate on the surface of roots and hypocoty1 and other subaerial parts and penetrate directly. The penetration may or may not be preceded by the formation of an appressorium. McKeen and Rimmer (1973) have shown that barley leaves induce the conidial germ tubes of *Blumeria* (*Erysiphe*) *graminis* to grow longitudinally on leaves and stimulate the production of appressorium. The papilla, a result of host reaction to the fungus, begins to form in the epidermal cell below the appressorium-arm as the appressorial infection pore develops. The tip of the blunt infection-peg lacks a cell wall and the host epidermal wall dissolves only in the region of the tip. Mechanical pressure is thought to force the peg through the host cell wall and papilla to invaginate the cytoplasm.

According to Yang and Ellingboe (1972), the primary infection of wheat and barley by *P. graminis* consists of a number of morphologically indentifiable stages of development, that is, spore germination formation of appressoria, penetration into host cells, formation of haustoria in host cells and formation of elongating secondary and tertiary infections. The wax layer of the cuticle surface is a major factor for the formation of appressoria.

The direct penetration of epidermal cells, by fungi has been described by Dickinson, (1960), Gaumann, (1950), Butler and Jones, (1949) and others. In direct penetration of a leaf, the infection hypha (peg or thread) which arises from the underside of an apressorium has to pass through the cuticle and underlined tissue and sometimes through the thickened outer wall of the epidermal cell, although at times it passes between the epidermal cells. The process has been studied in many fungi, including *Cladosporium carpophilum* [Fig. 3.7 (a, b)] on almond, *Venturia inaequalis* and *Venturia pirina* on pear, *Phytophthora infestans* on potatoes, *Puccinia graminis* on barberry, *Puccinia psidii* on pimento and *Ravenelia humphreyna* on *Caesalpinia*. The smut fungi usually penetrate directly into young ovaries, shoot buds, young seedlings or young tissues, such as the loose smut of wheat and barley, sugarcane smut (*Ustilago scitaminea*), bunts of wheat, flag smuts of wheat and rye, covered smuts of barley and oats and common smut of maize (Fig. 3.7c). Batts (1965) observed the direct penetration of the ovary by the loose smut pathogen.

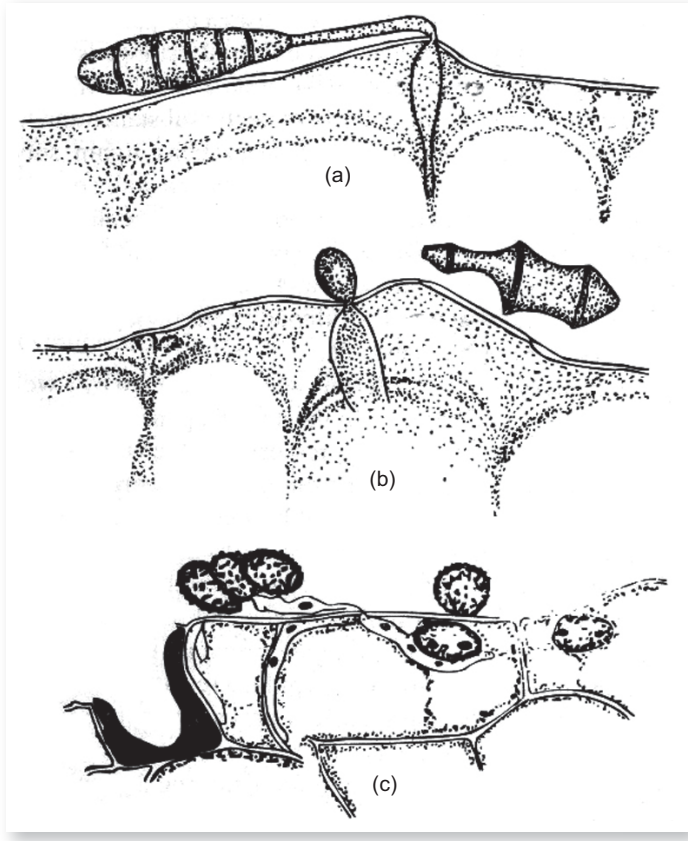


Figure 3.7 Direct penetration of cuticle. Penetration of almond leaves by germ tubes of *Cladosporium carpophilum,* the penetration hypha passing **(a)** between epidermal cells **(b)** passing into the epidermal cells **(c)** direct penetration of corn cells by the smut fungus, *Ustilago maydis,* the germ tube penetrating directly through an epidermal cell and making contact with host nucleus

In the case of infection due to *Blumeria* (*Erysiphe*) *graminis* on wheat leaves, a fine stylar process from the germ tube (or from a small subapical appressorium) pierces the cuticle, and in its passage through the cellulose layer it is preceded by a local thickening of the layer into a papilla which it eventually pierces at the apex. Inside the host cell it becomes dilated into a branched haustorium (Fig. 3.8).

The initiation, formation, and action of apressoria are integral parts of the infection process of many parasitic fungi. The formation of appressoria may be obligatory for infection, while sometimes it may be unnecessary. Appressoria had been discussed in a review article by R W Emett and D G Parbery (1975). The term appressorium was introduced by Frank (1883) for spore like "organisms" formed on the germ tubes of *Colletotrichum lindemuthianum, Polystigma rubrum* and *Fusicladium tremulae*. He believed that appressoria are adhesive discs attaching a parasitic fungus to its host during the early stages of infection. Hasselbring (1906) confirmed Frank's belief by experimenting with *Gloeosporium fructigenum.* He rejected the interpretation of many early authors that appressoria were also spore-like in function. The conflict of ideas about the nature of appressoria had been reviewed by Emmett (1969).

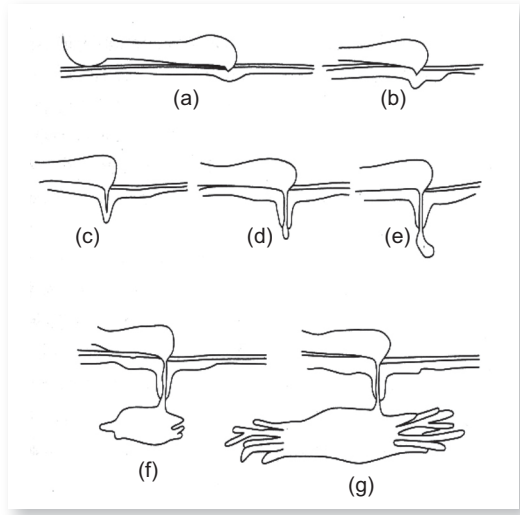


Figure 3.8 Germination of conidia of *Erysiphe graminis* on the epidermis of wheat: **(a)** germination of conidium **(b)** a fine stylar process from the germ tube (or from a small sub apical appressorium) pierces the cuticle **(c)** local thickening of the layer to form a papilla **(d)** papilla eventually pierces at the apex **(e)** dilatation inside the host cell **(f)** development of a lobed haustorium **(g)** branched haustorium and invagination of the lining layer of cytoplasm (x2000). (From S.A.J. Tarr, *Principles of Plant Pathology,* The Macmillian Press, London and

Appressoria may be simple or compound. Some fungi produce appressoria at different stages of growth; some produce more than one kind. The dematiaceous epiphytic parasites mostly produce appressoria on germ tubes identical to those produced latter on hyphae. In the members belonging to Erysiphaceae, most species produce appressoria which are different in form on hyphae than those on germ tubes, each type being morphologically constant for a species.

Emett and Parbery (1975) had surveyed the variety of appressoria formed by various ecological and phylogenetic groups of fungi. The different types can be divided into two basic groups: simple appressoria formed from a single modified cell and compound appressoria made up of many cells. Traditionally, only the structures produced on germ tubes are known as appressoria, while adhesion and penetration structures produced on hyphae have been described as "hyphopodia".

The main role of appressoria is the direct penetration of a host. To achieve this, appressoria must attach themselves firmly to the host surface and produce infection pegs that pierce the cuticle and usually the epidermal cell wall. Appressoria produced by germinating urediniospores of rusts and spores of *Mycosphaerella citri* penetrate through stomata. Few authors disagree that this is the primary role of the appressorium, but there are suggestions that appressoria may play a secondary role, and not the auxiliary role (Emmett and Parbery, 1975). Simple appressoria occur terminally on germ tubes, but may be terminal, lateral or intercalary when produced by mycelial hyphae. Appresoria produced on germ tubes are epiphytic and three types can be distinguished. The first: "proto appressoria" are little more than slightly swollen, hyaline, germ-like apices adhering to the host and giving rise to an infection peg. They are rarely delimited by a septum and occur in *Venturia inaequalis, Pythium* spp. and various other fungi. Hyaline appressoria occur in the Erysiphales, Uredinales and other species in the second group. They become swollen and are usually delimited from the germ tube by a septum. The third type: the dark appressoria, may be sessile or subtended by a germ tube from which they are delimited by one or more

septa much has been written on the ways in which infection hyphae penetrate the cuticle, particularly as to whether penetration is a totally physical process or cuticle softening enzymes are involved. It may be that slight localized softening of the cuticle occurs around the infection hypha, thus facilitating penetration. In the presumed absense of cuticle softening enzymes, it is probable that penetration is largely by a physical force.

Resistance of the leaves of *Berberis* and *Odostemon* spp. to basidiospores of *Puccinia graminis* was partly associated with their resistance to the combined thickness of the epidermal walls and cuticle (Melander and Craigie, 1927). Cuticle thickness has been correlated with resistance to foot rot of peas caused by *Ascochyta pinodella*. Butler and Jones (1949) reported that older barley leaves with cuticles 2.5-5 mm thick were resistant to mildew as compared to younger leaves with 0.4-1.5 μm cuticles in thickness. Resistance of the strawberry varieties to *Sphaerotheca macularis* was shown both by Peries (1962) and Jhooty and McKeen (1965) to vary with cuticle thickness.

Penetration of the Cell Wall

After passing through the cuticle, the infection hypha has to grow through the outer wall of the epidermal cells which consists of a network of cellulose microfibrils in a matrix consisting principally of pectic materials. The infection hyphae of some fungi pass between the radial walls of the epidermal cells without entering them. In *Diplocarpon rosae*, the cause of the black spot of rose leaves—the mycelium—develops between the cuticle and epidermis and enters the cells of the latter. The penetration of the walls is largely enzymic. The infection hyphae grow quickly through the softened materials. Pectolytic enzymes are important in this but there is doubt as to the part played by cellulases. Penetration of the cell walls may occur within a minute and is probably a combination of physical pressure and enzymic softening. Swelling of the invaded cell wall and of walls some distance away may occur due to the diffusion of enzymes. Such swellings very considerably in extent, ranging from sharply localized (*Ustilago segetum* (*tritici*) on wheat; Batts, 1955) to pronounce thickening which almost obliterates the lumen of the cell, as in the case of *Venturia inaequalis* attacking the epidermal cells of the stem of apple (Marsh and Walker, 1932).

Infection can be as readily caused by mycelial hyphae of many fungi as on germ tubes from spores. In *Armillaria mellea*, a root parasite of many trees, infection is restricted to the rhizomorphs.

The germ tubes of aeciospores of *Puccinia menthae* (mint rust) produce infection structures resembling appressoria when exposed to thymol at its own saturated vapour pressure on glass but not on glass in the absence of thymol. Thymol also reduces spore germination and elongation of germ tubes. It is thought that it acts as a morphogenetic switch in bringing about the formation of appressoria (Cox, 1969). Studies on different strains of *Corticium solani* on roots, stems, and leaves of several plants by Flentje and his co-workers in Australia indicated that appressoria developed on susceptible but not on resistant plants (Flentje, 1957; Kerr and Flentje, 1957). The formation of appressoria is probably affected by the nature of the surface on which the hypha grows and also by diffusable substances which cause the arrested growth and multiple branching of side branches leading to the development of appressoria.

In the case of infection by *Rhizoctonia solani*, infection cushions are seen on the roots or hypocotyls of the infected plants. Multicellular infection cushions are produced from a branch hypha in which normal elongation has ceased but prolific side branching now takes place. Strains of *Rhizoctonia solani*, non-pathgenic to seedlings of a particular species failed to become attached to the hypocotyl and organize the infection cushions.

Entry Through Wounds

All bacteria, most fungi, some viruses and all viroids can enter plants through various types of wounds. Penetration of viruses, mollicutes, fastidious bacteria and protozoa through wounds depends on the deposition of these pathogens by their vectors in fresh wounds created at the time of inoculation.

Many so-called low grade pathogens are not able to penetrate the host surface in the absense of a wound. However once they are in the plant, they are often able to cause severe disease symptoms. Wounds may be natural or they may be caused by the activities of man or other agencies, such as insect bites or nematodes. Many horticultural and agricultural practices involve accidental or even deliberate wounding. Entry through natural wounds (lenticels and abscission areas) has been dealt with earlier. Other natural wounds are those that occur due to the emergence of laterals from main roots. This is a means of entry for the tobacco root-rot fungus *Thielaviopsis basicola* and for *Fusarium solani f. phaseoli* into bean roots. Many important forest pathogens also enter through wounds. *Heterobasidion* (*Fomes) annosus*, a destructive pathogen of conifers, naturally colonizes wounds caused by high winds, snow or other natural agencies. It becomes a very damaging pathogen in plantations where it enters through the stumps left after felling. Man's activities are responsible for many of the wounds through which fungal penetration occurs. Many fruit and vegetable products are quite succulent when they are ripe for marketing. In this condition they are most susceptible to wounds by rough and careless handling. The *Rhizopus* rot of sweet cherries and peaches develops in wounds in this manner. The size of the wounds necessary for the infection of oranges by *Penicillium digitatum* varies with the degree of maturity and variety of the fruit. Wounds need to be deeper for the infection of Valencia oranges than for Naval and Jaffa oranges and also for the infection of immature fruits than for mature ones.

An unusual type of entry of a variety of microorganisms occurs through wounds in flax and maize seeds. The damage is caused mechanically during or after harvesting. Somewhat similar is the infection of wheat seedlings by *Cephalosporium gramineum* which enters through wounds caused by the breaking of the pericarp and coleorhiza as the root emerges on germination (Otieno, 1962). Wounds caused by insects are important in many diseases. In some diseases, the relationship between the insect and parasite is non-specific while in others the relationship may be more specific, the distribution of the disease coinciding with the appearance of a particular insect species.

Among the diseases caused by bacteria, vascular wilt of cucurbits is one where the relationship is highly specific. This wilt is caused by *Erwinia tracheiphila* which overwinters in hibernating adults of the cucumber beetles *Diabrotica vittata* and *D. duodecimpunctata*. When adults feed on plants, they introduce the bacterium into the vascular system, where it spreads to cause a typical vascular disease.

There is a similar relation in bacterial wilt and Stewart's leaf blight of corn caused by *Pantoea* (*Xanthomonas*) *stewartii*. The bacteria overwinters in the intestinal tracts of insects, in this case the flea beetles (*Chaetocnema pulicaria* and *C. deniticulata*), and plants become infected when beetles begin to feed in spring.

In another disease, the relationship is more casual, for example, in fire blight of apple and pear leaves caused by *Erwinia amylovora* where the bacteria overwinters in cankers. With the return of suitable growing condition in spring, bacteria multiplies in the cankers and are carried to the blossoms by insects.

A large number of fungal diseases are disseminated by insects and are instrumental in their entry into the host. In the ergot disease of rye caused by *Claviceps purpurea* conidia are disseminated by species of *Diptera* and *Coleptera*. In oak wilt caused by *Ceratocystis fagacearum*, the relationship between the fungus and insects is also relatively non-specific.

A variety of insects also cause feeding wounds, through which species of *Sclerotinia* invade stone and pome fruits.

In the well-known Dutch elm disease, there is a highly specific relationship between the fungus and insect vector. The pathogen *Ceratocystis ulmi* is carried and transmitted to elm trees by feeding beetles (*Scolytus scolytus, S. multistriatus*). Conidia are introduced directly into the sap wood as the beetles feed on the bark. In this example, the feeding tunnels not only breach the external protective layers of bark but also provide direct access to the vascular elements in which the pathogen can flourish.

Wounds Caused By Other Fungi

Resistance to infection can be modified by other fungi. Tubers highly resistant to *Phytophthora infestans* become susceptible when the parasite enters through wounds caused by *Spongospora subterranea.* The apple canker fungus, *Nectria galligena*, may enter through lesions on a stem caused by *Venturia inaequalis*. Similarly, *Fusarium* species which rot potato tubers may enter through wounds caused by *Spongospora subterranea* and *Phytophthora infestans.*

Wounds Caused by Nematodes

It is now well known that infection of plants by some bacteria and fungi depends on or is increased by damage caused by nematodes. Wilt of solanaceous plants caused by *Pseudomonas solanacearum* is introduced by *Meloidogyne incognita* var. *acrita*. In the case of yellow ear rot of wheat caused by the bacterium *Clavibacter* (*Corynebacterium*) *tritici*, recently renamed as *Rathayibacter tritici*, another nematode *Anguina tritici* plays an important part in the transmission and infection process. Several vascular wilt pathogens enter into the vascular plants through the injuries caused by the attack of nematodes; for example, *Fusarium oxysporum* f. sp. v*asinfectum* gains entry into the roots of susceptible cotton plants through the injuries caused by the root-knot-nematode, especially *Meloidogyne* species.

Entry Through Root Hairs

Root hairs are particularly vulnerable to invasion by pathogens. Among the pathogens which enter through root hairs, are *Plasmodiophora brassicae* (finger and toe disease of crucifers), *Phymatotrichum omnivorum* (Texas root rot of cotton), *Fusarium oxysporum* f. sp. *lini* (flax wilt), *F. oxysporum* f. sp. *conglutinans* (cabbage yellows). The mode of entry by *Plasmodiophora brassicae* appears to be quite unique. This process involves several unique structural features. Zoospore of the pathogen encysts on the wall of a root hair. The contents of the zoospore are injected into the host cell by a fine puncture or hole made in the cell wall of the host. The puncture is the result of the production of a bullet-shaped structure which suddenly forces its way from within the cyst through the wall into the interior of the root hair cell. This is an example of mechanical penetration and the actual infection process takes only a few seconds (Williams *et al*., 1973). Another interesting example of entry through root hairs is by the root nodule bacterium (*Rhizobium*) of leguminosae which enters through root hairs in the form of an infection thread.

Entry Through Buds

Some rust fungi, including *Uromyces pisi* enter through unfolding buds. *Taphrina cerasi* attacks cherry buds and causes them to develop into typical witches' brooms. The peach leaf curl fungus *T. deformans* enters its host through the unfolding leaf-buds. An unusual mode of entry is that of the pathogen *F. oxysporum* f. sp. *pisi* which enters through cotyledonary bundles which are exposed when cotyledons are rotted by soil saprophytes (Hepple, 1963).

Development Inside Host Tissue

Following penetration, there are very wide variations in the subsequent pattern of development and colonization of host tissues. In certain pathogens, such as powdery mildews, the growth of the pathogen is superficial (i.e. ectotrophic). The mycelial development of the apple scab fungus (*Venturia inaequalis*) is restricted to the subcuticular layer of the leaf and fruit, at least until the onset of general tissue senescence. Some parasitic fungi colonise the interior of the plant and also produce an external aerial mycelium which grows superficially and enters the host repeatedly, for example, *Corticium solani* is responsible for stem canker of potato and *G.* (*Ophiobolus) graminis* is responsible for take-all diseases of wheat. Many pathogens colonise the parenchyma of mesophyll and cortex; their further spread into the vascular bundles is prevented by the endodermis, although there may be an entry into the pith. In *Taphrina* the hyphae are entirely intercellular, obtaining nutrients through the membranes of the adjacent cells and this indirect method perhaps resuls in some degree of systemic development. The hyphae of less specialized pathogens are often intracellular, progressing with branches from cell to cell until the tissue is more or less completely colonized. Toxins and enzymes may be secreted and bring about rapid softening and necrosis of tissues ahead of the pathogen. Some parasites enter living cells, which however, may die soon after penetration. In diseases caused by obligate parasites and facultative saprophytes and in a few caused by facultative parasites, the cell wall is penetrated by short branches which usually grow to a limited extent in the lumen of the cell before their ends are transformed into structures which are simple-to-relatively complex in form in different parasites. These structures are haustoria, they are presumed to have an absorptive function and are the main means of metabolic interchange between the host and parasite. Haustoria almost always occur in parenchyma but in most powdery mildews they are confined to epidermal cells.

A comprehensive account of haustoria, as revealed by a light microscope, had given by Fraymouth (1956) for downy mildews, for rusts by Rice (1927), for powdery mildews by Smith (1900) and Hirata (1937). Haustoria of *Phytophthora infestans* were described by Blackwell (1953). The general picture revealed by light microscopy has been confirmed by electron microscopy. A more substantial account of the fine structure of haustorium is that of Ehrlich and Ehrlich (1963) for *Puccinia graminis tritici* on wheat.

The haustorium originates as a slight bulge on the inside wall of the haustorium mother cell and it becomes closely appressed to the host cell wall at the point it is to be penetrated. Here there is formed a conical opening, wide at the base and tapering at the apex, through which the haustorium passes. As it emerges, it is bounded only by a membrane continuous with that of the haustorium mother cell. The fact that the haustorium is not surrounded by a cell wall at this stage suggests that penetration is primarily a chemical and not a mechanical process and that the haustorium mother cells produce substances that

dissolve both their own walls and those of the host cells. After penetration, the haustorium elongates to form a neck around which a wall is formed. The apex of the neck expands to the characteristic final structure. There is some (but not conclusive) evidence of the invagination of host plasma membrane by the developing haustorium. The mature haustorium containing nuclei, mitochondria and endoplasmic reticulum is more complex than in the haustorium mother cell. The plasma membrane is invaginated at certain places. Outside is the cell wall which is thick and probably consist of at least two zones. Between the cytoplasm of the host and the wall of the haustorium is an encapsulation or "zone of apposition", which is not found around the parent hyphae or around the developing haustorium. The encapsulation is enclosed by a membrane which may be the host plasma membrane or a new structure. Ehrlich and Ehrlich (1963) interpret this to mean that haustorial protoplasm probably oozes out through ultramicroscopic pores in the walls and comes in intimate contact with the protoplasm of the host cell. At some points, the surface of the encapsulation is disrupted by small vesicles which project into the host cytoplasm and which contain particulates similar to those of encapsulation. These vesicles or secretory bodies are formed in the host cytoplasm, which then move to the encapsulation and discharge their contents.

Encapsulation is also well developed in downy mildews. Berlin and Bowen (1964) suggest that this term should be used instead of sheath which was used by Fraymouth (1956) and earlier workers, and that sheath should be applied to the inward projection of the host wall which surrounds the base of the haustorium as a collar. This is illustrated in Fig. 3.9 which shows diagrammatically the complex of host cell wall and haustoria for *Peronospora manshurica* on leaves of soybean (Peyton and Bowen, 1963) and for *Albugo candida* on leaves of radish [Berlin and Bowen, 1964 (Fig. 3.10)].

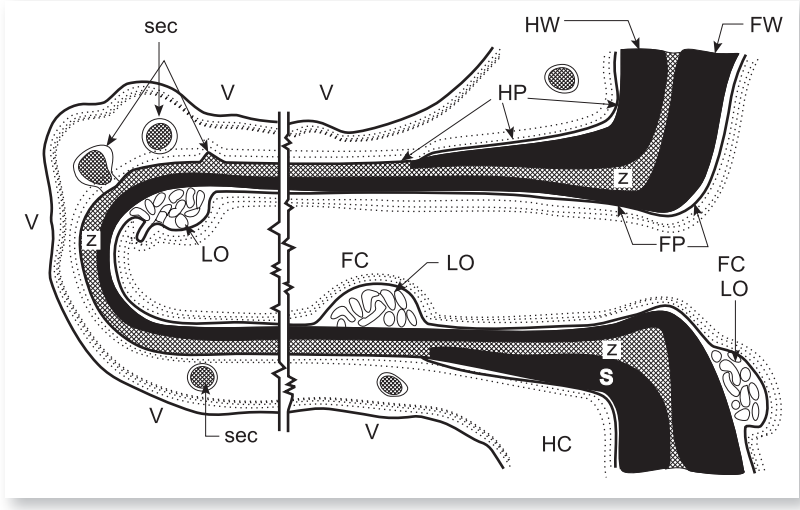


Figure 3.9 Diagram of the structure of *Peronospora manshurica* infecting *Glycine max* as revealed by electron microscopy The cytoplasm (FC) of the fungus is bounded by plasma membrane (FP), lomasomes (LO) and cell wall (FW). The host cell shows vacuole (V), cytoplasm (HC), plasma membrane (HP) and cell wall (HW), ending in sheath (S). A zone of apposition (Z) separates haustorium from host plasma membrane. Invaginations (sec) of host plasma membrane and vesicular host cytoplasm suggest secretion by host cell (Adapted from G.A.Peyton and C.C.Bowen, *Am.J.Bot.*, **50**: 787–797, 1963).

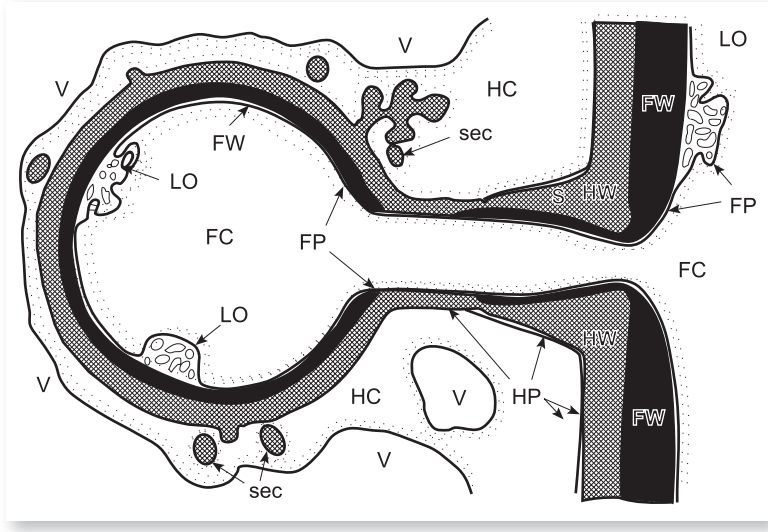


Figure 3.10 Diagram of the structure of *Albugo candida* infecting *Raphanus sativus* as revealed by electron microscopy The cytoplasm (FC) of the fungus is bounded by plasma membrane (FP), lomasomes (LO) and cell wall (FW). The host cell shows vacuole (V), cytoplasm (HC), plasma membrane (HP) and cell wall (HW), ending in sheath (S). A zone of apposition (Z) separates haustorium from host plasma membrane. Invaginations (sec) of host plasma membrane and vesicular host cytoplasm suggest secretion by host cell (Redrawn after modification from J.D. Berlin and C.C.Bowen, *Am.J.Bot.*, **51**: 445–452, 1962)

The basic structure is similar in both. The only important difference is the absense of fungal cell wall around part of the neck beneath the head of the haustorium and the absence of nuclei in *Albugo candida*.

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Questions

1. What is the recent concept of recognition by host of pathogens and non-pathogens?
2. What is chemotaxis and electrotaxis?
3. Distinguish between penetration and infection.
4. Name at least two pathogens which enter their hosts through the following:
   i. Stomata
   ii. Lenticels
   iii. Wounds caused by natural agencies
   iv. Directly through cell walls
   v. Wounds caused by nematodes
   vi. Wounds caused by fungi
   vii. Root hairs
   viii. Through unfolding buds
5. Name some pathogens which grow superficially (i.e. ectotrophic), subcuticularly and, endophytically in their hosts.
6. Distinguish between appressoria and hyphopodia. What is the function of appressoria? What are the different types of appressoria produced by plant pathogens?
7. Give examples of plant diseases in which insects play an important role in the entry of pathogen.
8. How do the following pathogens enter their hosts:
   Heterobasidion (*Fomes*) *annosus*
   Gaeumannomyces (*Ophiobolus*) *graminis*
   Penicillium expansum

Enzymes and Toxins in Plant Diseases

4

Enzymes in Plant Diseases

For successful infection by a pathogen, it should first enter the body of the host on which it feeds. Although some pathogens may use mechanical force or enter their hosts through natural openings or mechanical injury, the activities of pathogen in plants are largely chemical in nature which includes enzymes, toxins, growth regulators, and polysaccharides. Here in this chapter, the role of enzymes and toxins will be discussed. The role of growth regulators and polysaccharides etc., will be discussed in the next chapter (Chapter 5). According to Wilson (1973), hydrolytic enzymes are extremely important in pathogenesis because they provide the pathogen chemical means of entrance into the host and a process whereby nutrients can be digested. The plant cell is surrounded by a cellulose wall which is comparable to a closed box. If the parasite is to feed successfully, it should break this barrier which acts like a fortress wall. Decomposition and disintegration of host tissues are the constant components of plant disease syndrome. Enzymes and toxins are the main aspects under which the mechanism of disease has been studied in the last 80 years or so. The concept that cell wall degrading enzymes may be involved in pathological manifestations induced in plant tissues by biotic agents was introduced for the first time by de Bary (1886). He described the pathogen action on host tissues in two ways: one initiates the dissolution of certain constituents of the cell walls and subsequently the loss of tissue coherence; and the other kills the protoplast itself. The former effect was traced to some heat labile metabolites, possibly enzymes. The second effect could be due to the cell wall degrading enzymes themselves or it may be brought about by a second factor, possibly toxins. This view was substantiated by the researches of Ward (1888), Jones (1909), Brown (1915, 1917) and Hutchinson (1913) and they called these pectic enzymes. Pectolytic and cellulolytic enzymes are now implicated almost routinely as a feature of host-parasite interactions and their involvement in the degradation of the pectin and cellulolytic constituents of cell walls and of the middle lamella in plant tissues has been reported for such diverse types of diseases as soft rots, dry rots, charcoal rots, damping off, wilts, blights and leaf spots, for such diverse pathogenic agents as fungi, bacteria and nematodes; even virus infected tissues have been reported to undergo alterations in their pectic constituents. This ability to elaborate pectic enzymes is widely distributed amongst organisms (Brown, 1965; Mahadevan, 1970; Bateman and Basham, 1976; Cooper, 1983; Collmer and Keen, 1986). However, the ability to produce these enzymes does not indicate that an organism possesses pathogenic capabilities. In most plant diseases caused by microbial agents, cell walls are

penetrated, tissues are colonized and permeability of host cells is altered. An understanding of the cell wall and membrane structure, together with knowledge of the enzymes capable of degrading the components of these structures is essential for our understanding of disease physiology. Singh *et al.* (1994) have brought out three volumes on pathogenicity and host specificity in plant diseases discussing in detail histopathological, biochemical, genetic and molecular bases. Barras *et al*. (1994) have ably reviewed the work done on extracellular enzymes and pathogenesis of soft rot erwinias.

Composition of Cell Wall Materials and Middle Lamella

Cuticular Wax

Plant waxes are formed as granular or rod-like projections, or as a continuous layer outside or within the cuticle of many aerial plant parts. Wax formation is a continuous process and not a terminal phase in the development of a leaf. Recent indications are that minute pores exist beneath each wax projection and it is through these minute pores that wax is secreted. Cuticular waxes are made up of long chain molecules of paraffin hydrocarbon, alcohols, ketones, esters, and acids. They differ from fats in that glycerol is replaced by monohydric alcohols.

Cutin

Cutin is the main component of the cuticular layer. The upper part of the layer is admixed with waxes, while in its lower part, in the region where it merges with the outer walls of the epidermal cells, cutin is admixed with cellulose and pectin.

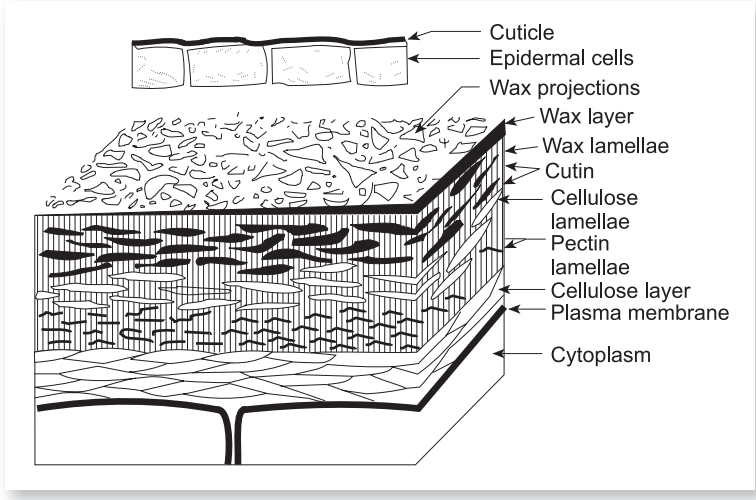


Figure 4.1 Schematic representation of the structure and composition of the cuticle and cell wall of foliar epidermal cells (redrawn from R.N. Goodman, Z. Kiraly and M. Zaitlin, *The Biochemistry and Physiology of Infectious Plant Diseases*, D. van Nostrand Company, Inc., 1967)

Cutin is believed to be an insoluble polymer of hydroxylated octa and hexadeconic acids of which phloinolic and $C_{17}H_{32}(OH)_3COOH$ is characteristic. Each chain of the polyester consists of 16–18 carbon

atoms and 2–3 are hydroxyl groups. The hydroxyl groups of one chain are linked to the carboxyl or hydroxyl groups of other chains which results in a three dimensional complex polymeric structure (Fig. 4.1 and 4.2). Cutler *et al.* (1982) have given a detailed account of plant cuticle. Gevens and Nicholson have reviewed "Cutin Composition: A Subtle Role for Fungal Cutinase" in 2000. "Host Wall Alterations by Parasitic Fungi" has been exhaustively discussed by Petrini and Ouellette in their book in 1994.

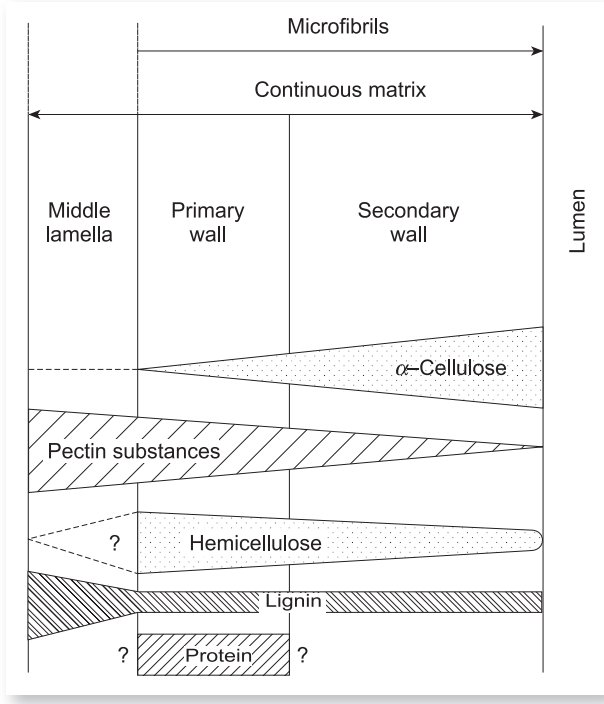


Figure 4.2 Distribution in the plant cell wall of the major wall constituents. (Adapted from D.F. Bateman and H.G. Basham, [1977], "Physiological Plant Pathology", in: Encyclo. of Pl. Physiol. (New Series), R. Heitefuss and P.H.Williams (Eds.), Springer-Verleg, Berlin, Heidelberg and New York.)

The Cell Wall

The cell wall of higher plants comprises a middle lamella and primary cell wall, and in some cases, a secondary cell wall. Pectic substances are the basic constituents of wall layers and form almost the entire middle lamella, whereas in the other layers, cellulose is also found in good amounts. Besides these two major groups of compounds, other components are hemicelluloses, lignins, proteins and limited amounts of other materials. In the classical view of structure, cell walls are a mesh of crystalline cellulose micro fibrils embedded in an amorphous matrix of hemicellulose with the pectic compounds localized in the middle lamella and serve to cement cells together. The serious challenge to this view came from Lamport (1967) who demonstrated that the cell wall contains protein rich in hydroxyproline. Functionally, the cell wall may be divided into three regions; middle lamella, primary wall, and secondary wall. The middle lamella acts as"intercellular cement" that binds cells together in the tissue system. A schematic representation of the structure and composition of a typical cell wall of higher plants is given in Fig. 4.3.

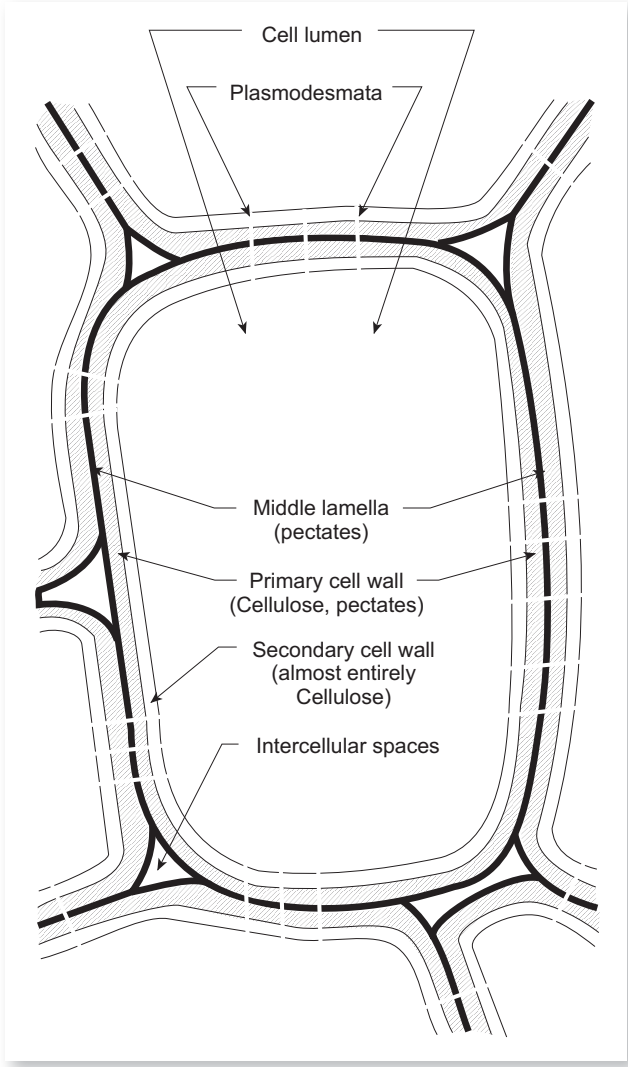
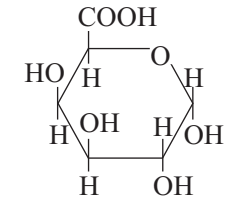


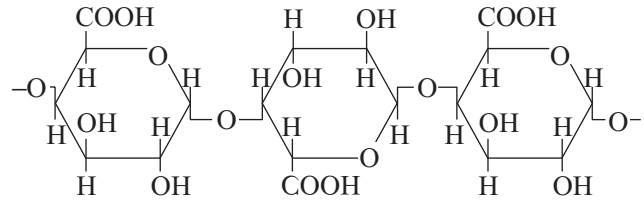
Figure 4.3 Schematic representation of structure and composition plant cell walls (From G.N. Agrios, *Plant Pathology*, Academic Press, London, New York, 1969).

Pectic Substances

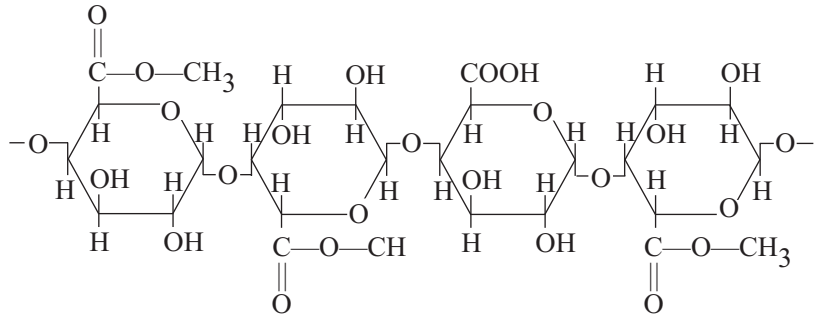
Pectic substances are a group of complex, colloidal carbohydrate derivatives which occur in or are prepared from plants and the basic structure of which is a linear polymer of D-galacturonic acid units in α 1-4 glycosidic linkages, esterified with a methyl group or linked with other carboxyl groups in calcium or magnesium salt bridges. The esterification varies with age, organ or species of the plant. The term *protopectin* is applied to substances which are insoluble in water and which, upon restricted hydrolysis, yield pectinic acid. Pectinic acid contains a variable quantity of methoxyl groups, though less than pectin, which may have 75% or more of the uronic carboxyls methylated. Pectic substances, essentially free from methyl ester groups, are classified as pectic acids.



α d–galacturonic acid



Pectic acid

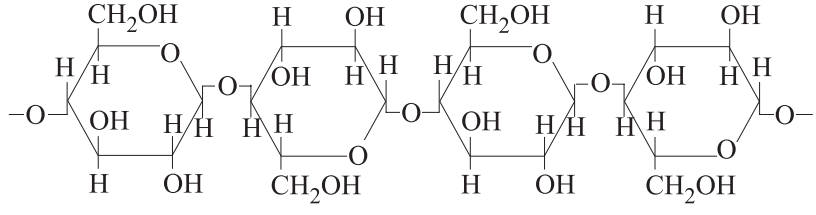


Pectinic acid

The proportions of carboxyl and methyl ester groups have profound effects on the properties of polymers. Calcium salts of pectin occur in the middle lamella of plant cells and represent a large portion of the pecitc substances in plants.

Cellulose

Cellulose is a polysaccharide which consists of an aggregation of β-D-glucopyranose from where C_1 is linked to C_4. Cellulose is the major constituent of the cell walls of higher plants. Cellulose is an insoluble, crystalline substance in its native form (Fig. 4.4).



Cellulose

Callose differs from cellulose in that it consists of (1-3) β-D glucane chains that can form duplexes and triplexes.

Hemicelluloses (Cross-Linking Glycans)

In addition to celluloses, these also occur in cell walls. Cross-linking glycans known earlier as hemicelluloses include several polymers. Xyloglucan is an important hemicellulose in the primary wall region. It is made up of a β-1, 4 linked glucopyranose chain with terminal branches of 1, 6 linked D-xylopyranose.

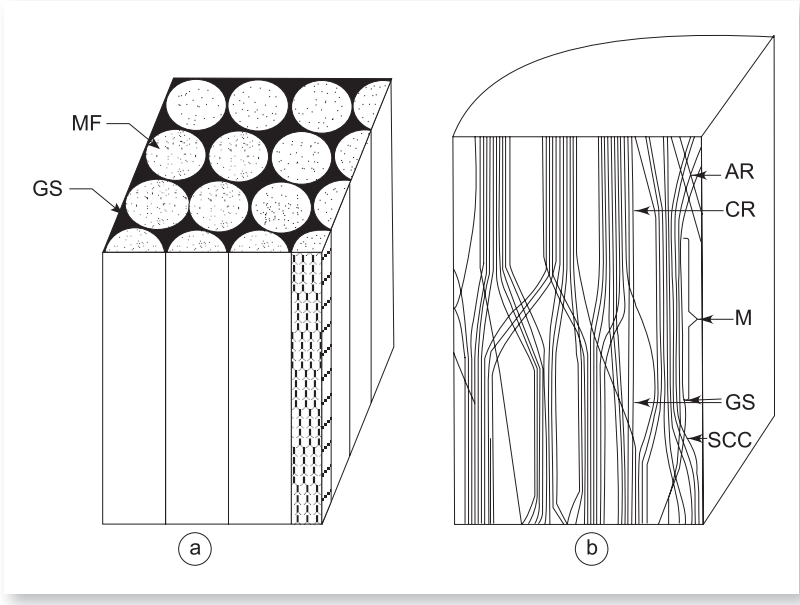
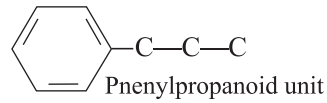


Figure 4.4 Schematic diagram of the gross structure of cellulose and microfibrils (a) and of the arrangement of cellulose molecules with in a microfibril (b) MF = microfibril, GS = ground substance (pectin, hemicelluloses or lignin), AR = amorphous region of cellulose, CR = crystalline region of cellulose, M = micelle, SCC = Single cellulose chain molecule (From G.N. Agrios, *Plant Pathology*, Academic Press, London, New York, 1969).

Other hemicelluloses include glucomannan, a heteropolymer of D-glucopyranose and D-mannopyranose linked β-1, 4 and glactoglucomannan, a glucomannan with 1, 6 linked D-gluctopyranose side branches.

Lignin

This is a high molecular weight, structurally complex, amorphous polymer that is different from carbohydrates and proteins. Its structure is still not known but the basic unit which forms a polymer is phenylpropanoid. Lignin is generated from the oxidation products of sinapyl, confiferyl and p-hydroxycinnamyl alcohols which condense by free radical mechanism to form an amorphous, three-dimensional branched polymers.



Pnenylpropanoid unit

Lignin is found in the middle lamella, the cell walls of xylem vessels and the sclerenchyma fibers as secondary thickening that strengthen plants. It may also be formed in other cells such as bundle sheath cells and occasionally epidermal cells. The lignin content of mature woody tissues may vary from 15–38% and is second only to cellulose. Lignin is formed by C-C and C-O bonds between the carbon and/or the hydroxyls of the aromatic ring and of the side chains of different phenyl propanoid or similar units. The polymer (three-dimensional) may consist of 103 or more phenyl propanoid units. Lignin can from

covalent linkages with polysaccharides and confers upon them resistance to cell wall dissolving enzymes. Its structure, formation and role in resistance are considered by Ride (1983).

Suberin

It is a complex material containing mixture of hydroxycinnamic acid and is very resistant to microbial attack. Suberin is found in certain tissue of various underground organs such as roots, tubers and stolons, and in periderm layers such as cork and bark tissues. Suberins are also found in response to wounding and to pathogen-induced defences of certain organs and cell types.

Cell Wall Protein

Proteins are also present in all plant cell walls in the region of the cuticle and possibly in the middle lamella. The structural wall proteins seem to be similar to other cell wall proteins in all aspects, except that an amino acid, hydroxyproline is also present, which is not present in other plant proteins. Mucopolysaccharides are formed as a result of the covalent bonds between the protein molecules and cell wall polysaccharides and give a complex called *extension* and are envisaged as playing a part in cell wall extension. Five classes of structural proteins have been formed in cell walls: (i) extensions, which are glycoproteins. There are upto 0.5% of the cell wall mass in healthy tissues but may increase to 5–15% in infected tissues. (ii) Proline rich proteins (iii) glycine rich proteins (iv) solanaceous lectins and (v) other structural proteins (Agrios, 1997, 2005).

Lipids

Phospholipids and glycolipids, along with proteins, are the main constituents of all plant cell membranes. The common characteristic of all lipids is that they contain fatty acids that may be saturated or unsaturated. Oils and fats are formed in many cells especially as storage compounds especially in seeds. Wax lipids are formed on aerial epidermal cells.

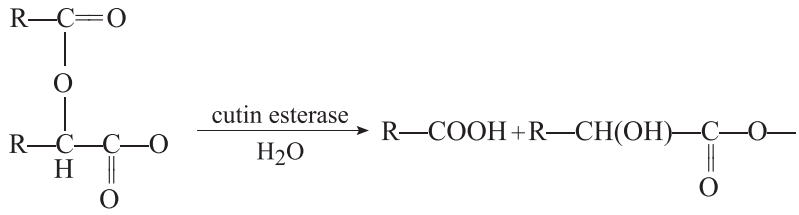
Enzymes for Waxes and Cutins

It was earlier thought that no pathogens are known to produce enzymes that can degrade waxes (Goodman *et al.,* 1967). Wax layers are mainly penetrated by mechanical force alone. However, electron microscopic studies suggest that some pathogens such as *Puccinia hordei* produce enzymes that can degrade waxes (Agrios, 1997, 2005). Sugui *et al.* (1992) reported that *Pestalotia malicola* which attacks fruit of Chinese quince grows on, within and beneath the fruit cuticle.

The significance of cuticle-softening enzymes in plant diseases is uncertain and needs more investigation. The cuticle consists of a cutin framework with waxes embedded within it and extruded from its surface to give a water proof "bloom". Until 1961, no enzymes were known which could degrade cutin. Heinen and Linskens (1961) demonstrated that the fungus *Penicillium spinulosum* from rotting leaves produced at least two types of cutinolytic enzymes.

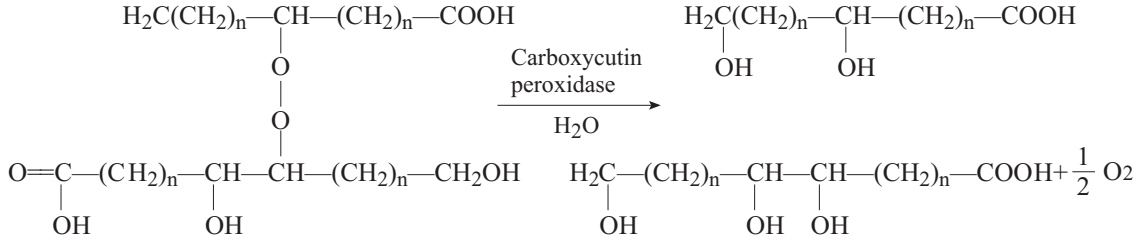
Cutin Esterase

This catalyzes the hydrolysis of ester bonds occurring between free hydroxyl and carboxyl groups of cutin bonds.



Carboxycutin-Peroxidase

This catalyzes the hydrolysis of the peroxide group of cutin.



Chaudhuri (1935) reported that the germinating spores of *Colletotrichum gloeosporioides* degrade the cuticle of orange leaves. Graniti (1962) reported the cuticular breakdown in olive leaves by *Spilocaea oleagini*.It is possible that in organisms like *Spilocaea oleagini*, which live sub- or intracuticularly, the entire cuticle is also used as a food source. Roberts *et al*., (1960) noted a considerable reduction in the content of cutin in rose leaves infected by the fungus *Venturia inaequalis*. However, some fungal pathogens may not produce sufficient quantity of cutin dissolving enzymes so as to play an important role in the penetration of cuticle. A localized secretion of cutin dissolving enzymes is significant as it may aid in the penetration process. The work of Edwards and Allen (1970) is significant in this connection. They visualized a localized secretion of cuticle degrading enzymes from the tip of the infection peg by the fungus *Blumeria* (*Erysiphe*) *graminis* f.sp.*hordei* attacking barley leaves. Cutinolytic enzymes and pathogenesis have been reviewed by Van den Ende and Linskens (1974). Fungal penetration of the first line defensive barriers of plants has been critically discussed by Kolattukudy and Koller (1983). Enzymatic penetration of the plant cuticle by fungal pathogens has been reviewed by Kolattukudy (1985). It is believed that fungal pathogens constantly produce low levels of cutinase which on contact with cutin release small amounts of cutin manomers (single molecules). These small manomers gain entry into the fungal pathogen which then trigger further expression of cutinase gene and stimulate the fungus to produce enormously large amounts of the cutinase enzyme. Fatty acids present in the wax can also stimulate the production of cutinase enzyme. Glucose on the other hand suppresses the production of cutinase enzyme. Involvement of cutinase in the penetration of host cuticle by plant pathogenic fungi comes from several indirect evidences. It has been shown that the production of the enzyme is highest at the point of penetration of the germ tube of the pathogen.

To prove the involvement of cutinase in penetration of pathogen into its host, secretion of cutinase by the penetrating fungus must be conclusively shown and conclusive evidence that cutinase is required for infection must be provided. Both of these have been accomplished for infection of pea by *Fusarium solani* f. *pisi* under controlled conditions.

Ferritin-conjugated antibodies prepared against cutinase were used to determine whether the fungus penetrating into intact pea stem, secretes cutinase. Scanning electron microscopy showed that the spores placed in suspension on pea stem germinated and penetrated into stem in 18 hours. Treatment of the penetrating area with ferritin labelled anticutinase followed by electron microscopy demonstrated the presence of cutinase antibody complex, showing that the penetrating fungus (pathogen) secreted cutinase. Chemicals known to be inhibitors of cutinase such as diisopropyl fluorophosphate also prevented infection, although such chemicals did not appear to affect the growth of the pathogen. Similar approach had been used to demonstrate that cutinase is essential for infection in case of papaya fruits infected with *Colletotrichum gloeosporioides*. The fungus *Botrytis cinerea*, the cause of many diseases on many plants produces a cutinase and a lipase, both of which breakdown cutin (Agrios, 2005).

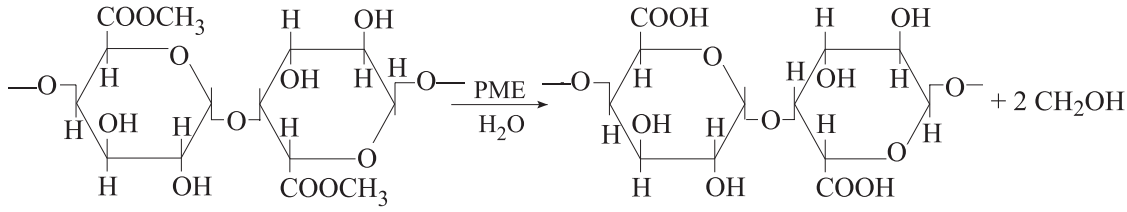
Pectic Enzymes

The role of these enzymes in wilt diseases, storage rots and fruit rots have been extensively studied. The importance of these enzymes in some anthracnose diseases and leaf spot diseases has also been demonstrated (Bateman and Miller, 1966; Bateman and Basham, 1976; and Mahadevan, 1976). Vidhyasekaran *et al*., (1973) emphasized the role of pectolytic enzymes in the pathogenesis of two obligate parasites (*Puccinia purpurea* and *Sclerospora sorghi*) and two facultative parasites (*Helminthosporium turcicum* and *Cercospora sorghi*).

The capacity to produce pectic enzymes is widespread in fungi, bacteria, and nematodes. According to Agrios (2005) the root knot nematode *Meloidogyne javanica* produces pectolytic enzymes for the penetration of root tissues and movement between plant cells and along the middle lamella and possibly in the formation of multinucleate giant cells on which it feeds throughout the rest of its life. The pectic enzymes which are currently known are classified into two major groups:

Pectinesterases (PE or PME)

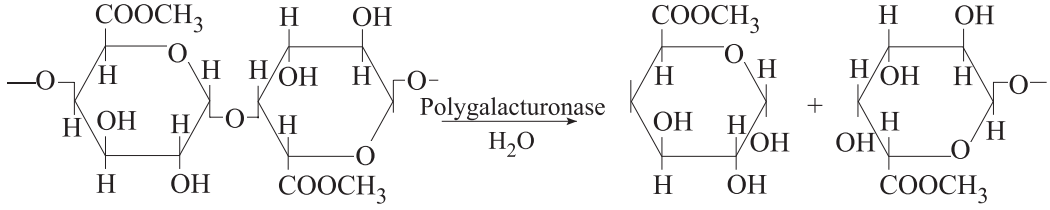
Pectin esterases or pectin methyl esterases catalyze the hydrolysis of methyl ester groups of pectinic acids to methyl alcohol and pectinic acids of reduced methoxy content and eventually to pectic acid. PME is also found in most of the higher plants and in microorganisms. The microbial PME and plant PME may, however, differ in their chemical and physical properties. It is of interest that the rate of cleavage of the α-1, 4 bond of the pectic substance is enhanced by the removal of the methoxyl groups. This is illustrated by the fact that endopolygalacturonase prefers demethylated pectin.

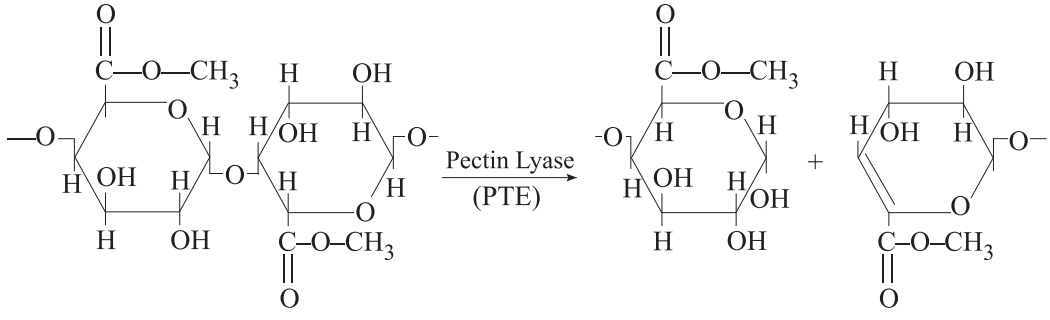


Polygalacturonases (PG)

Polygalacturonases or pectic glycosidases and layses are chain splitting enzymes which break the links between adjacent galacturonic acid units in pectic substances. This can occur by a hydrolytic mechanism

(hydrolases) or an eliminative mechanism (lyases). There has been some confusion in the names of various chain splitting enzymes of pectinous materials. However, Wood (1960) followed the classification proposed by Demain and Phaff (1957) who distinguished between the polygalacturonses (PG) which prefer pectic acid of the de-esterified parts of pectinic acid as substrates, and pectin methyl galacturonases (PMG) which prefer esterified chain parts. They further distinguished these polygalacturonases according to whether they cause cleavage is glycosidic linkages at random by an endo-type of polygalacuronase or polymethyl-galacturonase, or by an exo-type of action in which the end linkages are preferred.





Pectin Transeliminase (PTE)

A significant advance was the discovery of the pectin transeliminases (PTE) by Albersheim *et al*. (1960) which split the pectin chain by a transeliminative mechanism. This enzyme breaks the glycosidic linkage of pectin at C_4, accompanied by a simultaneous elimination of H^+ from C_5. Wood (1960) further classified these enzymes which preferably attack esterified or nonesterified parts of the chain and determined whether enzymes attack chains terminally (exo-) or at random (endo-).

Macerating Enzymes

Protopectinases (hypothetical enzymes) attack protopectin which is a native substance in cell walls. Although the name protopectinase is widely used for macerating acitivity, Demain and Phaff (1957) devised a new system of classification where the macerating principle was shown to be associated with chain splitting enzymes, namely, polygalacturonases (PG and PMG). Protopectinases can bring insoluble substances into solution and can include the enzymes degrading the middle lamella pectates. Maceration of plant tissues has often been associated with hydrolases and lyases which degrade in a

random manner the α -1, 4 linkages in the galacturonic acid polymers of pectin substances. Several workers have reported the "macerating enzymes" from the pectic enzymes. Byrde and Fielding (1962) separated the endopolygalacturonase from the macerating enzymes produced by *Sclerotinia fructigena* and subsequent studies have revealed that it is pectin methyl transeliminase. The work done by McClendon (1974), Zaitlin and Coltrin (1964) on purified pectic enzymes suggests that macerating enzymes are not of only one type. Looking at these discrepancies, Brown (1965), Bateman and Millar (1966) emphasized that the non-specific term, macerating enzymes, should be retained. But all the macerating enzymes which have been purified and identified to date have proven to be pectic enzymes.

The capacity to produce pectic enzymes seems to be widespread among living organisms, especially higher plants, microorganisms and animals feeding on plants. Thus, the presence or secretion of these enzymes by any microorganism is not indicative of its pathogenic capability. Pectin esterases occur in fruits, especially in ripening fruits. Bateman and Millar (1966) reported the occurrence of endo pectin-trans-eliminase in healthy pea tissue.

Many fungi and bacterial diseases particularly those characterized by the soft rotting tissues produce pectic enzymes especially PME in culture, and this may be stimulated by the presence of specific substrates. In some disesases, eg., the bacterial wilt of solanaceous plants caused by *Ralstonia solanacearum*, pectinolytic enzymes collectively are essential for the development of the disease. Although some of them individually seem to not be required for disease but rather for accelerated colonization of the host substrate and enhanced aggressiveness of the bacteria. Production of pectic enzymes by a pathogen in culture does not necessarily mean that they are produced in the host plant or that they play a part in pathogenesis. Damle (1952) working on *Pythium* spp. was able to establish a correlation between the pathogenicity and pectolytic enzyme secretion. In addition to the correlation between pectinase secretion and pathogenicity, the degree of pectolytic action varied with the relative rapidity of symptom development. Subramanian (1960) compared the pectolytic enzyme secretion *in vivo* in wilting cotton (*Gossypium arboreum* –K2), pigeon pea (*Cajanus cajan*-NP24) and foot rot infected rice (*Oryza sativa*-MT09). There was a good correlation in the wilt caused by *Fusarium oxysporum* f. *vasinfectum* and *F. oxysporum* f. *udum* in the development of wilt symptoms and pectolytic enzyme production. However, in the case of foot rot of rice caused by *Fusarium moniliforme* there was no such correlation. Pectolytic enzymes produced in culture by virulent and avirulent strains do not have any correlation with their pathogenic ability (Chan and Sackston, 1972; Goel and Mehrotra, 1974). Hancock *et al.* (1964) have demonstrated that different types of enzymes are produced by three *Botrytis* species pathogenic to onion, depending on whether the fungi are grown in culture on detached leaves or on leaves of infected plants. Hence, it is apparent that the determination of pectic enzymes produced by the pathogens *in vitro* may not be a reliable indication of the types of enzymes produced by the pathogen during pathogenesis.

Vascular pathogens, such as *Fusarium, Verticillium* and some bacteria produce pectolytic enzymes. *F. oxysporum* f.sp. *lycopersici* produces a pectin depolymerase, pectin methyl esterase, and cellulase and these appear to be involved in pathogenesis. Juice extracted from stems of tomato plants infected with *Verticillium albo-atrum* was found to contain higher amounts of PME and chain splitting pectolytic activity than healthy plants. Still, no direct evidence has been suggested for the involvement of pectins in reduced water transport.

Pectic enzymes may partially hydrolyse pectic substances along with walls of the vessels. The action of depolymerase and pectin methyl esterase together might free pectic acids probably cause plugging. In the presence of calcium ions, these materials could form calcium pectate gels. PME has been detected in higher quantity in the tracheal fluid of infected plants than in that of healthy plants. As a result of the

action of these enzymes in the vascular region, phenols are released into the xylem vessels and the phenol oxidase system of the plants aids in their conversion to melanins which are absorbed by the liquified xylem walls. The basis of the theory is that browning results from phenols produced in the xylem as a result of the action of the pectolytic enzyme (Gothoskar *et al.,* 1955).

Pectic enzymes are of great importance in soft rot diseases in which the parenchymatous tissue of the host parts is rapidly invaded. There is the dissolution of the middle lamella which results in a mass of soft, disorganized, water-soaked tissue. Although soft rot organisms produce pectic enzymes in culture, it is likely that other enzymes are also involved in the maceration of plant tissues. Some of the organisms responsible for soft rot in this manner are *Pectobacterium carotovorum, Pseudomonas marginalis, Penicillium expansum*, *Botrytis cinerea, Choanephora cucurbitarum, Sclerotinia fructigena, Pythium* spp. *Phytophthora* spp. *Rhizopus* spp., *Rhizoctonia solani* and *R. bataticola*.

The mechanism involved in the killing of plant cells by pectic enzyme and specifically the factors that regulate their activities in plant tissues are not currently understood. Pectic enzymes are relatively stable and are resistant to many classical enzyme inactivators or inhibitors and may act as a basis for the self-limited lesions in various plant diseases (Byrde, 1963). The cells of macerated tissues soon die, although the reasons for the death of the cells are obscure. Brown (1965) distinguished a 'macerating principal' from a 'lethal principle'.

The death of protoplasts (often the action of the pectolytic enzymes) has been ascribed by Wood (1967) to the bursting of the protoplasts following their rapid expansion on absorbing water. This is supported by the results of the plasmolysis experiments performed by Tribe (1955) in which the cells in the discs of a potato tuber exposed to macerating enzymes of *Erwinia aroideae* and *Botrytis cinerea* remained alive much longer when plasmolysed than when unplasmolysed, although maceration was only slightly affected by plasmolysis. Microscopical examination failed, however, to support the bursting theory. Hall and Wood (1973) suggest that cell death caused by pectic enzymes may be an osmotic effect which results in the rupture of plasma membrane.

The role of pectic enzymes has now begun to be appreciated in certain specialized plant pathogens, such as *Puccinia graminis tritici* where it has been shown that the germinating urediniospores of *P. graminis tritici* produce pectic enzymes which are thought to be involved in the growth of the hyphae between host cells and in the penetration of the epidermal cell wall.

Cellulolytic Enzymes

Although bulk of cellulose (cell wall polysaccharide) is broken down by numerous enzymes produced by pathogens, a portion of them appears to be broken by non-enzymatic oxidative systems, such as activated oxygen and hydroxyl radicles (OH) produced during infection process.

These enzymes have been investigated in those organisms which bring about the deterioration of textiles and wood but not much attention has been given to their significance in plant diseases. The enzymic degradation of cellulose is imperfectly understood. Cellulose degrading enzymes are produced by fungi, bacteria and nematodes and also by parasitic higher plants. They cause:

1. The break-up of the wall barrier for entry.
2. Provide food in the form of glucose from cellulose on hydrolysis.

The enzymes cellulases degrade cellulose and its derivatives. Cellulases which degrade native cellulose by destroying its crystalline structure are designated as C_1 enzymes. A second cellulase also attacks native cellulose and breaks it into shorter chains and expsoe the glucan chains to a third group

to enzymes β-1,4 endoglucanases, termed C_x enzymes, which degrade the glucan chains to cellobiose. The conversion of cellulose to glucose also requires a cellobiase or β-glucosidase. The presence of cellulolytic enzymes and the degradation of cellulose by bacteria and fungi have been reviewed by several workers. Host wall alterations by parasitic fungi have been reviewed by Cooper (1983) and Petrini and Quellete (1994).

Wood (1967) proposed a scheme of cellulolytic enzymes when native cellulose is degraded. However, this has been slightly modified, which is as follows:

Native cellulose

↓ One cellulase (C_1) attacks native cellulose cleaving cross-linkages between chains

Native cellulose (modified without cross linkages)

↓ Second Cellulase (C_2) also attacks native cellulose or modified cellulose into short chains

Native cellulose broken into shorter chains

↓ These shorter chains of cellulose are then attacked by a third group of cellulases (C_x) which degrade them to disaccharide cellobiose

Cellobiose

↓ Finally cellobiose is degraded by the enzyme β-glucosidase into glucose

Glucose

Cellulases are generally adaptive in their formation because the latter is stimulated by the presence of cellulose. The capacity to degrade native cellulose is confined to a few organisms, while soluble cellulose is degraded by a large number of microorganisms. Saprophytic fungi, mainly certain groups of basiodiomycetes and to a lesser extent saprophytic bacterium, bring about decomposition of cellulose in nature. However, in living plants cellulolytic enzymes secreted by the pathogens in the host are responsible for the collapse and disintegration of the cellulose.

It is generally believed that cellulases are not of much importance in the early stages of pathogenesis; perhaps cellulose is first protected by the enveloping matrix. Many soft rot organisms produce cellulases as well as pectolytic enzymes. Members belonging to the family pythiaceae do not form cellulases *in vitro*, whereas others, such as *Rhizoctonia solani, Fusarium moniliforme* are reported to produce both cellulolytic and pectic enzymes. *Sclerotium rolfsii* produces cellulolytic enzymes and has got the capacity to cause soft rot as well as degradation of hardened tissues (Husain and Kelman, 1957). In the root rot and foot rot of many plants and in leaf spot diseases, cellulases seem to play as important part in pathogenesis.

Cellulolytic enzymes have been implicated in wilt diseases (Husain and Kelman, 1957, 1958; Talboys, 1958; Husain and Dimond. 1960) caused by *Pseudomonas solanacearum, Verticillium albo-atrum* and *Fusarium oxysporum* f. sp. *lycopersici*. Wood (1960) suggested that the conditions in the trachea favour the production of cellulases by vascular wilt pathogens and that local action of these enzymes might result in the formation of the compounds of high molecular weight which could impede the flow of sap in the transpiration stream.

The production of cellulolytic enzymes *in vitro* is often reduced by sugars and by various inhibitors, such as polyphenols. Germinating uredospores of *Puccinia graminis tritici* are known to produce a cellulase but its role is uncertain.

There is increasing evidence that a number of fungi, particularly the wood rotting fungi, produce several cellulolytic enzymes. *Polyporus versicolor* produces a multiple component cellulase system.

Hemicellulases

Many microorganisms—saprophytic and parasitic—produce hemicellulases and can convert hemicelluloses into pentoses and uronides. Depending on the manomer released from the polymer on which they act the particular enzymes are celled as xylanase, galactanase, arabinase, mannose, and so on. Activated oxygen, hydroxyl, and other radicals produced by attacking pathogens also bring about non-enzymatic break down of hemicelluloses (Agrios, 1997). Although it is well known that pathogens or non-pathogens produce hemicellulases and oxidative agents, yet the exact manner in which these enzymes contribute to the breakdown of the cell walls or the ability to cause disease is not well understood. It is indeed surprising that so little attention has been given to cellulases and hemicellulases as compared with the pectolytic enzymes of plant pathogens. Hancock (1967), in the case of sunflower hypocotyls infected with *Sclerotinia sclerotiorum*, reported the production of xylanase and arabinase by the pathogen. Byrde and Fielding (1962, 1965) reported an arabino-furanosidase in culture filtrates of *S. fructigina*, while Strobel (1963) reported the production of at least two extracellular xylan degrading enzymes by *Diplodia viticola* when grown on autoclaved grapes. Van Etten and Bateman (1969) found that the pathogen *Sclerotium (Corticium) rolfsii* produced exogalacturonase, endomannase, galactosidase and endoxylanase in culture and these enzymes have also been detected in bean tissues infected by the pathogen. Hemicellulases will no doubt be investigated in many other plant pathogens and their role elucidated in plant diseases. According to Agrios (2005) it is still not clear how the hemicellulases and oxidative agents contribute to cell wall breakdown or the ability of the pathogen to cause a disease.

Lignolytic Enzymes

Lignin is a resistant material and only about 500 species of basidiomycetous fungi are capable of degrading it. Bacteria are seemingly not important in the degrading of lignin. The nature and action of enzymes which decompose lignin have not been properly understood. It has been recognized for many years that the white rot fungi, together with carbohydrates, decompose lignin in plant tissues (Campbell, 1952). Cowling (1961) showed that *Polyporus versicolor* can destroy over 97% of the lignin in sweetgum wood, and many reports of the removal of most of the lignin form wood by white rot fungi is found in the literature. There are several hundred species belonging to a number of families of Hymenomycetes, namely, Agaricaceae, Corticiaceae, Hydnacece, Polyporaceae, and Thelephoraceae that can bring about white rot. In addition, a few wood inhabiting Ascomycetes in the order Sphaeriales cause a white rot type of decay (e.g. *Hypoxylon deustum* and *Xylaria polymorpha*). There is very little experimental data available on the role of lignolytic enzymes in disease syndrome.

It appears that white rot fungi secrete an extracellular polyphenol oxidase, possible laccase, which enables them to use lignin. On the other hand, all type of wood rotting fungi seem to produce *transmethylase* that removes methoxyl groups form lignin, thus "modifying" lignin and increasing the exposure

of the embedded cellulose and other polysaccharides to the action of fungal enzymes. Then there are brown rot fungi, like most white rot fungi, they are the members of Holobasidiomycetes and unlike white rot fungi, which decompose both carbohydrates and lignin in wood, brown rot fungi decompose the carbohydrates with only a slight decrease in lignin content. The chemistry and breakdown of lignins are described by Brauns (1952, 1962), Brauns and Brauns (1960), Kremers (1959), Neish (1960), Pearl (1967). Husain and Kelman (1959) have pointed out that lignolytic enzymes might be playing an important role in many foot rot diseases of monocotyledonous plants which contain a high degree of lignin content in plant tissues. Certain species of *Fusarium,* such as *F. lactis* and *F. nivale* have been reported to degrade lignin. Kirk (1971) has discussed the effect of microorganisms on lignin.

Delignification of wood by wood decay fungi has been reviewed by Blanchette (1991).

Proteolytic Enzymes

Cell walls contain a protein rich in hydroxyproline (Lamport, 1973). Pathogens are known to degrade proteins enzymatically, and since no particular enzyme responsible for the degradation of cell wall protein is known, it is presumed that its breakdown follows the same steps as the degradation of cytoplasmic proteins. Some pathogens are known to produce proteolytic enzymes in culture and in the infected plant. Some of the pathogens which are known to produce the cell wall dissolving enzymes have also been shown to produce proteolytic enzymes. Kuc (1962) believed that the proteases might be important in view of the proteinaceous gel existing in cell wall.

One of the well documented studies on the secretion of proteolytic enzymes was on *Pyricularia oryzae* (now *P. grisea*) by Otani (1959). Protease was detected in the mycelium of the pathogen and in culture fluids. The enzyme was also located inside the diseased tissues. Degradation of RNA by *Phytophthora infestans*, the late blight pathogen, was demonstrated by Page (1959). Hancock and Miller (1965) detected protease activity in alfalfa plant tissues infected with *Stemphylium botryosum*.

Mahadevan (1970) reported the production of proteolytic enzymes and the existence of an RNAase in *Pyricularia oryzae* (now *P.grisea*). He also reported the production of proteolytic enzymes by the cotton wilt fungus (*F. oxysporum* f. *vasinfectum*) and *Helminthosporium oryzae* responsible for the brown leaf spot of paddy. In the three organisms—*Pyricularia grisea, F. oxysporum* f. *vasinfectum*, and *Cochliobolus* (*Helminthosporium*) *oryzae*-strong exocellular amino acid oxidases are present. Both amino acid oxidases and proteases help the pathogen to invade the tissues easily and effectively.

Lipolytic Enzymes

Various types of lipids occur in plant cells; the most important of them being the neutral lipids functioning as energy sources, while phospholipids and glycolipids are the main components of cell membranes. Several fungi, bacteria and nematodes are capable of degrading lipids. Plant pathogens produce enzymes that attack major membrane constituents. Lipolytic enzymes, called lipases, phosholipidases, etc., hydrolyse the liberation of fatty acids from the lipid molecule by hydrolysing the acyl ester and phosphate ester bonds of phospholipids. Phospholipase A is not known to be produced by plant pathogens. Phospholipase B is produced by *Thielaviopsis basicola, S. rolfsii,* and *Botrytis cinerea*. These enzymes and proteaces might affect cell membranes since phospholipids and proteins are the major constituents of plant membrane systems. According to Bateman and Basham (1976) more information

is needed before any conclusions can be drawn about the importance of phospholipases and proteinases in the destruction of intact plant membranes.

Inactivation of Enzymes

In some host parasite combinations resistance may be related to inactivation or inhibited synthesis of enzymes by the parasite.

In several instances of fungal enzyme inactivation, there is evidence that the inhibitors are of phenolic origin. Phenols and their oxidation products inhibited a wide range of enzymes and their effects on pectic enzymes have been studied by several workers. Byrde *et al.* (1960) suggested that in *Sclerotinia* attack on apple fruits, the hyphae grow through the tissue, macerating and killing the cells as they advance. From the time any cell is killed, the intermingling of phenolic oxidizable substances contained in the cell with the oxidizing enzymes supplied by both the host and fungus, leads to the formation of oxidized phenolic substances that inactivate the macerating enzyme.

Natural inhibitors of fungal enzymes and toxins in disease resistance have been discussed by Byrde (1963). Deverall and Wood (1961) have suggested that the self-limited lesions of chocolate spot of broad bean are due to the inactivation of the enzymes of the pathogen *Botrytis fabae*. As *Botrytis fabae* through its pectolysis of host cells begins to macerate, the latter products of pectin hydrolysis activate a latent phenolase in leaves. This phenolase then oxidizes polyphenols in host cells and the oxidation products inactivate the pectolytic enzymes.

Varietal resistance of certain varieties of cider apples to brown rot has been attributed to their high content of polyphenols in parenchyma of the fruit. Patil *et al*. (1964) studied the relation of chlorogenic acid free phenols in potato roots to infection by *Verticillium alboatrum*. They related the resistance of potatoes to *Verticillium* wilts to the content of chlorogenic acid in the superficial cells of roots. It is believed that this polyphenol inactivates hydrolytic enzymes during the early phases of host penetration.

In the wilt of tomato, there is inhibition of pectolytic activity in hyphae invading parenchyma, and the lack of inhibition of mycelium in vessels may account for mycelium being restricted primarily to vessels.Oxidized phenols, quinones, and related substances have been employed as chemotherapeutic agents in wilt diseases. Grossmann (1962a, b, and c) used rufianic acid (1, 4-dioxy anthraquinone sulfone) which inactivates both poly-galacturonases and pectinmethyl esterases. Patil and Dimond (1968b) have shown that polygalacturonase synthesis is inhibted by rufianic acid in *Verticillium.* Mahadevan *et al*. (1965) have demonstrated that the extracts of a number of plant species inhibit pectic enzymes *in vitro* and in some instances, occurrence of such inhibitors *in vivo* may be associated with resistance to pathogens.

Turner (1961) made an interesting contribution in the field of molecular phytopathology while studying the take all disease of oats caused by *Ophiobolus graminis*. Oat leaves and roots contain a fluorescent glucoside (avenacin) which inhibits the growth of several fungi. *Ophibolus graminis* var. *avenae* is pathogenic because it produces a specific enzyme (glucosidase) which hydrolyses avenacin. Thus, the pathogenic capability of *O. graminis* var. *avenae* is dependent on the production of an inhibitor.

Albersheim *et al*. (1969), while discussing the biochemistry of the cell wall in relation to the infective process, state that the repressed synthesis of the polysaccharide-degrading enzymes, which enable some pathogens to attack plants, is the possible mechanism which brings about resistance in certain host

parasite combinations. *Colletotrichum lindemuthianum* is capable of producing a variety of polysaccharide-degrading enzymes, and the virulence of the isolates has been correlated to their ability to produce α-galactosidase. When the alpha or gamma strain of the fungus is grown in a medium containing galactose as the sole carbon source, large quantities of α-galactosidase are secreted. In the presence of either glucose or xylose, α-galactosidase synthesis is suppressed. β-galactosidase appears to be produced constitutively in the alpha strain of *C. lindemuthianum*, while β-galactosidase is induced when either the alpha or gamma strain of the pathogen is grown in culture with xylose as the sole source of carbon.

Patil and Dimond (1968a) have demonstrated that the addition of glucose to the cut stems of *Fusarium oxysporum* f. *lycopersici* infected tomato plants reduces both the rate of symptom development and the level of vascular system polygalacturonase activity. Glucose also represses the synthesis of polygalacturonase activity.

Horton and Keen (1966) have studied the endo-polygalacturonase and cellulase formed in extracts of onion roots infected with *Pyrenochaeta terrestris*. The enzyme production in culture is repressed by the addition of glucose. Horton and Keen (1966) have shown that, upon the removal of the cotyledons from young onion seedlings infected with *P.terrestris* the sugar content of the seedling is reduced to about one fourth. This is accompanied by an increase in both endo-polygalacturonase and cellulase activities and acceleration in disease development. If plants are sprayed with glucose solution or maleic hydrazide, resulting in increased glucose levels in the tissues, there is reduced enzyme accumulation.

Bateman (1964) had shown that in the case of bean seedlings infected by *Rhizoctonia*, infection causes accelerated respiration and these results in accumulations of Ca^{++} and other cations in the infected region. The increased level Ca^{++} activities pectin methylesterase acitivity which in turn de-esterifies the carboxyl groups of polygalacturonide, converting them to pectic acid. Pectic acid then reacts with available Ca^{++} to form calcium pectate. The invaded tissue then becomes resistant to attack by polygalacturonases. This limits the size of the lesion.

Another way in which plants are protected against cell wall degrading enzymes secreted by pathogens is by a modification of the primary cell walls.The development of resistance in the hypocotyls of maturing Red Kidney beans to infection by *Rhizoctonia solani* is correlated with the conversion of pectin to calcium pectate in the hypocotyl cell walls. Calcium pectate is resistant to the polygalacturonase secreted by the fungus. Albersheim and Anderson (1975), while discussing the cell wall and wall degrading enzymes in the infection process, state that a pathogen is incapable of attacking a plant unless the former finds the proper environment to permit it to secrete sufficient amounts of endopolygalacturonase to overcome the amount of inhibitor present in the cell walls of the plant. Although there are several endopolygalacturonase inhibitors in the cell walls of dicotyledons, each of them appears to be able to inhibit the endopolygalacturonases of a variety to unrelated plant pathogens.

The ability of plants to produce enzymes that are able to degrade the cell walls of pathogen may represent another general defense mechanism. Plants can synthesize large amounts of lysozyme, chitinase and endo-β-1, 3 glucanase. The chitinase of tomatoes appears to participate in the lysis of *Verticillium albo-atrum* mycelia that occur during the infection of tomatoes by this pathogen. In some cases pathogens have also evolved their own defense against the potentially lytic enzymes of their hosts. *C. lindemuthianum* which infects beans has been shown to secrete a protein that can specifically inhibit the action of such a wall degrading enzyme present in beans. The inhibitor, an endo-β-1, 3, glucanase is produced in a large amount in bean tissues.

Toxins and Plant Diseases

There is an enormous amount of literature on toxins and their role in plant diseases. Some of the important contributions in this area are by Gaumann (1954), Ludwig (1960), Deverall (1964), Wheeler and Luke (1963), Dimond and Waggoner (1953) on vivotoxins; host specific plant toxins have been discussed by Pringle and Scheffer (1964); and toxins and plant diseases have been discussed by Kalyansundram and Charudattan (1966), and Husain and Janardhanan (1976). Toxins in plant diseases, their structure, and mode of action have been ably discussed by Owens (1969). Wood *et al*. (1972) have compiled papers on phytotoxins and plant diseases. Host specific toxins in relation to pathogenesis and disease resistance have been discussed by Scheffer (1976). Phytotoxins produced by plant parasites are reviewed by Strobel (1974). Toxins produced by phytopathogenic bacteria have been discussed by Patil (1974).

Some recent reviews are those of Van Alfen (1989) "Reassenment of Plant Wilt Toxins"; Walton and Panacione (1993) on "Host Selective Toxins and Disease Specificity". There are some edited books such as those of Daly and Deverall (1983) *Toxins and Plant Pathogenesis*, Graniti *et al*. (1989) *Phytotoxins in Plant Pathogenesis*; and a review on "Bacterial Phytotoxins: Mechanism of Action" by Durbin (1991). Markhan and Hille (2001) have ably reviewed "Host Selective Toxins and Agents of Cell Death in Plant Fungus Intractions".

Although the germ theory of disease was known earlier to the science of plant pathology than to human pathology (Prevost, 1807), yet the role of toxins in infections and in diseases was first established in human medicine. The term toxin in plant pathology has actually been adopted from the conceptual sphere of human medicine. The idea the pathogenesis might by due to the production of poisons by the pathogens is by no means new, but it is only in recent years that it has been put on a scientific footing.

De Bary (1886) showed that plant pathogens produce toxins but experimental evidence to support this hypothesis was available only in 1913 (Hutchinson). The credit for the discovery of toxin is given to Roux and Yersin (1888) who visualized the presence of the toxin in the disease caused by *Corynebacterium diptheriae*. In the diseased animals he observed symptoms very far from the seat of infection. He conceived that this may not be directly due to the microbe itself but to its metabolic product which gets translocated and reveals symptoms at distant places from the actual site of infection.

A toxin can be defined as a substance of microbial origin involved in host pathogenesis. Toxins are normally proteins which are antigenic in nature and have antitoxins. In addition, by introducing small doses of a toxin it is possible to develop immunity in the host against the toxin, and the organism producing it. However, this was not known when Roux first visualized the presence of a toxin in the disease caused by the bacterium *Corynebacterium diphtheriae*.

Plant pathologists have raised doubts of there being toxins in phytopathology. Actually, we do not have toxins in the real sense playing their part in the plant disease syndrome as in human or animal pathology because none of these satisfy the conditions of a toxin in mammalian pathology.

Gaumann (1954) stated, "Microorganisms are pathogenic only if they are toxigenic: in other words, the agents responsible for diseases can damage their hosts only if they form toxins—microbial poisons—that penetrate into the host tissues." The term toxin was used by Gaumann for all substances produced by the pathogens, including enzymes.

The *Concise Oxford Dictionary* defines a toxin as, "a poison, especially one secreted by a microbe and causing some particular disease", and a poison as, "a substance when introduced into or absorbed

by a living organism destroys life or injures health, especially (popularly) one that destroys life by rapid action and when taken in small quantity".

Dimond and Waggoner (1953) regarded a toxin as a phytotoxic compound secreted by a microorganism. This excludes toxic substances secreted by plant parasites other than microorganism. Such restriction is unnecessary. They also recognized vivotoxin as a substance produced in the infected host by the pathogen and/or its host which functions in the generation of a disease, although it is not itself the initiating agent of the disease.

Ludwig (1960), in his review, also regarded a toxin as a product of a microorganism, or of a microorganism host complex which acts on living host protoplasts to influence disease development or symptoms.Wheeler (1975) visualizes toxins as injurious substances produced by organisms. According to him, the term is generally restricted to substances active at physiologically low concentrations. Beyond these basic characteristics, there is little agreement on how toxins, with or without modifying prefixes, should be defined. Some follow the practice of excluding enzymes and growth regulators, others believe that one or both of these should be included in the toxin category. Still others restrict the term toxin to low molecular weight substances or to substances produced by microorganisms.

Toxins are different from enzymes in that they do not attack the structural integrity of the tissues but affect the metabolism in a subtle manner. It is the subtility of action that differentiates toxins from enzymes. Toxins act directly on the protoplasts of cells and substances with the same end effect but an indirect method of action are not toxins. The toxin of *Clostridium welchii*, lecithinase, even though an enzyme, is a toxin because it disrupts cell membranes. Such enzymes are toxins.

Classification of Toxins

Wheeler and Luke (1963) defined three broad classes of toxins, namely, phytotoxins, vivotoxins and pathotoxins according to their source of origin.

Phytotoxin

Any compound produced by a microorganism which is toxic to plants is a phytotoxin. Phytotoxins are nonspecific, incite few or none of the symptoms that are incited by the pathogen, and as happens in most cases, show no relation between toxin production and pathogenicity. Lycomarasmine and alternaric acid are examples of phytotoxins.

Vivotoxins

Dimond and Waggoner (1953), who coined the term, defined vivotoxin "as a substance produced in the infected host by the pathogen and/or its hosts which functions in the production of the disease but is not itself the initial inciting agent of the disease". They laid down certain criterias which a substance must fulfil before being called a vivotoxin. The requisites of a vivotoxin are:

1. It must be isolated from the diseased plant but should not be present in the healthy plant.
2. It must be characterized chemically.
3. When introduced in a pure form into a healthy host, it must produce the symptoms of the disease or a portion of the syndrome. Fusaric acid and piricularin are typical examples of vivotoxins.

Pathotoxins

Pathotoxins play a causal role in disease production, and produce symptoms characteristic of the disease in susceptible plants (Wheeler and Luke, 1963). These toxins may be produced by the pathogen, host or interaction between them. Piricularin produced by *Pyricularia grisea* (*oryzae*) is a typical pathotoxin.

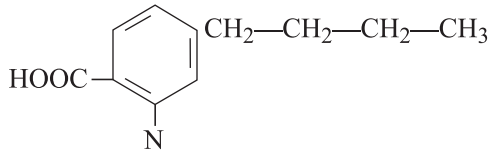
Fusaric Acid

Fusaric acid (5-n-butyl pyridine-2 carboxylic acid) is produced by several species of *Fusarium*, mainly belonging to the group Elegans Woolenweber [*F. oxysporum* (Schl.) Sny. et Hans.] and causing wilt diseases in tomato, cotton, pea, banana, and other plants.

Fusaric acid was first isolated as the metabolic product of *Fusarium heterosporum* Nees, a non-specific parasite, by Yabuta *et al*. in 1934. In 1952, it was recognized as an important toxin of *Fusarium oxysporum* f.sp. *lycopersici* Sacc., *Fusarium vasinfectum* Atk., and *Gibberella fujikuroi* (Saw.) Wr. It is now known to play a pathogenic role in the wilt of tomato and cotton. Subsequently, there has been evidence to show that fusaric acid is involved in the Panama disease of banana caused by *Fusarium oxysporum* f. sp. *cubense* E.F. Smith. A possible correlation has been demonstrated between pathogenicity and the amount of fusaric acid production *in vitro* in respect of several strains of *F. oxysoporum* f. sp. *niveum* and *F. oxysporum* f.sp. *pisi.*

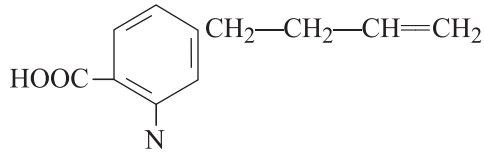
Fusaric acid is called a wilt toxin as it is now known to play a more distinct role in the wilt of tomato and cotton plants than in any other disease. Even when the pathogen is restricted to the roots, symptoms like vein clearing and epinasty are manifested on the leaves. This appears to be due to toxic metabolites translocated to distant parts of the plant from the locus of infection. Some of the toxic symptoms on diseased plants could be produced by pure toxin fusaric acid. Moreover, it has been firmly established that a concentration of 10^{-4}M of fusaric acid is reached in the infected plants, a concentration lethal to the host tissues. The symptoms of fusaric acid injury appear first in the stems and soon after in the leaves in the form of intercostal necrosis of leaves. In the stem it is mainly the cortical tissue over the vascular bundles that are destroyed and light grey-green sunken furrows appear on the stems and gradually spread to the petioles. Next the petioles start to bend downwards. This is called epinasty and this injury is similar to that caused by ethylene. In contrast, the conducting strands in the stems and petioles are apparently uninjured and are not browned (Gaumann, 1957). With higher concentration, the whole stem tissue is weakened and necrosis of the cortical tissues overlying the vascular bundles in the stem sets in. In the leaves, necrosis extends in the interveinal areas, and subsequently, along the whole length of the veins. About 24 hours after toxin intake, the leaves curl and become flaccid.

Fusaric acid has been detected in various plants after inoculation with their wilt pathogens. Fusaric acid (5-n butylpyridine-2-carboxylic acid) has the empirical formula $C_{10}H_{13}O_2N$ and molecular weight 179. It has the following structural formula:



Fusaric acid

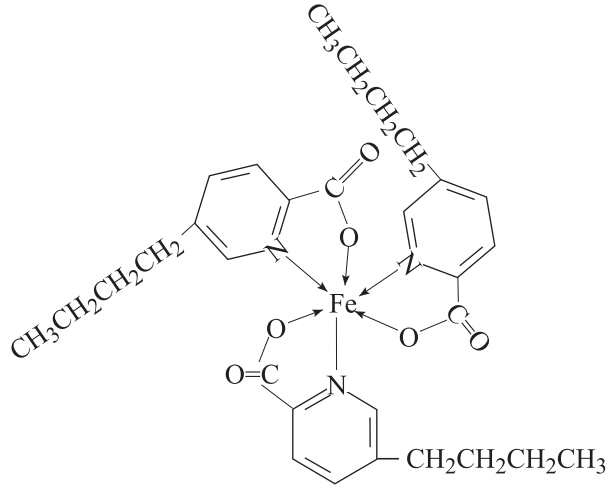
Pure fusaric acid has a melting point of 98–100°C. But biogenic preparations often contain traces of dehydrofusaric acid, and therefore, possess a higher melting point (up to 109°C). The existence of dehydrofuisaric acid in the preparation of fusaric acid has been demonstrated by Stoll (1954). It has the empirical formula $C_{10}H_{11}O_2N$ and molecular weight 177 with melting point 118–120°C. It has the following structural formua:



Dehydrofusaric acid

Surprisingly, the unsaturated dehydrofusaric acid appears after the appearance of the saturated fusraic acid in culture filtrates of *F.oxysporum f.sp.lycopersici*. Fusaric acid is toxic to higher plants and microorganisms interfering with their metabolism in various ways. Before the appearance of the visible symptoms—necrotic spots on the leaf blades, the shriveling and drying of leaves, and the shrinking and wilting of stem and petioles in tomato cutting—the water permeability of protoplasts and the water balance of the whole plant are altered and the respiratory rate decreases. Fusaric acid inhibits polyphenol oxidase competitively in much lower concentrations than other enzymes and may also react with various phenol derivatives.

A comparison of fusaric acid with other derivates shows that two parts of the molecule are essential for toxicity. Inhibition of fungal spore germination, yeast respiration and polyphenol oxidase depends on the presence of a carboxyl group in α-position to the nitrogen. The aliphatic side chain in the β-position determines primarily the effects on the water permeability, the severity of the damage increasing with the length of the side chain methyl to butyl.



Iron chelate of fusaric acid

Fusaric acid forms stable chelate rings, catching a metal ion between the N atom of the pyridine ring and carboxyl radical in the α-position, forming 5-membered coordinate link. Of the metal ions, ferric

ion is most strongly chelated with it. On the basis of the antagonism reported between ferrioxamine B and fusaric acid, it could be conjectured that fusaric acid specifically interferes with the incorporation of Fe^{+++} in the porphyrin skeleton. The mode of action of fusaric acid has been discussed by Kalyansundram (1970). Sanwal (1961) described the physiological effects of this toxin, and suggested that it could cause a partial uncoupling of oxidative phosphorylation. According to Deverall (1964), it plays no part in the development of wilt symptoms, although it might act in conjunction with other metabolites.

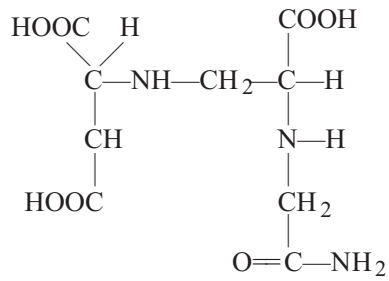
According to Nishimura (1962), fusaric acid is only one of the toxic substances which contribute to the fusarial wilt syndrome. He suggested that one of these—phytonivein—caused wilting; pectic enzymes, caused stem flaccidity; and fusaric acid caused leaf necrosis. All these must be present to reproduce the complete wilt disease syndrome.

Lycomarasmin

Lycomarasmin is a wilt toxin of the tomato wilt pathogen *F. oxysporum* f.sp. *lycopersici* Sacc. Described by Clauson-Kass *et al*. in 1944, this toxin, unlike fusaric acid, appears to be specific to *F. oxysporum* f.sp. *lycopersici.*

Lycomarasmin, when administered to tomato plants, produces injury in the intercostal fields of the leaves, and rolling of the leaf tips. The symptoms of tomato wilt simulate the toxic injury manifested by the synergistic action of lycomarasmin and fusaric acid.While fusaric acid has been demonstrated in infected tomato plants, no such direct evidence exists for lycomarasmin.

Lycomarasmin is a polypeptide with molecular weight 277.3 and empirical formula $C_9H_{15}O_7N_3$.



Lycomarasmin

Using the powerful techinique of NMR spectroscopy in a comparative study of lycomarasmin with aspartic acid, N-acetyl aspartic acid and (β carboxy-b-amino ethyl) aspartic acid, Hardegger *et al*. (1963) determined what is the currently accepted formula for lycomarasmin.

The toxicity of this substance seems to arise from fact that it can form metal chelates. Thus, the injury caused by lycomarasmin when administered to tomato plants causes a shock phase within the first few hours when there is a depressed rate of water intake as well as transpiration. Soon this is reversed, and the rate of transpiration far exceeds the normal rate, bringing about the wilt. The pathogenic effect of this toxin is partly due to the fact that its molecules, on their way through the tomato shoots, chelate the iron ions of the host, which would not be able to migrate themselves, and transport them into the leaves in the form of a lycomarasmin-iron complex. Here, part of the complex is decomposed due to photochemical effects or due to exchange by more potent chelating agents in the cell itself. Thus, on one hand, part of the lycomarasmin molecules are regenerated as chelating agents and cause appropriate local

deficiencies, while on the other hand, iron ions set free in the 'wrong' place lead to iron plethora. Gaumann and Naef-Roth (1956) assume that the excessive transpiration which occurs due to lycomarasmin poisoning is mainly due to iron injuries. Lycomarasmin without iron has no toxic effect on transpiration.

Another aspect of the study of the toxin has been its antagonism to the yeast growth factor, strepogenin which is an oligopeptide containing glutamic acid. As against glutamic acid which is present in strepogenin, lycomarasim has aspartic acid, and to this has been ascribed its antagonistic action. This growth factor, when supplied externally reserves some of the toxic symptoms of the lycomarasim injury in tomato, leading to the conclusion that it may interfere with the strepogenin metabolism of the host. The role of toxin lycomarasim in the development of disease symptoms has been disputed by a number of workers.

Pyricularin

Pyricularia grisea (formerly *P.oryzae*), belonging to the group of Hyphomycetes, is the causal organism of the blast disease of rice and has a wide distribution in all rice growing areas of the world. The symptoms of rice blast disease indicate that the causal fungus produces some very effective toxins.

It appears that two of the metabolic products of the fungus are responsible for the characteristic symptoms of the disease. One of them is identified as α-picolinic acid and the other is piricularin. Pyricularin is more active and semispecific in that it affects blast susceptible rice plants more than resistant ones. Low concentrations of piricularin inhibit the germination of the conidia of *P. grisea*, and the fungus has been shown to produce a pyricularin binding protein, a copper oxidase which binds piricularin and destroys its fungitoxicity but not its phytotoxicity. The ways in which piricularin affects plant tissues are not understood but it induces an increase of polyphenols and oxidases and its toxicity is counteracted by chlorogenic acid, one of the principal polyphenols of the rice plant. It increases respiration and growth at low concentrations but inhibits them at higher ones. Asparagine was found to be the best nitrogen source for the *in vitro* production of toxins by *P. grisea* followed by urea, ammonium sulphate and sodium nitrate. *P. grisea* tends to lose toxin-producing ability after repeated serial cultures.

Pyricularin has been assigned a tentative empirical formula of $C_{18}H_{14}N_2O_3$, melting point of 73.5°C and a molecular weight of 306. It is highly stable in water, especially at low *p*H.

The toxic action of piricularin appears to be due to its inhibitory effect on the activity of several enzymes such as peroxidases, catalase, cytochrome oxidase and ascorbic acid oxidase, even at low dilutions (1/100,000/200,000). The toxic effect of piricularin is removed by chlorogenic acid or ferulic acid, each of them being the natural phenolic constituent of the rice plants. It is presumed that the combination of piricularin with these substances is a natural detoxifying mechanism. The inhibitory activity of the toxin to a test organism, *Bacillus subtilis*, was considerably reduced by the presence of catechol, chlorogenic acid and caffeic acid in the toxin extract. Toxin produced *in vitro* inhibited plumule elongation and oxygen uptake in varieties susceptible to blast but not in those resistant to it.

A third substance, pyriculol ($C_{14}H_{16}O_4$) was isolated from cultures of *P. grisea* which cause natural blast lesion and also inhibits the growth of rice seedlings. The compound is a heptaketide (Turner,

1971). Rao and Suryanarayanan (1971), who earlier failed to detect piricularin and - picolinic acid, were able to identify pyriculol in the culture filtrates of six isolates of *P. grisea.*

In addition to the above three toxins, two more toxic substances have been recorded by Iwasaki *et al.* (1972), namely, 3,4 dihydro 3,4,8 trihydroxy-1 (2H) naphthelenone and Tenuazonic acid. The latter substance has been isolated from infected tissues also, and thus, is a vivotoxin.

Alternaric Acid

Alternaric acid occurs in the early blight of tomato and potato and is caused by *Alternaria solani*. Some of the lesions appear to be devoid of the fungus under conditions, suggesting that a mobile toxin is involved. Cultures of *A. solani* produced a dibasic acid-alternaric acid which caused necrotic lesions similar to those caused by the fungus. The toxin also inhibits or severely restricts growth of the fungal germs tubes without actually preventing germination. Alternaric acid is thought to be fairly nonspecific and its significance in early blight is uncertain. Thus, some highly pathogenic strains of the fungus failed to produce it in culture and it is clear that other factors and possibly other toxins are involved.

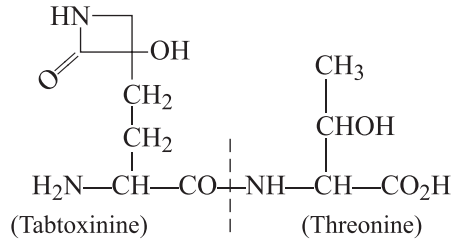
Alternaric acid produced by *A.solani* has antifungal but not antibacterial activities. In addition, it is highly fungitoxic. This substance is an optically inactive, unsaturated diabasic acid with an empirical formula of $C_{21}H_{30}O_8$. The effect of alternaric acid on the water economy of host plants is similar to that observed with the toxin lycomarasmine but it is highly potent. This substance or a substance closely related to it has been detected in the naturally infected plants and there is considerable evidence to show that it is involved in the disease caused by *A. solani*.

Tabtoxin (or Wild Fire Toxin)

Pseudomonas syringae pv. *tabaci* produces a pathotoxin called tabtoxin which is host non-specific. The pathogen causes a disease known as wild fire disease of tobacco. This toxin is also produced by other strains of pv. *tabaci* attacking other hosts such as bean and soybean and other pathovars (subspecies) of *P.syringae* such as those occuring on maize, oats and coffee. The pathogen causes necrotic spots on the leaves with a yellow hallow surrounding the necrotic spots. Sterile culture filtrates of the organism as well as purified toxin produce symptoms which are similar to those characterstic of wild fire disease of tobacco and other hosts.

It is interesting to note the mutants that have lost the ability to produce the toxin (Tox^-) show reduced virulence and cause necrotic spots without a yellow halo. Tox^- strains can not be distinguished from *P.angulata* which causes angular leaf spot of tobacco. *P.angulata* is now believed to be a non toxic form of *P.syringae* pv. *tabaci*.

Tabtoxin is a dipeptide composed of a common amino acid threonine and the amino acid tabtoxinine (not known previously). Tabtoxin itself is not toxic as such but in the host cells the toxin is hydrolysed with the release of the toxic moiety tabtoxinine (which is the active toxin) and threonine. Tabtoxinine is toxic to cells because it inactivates the enzyme glutamine synthetase which leads to depleted levels of glutamine with subsequent accumulation of ammonia which reaches toxic levels. Ammonia accumulation leads to the uncoupling of photosynthesis and photorespiration. The thylakoid membranes of the chloroplasts are destroyed which result in the chlorosis and necrosis of the tissues in the leaves.



TABTOXIN

Cercosporin Toxin

It is a non-specific toxin and is produced by most of the species of *Cercospora* such as *C. zea maydis* causing gray leaf spot of *Zea maydis* and *C. zinniae* causing leaf spot of *Zinnia elegans* along with leaf spot and blight diseases of many vegetable and ornamental plants. This toxin is activated in the presence of light and becomes toxic to plants by generating single oxygen, i.e., reactive atomic oxygen in the cell. This generated single oxygen destroys the membrane of infected host plants which in turn provides nutrients to the intercellular pathogen. Cercosporin is a photosensitizing perylenequinone that absorbs light energy and forms activated oxygen.This activated oxygen interferes with host lipid, protein and nucleic acid synthesis, thereby increasing the virulence of the pathogen that ultimately kills the plant cells. The fungal spores and mycelium are resistant to Cercosporin toxin due to production of pyridoxine (Vitamin B_6) by the fungus. This pyridoxine reacts with activated single oxygen and is neutralized during the course of infection. Thus, this toxin incites the production of reactive oxygen in the cells which causes disruption of cell membrane and loss of electrolyte from cells.

Phaseolotoxin

Phaseolotoxin (host non-specific toxin) is produced by the bacterium *Psendomonas syringae* pv. *phaseolicola* which causes halo blight of beans and some other legumes. The localized and systemic cholorotic symptoms produced in infected plants are identical to those produced on plants treated with the toxin alone. Plants treated with toxin alone or those infected with the bacterial pathogen show reduced growth of newly formed and expanding leaves, disruption of apical dominance and the accumulation of the amino acid ornithine.

Phaseolotoxin is a modified ornithine-alanine-arginine tripeptide carrying a phosphosulfinyl group. The toxin affects cells by binding to the active site of and inactivating enzyme ornithine carbamoyl transferase which normally converts ornithine to citrulline, a precursor of arginine. Thus the toxin causes accumulation of ornithine and depleted quantities of citrulline and arginine.

Phasaelotoxin also inhibits the synthesis of pyrimidine nucleotide biosynthesis, reduce the activity of ribosomes, interfere with lipid synthesis, change the permeability of the membranes and result in the accumulation of large starch grains in the chloroplasts. It is believed that the toxin breaks the disease resistance of the host.

Tentoxin

Tentoxin (host non-specific toxin) is produced by the fungus *Alternaria alternata* (previously called as *Alternaria tenuis*). The pathogen causes chlorosis in seedlings of many plant species. Tentoxin is a cyclic tetrapeptide that binds to and inactivates a protien (chloroplast- coupling factor) involved in energy transfer into chloroplasts, and also interfers or inhibits the light dependent phosphorylation of ADP to ATP.

An additional but apparently unrelated effect of tentoxin is that it inhibits the activity of polyphenol oxidases, enzymes involved in resistance mechanisms of plants. There are several areas such as the molecular site of action of tentoxin and the exact mechanism by which it brings about these effects are still not known.

There are several other non-host-specfic toxin such as fumaric acid produced by *Rhizopus* spp. in the almond hull disease; oxalic acid produced by *Sclerotium* and *Sclerotinia* spp. in various host plants in which these infect; and by *Cryptonectria parasitica*, the cause of chestnut blight disease, Ceratoulmin produced by *Ceratocystis ulmi* in Dutch elm disease, fusicoccin produced by *Fusicoccum amygdali* in twig blight disease of almond and peaches, cercosporin produced by *Cercospora* spp. and several others which are involved in host pathogenesis but are not host specific.

Host-Specific or Host-Selective Toxins

A host specific or host selective toxin is one which only affects plants susceptible to the pathogen producing it. There are several host specific toxin such as victorin (HV toxin) on victoria variety of oats, T-toxin produced by *H. maydis* now called as *Cochliobolus heterostrophus* on maize lines having Texas male-sterile (Tms) cytoplasm, HC-toxin produced by *Helminthosporium (Cochliobolus*) *carbonum* on specific maize lives, AM-toxin produced by apple pathotype of *Alternaria alternata* (previously called as *A mali*), HS-toxin produced by *H* (C*ochliobolus) sacchari* affecing sugarcane, ACL-toxin produced by *Alternaria citri* (lemon race) affecting rough lemons, ACT-toxin produced by *A. citri* (tangerine race) affecting Dancy tangerine, AK-toxin on Japanese pear, AT-toxin on tobacco. PC-toxin produced up *Periconia circinata* in sorghum, PM-toxin in corn that has Texas male cytoplasm, and CC-toxin produced by *Corynespora cassicola* in tomato.

Some of the evidences that a particular host specific toxin is involved in disease can be deduced from the following:

(i) Purified toxin reproduces all disease symptoms in the host.
(ii) Toxin affects susceptible but not resistant genotypes.
(iii) Tox^- strains are non pathogenic and
(iv) In crops between Tox^+ and Tox^- strains there is co-segregation among progency for toxin production and pathogenicity

Some of the host specific toxins will be discussed here in some details:

Victorin or Hv Toxin In the late 1940s, oat crops in the USA were affected by a seedling blight caused by *Cochliobolus (Helminthosporium) victoriae* in the variety victoria or its derivatives. Although the pathogen was typically localized in the basal portion of infected plants, symptoms appeared on the leaves which often collapsed. Litzenberger (1949) demonstrated that the culture filtrates of the fungus

contained a toxin that can reproduce accurately the visible symptoms of the disease. What was particularly interesting about this toxin was that resistant oat cultivars were not affected by it. The name "victorin" was given to this toxin by Wheeler and Luke (1954). The toxin is highly specific in its activity and is claimed to produce the symptoms only in the pathogen-susceptible variety of oats. Thus, victorin was the first host specific toxin to be deseribed. Since then a number of other toxins with host specificity and an ability to incite all of the symptoms associated with a particular disease have been identified. These are known as host-specific toxins to distinguish them from those which are non-specific.

Its chemical structure has been determined. It is a complex chlorinated, partially cyclic pentapeptide (tricyclic secondary amine), with molecular weight of around 2000.The earliest effect detected was in permeability. This led to the suggestion that the disruption of cell permeability could be an initial event which triggers subsequent pathological changes in many diseased plants.Victorin causes a decrease in C6/C1 ratio of susceptible oat leaves. This provides additional evidence that victorin induces metabolic alterations similar to those found in other diseased plants.

Victorin is able to incite toxic symptoms only in the blight susceptible variety of oats. Perhaps this could be attributed to the presence of a specific enzyme on the surface of the cells of the susceptible and not the resistant variety (Sadasivan and Kalyansundram, 1966). Cell wall modifications and increased secretory activity were the earliest ultrastructural changes found in victorin-treated tissues. The primary target of the toxin seems to be cell membranes (plasma membranes) where the toxin is believed to be binding to several proteins. But the exact mechanism of its action is still not clear.

The widespread use of new crop varieties often results in new disease problems. Thus, the black spot disease of Japanese pears (*Pyrus serotina*) appeared after the new natural mutant variety *Nijisseiki* was introduced. The causal organism was identified in 1917 as a species of *Alternaria*, later named as *A. kikuchiana* (Tanaka, 1933). Tanaka, in 1933, reported a host labile-a host selective factor produced by the causal fungus in culture. Convincing evidence for a host specific toxin produced by *A. kikuchiana* was supplied by Hiroe *et al*. (1958) and confirmed by Mori (1962). The host-specific toxin was extracted from narurally infected leaves (Ohkawa and Torikata, 1967).The effect of AK toxin on pear appers to be similar to the effect of HV toxin on oats. AK toxin caused an increase in electrolyte loss from susceptible but not resistant leaves within 20 minutes of initial exposure.

T-Toxin T-toxin is produced by race T of *Cochliobolus (Helminthosporium) heterostrophus* the cause of southern corn leaf blight in the USA. It attacks corn that have Texas male- sterile (Tms) cytoplasm. Corn with normal cytoplasm is resistant to the pathogen.

T-toxin is a mixture of long (35–45 carbon) polyketols. The toxin acts specifically on mitochondria of susceptible cells and inhibits the synthesis of ATP. The toxin reacts specifically with a specific receptor protein molecule (URF 13) that is located on the inner mitochondrial membrane of sensitive mitochondria (of Texas male sterile cytoplasm).When T-toxin is present, it forms pores in the inner mitochondrial membrane of maize lines having cytoplasmic male sterlity. The pores cause loss of mitochondrial integrity which results in selective permability of the mitochondrial membrane and disease.

There are several other toxins which have been reported during the last few decades. Some of these are host specific while others are non-specific. A list of various toxins with the effects they produce on the host plants or tissues, and the possible mechanism of their action are given in Table 4.1.

Table 4.1 Various toxins, the organisms producing them, their chemical structure and their effects on host plants.

Fusaric acid	*Fusarium oxysporum* f.sp. *vasinfectum*, *F. heterosporum*, *F. cubense*, *F. moniliforme*, *Nectria cinnabarina*, *F. oxysporum lycopersici* (host: cotton, tomato, banana, rice)	5 n butylpyridine carboxylic acid	Non-specific vivo-toxin	Affects permeability of plant membranes, water intake, depresses respiration and polyphenoloxidase, disturbs oxidative phosphorylation, inhibits Fe-Porphyrin oxidases, disturbs balance of inorgainc ions in diseased plants
Lycomarasmin	*F. oxysporum lycopersici* (host: tomato)	Amino acid derivative	Non-specific	Causes strepogenin deficiency, chelates Fe^{+++} free and bound, injures permeability of leaf cells.
Pyricularin	*Pyricularia grisea* (formerly *oryzae*) (host: rice)	Empirical formula $C_{18}H_{14}N_2O_3$	Non-specific activity	Increases respiration and growth at low concentration and inhibits them at high doses, inhibits the enzyme peroxidase, catalase, cytochrome oxidase and ascorbic acid oxidase
α-Picolinic acid	*P. grisea* (formerly *oryzae*) (host: rice)	α-Picolinic acid		Affects catalase, chelates iron in respiratory enzymes
Pyriculol	*P. grisea* (host: rice)	$C_{14}H_{16}O_4$		Unknown
Victorin	*Helminthosporium victoriae* (host: oat)	Polypeptide linked to a tricyclic-secondary amine	Host-specific toxin, not yet detected in the host tissues	1. Primary effect on plasma membrane-damages permeability 2. Increases respiration and has secondary effects on several other metabolic systems. Loss of electrolytes from host cells, disruption of chloroplasts, disruption of internal membrane

(Contd.)

				systems,effects on permeability of the cell membranes, leading to the loss of cellular contents, inhibition of root growth, transpiration and auxin induced cell elongation, victorin acts by uncoupling oxidative phosphorylation
Periconin or PC toxin	*Periconia circinata* (which causes milo disease of *Sorghum vulgare* var.*subglabrescens*)	Probably a polypeptide of fairly low molecular weight. Two toxins—toxin A: peptide ± base, B: peptide	Host-specific toxin, although it remains to be shown that the toxin is a vivotxin	Increased respiration, loss of cellular materials, decreased ability to incorporate amino acids, interference with membrane functions.
Alternaric acid	*Alternaria solani* (host: potato, tomato)	Dibasic acid Alternaric acid	Non-specific phytotoxin	Role in disease doubtful, wilting, necrosis and chlorosis
Colletotin	*Colletotrichum fuscum* (anthracnose of *Digitalis*)	Similar to alternaric acid, polysaccharide and peptide characteristics	Non-specific phytotoxin	Disrupts cell permeability or affects pectic enzymes
Diaporthin	*Endothia parasitica* (host: Chestnut)	Structure similar to isocoumarins	Phytotoxin	Necrosis of the conducting vessels.
Fusicoccin	*Fusicoccum amygdali* (host: almond, peach)	A terpenoid (a glucoside of carbotricyclic terpene)	Phytotoxin	Possesses growth regulatory properties, causes stomata to open
Tentoxin	*Alternaria tenuis*	Cyclo-N-methyl dehydrophenyl alanyl-L-n-methyl alanyl	Non-selective phytotoxin	Reduction in chlorophyll content, inhibits photophosphoylation, causes stomatal closure
Altenuene	*A.tenuis* (brown spot of tobacco)	Structually related to alternariol	Selective	Induces chlorosis on the leaves

(Contd.)

Wildfire toxin (Tabtoxin)	*Pseudomonas syringae* pv.*tabaci,* *P. angulata*, *P. coronofaciens* (host: tobacco)	Wooley *et al* (1952, 1955) proposed that the widfire toxin is composed of an amino acid tabtoxinine linked to lactic acid by a lactone ring, Sinden and Durbin (1970) failed to detect lactic acid but did find that threonine and serine were present	Non-specific pathotoxin	Formely thought to interfere with methionine metabolism but recent studies indicate that it may act by inhibiting glutamine synthetase activity
Cercosporin	*Cercospora zea maydis* (Host: Maize, *Zinnia*)	Photosensitizing perlenequinone	Non-specific	Distruption of cell-membrane and loss of electrolyte from cells
Rhizobito-xin	*Rhizobium japonicum* (host: tobaccoo)	Basic sulphur containing amino acid presumably composed of two molecules of an ether derivation of homoserine linked through thioester bridge	Phytotoxin not host specific	Sole cause of chlorosis in plants infected, inhibits B-crystathionase
Alternariol Monomethyl ether	*A. tenuis*	Alternariol monomethyl ether	-	Inhibits phenol metabolism of the diseased plants
Tenuazonic acid	*Alternaria longipes* (leaf spot of tobacco), *A. alternata* (leaf blight of *Datura innoxia*), *Pyricularia grisea (oryzae)* (blast of rice)	Tenuazonic acid	Vivotoxin	Chlorotic haloes, inhibits the release of newly formed proteins from the ribosomes and thus inhibits further synthesis.

(Contd.)

Amylovorin	*Erwinia amylovora*	A refind polypeptide antibiotic	Non-specific	Broad spectrum, antibiotic, disrupting cellular permeability, induces shoot cankers and necrotic leaf spots
HC toxin	*Helminthosporium carbonum* (host: corn)	Cyclic polypeptide	Host-specific phytotoxin	Effect on nitrate uptake, reduction of corn tissues
HS toxin	*Helminthosporium sacchari* (host: sugar cane)	Glycoside helminthosporiside (2 hydroxy cyclopropyl-D galactopyran-oside)	Host-specific selective	Disruption of chloroplast lamellae. Plasmalemma disruption
HM-T toxin	*Helminthosporium maydis* race T (host: corn)	Chemical structure not yet established (appears to be a ninhydrin positive low molecular weight substance)	Host-specific	Swelling of mitochondria and loss of respiratory control, initially affects respiratory centres, alters membrane permeability. HM-T toxin stops light induced K^+uptake by guard cells, thus preventing opening of stomata.
AK toxin (phytoalternarin)	*Alternaria kikuchiana* (host: pear) *Phyllosticta maydis* (host: corn)	Has not been identified chemically	Host-specific	Perhaps causes damage to cellular membranes
PM toxin	*Phyllosticta maydis* (host: corn)		Host-specific	Effects similar to T-toxin, plants with Tms cytoplasm are more susceptible, mitochondria are affected
Helmintho-sporal	*Helminthosporium sativum* (host: wheat, barley)	Sesquiterpenoid	Phytotoxin, non-specific	Inhibits respiration in root tissues of barley and wheat
Ophiobolin A formerly known as cochliobolin	*Helminthosporium oryzae* (host: rice)	Oxalic acid	Phytotoxin, non-specific	Phenol metabolism is affected

(Contd.)

Hydrogen cyanide	Unidentified psychrophilic basidiomycetous fungus responsible for white crown rot (snow mold of alfalfa)	HCN	Phytotoxin	Symptoms develop in winter when snow and ice might prevent the gas from escaping
Fumaric acid	*Rhizopus* sp. (in the hull rot of almonds associated with the rotting of the fruit mesocarp of almond)	Either fumaric acid or its derivative	Non-selective pathotoxin	The toxin is translocated from the affected fruit and causes blighting of adjacent leaves and twings
Pseudomonas phaseolicola toxin	*Pseudomonas phaseolicola* (host: bean) *Pseudomonas phaseolicola* (host: bean)	Polysaccharide	Pathotoxin	Inhibits ornithine carbamoyl transferase activity
Pseudomonas solanacearum toxin	*Pseudomonas solanacearum* (host: potato)	Polysaccharide	Pathotoxin	Blocks water flow in the vascular system

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For Toxins β

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Questions

1. What do you understand by cell wall degrading enzymes? How are these involved in the production of disease syndrome? Explain their role with reference to soft rots in vegetables or fruits.
2. How will you classify cell wall degrading enzymes on the basis of their substrate utilization and mode of action?
3. What do you understand by the mechanism of disease development in soft rots?
4. What are "macerating principles"? Explain their role in disease development.
5. What are toxins? How are these involved in the development of vascular wilt syndrome?
6. Explain the following:
   i. Host-specific toxins
   ii. Vivotoxins, phytotoxins and pathotoxins
   iii. Role of proteolytic enzymes in pathogenesis
   iv. Role of cutinolytic enzymes in pathogenesis
   v. Role of lignolytic enzymes in pathogenesis.
7. What is role of toxins in host-specificity?
8. What do you understand by mycotoxin, phytotoxin, pathotoxin and vivotoxin? Explain by giving suitable examples.
9. Write in brief about the chemical 'weapons' utilized by the pathogens in disease development.
10. What do you know of the detoxification of enzymes and toxins?
11. What is the mechanism of action of the following host-specific (selective) toxins:
    i. Victorin
    ii. T-toxin
    iii. Ak-toxin
12. The discovery of host-specific toxin raised hopes that biochemical basis of pathogenicity, and conversely host resistance, might turn out to be simple, determined by a right compound of major effect. Overall these hopes have not been fully realized. Why?
13. What can be the evidences for involvement of a host specific toxin in plant disease?

Alteration in Plant Physiological Functions Due to Plant Pathogen Interaction

5

Permeability Changes in Diseased Plants

Permeability phenomena in plant diseases have been reviewed by Wheeler and Hanchey (1968). The opening paragraph of their review article is of great relevance: "Ignorance rarely goes unmasked. Thirty years ago, our ignorance of the fundamental nature of plant disease wore a physiological mask. Today, permeability is in fashion tomorrow nucleic acids, masked or unmasked, will probably be the vogue." There is also a review by Wheeler (1976). In addition, a number of reviews have dealt completely or in part with the effects of phytotoxins on permeability (Wheeler and Luke, 1963; Owens, 1969). Dube and Srivastava (1975) have discussed altered permeability as a trigger for pathogenesis.

Membranes are composed primarily of lipids and proteins, with small amounts of carbohydrate. These are arranged as a bilayer of phospholipid molecules with the ionic groups facing outwards and the non-polar ends of the phospholipids facing inwards with embedded protein molecules protruding on either side. These proteins may move within the fluid bilayer. It is generally accepted that all water relations are controlled by the plasma membrane although in plant cells the rigid cell wall is also important in maintaining turgor. Due to its semipermeable nature, the plasma membrane is of central importance in regulating the passage of ions and organic molecules into and out of the cell. Thus, plasma membrane is a very complex and dynamic structure which maintains a suitable intracellular environment for various activities of the cell. Similar membranes also occur in cell cytoplasm as endoplasmic reticulum and delimiting various organelles. Membranes are responsible for ion transport. There are two methods of transport of ions. One is by passive diffusion in response to concentration gradients. The other method of transport of ions is against concentration gradient and against electrochemical gradients by active transport in which ions are pumped across membranes using metabolic energy, in the form of ATP, and mediated by ATPases. Membrane permeability plays a vital role in this inward and outward movement of the substances.

Among the several disturbances that a diseased plant shows, some are primary while others are the secondary effects of the former. Regardless of the disease type or the nature of the pathogenic agents, changes in permeability are present in all diseased tissues which have been examined. Changes in permeability have been detected before any other symptom of the disease appeared or in advance of the pathogen. This, plus the fact that changes in permeability can be caused by a variety of biological, chemical, or physical agents, led to the suggestion that a change in cell permeability might be the initial event in pathogenesis.

The first case of altered permeability was reported by Hutchinson (1913) for Rangpur tobacco wilt caused by *Pseudomonas solanacearum* (now *Ralstonia solanacearum*). Thatcher (1939, 1942, 1943), however, made the first comprehensive study of the extent of permeability changes in plant diseases, that is rusts, powdery mildews, soft rots, dry rots, and wilts and concluded that:

1. Cells of the fungus pathogens have higher osmotic pressure than those of their hosts.
2. Altered permeability of the host cells is consistently associated with disease.
3. For rusts, which were most extensively studied, an increase in host cell permeability characterizes susceptibility, while decreased permeability results in resistance.

Biotrophic fungus which forms haustoria establishes a very specific relationship with the host cell membranes. These fungi do not cause massive membrane damage since this would kill their host cells. The plasma membrane of the host and the pathogen remain in very close contact near the point at which the cell wall is penetrated. As the fungal haustorium grows, the host membrane becomes invaginated (extrahaustorial membrane) to accommodate the fungus. The fungal cell wall around the haustorium remains intact and is completely enveloped by an encapsulating zone or sheath, possibly of host origin, but which differs in composition from the host cell wall through which the haustorium has penetrated. The extra haustorial membrane lacks ATPase, membrane bound enzymes which control the pumping of H^+ ions out and K^+ ions in, across the membrane. The haustorium of the pathogen can deplete solutes from the matrix which surrounds it, and maintains a high concentration gradient. The fungus thus maintains a tight control over membrane permeability. Thus, biotrophic pathogens also increase the permeability of host tissues. Cells penetrated by haustoria can still be plasmolysed, so the integrity of the membrane is maintained but there is a definite change in the semipermeable properties of the plasma membrane which is related to the uptake of nutrients by the pathogen. The extra haustorial membrane loses control of nutrient transport so that sugars pass freely to the pathogen.

Ionic imbalance has been reported in Verticillium wilt of cotton and blast disease of rice by Dube, (1971, 1973). Bateman and Millar (1966), Beckman (1964), and Kuc (1966) related calcium to resistance in plants infected by *Rhizoctonia solani.* Membrane damage has been observed in the electronphotomicrographs of infected tissues. Hall and Wood (1970) and Mount and Bateman (1970) have reported that endo-PGTE, a pectic enzyme, damages plasma membranes and causes the leakage of ions. The rupturing of plasma membrane occurs at plasmodesmata as a result of excessive influx of water from the hypotonic solution (formed by the enzymic dissolution of middle lamella) into the host cells. Spalding and Abdul-Baki (1973) have shown that maceration and celll death were closely correlated when potato discs were treated with a purified pectinmethyl trans-eliminase produced by *Penicillium expansum.* Tissues exposed to pectic enzymes undergo changes in permeability before cells are separated or killed; the relationship among these phenomena is not clear at all. If tissues are plasmolysed in hypertonic solutions, cells death and permeability changes can be greatly delayed during exposure to pectic enzymes, but such conditions have little effect on the maceration.

Attempts to correlate tissue permeability with disease resistance have yielded variable results. It had been shown that when the leaves of Minden wheat were exposed to chloroform vapour, there was an increase in the permeability of the tissue and there was definitely an increase in the susceptibility to rusts. However, Johnson and Johnson (1934) found no correlation between osmotic pressure and resistance to rusts. The susceptibility of several varieties of wheat to rust and cotton of *Fusarium* wilt has been correlated to the quantities of organic substances secreted.

In an interesting work, Stall and Cook (1979), recorded the levels of conductivity measuring leakage of electrolytes released from leaves of pepper plants inoculated with three races of the bacterium

Xanthomonas campestris pv. v*esicatoria.* Disruption of membranes and electrolyte leakage occurred much earlier and at a much greater rate when the leaves were inoculated with bacterial races carrying virulent genes, while release of electrolytes occurred later and at a slower ratio with avirulent race of the pathogen.

The substitution of pathogens by toxins has greatly helped in understanding the various steps in pathogenesis. Linskens (1955) treated tomato tissues with fusaric acid and lycomarasmin. Ions of Na^+, K^+, Ca^{++}, as well as free amino acids were detectable in leaf exudates after treatment with fusaric acid and lycomarasmin. This gave support to Gaumann's theory that damage of plasma membranes was the primary cause of pathological wilting of plants. Victorin, a host-specific toxin (pathotoxin), produced by *Cochliobolus* (*Helminthosporium*) *victoriae*, which causes victoria blight of the oats, provided a lot of information (Wheeler and Luke, 1963). Wheeler and Black (1963) noted the passive leakage of large quantities of electrolytes and increased oxygen uptake in victorin treated leaf tissues. However, victorin has no effect on isolated mitochondria. Thus, increased respiration of tissues under toxemia was not the result of direct action of victorin on mitochondria (Black and Wheeler, 1966; Wheeler and Hanchey, 1968). Apparently some site other than the Krebs cycle is affected which brings about increased oxidation rates in an unknown way. The electrolyte leakage from a victorin treated tissue was very quick (within two minutes) and it was almost an instant response (Samaddar and Scheffer, 1968). That permeability damage is a critical event which ensues much before the loss of respiratory control is proved beyond doubt in the experiment with victorin. Respiratory changes occur as the secondary effects of toxin action.

Mechanisms responsible for membrane permeability alterations under pathogenesis still remain unknown. According to one hypothesis these toxins combine with some unknown component or receptor in the susceptible cell and thus bring about its disorganization. Phosphatidase, tyrosinase and also pectic enzymes have been implicated in membrane damage. The rupture of the plasma membrane at the site of plasmodesmata has been suggested by Hall and Wood (1970) to explain the leakage of electrolytes from endo-PGTE treated tissues. It has been suggested that in the bacterial hypersensitive reaction, an inducer disrupts the tertiary structure of the membrane protein by breaking the disulfide bond. SH-containing compounds, such as mercaptans, bring about similar splitting. Thus, membrane stability and self-repair may be crucial determinative factors in susceptibility and resistance. Some phytoalexins have been shown to cause membrane damage as well as other effects on the host plant. The phytoalexin pisatin influences stomatal opening by affecting membrane function. Leakage of electrolytes has also been reported in virus infected tissues, especially roots but this does not seem to be common symptom in other diseases.

Effect of Plant Pathogens on Translocation of Water and Nutrients in Host Plant

Many plant pathogens interfere in one or more ways with the absorption of water and uptake of nutrients and their translocation in the plants. In many diseases where the root rotting fungi, bacteria, nematodes, and viruses there the pathogens bring about extensive damage or destruction of the roots absorption of water is considerably reduced. Root injury directly affects the amount of functioning roots and decreases proportionately the amount of water absorbed by the roots. In vascular wilt diseases there is resistance to water flow which may be due to physical blockages which obstruct the vascular system by the fungal

biomass (as in *Verticillium spp. Fusarium oxysporum* and *F. solani*), or action of fungal toxins and enzymes. There may be host plant responses which may lead to xylem vessel blockage. The formation of tyloses is often reported, particularly as host response to vascular wilt infections. Xylem parenchyma cells balloon through pit membranes and very effectively clog or close xylem vessel lumen. Gels and polysaccharide gums are also cell wall degrading enzymes by the invading pathogens. These may also obstruct xylem vessels, often accumulating at perforation pits. Dysfunction of the water system has been discussed by Talboys (1978) and Isaac (1992) and dysfunction in the flow of foods by Zimmerman and McDonough (1978). Disturbed mineral nutrition has been discussed by Huber (1978).

Ion transport is also interfered with as a result of infection. Ions diffuse differently through solution than through pure water their diffusion coefficient is greatly reduced in soil. PO_4^{3-} diffuse through soil very slowly and a depletion zone may occur around the active absorbing roots. It is therefore, very essential that new root growth should occur in the growing plants. Ca^{+2} moves through plant tissues with water and enters the symplast at the casparian strip**.** Suberization blocks the uptake of Ca^{2}+, Mg^{2}+ and Fe^{2}+ions. K^{+} and PO_4^{3-} ions are taken up by the symplast in the root cortex, so that increased suberization of maturing roots does not affect the increased ion uptake and such as K^{+} which accumulate at the infection sites. In take all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* infected plant are seen to lead to the reduction of K^{+}, P and Ca^{+2}.

As far as organic nutrients are concerned, these are produced in the leaf cells through photosynthesis and move through plasmodesmata into adjoining phloem elements, from there through plasmodesmata into the protoplasm of living photosynthetic cells, where these are either utilized or stored into storage tissues. Plant pathogens may interfere with the movement of organic nutrients from the leaf cells to the phloem, with their translocation through the phloem elements, or possibly, with translocation through the phloem elements, or possibly, with their movement from the phloem into cells that will utilize them.

Biotrophs such as powdery mildew fungi and rusts cause an accumulation of photosynthetic products, as well as inorganic nutrients, in the areas invaded by the pathogens. As indicated earlier in these diseases, the infected areas are characterized by reduced photosynthesis and increased respiration. However, it has been seen that the synthesis of starch and other compounds as well as dry weight are temporarily increased in the infected areas. In some virus diseases such as leaf curling type and some yellows diseases, starch accumulation in the leaves is mainly the result of degeneration of the phloem of infected areas. There is another possibility in the interference with translocation of starch in that the virus inhibits the enzymes involved in the breaking down of starch into smaller translocable molecules.

Effect of Pathogens on Translocation of Nutrients and Water

In order to survive and carry out various physiological and metabolic processes in the plant, all living cells require water, and organic and inorganic nutrients. The roots of the plant help in the absorption of water and mineral from the soil. These substances are generally translocated upward through the xylem vessels of the stem and into the vascular bundles of leaves. As a result of photosynthesis, these organic nutrients are then translocated downward and distributed to all the living cells through the phloem tissues. As a result of pathogen infection to the plant, the upward and downward translocation of nutrients is inhibited and thereby enabling the plant to carry out its normal functions and thus causing disease of the entire plant.

Interference with Upward Translocation of Water and Inorganic Nutrients

Some of the pathogens affect the roots and cause them to absorb less water, or some of pathogens growing in the xylem vessels interfere with the translocation of water through the stem, or some pathogens cause excessive transpiration. Many pathogens, such as root rot of black pepper caused by *Phytophthora capsici*, wilt of leguminous plants with the fungus *Fusarium*, galls caused buy the bacterium *Agrobacterium tumefaciens*, and root knot galls caused by the nematode *Meloidogyne* sp. cause destruction of the root before any symptoms appear on aerial parts of the host. Such abnormality in the roots will interfere with the normal absorption of water and nutrients by the roots. Apart from this, some pathogens also inhibit root hair production and also alter permeability of root cells which will ultimately interfere with the normal absorption of water by roots.

In case of damping off, wilts and stem rots, bacterial and fungal pathogens may reach the xylem vessels in the area of infection and may cause death of the young plants. In vascular wilts caused by *Fusarium* spp. the reduction in water translocation may vary from little to complete depending upon the susceptibility or resistance of the host. Apart from this, the affected vessels may be filled with the microconidia, macroconidia, or chlamydospores of *Fusarium* spp., and with the substances like Fusaric acid or polysaccharides secreted by the pathogen and may become clogged. Such clogged vessels cease to function normally and allow little or no water to pass through them. Some bacteria and nematodes cause root galls or root knots and these enlarged and proliferating cells around the xylem exert pressure on the xylem vessels which may be crushed, thereby becoming less efficient in water uptake.

In all cases, in infected hosts the flow of water is reduced through reduction in the size or death of xylem tissues due to infection or development of tyloses in the xylem vessels or release of some molecules in the vessels as a result of breakdown of cell walls by the enzymes of the pathogens.

Photosynthesis

Photosynthesis is a basic function of green plants that enables them to transform light energy into energy of chemical bonds, literally, photosynthesis means "synthesis with the help of light". This covers a variety of processes in organic and inorganic chemistry. However, the term is usually applied to one reaction only—the synthesis of organic matter by plants in light—a process also called carbon assimilation.

In the basic reaction of photosynthesis, carbon dioxide from the atmosphere and water from the soil are brought together in the chloroplasts in the presence of light which react to form glucose with the concurrent release of oxygen.

$$6CO_2 + 12H2_O \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6O_2 + 6H_2O$$

$\Delta G^o = 686$ kcal.

Carbon dioxide is reduced to the oxidation reduction level of carbohydrate, as symbolized by CH_2O. Each molecule of CO_2 fixed into carbohydrates requires a net input of 1, 18,000 calories from light. As an inherent feature of this process, light energy is also converted to chemical energy by a process known as photosynthetic phosphorylation, where light energy is stored in the useful high energy organic phosphate compound—adenosine triphosphate (ATP).

The details of the perturbations in the phenomenon of photosynthesis by plant pathogens have received little attention and there is scanty information available on this subject. The carbon balance of

diseased plants had been reviewed by Daly (1976) and bioenergetic and metabolic disturbances in diseased plants by Hutcheson and Buckanan (1983). The principal attention has been paid to the effect of the pathogen on the overall rate of the photosynthesis of plants because the photosynthetic apparatus—the chloroplast—is destroyed or disturbed by viral, fungal or bacterial pathogens. Our understanding of the perturbations in photosynthesis as a result of infection will not be complete unless we study the mechanism in a healthy plant. Our description of the process of photosynthesis in healthy plants will be very brief. The pigments, enzymes and cofactors of photosynthesis are all located in the chloroplast. The chloroplast is an ellipsoidal structure with an axis 3.5 mm long. It is a highly organized structure in which the pigments are found in a series of concentrated lamellae in the regions termed grana. A smaller number of lamellae are also found between the grana.

The lamellae consist of an ordered array of chlorophyll, lipid, and accessory pigment molecules (such as carotene) oriented in such a way as to allow the transfer of light from the pigment molecules which absorb the photons of light to the pigment molecules in which the energy is ultimately utilized in photosynthesis.

We know that photosynthesis is a two-phase process. In the first phase which is completed in the presence of light, Water is broken down (photolysis) and an energy-rich bond or ATP is generated, together with a molecule of NADP which is reduced to $NADPH_2$. ATP and NADPH together form the assimilatory power.

In most plants, two light reaction systems—Photo system I and Photo system II—are responsible for the trapping of light energy (Fig. 5.1).

The mechanism by which cyclic and noncyclic photophosphorylation are generally considered to be effected by the thylakoid membrane of chloroplasts is shown in figure 5.1. The heart of the system is a light driven transport of electrons from two different active chlorophylls (P680, P700) to each of the two acceptors A_{II} and A_I. In the non-cyclic reactions, electrons from water flow through two photochemical reactions (photo system II and I) to ferredoxin and then the NADP. In the cyclic reactions, electrons flow through a single photochemical reaction (Photo system I) and cyclic in a closed system without net oxidation or reduction.

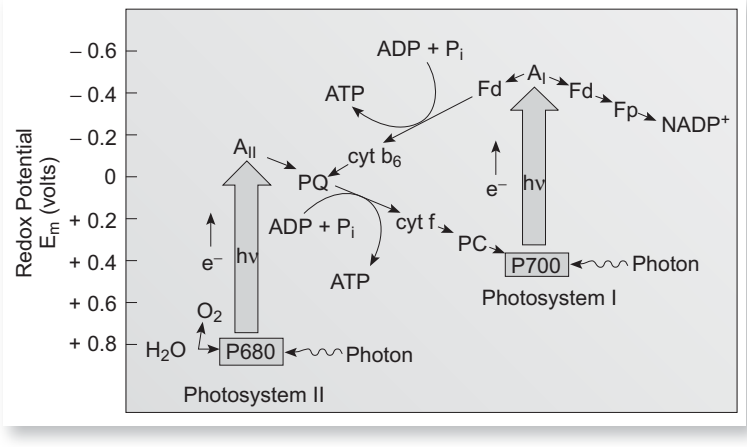


Figure 5.1 Mechanisms of non-cyclic and cyclic photophosphorylation in chloroplasts. A_{II}, primary electron acceptor of photosystem II; PQ, plastoquinone, cyt. f, cytochrome f, PC, plastocynin; P 700, reaction center chlorophyll of photosystem I, A_I primary electron acceptor of photosystem I; F_d, ferredoxin; Fp, favoprotein (Ferredoxin-NADP reductase), $cytb_6$, cytochrome b_6

Carbon-Reduction Reactions

The second phase of photosynthesis is completed in the absence of light. CO_2 is reduced to form a carbohydrate molecule through a series of reactions by the utilization of assimilatory power generated in light period. The dark reactions are called the Calvin-Benson Cycle.

Alternate Paths of Carbon Fixation

The Calvin-Benson cycle of CO_2 fixation into carbohydrates is not the only means by which plants can fix CO_2. In 1966, Hatch and Slack discovered that in sugarcane the first stable intermediate is not a 3-carbon atom compound such as the one which occurs in the Calvin-Benson cycle but a 4-carbon atom compound, oxaloacetate, another intermediate in plant and animal glucose metabolism. The key feature of this C_4 cycle is that the primary carboxylation reaction is catalyzed by PEP carboxylase so that the resulting product is oxaloacetate. This 4-carbon acid is formed by a completely different pathway in which a CO_2 molecule is connected with a 3-carbon atom precursor to form the 4-carbon acid.

This discovery of Hatch and Slack has added a fascinating page to the history of photosynthesis research, in as much as it is apparent that plants using the 4-carbon atom cycle have a very fundamental difference in physiology as compared to plant using the 3-carbon atom pathway as their main carbon fixation device. The 4-carbon atom pathway plants (sugarcane, corn and, certain grasses) have been termed efficient plants by some recent workers for the reason that unlike that 3-carbon plants (or non-efficient plants) they are able to grow fast at high temperatures and light intensities.

Furthermore, there is a phenomenon common to many non-efficient plants whereby as much as 30% of CO_2 initially fixed into carbohydrates compounds is released as CO_2 through a light dependent process called photorespiration. The efficient plants also have a photorespiration pathway that can degrade carbohydrates back to CO_2 and water, but it is compensated for by a very efficient system that traps any CO_2 released by photorespiration and incorporates it into the 4-carbon oxaloacetate. Therefore, all the CO_2 fixed into carbohydrate remains and can be stored as starch or used for plant growth. Many of the tropical plants growing in the region of high light intensity and temperature have been found to employ the efficient 4-carbon pathway has obvious evolutionary advantages.

It is interesting to find that the efficient plants have certain cells with chloroplasts that exhibit different membrane structures as compared to chloroplasts in the cells of non-efficient plants. The efficient plants have the Calvin-Benson 3-carbon pathway along with the 4-carbon path, but the latter predominates. The most diagnostic and easily determined feature of all plants with the C_4 pathway photosynthesis is a Kranz or wreath-like leaf anatomy. The anatomy of the typical C_3 dicot is characterized by a distinct dorsiventrality of the internal leaf structure as a result of a well defined palisade and spongy mesophyll cell layers, both of which contain numerous chloroplasts. In marked contrast to the wreath-like anatomy of the C_4 grass, such as maize, in which the vascular tissue is surrounded by a concentric layer of large bundle sheath cells containing numerous starch-filled chloroplasts, this layer is surrounded by one or more rings of mesophyll cells which also contain numerous chloroplasts.

The growth of the host is dependent on its ability to synthesize food materials from CO_2 and water in the presence of light. Many plant pathogens affect this photosynthesis. Some do so because they kill the tissues, such as *Phytophthora infestans* on potato, while other leaf infecting pathogens, such as viruses, the powdery mildews, and rusts affect this in a more subtle manner. Kosuge (1978) had reviewed the work on the capture and use of energy by diseased plants. Photosynthesis, as influenced by viral, bacterial and fungal pathogens is discussed below.

Symptoms of viral infection in leaves of plant often include characteristic symptoms of mosaics of green and yellow areas in which chloroplasts are reduced in number or show ultrastructural abnormalities such as swelling and fewer lamellae which are the actual sites of photochemical reactions.

Photosynthesis as Influenced by Virus Infection

Viruses affect the photosynthesis of their host plants considerably, and in advanced infections, photosynthetic rates might be 75–80% of those of uninfected plants. The characteristic symptom of a virus disease often is chlorosis. The intracellular manifestation of chlorosis is either loss of chlorophyll or the breakdown of the chloroplast or both. Peterson and McKinney (1938) reported less chlorophyll in diseased than in healthy tissues as a result of the influence of four mosaic diseases. Roberts *et al.* (1952) reported a reduction in photosynthetic rates in the case of those virus diseases in which the symptoms were mild or absent as well as the induction of chlorosis, mosaic or local lesion formation.

Roberts and Corbett (1965) reported reduced photosynthesis in tobacco plants infected with Tobacco Ring Spot Virus. They demonstrated that a symptomless tissue of low virus content showed reduced photosynthesis (O_2 evolution) per unit of chlorophyll. This suggests that in this case, the perturbation of photosynthesis does not result from chlorophyll loss; in fact, this type of tissue has slightly increased chlorophyll content per unit leaf area and appears greener than the uninfected tissue. Apparently, other components of photosynthetic apparatus are affected before the conversion of chlorophyll to the chlorine ring containing magnesium (the chlorophyllide) and chain alcohol (the phytol).

$$\text{Chlorophyll} \xleftrightarrow{\text{Chlorophyllase}} \text{Chlorophyllide} + \text{phytol}$$

Chlorophyllase enzyme located in the chloroplast is involved in what is probably the fist step in the destruction of chlorophyll *in vivo.* Chlorophyll is removed from the lamellar structure of the chloroplast and chlorophyllide crystallizes within the cell.

Owen (1957b) reported an increase in the photosynthetic rate of tobacco leaves infected with mosaic virus which was confirmed by Zaitlin and Hesketh (1965). However, Doke and Hirai (1969) found no difference until the development of mosaic symptoms. A reduction in photosynthesis in tobacco carrying tobacco etch virus was reported by Owen (1957a) who maintained that decline began two days after inoculation. This can be explained by a combination of the reduction in chloroplast numbers and the very low efficiency of fixation by chloroplasts. Tu *et al.* (1968) reported an 18% decrease in chloroplast size and number of leaves of corn systemically infected by maize dwarf mosaic and a decrease of 24% in total chlorophyll. Reduction in photosynthesis was 31%. Jensen (1968) found in barley plants infected with barley dwarf virus a reduction of 50% photosynthesis per unit of chlorophyll as compared to that of healthy leaves. This discrepancy may be partly explained by the reported increase in dark respiration (Tu *et al.*, 1968), while the data by Jensen (1969) cannot be explained in this manner. When expressed on a comparable dry weight basis, dark respiration, in contrast to photosynthesis, is unaffected by disease. Stimulated photorespiration during the infection of this C_3 plant may be the reason for this phenomenon.

According to Hutcheson and Buchanan (1983), viral infections can alter photosynthesis in either two ways depending on the disease:

(1) As exemplified by squash plants (*Cucurbita pepo*) systemically infected with squash mosaic virus in which there is a shift in the product from sugars to amino acids (particularly alanine), an increase in cytoplasmic ribosomes, and fewer chloroplasts. There was no difference between the healthy and

diseased chloroplasts with respect to most parameters tested: sensitivity of CO_2 assimilation to DCMU [(3-(3,4-dichlorophenyl)-1,1-dimethylurea)] and Antimycin A; ultra centrifugation profile; products of photosynthetic CO_2 assimilation; activity of the enzymes, ribulose 1, 5—biphosphate carboxylase/oxygenase and phosphoenolpyrurate carboxylase; chloroplast ultrastructure. Despite the presence of fewer chloroplasts, Hutcheson group found, on chlorophyll basis, a slight increase in the rate of CO_2 assimilation in infected plants.

(2) As exemplified by turnip yellow mosaic virus on photosynthesis, research groups of Bove (Goffeau and Bove, 1965) and of Matthew (Bedbrook and Matthews, 1972, 1973) used *Brassica pekinensis* in their studies and it was concluded that the chloroplasts were structurally and biochemically altered after infection. Bove's group reported an increase of both cyclic and non-cyclic photophosphorylation in chloroplasts from infected plants. Matthew's group observed that infection leads to a shift in the products of photosynthesis from sugars to organic acids and amino acids. Some other investigations have also reported such shift with other viruses. It is interesting to note that with turnip yellow mosaic virus, the viral nucleic acid is formed in the vesicles of the chloroplast envelope and the virus is thus a parasite of the chloroplast.

Deranged starch metabolism, with either an excessive accumulation or paucity of starch, is a common biochemical symptom of viral diseases. Starch accumulates in the chloroplasts which are affected by virus diseases. Holmes in 1931 observed that tobacco plants infected with TMV failed both to form and to metabolize starch at a normal rate. There was more starch in virus infected areas. This phenomenon was always consistent; leaves taken at the end of the period of active photosynthesis often contained less starch in virus-infected plants than those taken from the green house and placed in the dark under conditions favourable for the starch to be metabolized. Holmes (1931) found that the infected leaves have a reduced capacity for synthesizing and degrading starch. Robbins (1921) found an accumulation of starch in mosaic leaves of sugar beet, while Narayanaswamy and Ramakrishnan (1965) reported a reduced level of starch in pigeon pea mosaic infected leaves. Vidhyasekaran and Kandaswamy (1972) also observed that the mosaic disease incidence of *Phaseolus aureus* did not alter the starch metabolism. Starch accumulation is also characteristic of a number of other viral diseases, such as Potato leaf roll, Sugar beet yellows, and Barley yellow dwarf. It is not known where the virus affects these processes, although it can influence the loss of some enzyme activity from the chloroplast.

The inability of some virus-infected plants to degrade starch is also of interest. How starch is degraded within tissues still requires further investigation.

Photosynthesis as Influenced by Bacterial Pathogens

An attack by bacterial pathogen frequently involves chlorotic symptoms, for example, a number of leaves spotting xanthomonads where there is a direct effect on chloroplasts. This is perhaps by the toxic metabolites of the pathogen. Wildfire-toxin or tabtoxin produced by *Pseudomonas syringae* pv. *tabaci* suggests that the toxin affects photosynthesis.

Tabtoxin as such is not toxic, but in the host cell the toxin is hydrolysed and releases tabtoxinine which is the active toxic molecule. Tabtoxine inactivates the enzyme glutamine synthetase, which leads to depleted levels of glutamine and as a consequence accumulation of ammonia to toxic levels. The latter uncouples photosynthesis and photorespiration and destroys the thylakoid membrane of the chloroplasts, thereby causing chlorosis and eventually necrosis.

Photosynthesis may also be affected indirectly by the occlusion of the vascular system by a bacterial pathogen leading to water stress and wilting. This results in the closure of stomata, and consequently, there is inhibition of CO_2 absorption. Beckman, Brun and Buddenhagen (1962) ascribed the symptom of wilting in banana (Moko disease) induced by *Ralstonia (Pseudomonas) solanacearum* to one of the two causes: (1) vascular occlusion or, (2) a toxic effect which would influence water retention by host cells.

Photosynthesis as Influenced by Fungi

Fungal pathogens seem to affect the photosynthetic process by affecting the chloroplast. Microscopic observations of many fungal diseased tissues also suggest that the chloroplasts are affected. Rusted wheat leaves and leaves of bean infected with *Uromyces phaseoli* retained chlorophyll in regions termed *green islands* at the edge of the infection. Wang (1961) has shown in the case of bean rust, that these regions are photosynthetically active and starch tends to accumulate in them to a greater extent than in the surrounding areas infected by *Uromyces phaseoli*, while Schippper and Mirocha (1969a, b) showed the depletion of starch in the same infected tissues. Certain effects on the photosynthetic system may be observed anatomically. There may be chlorosis of the whole leaf. This is due to a reduction in the amount of chlorophyll per chloroplast rather than to a reduction in chloroplast number, at least in *Puccinia recondita* on wheat. Green islands may appear around a pustule when the remainder of the leaf is yellow. Bushnell and Allen (1962) found that *Erysiphe graminis* on barley caused the tissues around a pustule first to become chlorotic and then to redevelop chlorophyll. Green islands might also be induced by extracts of conidia of *E. graminis* or uredospores of *Puccinia graminis.* Auxins or kinetins may also induce green island formation. It had been shown that in barley kinetin induced island contain more starch but less nitrogen than mildew induced islands. Electron micrographs show that grana and intergranar lamellae of chloroplasts in leaves infected with *E. graminis* become progressively more disorganized with the progress of the diseases.

Figure 5.2 shows the net photosynthetic rate of oak leaves infected by *Microsphaera alphitoides* (powdery mildew pathogen). There is a slight initial stimulation of photosynthesis in inoculated leaves but this is followed by a gradual decline.

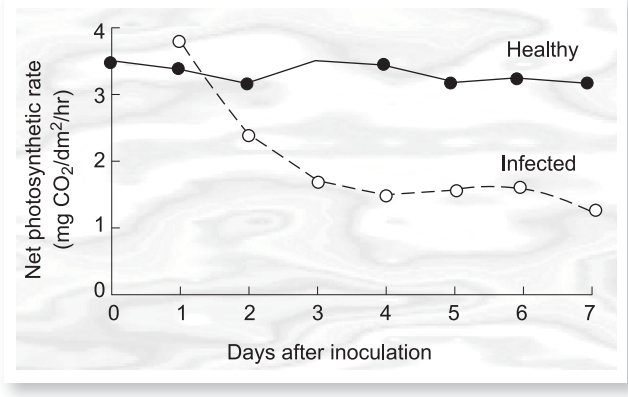


Figure 5.2 Changes in photosynthesis of oak leaves following infection by the powdery mildew fungus *Microsphaera alphitoides* (From C. H. Dicknison and J. A. Lucas, *Plant Pathology and Plant Pathogens,* Blackwell Scientific Publications, Osney Mead, Oxford, 1977, p. 94.)

According to Magyarosy *et al.* (1976), the rate of $^{14}CO_2$ uptake by leaf discs from sugar beet infected with powdery mildew (*E. polygoni*) is also reduced as compared to healthy tissues (Fig. 5.3). Chloroplasts isolated from mildewed beet leaves show a reduced capacity to form ATP by non-cyclic photophosphorylation. In powdery mildewed-infected barley leaves it has been shown that these are also a progressive reduction is the activity of several key enzymes of the Calvin cycle. This reduced capacity to form ATP by non-cyclic photophosphorylation may be linked to change in the concentration of soluble carbohydrates in infected tissues, which in turn influences the rate of photosynthetic CO_2 fixation. In *Arabidopsis* leaves infected by *Albugo candida,* the reduction in the rate of photosynthesis are paralleled by decrease in the amounts of Rubisco (ribulose biphosphate carboxylase) protein present the host.

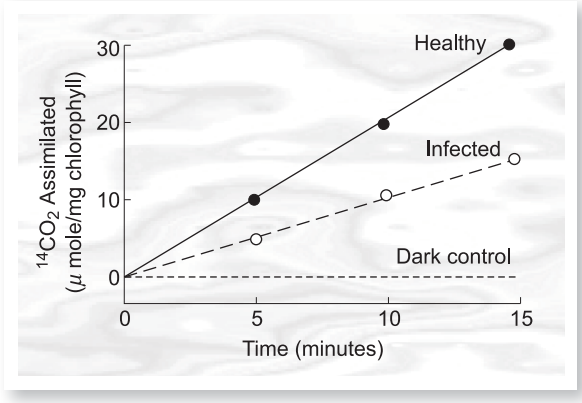


Figure 5.3 Effect of infection by *Erysiphe polygoni* on the rate of photosynthesis $^{14}CO_2$ assimilation by sugar beet leaf disc (From C. H. Dicknison and J. A. Lucas, *Plant Pathology and Plant Pathogens,* Blackwell Scientific Publications, Osney Mead, Oxford, 1977, p. 95)

Much of the reduced rate of photosynthesis has been attributed to reduction in the photosynthetic machinery of leaf cells. Chloroplasts show ultrastructural changes after infection, sometimes concentration has been sown to be reduced in mildewed barley plants and in barley with brown rust infection. One very interesting observation in case of barley plants with powdery mildew infections and for rusted bean plants is that uninfected leaves exhibits stimulated or enhanced CO_2 fixation. This suggests that uninfected leaves are able to compensate for the reduced capacity of infected leaves by increasing photosynthesis (Fig. 5.4).

In the case of facultative parasites, studies have shown a significant inhibition of photosynthesis within a day after the inoculation of wheat with *Septoria nodorum* and of ragi with *Helminthosporium nodulosum* (Vidhyasekaran, 1974). This early inhibiting effect suggests that there are other factors involved in the decline of photosynthesis for apparently healthy leaves with normal chlorophyll content.

Changes in starch content following infection have been observed in many foliar diseases. The general pattern is an initial decrease followed by a marked increase with heavy accumulations around the margins of lesions. Still later, the starch content again declines. Starch accumulates in wheat leaves infected with stripe rust. Work with wheat leaves infected with *Puccinia striiformis* led to the suggestion that changes in the activity of ADP-glucose photophosphorylase during disease development might account for the pattern of changes in starch contents (MacDonald and Strobel, 1970). MacDonald and Strobel (1970) assayed these enzymes in the presence of activators (sugar phosphates) and inhibitors (inorganic phosphates) at concentrations found in diseased leaves at various times after inoculation.

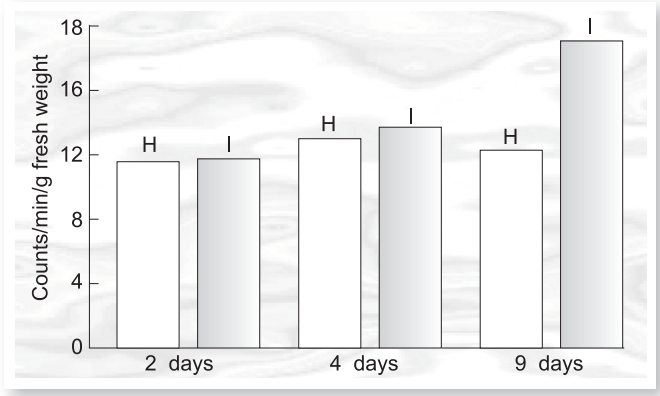


Figure 5.4 Comparision of $^{14}CO_2$ uptake by rust free leaves from healthy (H) and rust-infected (I) bean plants at different times after inoculation (From C. H. Dicknison and J. A. Lucas, *Plant Pathology and Plant Pathogens*, Blackwell Scientific Publications, Osney Mead, Oxford, 1977, p. 95)

They reported that the pattern of changes in the activity of this enzyme was similar to that of changes in starch contents. Changes in starch in leaves infected with *P. striiformis* were very similar to those observed in other diseases. In contrast, photosynthesis in infected leaves increases sharply, reaches a peak of twice the normal as symptoms appear, and remains above normal for at least six days after sporulation. These results which differed markedly from those obtained from other diseases, indicate that *P. striiformis* infected plants may represent a special case.

According to Vidhyasekaran and Kandaswamy (1972), the powdery mildewed (*Oidium* sp.) infected tissue of *Phaseolus aureus* showed a severe reduction in both starch and sugar content. The phenomenon of depletion of starch in mildew infected tissues has already been observed (Mirocha and Zakai, 1966) and the metabolism of starch seems to play an active role in pathogenesis, possibly supplying metabolites to support the growth of the fungus immediately after the penetration of the host as well as during spore formation (Schipper and Mirocha, 1969a). According to Schipper and Mirocha (1969a), this may be due to the activation of starch hydrolyzing amylase in the infected tissues.

The facultative parasites, such as *Cercospora,* also cause a depletion of starch. Kolandaisamy (1964) reported the reduction in starch content in banana leaves and an initial increase in major content of the groundnut leaves infected by *Cercosporidium personatum*. However, there was a sharp decline in sugar content. Vidhyasekaran and Kandaswamy (1972) observed in the case of *Cercospora cruenta* infecting *Phaseolus aureus* that even in the initial stages of infection, there was a sharp fall in the sugar content. Jayapal and Mahadevan (1968) also found a quick decline in the sugar content of the banana leaves due to Cercospora infection. Although increased respiration reported in the tissues infected with facultative parasites may be a factor in the depletion of sugars, it is also possible that, the sugars may form the basic materials for the growth and sporulation of the pathogen.

The most important exception to the usual sequence or chlorosis and reduced photosynthetic activity is also seen in diseases caused by the rust and powdery mildew fungi. In rust infection, colonized leaves become chlorotic but immediately around the infection sites, chlorophyll is retained, appearing as a green halo know as “green island”. In powdery mildew fungal infections, chlorophyll is initially lost form the leaf but later resynthesized around infection rites (sequencing). There has been much discussion on the significance of these green islands. The selective retention of chlorophyll around infection

sites suggests that the pathogen exerts some degree of control over host physiology. Photosynthesis in the green island areas occurs at a rate comparable with that in uninfected tissues or even slightly greater rate which it is believed serves to support the invading fungus through sporulation.

Respiration

Respiration is the *sine qua non* for life. In normal healthy plant cells, the function of respiration is two-fold: to provide (1) energy and, (2) carbon skeletons. Respiration is the oxidation (aerobic or anaerobic breakdown) of organic material, primarily carbohydrates, to simple compounds, H_2O and CO_2 with the production of utilizable energy for the cell in the form of ATP. In aerobic respiration this takes place with the uptake of oxygen from the air. In fermentation, the degradation of organic material is anaerobic. Energy is liberated through the process of respiration. Actually, the oxidation of carbohydrates and related compounds is the main source of energy but energy derived from photosynthetic phosphorylation is also a characteristic of plant metabolism. In the process of respiration energy is not "liberated" as such, it is first redistributed in molecules formed by oxidative reactions, creating bond energy. The energy rich group generated by oxidation is capable of initiating reactions requiring energy. Hence the coupling of the chemically bound energy to other systems is the key reaction in the biosynthetic processes of plants.

One of the simplest substrates for oxidation is glucose. The overall reaction involved in the respiration of glucose is as follows:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \Delta G^\circ = 686 \text{ kacl}$$

The respiratory process, however, can be divided into two portions:

1. The initial phase known as the Embden-Meyerhof-Paranas pathway or glycolysis, in which each glucose molecule is converted into two molecules of pyruvic acids.
2. The terminal phase (Citric acid cycle or Krebs cycle or Tricarboxyic acid cycle) in which pyruvate is oxidized to $CO_2 + H_2O$, utilizing oxygen as the final hydrogen acceptor.

The glycolytic phase can take place in the absence of O_2 (anaerobically) and is common both to aerobic respiration and to alcoholic or lactic acid fermentation. The terminal phase is strictly aerobic.

If an adequate amount of oxygen is lacking and if anaerobic pathways are favoured, this scheme is altered. Glycolysis occurs but intermediates of glycolysis acting as the final hydrogen acceptors are reduced to other end products. A typical overall reaction for anaerobic respiration is

$$C_6H_{12}O_6 = 2\ CH_3CH_2OH + 2CO_2 + 2\ ATP$$

(Ethyl alcohol)

Note that the energy yield of fermentation is less because the end product is incompletely oxidized, and still stores energy. The discussion of respiration is divided into (1) glycolysis, (2) fermentation end products, (3) CA-cycle.

Glycolysis

Glycolysis is the conversion of glucose to pyruvic acid by any biochemical pathway. The most common glycolytic pathway is the Embden-Meyerhof-Paranas pathway, although the Hexose monophosphate pathway (HMP) and the Entner Doudoroff (ED) pathway may provide alternate mechanisms.

The interrelationships between the EMP, HMP and ED glycolytic pathways are given in Figure 5.5. The EMP and HMP pathways occur in animals, plants, bacteria, and fungi, while the ED pathway is restricted to bacteria and fungi. The EMP pathway in fungi is the principal glycolytic pathway as in most of the other organisms and usually accounts for at least 50% of the glucose dissimilated; in some fungi it accounts for all the glucose dissimilated. However, the EMP pathway may be secondary in importance to either the HMP or ED pathway in a few fungi, or totally lacking as in the case of *Cladariomyces fumago*. The EMP pathway becomes increasingly important under anaerobic conditions as the HMP pathway accounts for all the glucose dissimilated.

The HMP pathway is common in plants and animals. The enzymes required to carry out the HMP pathway are widely distributed in fungi and may be universal. The presence of the enzymes, however, is not a proof that the HMP pathway occurs. It is found in a number of fungi, usually accounting for the less than 40% of the glucose dissimilated. The relatively low level of the HMP reactions is perhaps due to limiting amounts of NADP. The HMP pathway is the major route of glycolysis in 5 of the 15 fungi surveyed (Blumenthal, 1965).

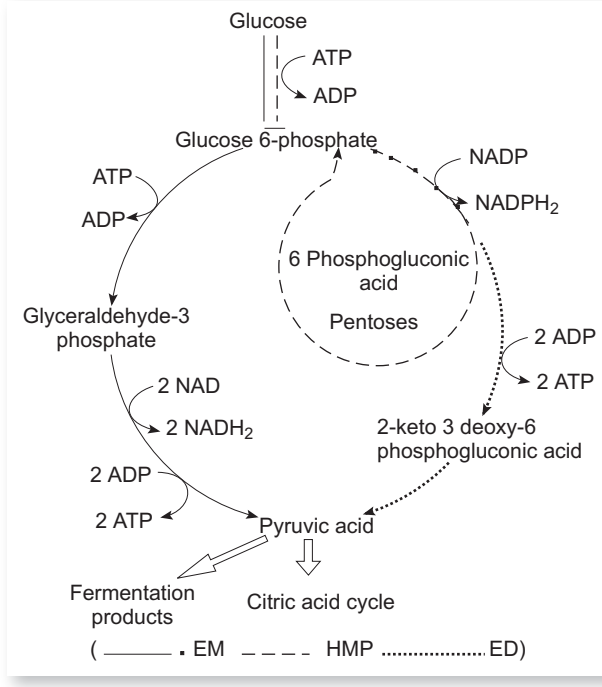


Figure 5.5 The interrelationship between EM, HMP and ED glycolytic pathways

Respiration in Plants Under Pathogenesis

Plant respiration as an index of disease has been a subject of investigation for a number of years. Increased respiration in tissues generally accompanies a variety of pathological conditions except in plants systemically infected with viruses. Merrett and Bayley (1969) have critically examined the special topic of virus infections. A study of the altered respiration of infected plants is also of interest to plant

physiologists and biochemists investigating the nature of the respiratory rate. There is some evidence that genotypically controlled resistance to disease depends on differences in metabolism and respiration and respiratory rates as indices of such differences seem to open up new lines of interest both to the pathologist and the plant breeder.

Prior to 1953, studies on respiratory stimulation in infected plants were confined mainly to the quantitative aspect of the increase. It was Allen (1953, 1954) who first pointed out that disease was the result of disturbances in host metabolism induced by the diffusible metabolites (toxins) of the parasite. He postulated that increased respiration in mildew disease was the result of a toxin produced by the fungus which uncouples phosphorylation from electron transfer in host cells. Attempts have been made by several workers to isolate toxic agents inducing stimulation of respiration. Figure 5.6 shows the rate of oxygen uptake in cabbage cotyledons during colonization by *Peronospora parasitica* as shown by Thorton and Cooke (1974).

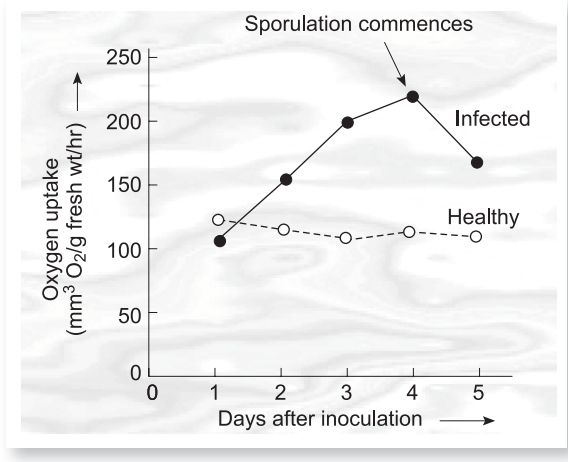


Figure 5.6 Oxygen uptake by healthy cabbage cotyledons and those infected by *Pernospora.* (From C.H. Dicknison and J.A. Lucas, *Plant Pathology and Plant Pathogens,* Blackwell Scientific Publications, Osney Mead, Oxford, 1977, p. 90)

Mechanism of Respiratory Increase

In a diseased plant there are a number of changes which accompany increased respiration, including changes to pathways used, increases in the concentration of enzymes involved, and changes to the uncoupling of respiration to other related cellular processes. There are a number of theories which seek to explain the enhanced respiration rate in diseased plants (Fig. 5.7). These are:

1. Uncoupling of oxidative phosphorylation
2. Augmented synthetic processes
3. Changes in respiratory pathways
4. Increased activity of terminal oxidases

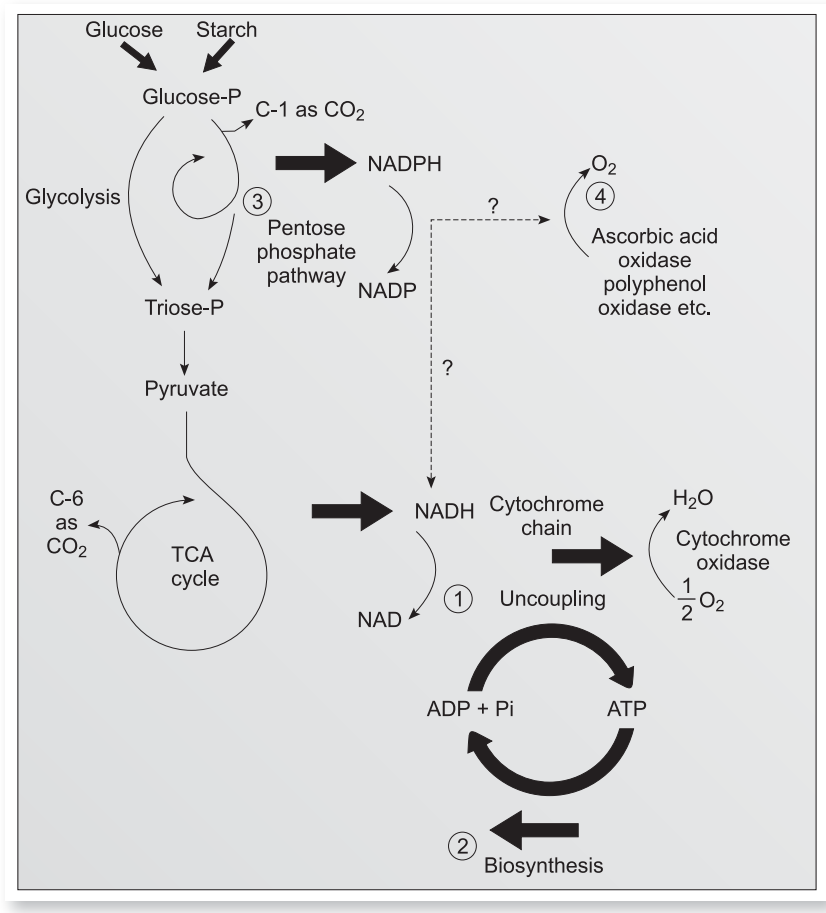


Figure 5.7 Possible mechanisms of stimulated respiration in diseased plants due to infection

The direct contribution of the parasite in the increased respiration rate is negligible.

Uncoupling of Oxidative Phosphorylation

It has been suggested that the pathogens may uncouple host respiration (oxidative phosphorylation). This uncoupling of electron transfer from phosphorylation can be brought about by chemical inhibitors such as 2–4-dinitrophenol (DNP). The synthesis of ATP accumulates via the electron transfer chain is prevented and ADP accumulates, stimulating respiration and increasing the rate of oxygen uptake. The hypothesis that pathological increases in respiration result from an uncoupling of phosphorylation from electron transport was put forward by Allen (1953). The effect has often been attributed to diffusible substances or toxins produced by invading pathogens, e.g., *Helminthosporium maydis, H. victoriae* and *Gibberella saubinetti*. The evidence that the pathogen or their toxins uncouple oxidative phosphorylation is proved by the fact respiration in decreased plants in not increased or stimulated even after treatment with DPN as the pathogen or its toxin has already done so.

In the presence of oxygen (aerobic condition) glucose is used more efficiently through normal respiration and fermentation is suppressed. This is known as Pasture effect. Under anaerobic conditions (i.e. in the absence of oxygen) however, pyruvate cannot be oxidized, instead it undergoes fermentation and yields lactic acid or alcohol. In diseased plant aerobic carbohydrate breakdown becomes less efficient, but at the same time energy requirements are high and ADP level rise. This results in an increase in respiration rate. The Pasteur effect is lost in a similar way as treatment with DNP. An important gain to the plant by the abolition of Pasteur effect is the accumulation of intermediates of carbohydrate breakdown which are utilized in the formation of phenolic compounds. Phenolic synthesis is triggered in the diseased plant.

Augmented Synthetic Processes

Another explanation offered for the mechanism of respiratory increase is ascribed to the active metabolism accelerating the ATP utilizing reactions of host tissues. The synthetic processes requiring energy accelerate ATP breakdown to ADP which in turn causes respiration to rise. In support of the above mechanism, many characteristic features indicating the activation of the metabolism of the host have been reported, such as:

1. Accumulation, mobilization, and synthesis of phosphorus and carbon compounds.
2. The growth of the host tissue.
3. Synthesis of proteins, including activation of enzymes and enzyme systems.
4. Increase in protoplasmic work, such as protoplasmic streaming (cyclosis).

According to the results of Daly and Sayre (1957), safflower infected with *Puccinia carthami* exhibited a pathological stimulated cellular growth and a concomitant increase in tissue respiration. The rise was at its maximum during the sporulation of the parasite. In supporting this abnormal growth of diseased host cells ATP is consumed; thus the breakdown of ATP is accelerated and eventually enhances the respiratory rate of the host.

Stimulated growth, accumulation and mobilization of metabolites occur in rust and bean plants, synthesis of proteins in rotted sweet potato and late blight of potato, and increased protoplasmic streaming in late blight of potato. All these demand energy that is ATP and thus respiration is automatically regulated through the control of the level of ADP (phosphate acceptor).

Besides the respiratory rate, the temperature of diseased tissues is also higher than that of healthy ones. The increase in temperature is from 0.3°C to 0.7°C for mildew and rust diseases (Yarwood, 1953). It is not yet known whether respiration of infected plants is less efficient than that of healthy plants. It is possible that the high rate of respiration of infected plants is in part at least, a wasteful one.

Changes in Respiratory Pathways

The EMP pathway is the most important pathway of carbohydrate breakdown but this respiratory pathway becomes altered in higher plant tissues as a result of biochemical transformations during differentiation and maturation and it is not surprising that infection with viruses, bacteria and fungi also bring about such changes. The HMP pathway is of minor importance, except in special conditions. The work down in this sphere on healthy and infected tissues strongly suggests that the HMP pathway (Pentose phosphate pathway) becomes quite important during infections. The work of Farkas and Kiraly (1955) was the first on this area.

Daly *et al.* (1957) have put forward three arguments for a change in the nature of respiratory pathways in rust diseases.

1. It was observed that at sporulation the ratio of anaerobic to aerobic carbon dioxide production fell below the limit of 0.33 which was expected if only glycolysis and TCA cycle were operating.
2. Sodium fluoride inhibits the reaction in the EMP pathway by which phosphoenol pyruvate is formed from phosphoglycerate through the activity of enolase. Inaction to sodium fluoride showed that the EMP pathway is not operative.

The tracer methodology has been utilized for this purpose by which radioactive carbon is incorporated into either the C_6 or the C_1 position of the glucose molecule. The ratio of labelled CO_2 released from each source during respiration is measured which indicates the predominating pathway. Activation of the pentose phosphate pathway leads to an increased contribution from the C_1 position, and hence, the C_6/C_1 ratio is lower. Infected plants, the C_6/C_1 ratio is typically lower than the value obtained from healthy tissues, suggesting the there is an increased participation of the pentose route.

A shift from the glycolytic to HMP pathway has been detected in several diseases caused by fungi, bacteria and viruses (only in local lesion type). Some of the examples are as follows:

1. Infection of *Datura stramonium* by TMV
2. Tomato tumour caused by *Agrobacterium tumefaciens*
3. Halo blight of tobacco cause by *Pseudomonas tabaci(* now *Pseudomonas syringae pv tabaci*)
4. Rusted and mildewed wheat
5. Potato tubers infected with *Phytophthora infestans*
6. Safflower hypocotyl infected with *Puccinia carthami*
7. Tobacco leaves infected with several strains of potato virus.
8. Bean hypocotyls infected with *Rhizoctonia solani*

The actual significance of the switch to the pentose pathway is linked to its role in the biosynthesis of various compounds in addition to providing pentoses for the biosynthesis of nucleic acid, pentose phosphate intermediates are involved in the production of numerous aromatic compounds, particularly phenols and their derivatives.

Changes in the operation of the Krebs cycle have been recorded in several plants by studying the effect of malonic acid which inhibits an enzyme system (succinic dehydrogenase) in the cycle on oxygen uptake. Some of the examples are as follows :

1. Rusted and mildewed wheat
2. Potato tuber infected with *Gibberella saubinetti*
3. In *Cercospora* infection on groundnut, particularly in later stages of infection.

Increased Activity of Terminal Oxidases

In addition to changes in reparatory pathways, alternate terminal oxidation systems may operate in diseased tissues. Apart from the usual cytochrome system terminating with cytochrome oxidase, system involving phenol oxidases and ascorbic acid oxidases has been detected in the plants.

The reduced coenzyme NADPH produced via PP pathway may be oxidised by non-cytochrome oxidative system in diseased plants; with the increase in oxygen uptake in diseased plants there may be an increase in the activity of polyphenol oxidases, e.g., in diseases caused by *Phytophthora*, *Ceratocystis* and *Fusarium* species. Ascorbic acid oxidation has also been reported to increase as oxygen uptake

increases, e.g., in cabbage infected by *Fusarium oxysporum* and rusted wheat plants or oat plants treated with *Helminthosporium victoriae*.

The precise mechanisms and significance of such oxidation systems are not clear but phenol oxidases play an importance part in the production of phenolics which have their bearing in bringing about resistance in plant. These alternative pathway oxidases are unable to participate in the formation of ATP during oxygen uptake.

Relationship of Resistance and Susceptibilty

Resistant and susceptible cultivars have been shown to react differently to infection by fungal pathogens and it has been suggested that different mechanisms may operate (Smedegaard-Peterson, 1984). In infected resistant cultivars (incompatible reaction) oxygen uptake has been shown to rise sharply quite early in infection, although at a later stage subsequently the level is reduced to normal. In infected susceptible cultivars (compatible reaction), there is much less change initially and a rise in oxygen uptake occurs a few days after infection.

Many workers have studied the relationship between respiration and resistance to plant pathogenic organisms. Samborski and Shaw (1956) observed that in wheat rust, the resistant variety recorded a steep rise followed by an equally steep fall in respiration while the susceptible variety had a continuous rise in respiration.

Some interesting correlations between the respiratory rate and resistance to parasitic attack have been observed at Madras laboratory. Andal (1959) studied the respiratory rate of three varieties of rice differing in their degree of susceptibility to *Fusarium moniliforme*. It was found that the respiratory rates of both root and shoot tissues were directly proportional to the degree of susceptibility. Similar correlations were obtained by Lakshmanan (1956, 1959) in cotton varieties resistant and susceptible to *Fusarium oxysporum* f. sp. *vasinfectum*. Lakshmanan (1956) has further shown that there is a correlation between respiratory activity and induced resistance.

Studies by Rubin and his co-workers in the USSR (Farkas and Kiraly, 1958) on potato-*Phytophthora*, cabbage-*Botrytis*, citrus-*Penicillium* and other diseases led them to conclude that resistant varieties respire more intensively than susceptible ones. Balasubramanian *et al*. (1971) studied the respiratory pattern of *Botrytis fabae* and *B. cinerea* on a common host. In the case of *B. fabae*, where lesions were spreading, the respiratory rate was three times after 24 hours than in the limited lesions caused by *B. cinerea*.

Effect on Transpiration

In infected plants, transpiration is usually increased due to an increase in the permeability of leaf cells and the dysfunction of stomata. The wheat leaf rust pathogen *Puccinia recondita* produces numerous lesions (Uredinia) and causes many breaks in leaf epidermis through which transpiration goes on uncontrolled. In grape berries infected with the powdery mildew fungus *Uncinula necator*, the mycelium of which penetrates and forms haustoria in almost every epidermal cell, the destruction of cuticle and epidermis results in uncontrolled loss of water from the affected areas.

Similarly the apple scab fungus *Venturia inaequalis* grows between the cuticle and the epidermis, causing the cuticle to break in numerous places, allowing transpiration to occur. If the transpiration is more than absorption of water, wilting of leaves occurs and leads to death of the host plant.

Changes in Nitrogen Metabolism of Infected Plants

In many diseased tissues, especially in those characterized by overgrowths, galls or other growth abnormalities, increased metabolic activity is accompanied by marked increases in nucleic acid and protein synthesis. Cytochemical evidence for major changes in nuclei, nucleoli and changes in ribosomes is available.

Nucleic Acid Metabolism

No field of biological science has developed and progressed to such an extent during the last few decades as have nucleic acids because they are involved not only in the transfer of genetic information but also in the regulation of gene activity. Nucleic acid metabolism in infections caused by obligate parasites had been reviewed by Heitefuss (1966). Nucleic acids in host parasite interactions had been reviewed by Heitefuss and Wolf (1977). Changes in the nucleic level have been reported in diseases caused by all kinds of pathogens. The host RNA content in TMV infected tobacco leaves increases shortly (8 hours after inoculation) but afterwards (70 hours) the viral RNA increases together with the total increase of RNA. Total RNA increases in infected tissues, while it decreases in the uninoculated control tissues. In various virus-host plant combinations, transitory increases, moderate declines, or no significant changes in DNA have been reported. Such variations probably reflect differences among the plants and viruses studied. In the case of tobacco leaves infected with TMV, one investigator reported that t-RNA increased fourfold after infection but another worker while, using similar methods, found no such increases. Thus, the explanation given earlier does not account for inconsistent data in this case. Similarly, a rapid breakdown in ribosomes following TMV infection has not been confirmed. Marked increases in ribosomes have been observed around necrotic areas on local-lesion host inoculated with TMV.

Gall cells induced by various fungi, bacteria and nematodes have been shown to contain a very large quantity of nucleic acids. In the case of *Pasmodiophora brassicae*, causing club root disease of crucifers, the infected cells each contains an enlarged nucleolus which may be as much as 30 or more folds in volume over nucleoli in non-parasitized cells and the DNA content may be sixteenfold greater than in the non-infected cells. Hypertophy of host cells together with increases in the sizes of nuclei have been reported for various other host parasite combinations. In the same disease, the nucleolus of root hair cells begins to enlarge within two hours after penetration and after twenty hours this volume has increased sixfold. The increase in nucleolar volume is accompanied by an increased nuclear RNA and non-histone protein. The lysine rich portion of histone decreases. Since histones have a regulatory role, any change in their quantity is related to a change in the regulation of gene action. It seems that the normal transcriptional processes of invaded cells are altered by parasites (Williams *et al*., 1973). A higher level of RNA in the rust-infected enlargement of the nucleolus has been reported by a number of workers. The higher level of RNA in rust infected wheat is indicated also by an increased incorporation of P^{32} into RNA during the first four to five days after inoculation (Fig. 5.8). An increase in the RNA content of the nuclei of the cells of diseased plants probably indicates an induced synthesis of proteins in the host. Experiments of Millerd and Scott (1963), and Quick and Shaw (1964) have shown a correlation between increased respiration and RNA synthesis in barley, powdery mildew disease, and rust infections of wheat. There is initial swelling and later degradation of the infected host nuclei in many fungal diseases of the plants. This is accompanied in every case by an increase in RNA and protein synthesis. Eventually the collapse of the nuclei is caused by proteolysis in the host. A decrease in the RNA and DNA contents of the

soybean leaves infected with *Peronospora manshurica* has been demonstrated by Millikan *et al.* (1965). Callow (1973) showed that an increase in the total RNA content of cucumber leaves during the period of leaf expansion is retarded by inoculation with *Erysiphe cichoracearum*. But when the expansion of the leaves went up to 75% of their final area, a very slight increase in cytoplasmic RNA was observed.

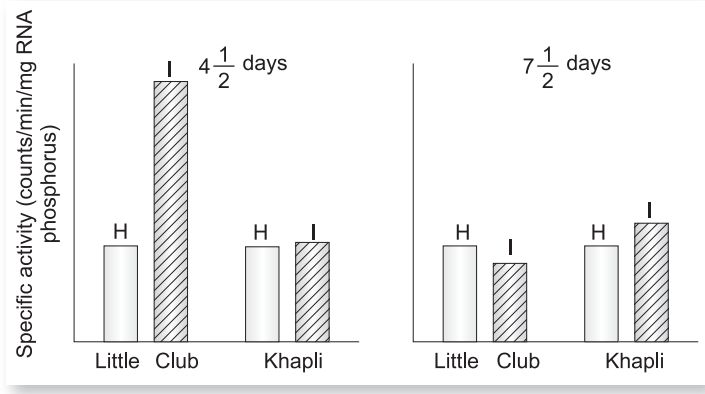


Figure 5.8 Amount of P^{32} incorporated into RNA of wheat leaves CV Little Club (compatible) and CV Khapli (incompatible) inoculated with *Puccinia graminis.* H = Healthy and I = Infected leaves (From C. H. Dicknison and J. A. Lucas, *Plant Pathology and Plant Pathogens,* Blackwell Scientific Publications, Osney Mead, Oxford, 1977, p. 109)

Haustoria of powdery mildew fungi only penetrate epidermal cells, which do not contain chloroplasts. Loss of nucleic acids from chloroplasts must therefore be caused by diffusible metabolites released by the pathogen. In incompatible reactions of soybean plants infected by *Phytophthora megasperma* f. sp. *glycinea* increases in m-RNA levels may precede the formation of phytoalexins.

Changes in Protein Metabolism

Uritani (1971) has surveyed the changes in the proteins in diseased tissues and compared them to those induced by mechanical injury. Changes in protein metabolism in plant tissue during the initiation of pathogenic infection and course of disease development have been reviewed by Uritani (1976). The total content of the protein (that of the plant and pathogen) usually increases during the early stages of infection, when the contribution of the pathogen is seen, little change, or in some cases, a decrease in plant protein has been found in most diseased tissues. In the case of crown gall tumours, there is much more protein in them than could be expected from synthetic activities of the pathogen. In late degenerative stages of disease, large decreases in protein, sometimes accompanied by increases in free amino acids, are often found.

It is not possible to generalize about the influence of virus infection on host-protein metabolism. A few viruses such as TMV and potato virus X, in connection with which this problem has been most thoroughly studied, may be atypical of plant viruses as a whole; these viruses multiply to such an extent that the virus protein contributes significantly to the total protein of the leaf. The synthesis of virus protein in turn must exert a considerable direct stress on host protein metabolism. Most viruses, however, contribute very little to the total plant protein.

Levels of protein synthesis measured in infected plants at the infection sites in susceptible plants are usually increased over levels in healthy or resistant plants. Much of this increase in protein level can be attributed to the synthesizing activities of the invading pathogen.

It is generally agreed that there are marked differences between the nitrogenous constituents of normal resting cells and actively dividing tumour cells incited to division by the crown gall bacterium *Agrobacterium tumefaciens* (Braun, 1962). Neish and Hibbert (1943) have reported that while both normal and tumour tissues contain about the same amounts of non-protein nitrogen, tumours have three times the protein of normal tissues and the difference in water soluble protein is 6:1 in favour of tumour tissues. This was interpreted as a greater tendency and capacity on the part of tumour tissues to synthesize protein.

According to studies by Klein (1952), the greatest increases in proteins and soluble nitrogen became apparent in tumour tissues late in their development—at the time of cell volume increase. He suggested that increased protein synthesis registered in the tumour tissues at this time did not occur entirely at the expense of free amino acids and amides in close proximity to the tumour. On the basis of observations of the stunted and nitrogen deficient appearance of tumorous plants, Klein stated that the nitrogen of the whole plant was being mobilized into the tumour.

Patel and Walker (1963) studied the amino acids and amide pool of the bean plants, susceptible and resistant to the halo blight bacterium *Pseudomonas phaseolicola*. The amino acids and amide content were not appreciably altered in resistant varieties as a result of infection. Chromatographic analysis of a healthy and inoculated leaf of a susceptible variety revealed 22 ninhydrin positive compounds, and of these, 15 were identified as amino acids and amides.

Changes in amino compounds were determined in inoculated and chlorotic tissues and again there was a large accumulation of ornithine as well as histidine and methionine in the inoculated leaf tissue. More than five times as much ornithine accumulated in the uninoculated chlorotic leaves.

Increase in the protein synthesizing activity of green island tissue is quite considerable whereas in the chlorotic tissues between these sites protein synthesis is depressed to low levels.

Some proteins novel to the host plant may be synthesized in resistant plants and are usually associated with hypersenitive response and resistant mechanism (Chapter 6). These novel proteins are termed as pathogenesis related proteins (PR protein). Such polypeptides are usually of low molecular weight. These have been detected in infected plants and also in tissues which have been treated with elicitor molecules from microbes or abiotic factors. Detailed aspects of pathogenesis related protein will be discussed in Chapter 6.

Phenols

Phenols are compounds, which contain one or more aromatic (benzene) rings with one or more phenolic hydroxyl groups. The simplest compound is phenol. Compounds having more than one hydroxyl group on the benzene ring are called polyphenols. Phenolic compounds are widely distributed in higher plants and fungi. Anthocyanins, leucoanthocyanins, and anthoxanthins, hydroxy benzoic acids, glycosides, sugar esters of quinones and Shikimic acids, esters or hydroxycinnamic acids and coumarine derivatives make up a vast class of compounds known as phenolics. Formerly, term tannin, and more recently, the term polyphenol, has been used to refer to these compounds. In their discussion on the

pathological darkening of the plant tissue, Rubin and Artsikhovskaya (1964) stressed on the diversity of physiological roles played by phenolic compounds which characteristically accumulate in diseased plants. The extensive literature on the role of phenolic compounds in disease development has been covered in proceedings and reviews by Pridham (1960), Wood (1967) and Kosuge (1969). Phenolic compounds function as hydrogen donors or acceptors in oxidation reduction reactions and they play an essential role in lignification. Some phenolics show antiauxin activities and are potent growth inhibitors. Others act synergistically with auxins in growth stimulation. Phenolic compounds have been shown to play key roles in the resistance of plants to disease causing microorganisms (Farkas and Kiraly, 1962; Cruickshank, 1963; Rohringer and Samborski, 1967; Kuc', 1972). Phenolic compounds and their role in disease resistance have been reviewed by Nicholson and Hammerschmidt (1992). In addition, phenolics may also serve as protectants against leaf eating insects, for example, they may influence the availability or digestibility of proteins which insects obtain from leaves. Phenolics may also interfere with growth and other energy dependent activities by uncoupling oxidative phosphorylation. The oxidation of phenols yields highly reactive quinones which inhibit enzymes by complexing with metal ions, reacting with sulfahydryl groups or binding nonspecifically to proteins. It is obvious that even small changes in phenol metabolism may severely disrupt many processes which are essential for normal plant growth and development.

Evidences from ^{14}C tracer elements indicate that the biosynthesis of phenolic compounds in plants may proceed via one or more of three different pathways:

1. Shikimic acid pathway.
2. Acetate malonate pathway.
3. Acetate-mevalonate pathway.

The most important of these appears to be the Shikimic acid pathway (Fig. 5.9) in which the initial reaction involves the condensation of phosphoenol pyruvate and erythrose-4-phosphate to yield a seven carbon intermediate which is converted by a series of reactions into shikimic acid.

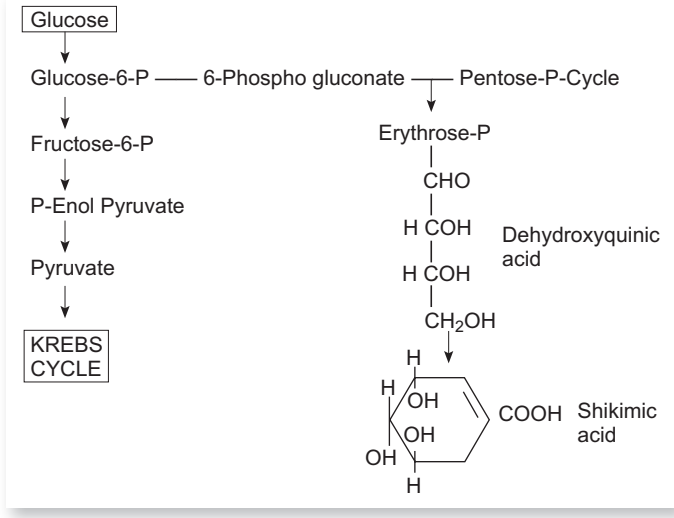


Figure 5.9 The Shikimic acid pathway

The Shikimic Acid Pathway

The Shikimic acid pathway, elucidated first in bacteria, appears to function in higher plants for synthesis of phenolic compounds. The initial reaction of this pathway involves the condensation of phosphoenol pyruvate and erythrose-4-phosphate.

Phosphoenol pyruvate is supplied by glycolysis, whereas erythrose-4-phosphate is derived from the pentose-phosphate pathway. One reaction, the reduction of 5-dehydroshikimic to shikimic, requires NADPH which is also a product of the pentose-phosphate pathway. Transaminations convert shikimic acid to phenylalanine or tyrosine. In dicotyledons, phenylalanine ammonia lyase (PAL) converts phenylalanine to trans-cinnamic acid and thus provides phenyl propane skeletons which can serve as building blocks for lignin or be utilized in the synthesis of flavonoids and various other phenolic derivatives. In cereal grasses, tyrosine ammonia lyase converts tyrosine to p-coumaric acid (4-hydroxycinnamic) and this, in addition to the PAL system, provides phenylpropane skeletons which serve the same functions as those provided by PAL. In several plant tissues, ammonia lyase activity has been found to be closely related to the biosynthesis of phenolic compound (Kosuge, 1969). PAL activity has been shown to increase in response to a number of stimuli, including light and mechanical injury as well as infections by viruses, bacteria and fungi. The level of PAL activity increases to a peak within 24 hours of infection by *Ceratocystis fimbriata* and this increase is followed by a rise in the concentration of polyphenols (Fig. 5.10).

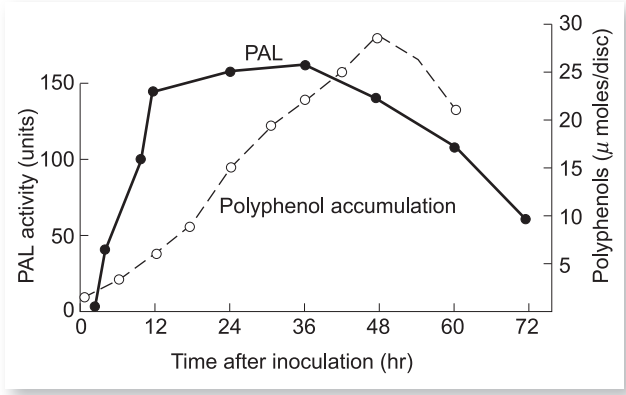


Figure 5.10 Activity of PAL and polyphenol accumulation in discs of sweet potato tissue following inoculation with *Ceratocystis fimbriata* (From C.H. Dicknison and J.A. Lucas, *Plant Pathology and Plant Pathogens,* Blackwell Scientific Publications, Osney Mead, Oxford, 1977, p. 115)

Acetate Malonate Pathway

Some phenolic compounds are synthesized by head to tail condensation acetate units. This pathway, know as the acetate malonate pathway, is thought to involve acetyl Co-A and malonyl Co-A as intermediates and resembles fatty acid synthesis. Isocoumarin (3-methyl, 6-methoxy, 8-hydroxy, 3-4 dhydro isocoumarin), which accumulates in carrot slices, is apparently synthesized via the acetate malonate pathway.

Acetate-Mevalonate Pathway

In a third pathway, the acetate-mevalonate pathway, acetyl Co-A units are condensed to form mevalonic acid which is then converted into cyclic terpenes or steroids. The synthesis of compounds with two or more aromatic rings often involves interactions among different pathways. For example, both the acetate malonate, and shikimic acid pathways contribute to the synthesis of the isoflavone and pisatin.

The current interest in phenol metabolism stems from the identification of a large number of compounds synthesized via the shikimic acid or acetate pathways which accumulate after infection or injury. Many of these compounds, called phytoalexins, have antibiotic properties and are thought to function in disease resistance. These have been discussed in Chapter 6.

Phenol Metabolism

Phenol oxidizing enzymes—laccase and tyrosinase—the two most important phenol oxidases occur both in higher plants as well as in fungi. The enzymes laccase catalyzes the aerobic oxidation of p-hydroquinones or p-phenylenediamines to p-quinones of p-quinonediamines. The other enzyme, tyrosinase, is actually composed of two enzymes. One of the enzymes possesses only catecholase activity, whereas the other shows cresolase activity. This enzyme (tyrosinase) complex is also known as phenolase, phenoloxidase, polyphenoloxidase, or DoP A-oxidase. Tyrosinase is also an aerobic oxidase which is able to oxidize many phenol compounds by oxygen. However, neither para nor meta-dihydroxyphenols are suitable substrates for this enzyme since it is able to oxidize only ortho-dihydroxyphenols.

The enzymatic activity which makes O-diphenol from monophenol oxidatively (monophenol + ½ O_2 = O-diphenol) is frequently referred to as cresolase activity. On the other hand, catecholase activity oxidizes the produced diphenol to quinone (O-diphenol + ½ O_2 = O-quinone + H_2O). These activities are given in figure 5.11.

O-Quinone produces "melanine", which is dark-coloured pigment produced in the course of further enzymatic or non-enzymatic reactions. Melanine is considered to be an end product but the details of the formation of this complex are not yet known.

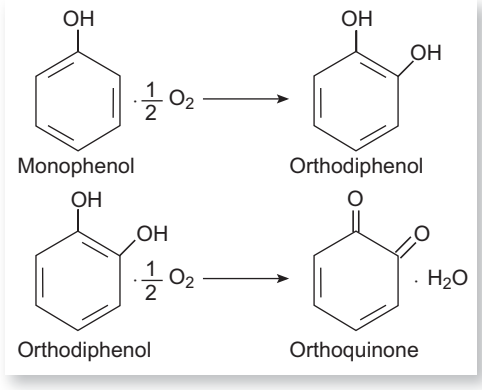


Figure 5.11 Catechol activity of the phenolase enzyme

Changes in the activity of phenol oxidizing enzymes, peroxidase and phenolase may also play a role in the regulation of metabolic pathways in diseased or injured tissues. Phenolase is a copper-containing

enzyme which catalyses the oxidation of mono- and O-dihydroxyphenols to phenols. The resistance to several fungal pathogens has been ascribed to a higher concentration of fungitoxic phenolic substances and their oxidation products, to increased phenolic substances and their oxidation products, to increased polyphenol oxidase activity which generally, but not invariably results from infection. More than 80 years ago, Dufrenoy (1936) observed that when the progress of some pathogens is checked, phenolic compounds, mostly tannins of the gallic group, develop in abundance within the vacuoles of adjacent cells, whereas in specialized parasites, tending to become systemic, tannin is formed very slowly in small amounts and only in vacuoles close to the parasite. Subsequently, it was suggested that the establishment of *Puccinia coronata* in oat leaves depended on the supply of phosphorus from the host tissue; on the depletion of this element, relatively innocuous host phenolic compounds were dehydrogenated by tyrosinase into toxic quinones, leading to the localized necrosis of the tissue.

Importance of Phenol Oxidation in Plant Diseases

The phenol oxidase system plays a role in respiration by transferring electrons from respiratory substrates to other hydrogen or electron acceptors. Oxidation products of phenols (quinones) may be reduced to their original phenol form by respiratory carriers. $NaDH_2$ and $NADPH_2$, generated by the oxidation of respiratory substrates, may reduce quinones in the presence of quinone reductase enzyme. Polyphenolases oxidize monophenol to polyphenols by means of cersolase activity in which the hydroxylation of monophenol leads to the formation of complicated polyphenols, such as flavonoids, tannins, lignins and others, whereas the catechol acitivity has a role in the browning reaction of plant tissues.

Phenolase may influence the amino acid metabolism of plants. It has been seen in many cases that a close correlation exists between the enhanced activity of phenolase and peroxidase and the concentration of phenolic substances. Phenolic substances in turn have been correlated with resistance to pathways. The role of phenolases in host resistance has been summarized in several reviews. The increased phenolase activity in diseased or wounded areas of plant tissues is generally accompanied by an increased concentration of phenolic substances.

Polyphenols, like chlorogenic and caffeic acids, are known to play a role in the inhibition of the activity of indole acetic acid oxidase. Phenolics and their oxidation products seem to play a role in the auxin metabolism of plants. Polyphenols, such as chlorogenic and caffeic acid, known to be powerful inhibitors of IAA-oxidase, may in turn be able to reverse the enzyme activity and confer resistance via an auxin-spring action on plants that produce them in response to infection.

The phenols synthesized by plant tissues after infection appear to have a broad antifungal spectrum. Oxidized polyphenols inhibit the pectinolytic enzyme produced by various fungi and they have early been associated with the resistance of apples to brown rot. It has been shown in many experiments that a correlation may exist between the degree of resistance and the phenol level in healthy plants. Chandramohan *et al*. (1967) showed that varieties of *Amaranthus tricolor* resistant of *Alternaria* sp. accumulate O-D phenols and the amount of the latter was many a times that of the susceptible varieties. Walker and Stahmann (1955) showed that onion varieties resistant to *Colletotrichum circinans* accumulate flavones, anthocyanins and simple phenolics, such as protocatechuic acid and catechol in the dried bulbs scales which are red pigmented.

It has been shown that oxidized phenols as well as oxidized phenolic glucosides are even more effective. In the case of apple leaves, resistance to *Venturia inaequalis* has been attributed to

phloretin and that of pea shoots to *Erwinia amylovora* is attributed to arbutin. This is due to the action of β-glucosidases, polyphenol oxidases and peroxidases.

Lee and Le Tourneau (1958) reported the accumulation of higher amounts of chlorogenic acid in the potato roots of varieties resistant of Verticillium wilt disease. Mahadevan (1965) showed that phenols and their oxidation products showed inhibitory activity on the pectolytic and cellulolytic enzymes of the pathogen, *Cladosporium cucumerinum*. He suggested that the defense mechanism in resistant plants should involve the importance of phenolic constituents and their oxidation products on the cell wall degrading enzymes of the parasite. The inactivation of extracellular microbial enzymes by the phenols or quinones present in plants after penetration is certainly a defense mechanism.

According to Johnson and Schall (1957), the periderm of tubers from a variety resistant to scab (*Streptomyces scabies*) showed much more chlorogenic and caffeic acid and were largely confined to the outermost tissues where the scab organisms proliferate. In the Verticillium wilt of potato, the resistance is ascribed to a greater quantity of chlorogenic acid in root tips, sprout tips and the vascular system of resistant varieties.

In the case of *Phytophthora infestans* infection, the leaves of resistant potato varieties show a more intense accumulation of toxic oxidative derivatives of polyphenols than do the leaves of susceptible varieties. According to Tomiyama *et al*. (1958, 1963), the resistance to *Phytophthora* is reversed by copper enzyme inhibitors that interfere with the formation of toxic quinones via polyphenol oxidases (PPO).

Following inoculation with *Phytophthora infestans*, both polyphenol and PPO increased in resistant potato varieties but not in susceptible varieties which suggests a resistance mechanism to the enzyme. Voros *et al*. (1957) have reported that when streptomycin is absorbed through the root, it gives resistance to potato against *Phytophthora*. This is explained by the fact that antibiotic activity generally enhances PPO activity, and hence, the resistance in plants.

The activity of PPO would seem to be important in that it can oxidize phenolics to quinones which may be more fungitoxic. Polymerisation of quinones to dark coloured tannins may occur. In tomato wilt (*F. oxysporum* f. sp. *lycopersici*) conjugated phenols such as glucosides, tannins and lignins may be hydrolyzed by enzymes to toxic quinones which then polymerize to cause characteristic vascular discolouration as suggested by Davis and Dimond (1954) and Davis *et al*. (1953), Waggoner and Dimond (1953). PPO produced by the pathogen might oxidize host polyphenols to more highly fungitoxic substance which prevent further development of the fungal pathogen, for example, a PPO inhibitor reduce the toxicity of catechol to *Cochliobolus miyabeanus* (leaf blight to rice) and prevents the development of melanin presumably by preventing enzymic conversion of catechol into fungitoxic quinones (Oku, 1967). The melanin is apparently fungitoxic although in other cases these insoluble pigments are relatively non-toxic to fungi. Such inhibition can thus result from the production of PPO by the pathogens.

The germinating uredospores of *Puccinia graminis* release phenols and PPO and the resistance of barely plants to *Erysiphe graminis* appears to be associated partly with an accompanying release of a phenolic substance which accumulates around the haustoria and inhibits further development of the fungus. Aromatic substances, such as polyphenols, phenolic glucosides, flavonoids, anthocyanins, aromatic amino acids and coumarin derivatives tend to accumulate in and around infected plant tissue and also in tissue adjacent to wounds, where presumably they might exert a fungitoxic effect. Kuc' (1963) described two types of reactions in apple varieties resistant to *Venturia inaequalis*. Phloridzin, a polyphenolic glucoside present in the bark and leaves of apple, was hydrolyzed to glucose and phloretin by a β-glycosidase and the phloretin were oxidized by PPO to a fungitoxic intermediate compound and later to melanins. These intermediate compounds, possibly quinones, may have been responsible for

resistance to the fungus. They are produced in susceptible and resistant apple leaves but their formation is much quicker in the latter, thus leading to rapid inhibition of the invading pathogen.

Kuc' (1963) suggested that a great variety of phenolic compounds occurring in plants might provide a partial explanation for the specificity of the host–parasite interaction. For example, certain plant species (resistant) might contain or produce phenolic substances toxic to a particular pathogen, whereas others (susceptible) might lack them. Other workers, however, are skeptical and point to the paucity of adequately documented cases. Cruickshank (1963) notes that there is little direct evidence that the brown pigments (broadly described as polyphenol oxidation products) often associated with infection are of any great significance in pathogenesis—they may be a diffuse secondary attendant phenomenon of defence reaction which has no causal relationship to resistance *per se*. The significance of phenolic compounds in host parasite interaction undoubtedly is complex and needs more critical investigations.

Growth Regulators in Plant Diseases

Role of Growth Regulators in Plant Disease

The growth regulators play vital roles in the growth and development of the plant. The concentration of various growth regulators in the plants is not uniform as it rises and declines very quickly as a result of the action of growth hormone-growth inhibitory systems present in the plant. These growth regulators ultimately regulate the synthesis of messenger RNA molecules which lead to the formation of specific enzymes that finally control the biochemistry and physiology of the plant.

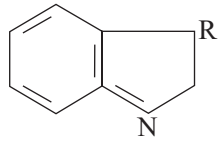
Plant pathogens as a result of infection to the host plants may produce more of growth regulators or more of growth inhibitors or may produce new or different growth regulators or inhibitors or may produce substances that stimulate or retard the production of growth regulators or growth inhibitors by the plants. Whatever the mechanism of action involved, pathogens often cause an imbalance of hormonal system of the plant which is incompatible for the healthy development of the plant. As a result of this abnormal secretion of growth regulators or inhibitors, the infected plant shows a variety of symptoms such as stunting, overgrowth, rosetting, stem malformation, leaf epinasty, and suppression of bud development in healthy plants. Here important growth regulators, their functions and role in disease development are discussed briefly.

Symptoms, such as galls, overgrowth or malformation of host tissues, epinasty, elongation, witches broom effects, etc., indicate abnormal growth activities in many plant diseases. The extracts of tissues which exhibit such abnormal growth patterns are often ten times more active in the tests for growth-regulating substances than are the extracts of healthy plants. Even in the absence of obvious growth abnormalities, diseased tissues often contain elevated levels of growth regulators. Four classes of compounds are known to be involved in plant diseases. All the major types of plant growth regulators (auxins, gibberellins, cytokinins and volatile hormone ethylene) are produced by viral, bacterial and fungal pathogens. An evaluation of the role of individual growth regulators in pathogenesis is complex because their physiological effects in many cases overlap. Growth regulators in plant diseases have been reviewed by Sequeira (1963). Endogenous cytokinins in healthy and diseased plants have been discussed by Dekhuijzen (1976). Endogenous auxins, gibberellins and ethylene in healthy and diseased plants have been discussed by Pegg (1976). Mahadevan (1984) has comprehensively discussed the role of growth regulators, microorganisms, and diseased plants. The role of auxin in plant disease development has been reviewed by Yamada (1993).

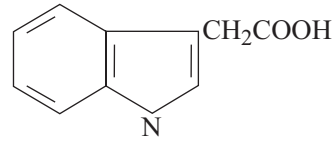
Auxins

The Auxin naturally occurring in plants is Indole-3-acetic acid (IAA). It is continually produced in growing plant tissues in which it occurs in concentrations of about 5mg per kg of green material. Perhaps, no other single compound in plants has received as much attention from plant biologists as has b-3-Indole Acetic Acid (IAA or auxin). One might also add that never have so many diverse primary functions been ascribed to a compound present in the plant in such a comparatively small amount. Of all the endogenous plant growth regulators, IAA is perhaps the nearest to being ubiquitous. Auxin is an organic substance that promotes growth along the longitudinal axis when applied in low concentrations to the shoots of plants which are freed, as far as possible, from their own inherent growth promoting substance.

Went (1926) first showed the effect of IAA on *Avena*, following which it was demonstrated that IAA has a widespread distribution in higher plants and especially in diseased tissues. Auxin is also produced freely by many species of bacteria and fungi. The synthesis of IAA in extremely small quantities takes place presumably in young leaves and root tips. IAA is required for cell elongation and differentiation. Cell elongation is thought to be brought about by the removal of Ca++ and Mg++ cation bridges from the pectate network which leads to the formation of straight chain pectic acids or pectinic acids and this by forming a complex with, and thus inactivating the enzyme pectin methyl esterase (PME) on the cell membrane and so further inhibiting the formation of sites suitable for the attachment of divalent cations. The absorption of IAA to the cell membrane also affects the permeability of the membrane. Many primary functions have been attributed to IAA. It has been linked with cell metabolism, phenol metabolism, increase in respiration, enhancement of the pentose phosphate pathway activity and membrane permeability. Evidence has also accumulated of the primary site of action of IAA being on the nuclear control of RNA synthesis or on RNA itself, thus affecting the level of certain key enzymes in cell growth.



The indole nucleus



Indole-3 acetic acid (IAA)

Changed Level of Auxin in Diseased Tissues The study of plant-growth substances in diseased plants is comparatively recent. This is somewhat surprising, since in many diseases, symptoms typically illustrate the effect of enhanced growth regulatory substances. There are some good review article on this subject such as the one by Sequeira (1963, 1973), Braun (1959, 1962), Veldstra (1968) and Van Andel and Fuchs (1972), Pegg (1977, 1981, 1984), and Yamada (1993).

Viral Diseases Grieve (1943) attributed the reduction in the growth of tomato plants infected with tomato spotted wilt virus to a decrease in auxin content and to reduced rate of translocation through the stem. Surprisingly, Jones (1956) did not find a decreased auxin content in the tomato tissues infected with spotted wilt virus. This can be explained by the observation made by Best (1936,1944) that such infected plants contain a high level of scopoletin which is a known inhibitor of IAA oxidase (Sequeira,1969). Symptoms of dwarfing in many viral infections are believed to be due to the lower auxin

content of the tissues. Lower auxin content has been associated with TMV and potato leaf roll virus. Lower endogenous levels of auxins have also been found in *Lycopersicon esculentum, Phaseolus vulgaris* and *Beta vulgaris* plants infected with curly top virus rather than in healthy plants.

Bacterial Disease The production and role of auxins in plant disease has been studied more extensively in some bacterial diseases of plants such as *Agrobacterium tumefaciens, Pseudomonas savastoni*, and *Pseudomonas solanacearum.* One of the best known plant diseases, displaying overgrowth symptoms, is crown gall caused by *Agrobacterium tumefaciens.* There is ample evidence that the abnormal amounts of auxins found in crown gall tumour are produced by the host. This disease has been studied by many investigators as a model system in cancer and tumour genesis. In crown gall, a disease caused by the bacterium on more than a hundred plant species, galls or tumours develop on roots and stems. Tumours develop when the crown gall bacteria enter fresh wounds on a susceptible host. The cells around the wound produce various phenolic compounds specially acetosyringone and are activated to divide. The pathogenic bacteria do not invade or enter cells of the host but simply attach themselves to the cell walls. In response to the phenolic compounds and other signals they begin processing the DNA in their Ti-plasmid (Tumour inducing plasmid). Intense cell divisions begin to take place wounding and after 2^{nd} or 3^{rd} days after wounding, the plant cells are somehow conditioned and made receptive to a piece of bacterial plasmid DNA (T-DNA, for tumour DNA). A part of the bacterial plasmid DNA is cut out from the Ti-plasmid and is transferred into the host plant cells as T-DNA-protein complex. This T-DNA then becomes incorporated or integrated into the nuclear plant DNA and transforms normal plant cells into tumour cells (Agrios, 1997). Tumour cells subsequently grow and divide independently of the bacteria, and their organization rate of growth, and rate of division can no longer be controlled by the host plants. The transferred (or T) region of plasmid DNA carries several genes which, when expressed in the host cell cause the characteristic tumour. The one as in oncogenic–cancer causing genes specify the synthesis of auxins, and cytokinins leading to uncontrolled cell division, while other genes code for the production of unusual amino acids known as opines. These unusual amino acids can not be utilized by the plant, but are instead catabolized by the pathogen.

In another hyperplastic disease, the knot disease of olive and oleander, the pathogen *Pseudomonas savastoni* produces IAA which induces infected plants to produce galls. *Pseudomonas solanacearum*, the cause of bacterial wilt of solanaceous plants induces a hundredfold increase in the IAA level of diseased plants compared to healthy plants. Increased plasticity of the cell walls as a result of higher IAA content may facilitate their degradation by cell wall renders the pectin, cellulose and protein components of the wall more accessible to degradation by the enzymes of the pathogen.

Fungal Diseases There are numerous reports about the production of IAA in quantity from culture media supplemented with tryptophan or other IAA precursors. This has been used as more than circumstantial evidence for the production of IAA *in vivo* by the particular pathogen. Critical studies have not been made to find out the relative roles of the fungus and host in the hyperauxiny syndromes.

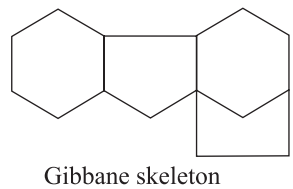
Many fungal diseases of plants cause an increase in the levels of auxin in tissues (hyperauxing) often in very high concentrations. The best known example of increased levels in plant trees infected by fungal pathogens is club root disease of crucifers caused by *Plasmodiophora brassicae*. The pathogen on entry to the roots through mobile zoospores upsets the plant's normal regulatory control of IAA synthesis which leads to root distortion in infected individuals. Increased level of IAA has also been found in rust infected tissues of safflower with *Puccinia carthami*. A ten fold increase has been measured in susceptible potato tubes tissues infected by *Phytophthora infestans* and in corn infected by *Ustilago zeae*.

Higher levels of IAA may also be due to the fact that in infected tissues IAA oxidase has been shown to be lower than in healthy tissues of *Euphorbia cyparissias* infected by *Uromyces pisi*. Thus there was a correlation between increase in IAA and the decrease in IAA oxidase in healthy plants.

Auxins are also involved in wilt diseases. Beckman *et al*. (1953) showed that cultures of the fungus produced a substance similar to IAA. It was claimed that the production of this substance *in vivo* increased the plasticity of xylem parenchyma walls allowing a pectic enzyme weakening of pit membranes and resulting finally in tyloses. IAA solution induced the formation of tyloses in tomato shoots. This supported the view that auxin is involved in the disease syndrome of the wilt. Increased levels of IAA have been reported in infected stems. Auxins have also been implicated in resistance to vascular wilting.

Gibberellins

Gibberellins are a group of chemically similar growth-regulating substances. They can be considered to be normal constituents of green plants. The existence of gibberellin in plants might still have been unknown if the "bakanae" or "foolish seedling" disease (which had devastating effect on the rice economy of Japan) had not broken out. Japanese farmers noted that plants affected with this disease out-grew normal plants but were thinner and paler than their normal counterparts and were sometimes devoid of fruits (Phinney and West, 1960). In the early part of this century, Japanese pathologists first demonstrated the connection between the bakanae disease and the fungus called *Gibberella fujikuroi* (the teleomorph of *Fusarium moniliforme*). Sawada (1912) postulated that disease may be caused by some secretion of the fungus. Yabuta and Hayshi (1939) were able to isolate the crystalline gibberellin from the infected rice plants. Since that time, gibberellins and gibberellin-like substances have been found in higher plants and implicated in disease symptoms. About 60 more are known and form a group of compounds which possess the same general carbon skeleton and all are able to promote either stem elongation or cell division or both in plants. They have a gibbane skeleton.



Gibbane skeleton

Gibbane skeleton

Numerous functions have been attributed to gibberellins. Thus, gibberellin is the chemical signal, that is, the hormone secreted by the embryo in the seed that activates the cells of the aleurone layer into secreting hydrolytic enzymes, such as amylase, which is responsible for liquifying the reserve starch. It also activates the enzymes that promote cell wall degradation, thereby aiding in the digestion of the endosperm cells and in weakening the seed-coats which subsequently allows the seeds to germinate. Besides inducing amylase activity in the aleurone layer of seeds, gibberellins exert a potent stimulus on terminal meristems, and thus in some resects, mimic the effects of auxins on plant growth. Gibberellin also activates or induces the synthesis of other enzymes, including proteinases. Proteinases break down the reserve proteins of the endosperm and release various amino acids, including tryptophan. Tryptophan

is then activated to form auxin (IAA). Gibberellins generally have striking growth promoting effects; they speed the elongation of dwarf varieties to normal size, promote flowering, elongate the stem and root and encourage the growth of fruit. Auxins and gibberellins may also act synergistically. The point of action of the gibberellins in the cells seems to be close to the nucleic acid system, somewhere between DNA and m-RNA. The involvement and role of gibberellins in plant disease has been studied in relatively few diseases. In the "bakanae" disease of rice, it is not known whether the infected plants contain more gibberellin than the healthy plants, or in what manner the fungal gibberellin causes the symptoms observed. Following the classical discovery of the "bakanae" disease, the first truly experimental investigations on endogenous gibberellins and infected plants were made by Radley (1961). It was shown by her that pea nodules infected with *Rhizobium* contain an increased level of GLS as compared to healthy roots. Gibberellic acid has effects in infection of cassava (*Manihot esculenta*) caused by *Sphaceloma manihotica*, which initially causes the formation of necrotic leaf spots and stem canker, but subsequently gives rise to massive elongation of internodes as a characteristic disease symptom.

Gibberellins have been studied more extensively in relation to viral diseases. The spraying of diseased plants with gibberellin has been shown to overcome some of the diseases caused by viruses. Thus, the stunting of corn plants infected with corn stunt virus and tobacco plants infected with severe etch virus was reversed after treatment with gibberellin. Axillary bud suppression caused by sour cherry yellows virus (SCYV) on cherry and leaf curl virus on tobacco was also overcome by gibberellin sprays. The fact that stunting caused by some viruses, can be reversed by applying exogenous gibberellins suggests that the reduced growth of these virus-infected plants might be due to decreased gibberellin content.

Cytokinins

Cytokinins are potent growth factors necessary for cell growth and differentiation. The first cytokinin discovered was kinetin by Skoog and Miller in the early 1950s. The main action of cytokinin, an exogenously applied kinetin, is on cell division. It also seems to be capable of orienting plant growth induced by auxin. According to Osborne (1965), cytokinins stimulate protein synthesis at the level of DNA dependent RNA synthesis. Cytokinins also delay senescence in plant tissues. The action of cytokinins is dependent on auxins. They are derivatives of adenine. Kinetin (6 furfurylaminopurine), a compound, has been isolated from yeast DNA by Miller *et al*. (1956). Kinetin was, however, isolated from herring sperm DNA and has not yet been shown to occur naturally in green plants. Several other adenine derivatives with cytokinin like activities have been synthesized and recently, similar compounds, that is, zeatin and N^6 (2-iso pentenyl) adenosin (IPA) have been isolated from young corn seeds and from spinach and peas respectively. Thrower (1965), Scott (1972), Sequeira (1963, 1973) and Dekhuijzen (1976) have reviewed the role of cytokinins in plant diseases.

Cells in cultures show no response when they are supplied with kinetin alone, and they increase in size but do not divide when supplied with only IAA. When both substances are supplied together, the cells enlarge and divide. The relative concentrations of kinetin seem to determine the organs that will differentiate, low concentrations inducing root formation, higher concentrations inducing bud formation. The mechanism of action of cytokinins is not yet known but the available evidence indicates that it is similar to that of gibberellins. Cytokinins act at the stage of m-RNA synthesis, possibly by preventing genes from being turned off and by activating genes that have been previously turned off. The role of cytokinins in plant disease has only now become a subject of study. A sixfold increase in cytokinin activity was detected in extracts from the galled roots of root knot nematode-infected tobacco plants compared

with extracts from healthy plants; the cytokinin, however, appear late in the infection since they can be detected in galled roots 75 days, and not 35 days, after inoculation. Root cells near a nematode's head display hypertrophy with repeated nuclear divisions together with the incorporation of neighboring cells, and this result in large, thick-walled multinucleate syncytia or giant cells. The endogenous levels of several hormones are reported to increase in several hosts infected by nematodes. Cytokinin activity also increases in rust-infected beans and broad bean leaves and seems to be related to both the juvenile features of the green islands around the infection centers and the senescence outside the green island. In the Helminthosporium blight disease of oats, cytokinins do not increase the toxicity of toxin victorin but they increase the quantity of toxin absorbed. It appears that cytokinins may enhance the effectiveness of the pathogens that produce pathotoxins. On the other hand, cucumber leaf discs treated with kinetin become resistant to the powdery mildew fungus, and tobacco leaves infected with the wild-fire toxin and treated with kinetin fail to develop the typical toxin induced chlorosis. A cytokinin has been implicated in the initiation and development of the fasciation of peas caused by *Rhodococcus (Corynebacterium) fascians* (Sequeira, 1973). Culture extracts of the pathogens and of infected tissues contained materials with cytokinin-like properties. The major active component obtained from the bacterium is 6-(3 methyl-2-butanylamino) purine, the riboside of which is found in t-RNA of many bacteria. Plants treated with a high concentration of kinetin develop symptoms similar to those infected by the bacterium. Although these findings suggest that cytokinins play a role in this disease, a low amount of cytokinin produced by the bacterium in culture indicates that other factors are probably involved. It has been suggested that cytokinins may be involved in the cell proliferation stage of tumour development in oleander infected with *Pseudomonas savastoni*. Studies on kinetin and plant virus multiplication have been stimulated by the work of Richmond and Lang (1957) and later by Osborne (1962) and Mothes *et al.* (1961) concerning the effect of kinetin on protein and nucleic acid metabolism and on amino acids accumulation in leaves. It will be recalled that kinetin tends to arrest the breakdown of proteins and nucleic acids which occurs when a leaf is removed from a plant and it tends to mobilize the accumulation of amino acids at the point of its application to a leaf. Osborne has suggested that the new RNA and proteins are even synthesized under these conditions which, without kinetin, greatly favour catabolism. Kiraly and Szirmai (1964) observed a decrease in TMV production in the leaves and leaf discs of a local lesion host *Nicotiana glutinosa* treated with kinetin (40–50mg/litre). The kinetin treatment to be effective has to be applied before or shortly after the infection. Pozsar and Kiraly (1964) showed that kinetin reduced virus multiplication in a systemic host.

There are conflicting reports of the role of cytokinin in the green island formation in infected leaves. It has been pointed out that the green island formation in mildew infected wheat leaf does not merely represent retention of chlorophyll; they actually are areas in which chlorophyll was destroyed and formed subsequently. On the other hand it had been suggested that the green island formation is the result of chlorophyll retention. The green island formation in Pinto bean leaves infected with *Uromyces phaseoli* depends on light conditions after the inoculation of the plants. The total green area which finally becomes visible is the result of retardation in chlorophyll breakdown and the resynthesis of chlorophyll. Brian (1967) speculated that the green island formation may be due to a release of cytokinin by the pathogen. This is, however, doubtful. Most probably the increased cytokinin level originates from host cells and not from the pathogen. Atkin and Neilands (1972) have speculated about the possible role of siderochromes in the formation of green islands.

An increase in cytokinin content has also been noticed in tumours caused by *Ustilago zeae* and *Synchytrium endobioticum*, club root disease of cabbage caused by *Plasmodiophora brassicae* and peach leaves infected with *Taphrina deformans*. However, peach leaf curl symptoms cannot be entirely attributed to changes in cytokinins, as the level of IAA also increases in infected leaves.

Ethylene

Ethylene (C_2H_4) is a plant growth regulator which is biologically highly active. It has an important role in regulation of plant growth and is implicated in a number of disease symptoms, eg., tissue swelling, stimulation of root formation, leaf abscission, epinasty and fruit ripening. It is produced by plants and some species of pathogenic organisms, is effective in very low concentration and is synthesized from methionine in the presence of light.

Ethylene stimulates the production of a number of enzymes. In healthy plants, ethylene is reported to influence the levels of hydroxyproline-rich proteins in cell walls which are important to wall structure. It has been seen that the ethylene treatment increases the levels of cellulase and b-1, 3-glucanase which are implicated in the degradation of cell wall polymers. According to Hadwinger *et al.* (1970) the role of ethylene in disease resistance may be due to non-specific induction of phytoalexins synthesis and the stimulation of enzymes which are active in the synthesis of phytoalexin, e.g., PAL, peroxidase, polyphenol oxidase and pectin esterase. These enzymes oxidize phenolic compounds and are involved in resistant reactions of the host plants. Ethylene is ubiquitously present in plant tissues and is produced in increased amounts in damaged or diseased plants. Applied exogenously, it is effective in very low concentrations (below 0.1 ppm) and produces a variety of plant responses. Some of this responses—epinasty, leaf abscission, premature senescence, fruit ripening and shedding of leaves—are similar to the symptoms of certain plant diseases. A number of bacterial or fungal pathogens produce ethylene and in the case of fusarial wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*, ethylene production by the pathogen is sufficient to account for the epinastic symptoms of a disease. The defoliating symptoms caused by *Verticillium* wilt of hop plants is attributed to increased levels of ethylene in infected hop plants. In *Verticillium* wilt disease ethylene has been attributed a dual role; acting directly as a toxin on plant tissues covering leaf drop, epinasty and premature senescence and also inducing disease resistance in infected plants.

The origin and possible role of ethylene in diseased plants has been discussed by Abeles (1973), Hislop *et al.* (1973), Sequeira (1973), Pegg (1976), Mahadevn (1984), and Broekaert *et al*. (2006). In a number of viral diseases, leaves with necrotic local lesions produce more ethylene than those of systemically infected plants without local lesions. Necrosis induced by toxic chemicals also results in increased ethylene evolution. These results suggest that ethylene is a product rather than the cause of necrosis.

Erwinia amylovora does not produce ethylene in culture but when cauliflower florets are inoculated with this bacterium, it stimulates ethylene production. This indicates that the evolution in diseased tissue most probably is host origin arising as a result of cellular damage in a still-living tissue. A pectate lyase in the culture filtrate seems to be responsible for the increased production by the plant.

Ethylene undoubtedly interacts with other growth regulating substances, and Van Andel and Fuchs (1972) have pointed out that changes in the balance of growth regulators as a result of such constantly changing interactions may be more important in symptom development than the quantity of any single component. It must be emphasized that it is still not clear whether ethylene is a primary factor in triggering metabolic charges or it is merely produced as a result of these changes.

Transcription and Translation in Diseased Plants

This subject has been dealt in two review articles by Samborski *et al.* (1978) and van Loon and Callow (1983). All living cells hold genetic information in DNA molecules which can be transcribed into RNA and translated into polypeptides according to the code of particular gene. Mechanisms involved in transcription and translation are now fairly well understood, at least in prokaryotes. Transcription is the process by which genetic information encoded in DNA is copied during the process of mRNA synthesis. Translation is the process by which the information is used to specify certain amino acids sequences during peptide synthesis. Several pathogens, particularly viruses and fungal biotrophs such as rusts and powdery mildews, affect the transcription process in infected cells. In diseases caused by viruses, the pathogen through its own enzyme or by modifying the host enzyme (RNA polymerase) that makes RNA utilizes the host cell nucleotides and machinery to make its own RNA.

Effect on Transcription

In some cases, the pathogens affect transcription by changing the composition, structure of, or function of the chromatin associated with the cell DNA. Ribonucleases have been investigated during obligate biotrophic infections such as rusts and powdery mildews. Ribonucleases in infected plants have been shown to exhibit different substrate preferences form those in infected plant tissues (host RNase) and from those of invading fungal mycelium (fungal RNase). Additionally, this type of novel activity was not detected in resistant hosts, which therefore suggests that the changes in RNases may be related to the expression of resistance in the host or to the modification of host RNase by the pathogen. It is generally believed that the greater RNA levels and therefore increased transcription in cells indicate increased synthesis of substances involved in defence mechanism of plants.

Effect on Translation

Infected plant tissues often show increased activity in several enzymes, particularly those associated with respiration or with the production of various phenolic compounds, some of which may be involved in defence mechanism of the plants. Although certain amount of these enzymes (proteins) may be present in the cell at the time of infection of plants by pathogens, several new enzymes or proteins are synthesized *de novo*. Thus there is an increased level of transcription and translation activity in the infected tissues. It has been commonly seen that the increased level of proteins in hosts are those which are resistant to the pathogens. If the resistant tissues are treated with Actinomycin-D an inhibitor of protein synthesis, their resistance to the pathogens is reduced. Thus, it is very clear that the increased activity of the protein synthesis in plants attacked by pathogens is related to the defence mechanism of the plants.

Polysaccharides

Production of polysaccharide appears to be necessary for many pathogens to cause normal symptoms in the plant or by promoting colonization of the pathogen in the host or by increasing survival rate of the pathogen.

In vascular wilts, slimy polysaccharides appear to play important role as these polysaccharides released by the pathogen in the infecting tissue (especially xylem) may be responsible for mechanical blockage of vascular bundles and thus cause wilting. Such a type of possibility may occur rarely in nature, but when macromolecules substances are released in the xylem vessels as a result of breakdown of host substances by the infecting pathogen through its enzymes activity, the possibility of polysaccharides' involvement in the blockage of vessels during wilt becomes obvious.

Detoxification of Antimicrobial Molecules

Several kinds of low molecular weight antimicrobial molecules like saponins which include the avenacins and tomatines are present in the plants are produced by as a result of host–pathogen interaction that provide plants with some degree of protection against the fungal pathogens.

Roots and leaves of oat contain avenacins that are resistant to *Gaeumannomyces graminis*, but this fungus infects other cereals that contain no avenacins. But a strain of fungus that infects oat, *G.graminis* f.sp. *avenae*, produces enzymes avenicinase that detoxify the avenacins by secreting some enzymes. Another saponin, tomatine is present in tomatoes, that protect the plant against infection but the fungus *Septoria lycopersici* produces tomatinase enzyme that detoxify tomatine and infects tomato plants. Similarly some strains of *Botrytis cinerea* can detoxify tomatine and can infect but not all the strains can detoxify tomatine.

Promotion of Bacterial Virulence by Avr Genes

Avr genes in bacteria are thought to direct the production of such molecules in the host plant that elicit the activation of defense response on resistant host plants. The presence of such avr genes among bacterial pathogens suggest that they not only provide advantage to the pathogen but also provide warning to host plant that they are about to be attacked. In many plant bacterial pathogenesis, the proteins (Avr proteins) coded for by avr genes promote pathogen growth and disease development in susceptible hosts. However, Avr proteins have been shown to interfere with the resistance mediated by avr gene. Different avr genes present in bacterial pathogen, contribute different degree of susceptibility/aggressiveness to bacteria. As Avr proteins are coded for by the avr genes, this shows that the particular Avr proteins present in the host cell can promote bacterial virulence. In the susceptible plants the particular avr genes acts as a virulence factor that promotes growth of the bacterium.

Effect of Type III Secretion In Bacterial Pathogenesis

Although the primary determinants of pathogenicity and virulence in many bacteria are due to the secretion of various enzymes like pectolytic, proteolytic and cellulolytic that macerates plant tissues. Now it is known that in case of Erwinia bacteria, the genes for hypersensitive reaction and pathogenicity (hrp genes) ultimately determine the secondary pathogenesis. In plant pathogens, hrp genes code for a type III secretion which is thought to transport bacterial effector proteins directly into the host cell.

Suppressors of Plant Defense Responses

It has been seen that in case of black rust of wheat caused by *Puccinia graminis tritici* and leaf spot of tea by *Mycosphaerella pinodes* there is production of substances called suppressors that suppresses the defense response in the host plant. In case of wheat stem rust, the defense suppressor is present in the germinating fungus and in the intercellular fluid of rust-infected wheat tissues. The suppressor interacts with the wheat cell plasma membrane and reduces binding the pathogen's glycoprotein elicitor of host defenses to the plasma membrane. In this way, the suppressor molecule suppresses the activity of phenylalanine lyase and normal development of defense responses. The *Mycosphaerella* suppressors seem to produce the protein pumping activity of the host cell membrane. ATPase and thereby temporarily lower the ability of cell to function and to defend itself. In case of ergot of rye caused by *Claviceps purpurea*, the fungus produces the enzyme catalase, which neutralizes the H_2O_2 (hydrogen peroxide) that is produced by host plant as a result of defense mechanism. Another way is that the fungus inactivates active oxygen produced by host cell through catalase enzyme.

Pathogenicity and Virulence Factors in Viruses and Viroids

Plant viruses and viroids have no genes that allow them to produce macerating enzymes, toxins or other components by which they can affect plant cells. The various types of symptoms produced by these viruses are as a result of action and interaction of various compounds present in infected cell. The capsid, the outer protein coat of viruses not only protect the viral nucleic acid but play a role in host recognition, release of nucleic acid, movement of viruses between cells by altering the properties of plasmodesmata, block the defense mechanism of host. Some viroids form complex substances with host protein that help the viroids to move freely in host cell through plasmodesmata.

Interference with Translocation of Organic Nutrients Through The Phloem

Organic nutrients produced in leaf cells move down through plasmodesmata into the phloem sieve tubes into the living non-photosynthetic cells where either they are utilized or stored. Plant pathogens may interfere with the movement of organic nutrients from the leaf cells to the phloem tissues. In obligate fungal parasites, the diseased plants are characterized by reduced photosynthesis and increased respiration but starch synthesis is increased temporarily in the infected areas indicating movement of organic nutrients from healthy leaves towards infected areas.

For example, in young canker of woody plants caused by *Nectria* in which cankers develop the pathogen attacks and remains confined to the bark for a considerable time. During that time the fungus may destroy the phloem tissues in that area, thereby interfering with the downward translocation of nutrients. Similarly in diseases caused by Phytoplasmas and phloem-limited fastidious bacteria, these microorganisms fill phloem sieve elements and block the downward translocation of photosynthesis.

Effect of Pathogens on Plant Growth

It has been seen that pathogens that destroy part of the photosynthetic area of plants and cause reduced photosynthetic output often result is smaller growth and yield of these plants. Similarly, pathogens also destroy or clog xylem or phloem elements of plants thereby interfering with the movement of water and inorganic or organic nutrients; often cause reduced growth and sometimes death of the plant. In some plants diseases like club root of crucifers caused by *Plasmodiophora brassicae*, potato wart caused by *Spongospora subterranean*, peach leaf caused by *Taphrina deformans*, crown gall of stone fruits caused by *Agrobacterium tumefaciens*, and root knot of vegetables caused by *Meloidogyne* spp. the infected plant part increases or there is abnormal growth without a clear cut explanation of how these changes are brought about. It is believed that growth regulators might be playing an important role in cell division and cell enlargement. Some of the diseases caused by the phloem inhabiting phytoplasmas may cause stunting of their host. Many viruses and viroids also cause stunting or dwarfing of infected plants.

Some of the pathogens have direct adverse effect on plant reproduction because they attack and kill flower, fruit, or seed directly or indirectly with the propagation of their host plant. For example, in the post bloom fruit drop of citrus, the fruit drops prematurely as a result of infection by the anthracnose fungus *Colletotrichum acutatum*. Similarly in ergot of rye caused by *Claviceps purpurea* and loose smuts of various cereals caused by *Tilletia* and *Ustilago* spp. the pathogen interferes with the reproduction by killing the embryo and replacing the embryo with its own spores. Finally, in phloem limited bacteria or phytoplasmas, no flowers are produced and therefore no fruit and seeds are produced.

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Transcription and Translation in Plant Disease

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Questions

1. What do you know about the changes in the permeability of membranes as a result of infection by a pathogen? Suggest various methods by which you can measure the altered permeability.
2. Define photosynthesis. How is this process affected as a result of infection by a pathogen?
3. Define respiration. How is this process affected due to infection by a pathogen?
4. What are the various mechanisms responsible for augmented respiration in diseased plant?
5. Explain the differences in respiration between resistant and susceptible varieties.
6. What are laccases and tyrosinases? How are these involved in the resistance mechanism?
7. What is transcription and translation? How does the disturbance in any of these lead to drastic changes in structure and function of the affected cell?

How Plants Defend Themselves Against Infection

6

A great variety of plant and animal life exists in a common environment but the parasitism of one form by another is rare. All plants have resistance to some pathogens under certain conditions. Host–parasite relationships may be inhibited or completely hindered if the host is able to ward off the pathogen by passive or active processes. Disease resistance appears to be the rule rather than the exception in nature. Its genetic constitution and the environmental conditions under which the genes operate govern the capacity or ability of a plant to defend itself against a pathogen. The attributes of the hosts that reduce the chances of infection or the development of the pathogen are considered to be defense mechanisms. Different plants defend themselves against pathogens in different ways. The defense mechanisms of plants against pathogens fall into two categories:

1. Morphological or structural defense mechanism.
2. Biochemical defense mechanism.

Morphological or Structural Defense Mechanism

The histology of defense in plants had been reviewed by Akai (1959), Akai and Fukutomi (1980), Dickinson (1960), and Royle (1976). Cell walls and other structural barriers in defence had been ably reviewed by Ride (1983).The first line of defense against pathogens is the surface barrier, which a pathogen must penetrate before it can cause infection. The entry of the pathogens may either be through the epidermal cell walls directly or through the natural openings in the epidermis, such as stomata, lenticels, and hydathodes, or through injured areas caused by living or non-living agencies. When once a pathogen has gained entry into the host, its movement and spread is further checked between or through succulent parenchymatous cells, and in the vascular diseases through the lumen of the xylem vessels. Certain structural features of the epidermis or its interior may greatly affect the ability of the pathogen to penetrate or to invade a host plant. Such morphological defense structures may be present before penetration or infection or may be produced afterwards as a result of the interaction of the host and the pathogen.

Defense Structures Existing Before Infection

Waxes and Cuticles The cuticle which, consists of cutin and waxes forms the outermost covering of the epidermal cells and appears as a non-cellular, membranous layer. Waxes play a defensive role by forming a hydrophobic surface which acts as a water repellent and thereby prevents the retention of

water on sloping plant surfaces on which pathogens might be deposited, and may subsequently further invade the host. The cuticle acts as a physical or chemical barrier to infection. In *Berberis* spp. infected with *Puccinia graminis*, Melander and Craigie (1927) have attributed disease resistance to the outer epidermal cell wall with a thicker cuticle. There are several examples where cutin acts as a physical barrier and enables a greater degree of resistance, for example, *Linum usitatissimum* (linseed) attacked by *Melampsora lini* and coffee attacked by *Colletotrichum coffeanum*. Wilson and Jarvis (1963) attributed the greater infection of white blister, *Albugo candida* on the leaves of mutant forms of Brussels sprout to the absence in the mutant of surface wax. The resistance of strawberry varieties to *Sphaerotheca macularis* was shown by Peries (1962) and Jhooty and McKeen (1965) to vary with the cuticle thickness. Martin (1964) has discussed the role of the cuticle in the defense of plants against diseases.

Structure of Epidermal Cell Wall The thick and tough outer wall of epidermal cells forms and important barrier for certain pathogens. Lignification or the presence of silicic acid in the epidermis of some plants acts as an important structural defense mechanism. In the case of the rice blast fungus *Pyricularia grisea (oryzae*), the outer walls of most of the epidermal cells of the leaves are lignified and the entry of the pathogen inside the host is generally through the thin walled motor cells having pectin rather than lignin. Suberin deposition in potato tuber cells enables better resistance to penetration by some pathogens.

Structure of Natural Openings Most pathogens enter plants through natural openings. In the case of the stem rust of wheat caused by *Puccinia graminis tritici*, the varieties in which stomata open late in the morning are resistant because the germ tubes of the spores germinating in the night dew desiccate. This is due to the evaporation of the dew before the stomata begins to open. This is some times called functional resistance. The kind of structure of the stomata of the host gives resistance to penetration in certain pathogenic bacteria. *Xanthomonas campestris* pv. *citri* is responsible for the citrus canker disease and affects grape fruits and mandarin oranges when artificially introduced into them. The mandarin variety, Szinkum, is resistant to the citrus canker bacterium because it possesses a broad cuticular ridge projecting over the stomata and a narrow slit leading to the stomatal cavity so that the rain-drops containing fungal spores and bacteria rarely reach the mesophyll of the leaf (Fig. 6.1).

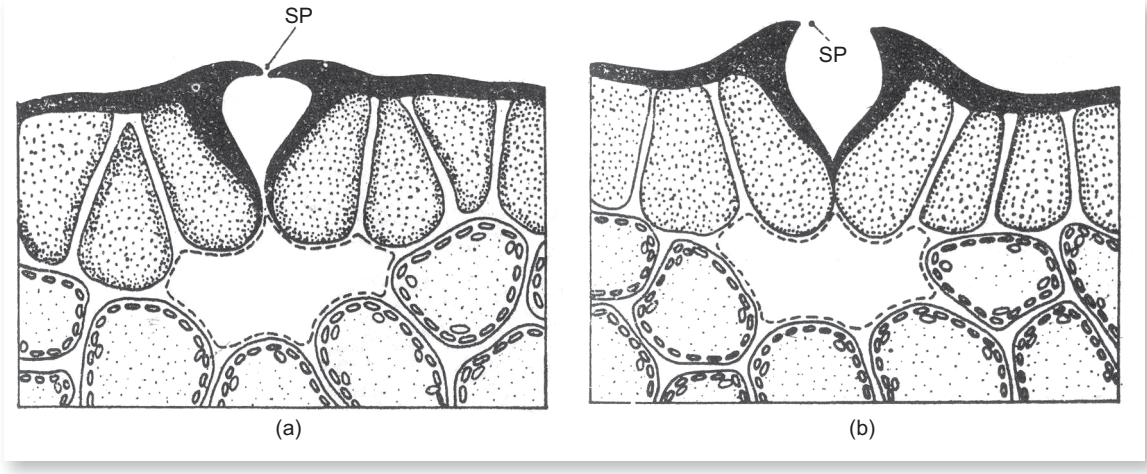


Figure 6.1 Stomata of Szinkum mandarin: (a) resistant to the citrus canker bacterium (*Xanthomonas campestris* pv. *citri*) and of a variety (b) susceptible to the pathogen. SP = stomatal pore.

Internal Structural Barriers to Pathogen Invasion In certain varieties of wheat, the presence of bundles or increased areas of sclerenchyma cells prevent infection in cereal rusts.

Defense Structures Formed After Infection

Defense structures are produced inside the host plant to block or prevent the spread of the pathogen such as the formation of cork layers, tyloses and abscission layers; deposition of gums, resins and tannin-like substances; swelling of the cell walls; sheathing of hyphae, etc.

Formation of Cork Layers In the *Rhizoctonia* diseases of potato tubers, following infection, cork layers are produced just below the areas of infection. This layer prevents further invasion by the pathogen (Fig. 6.2). It blocks the spread of toxic substances produced by the pathogen and checks the flow of nutrients and water from the healthy to the infected area and this results in the starvation of the pathogen. The speed of the formation of the defense barrier is also important. Cork layers are also formed between healthy and diseased areas on *Prunus domestica* leaves attacked by *Coccomyces prunophorae* (Fig. 6.3)

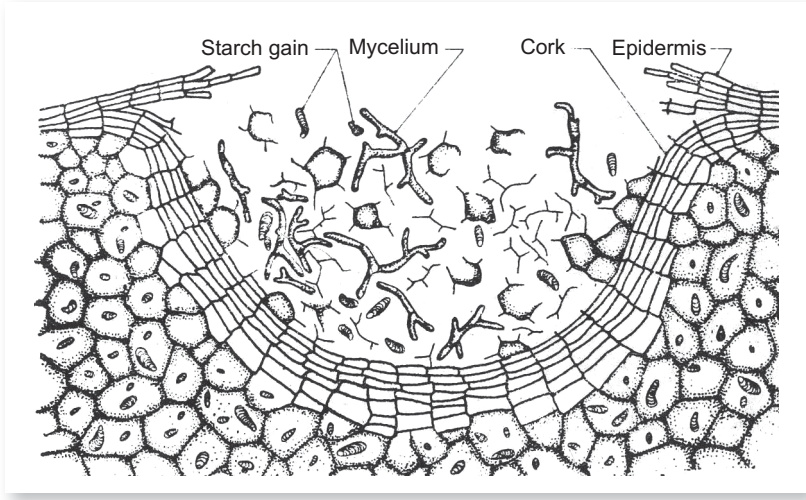


Figure 6.2 Formation of cork layer on potato tubers following infection with *Rhizoctonia*

Formation of Tyloses Tyloses are the overgrowths of the protoplast of adjacent living parenchymatous cells which, protrude into xylem vessels through half bordered pits and effectively clog xylem vessels. Tyloses are usually considered to be one of the factors which cause wilt. The time and rapidity of the formation of tyloses determine whether their role will be defensive or whether they themselves will be one of the factors for the causation of the disease (Fig. 6.4). In sweet potato, wilt caused by fungus *Fusarium oxysporum* f. sp. *batatas*, tyloses in some varieties are formed abundantly and quickly before the pathogen, and thus brings about resistance as this prevents further spread of the pathogen. Evidence of the importance of vessel tyloses as a defense mechanism is available for vascular wilt of tomatoes. Blackhurst and Wood (1963) reported that the percentage of vessels occluded by tyloses nineteen days after root inoculation with *Verticillium albo-atrum* was higher in the wilt resistant variety, Loran Blood (36%), than in the susceptible variety, Ailsa Craig (23%).

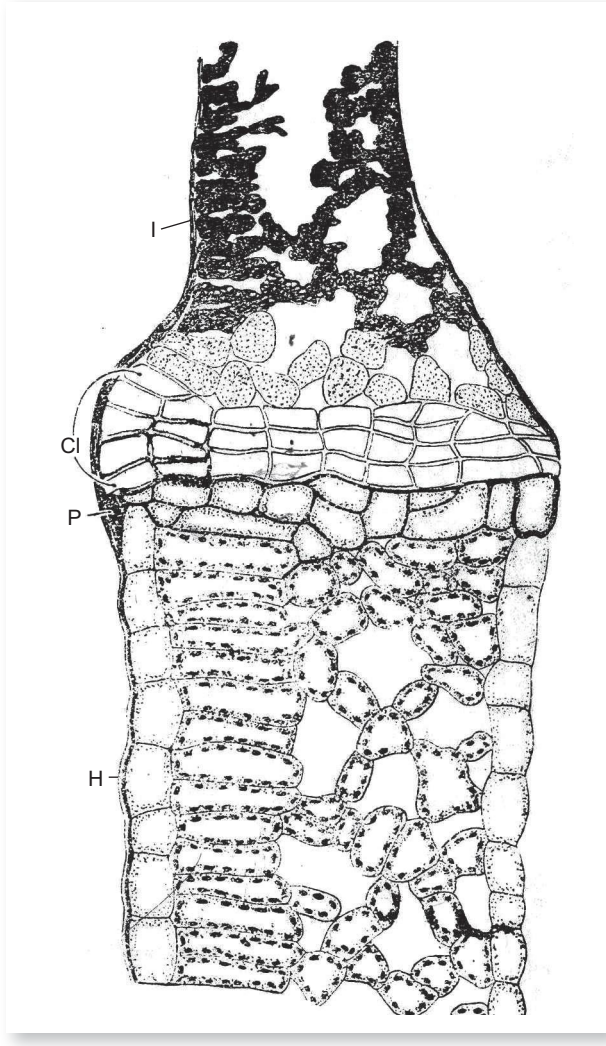


Figure 6.3 Formation of cork layer between infected and healthy areas of leaf. CL = cork layer H= healthy tissue, I= infected, P= phellogen

Formation of Abscission Layers As a result of infection by several fungi, bacteria or viruses, abscission layers are formed in the young active leaves of stone fruits. Due to the formation of abscission layer (upon infections) in peach leaves by the bacterium *Xanthomonas arboricola* (formerly *X. campestris*) pv. *pruni* or the fungus *Clasterosporium carpophilum*, and in sour cherry trees due to infection with necrotic ring spot and other viruses, there is the swelling of the two layers of cells surrounding the infected spot. They become thin-walled while the pectic materials of the middle lamellae get dissolved which results in the formation of abscission layers (Fig. 6.5). A shot hole effect results from the development of an abscission layer which extends from the upper to the lower epidermis around the infected tissue and causes the latter to drop out as in the shot holes of peach caused by *Clasterosporium carpophilum.*

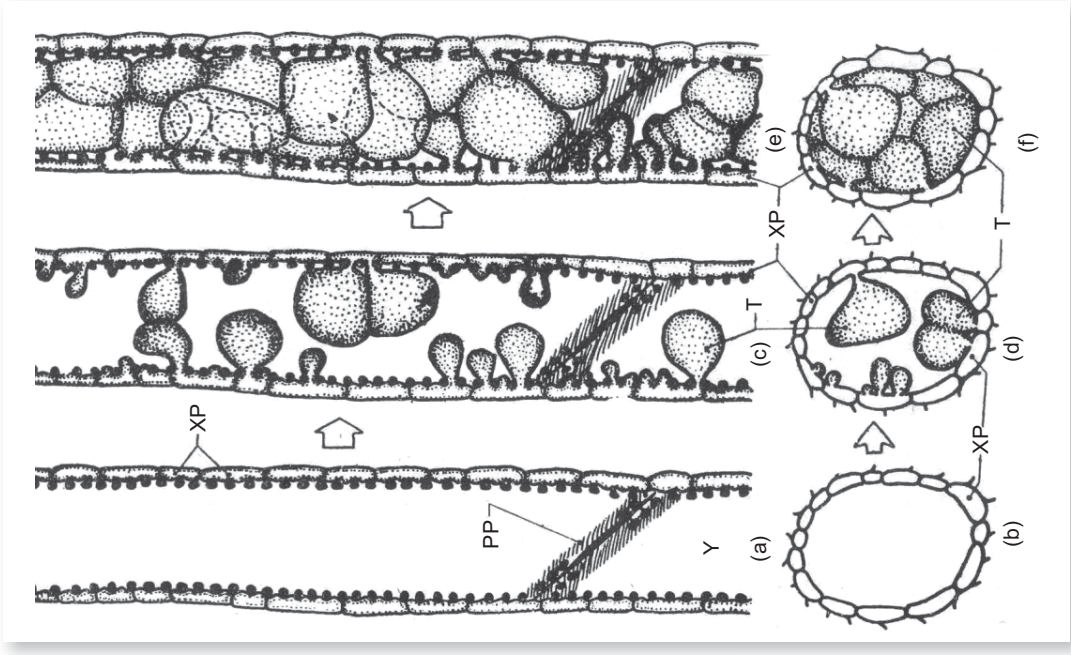


Figure 6.4 Development of tyloses in xylem vessels: **(a)** longitudinal and **(b)** cross section view of healthy vessels **(c)** longitudinal and **(d)** cross section of vessels with tyloses **(e)** longitudinal and **(f)** cross section of vessels completely clogged with tyloses PP= perforation plate, V= xylem vessel, XP= xylem parenchyma cell, T= tyloses

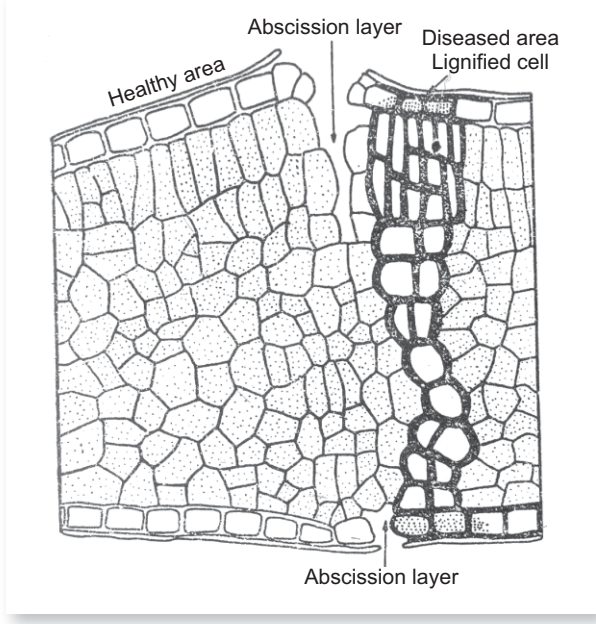


Figure 6.5 Abscission layer formation around a diseased spot of a *Prunus* leaf

Gum Deposition Gum deposition along the borders of diseased lesions often serves as a protective demarcation and constitutes a type of mechanical resistance. In the silver leaf disease in plums caused by *Stereum purpureum*, the wood of the host resistant variety, Pershore, produces so much gum in advance of the fungus in a relatively short time that the fungus becomes completely enclosed by an impenetrable gum barrier in comparison to the susceptible Victoria variety. Hesler (1916) has found a similar gum barrier in the diseased part of the apple twig infected with *Physalospora cydoniae* (Fig. 6.6). In varieties of rice resistant to blast disease or Helminthosporium leaf spot, gum deposits are formed in the intercellular spaces that aid in restricting the fungus to the area of primary invasion. These deposits are found to be highly developed in Shoemed rice (resistant to helminthosporiose).



Figure 6.6 Gum barrier in the apple twig infected by *Physalospora cydoniae. A*= wood parenchyma, B= xylem vessel, C= wood fiber

Sheathing of Hyphae It has been seen that the hyphae penetrating the cell walls are often enveloped in a sheath formed by the extension of the cell walls and prevent the pathogen from spreading (Fig. 6.7). The nature of the sheath is not clear. It consists of cellulose, callose substances or other materials, and in some cases, a sheath may be the deposition product of the cytoplasm rather than the cell wall. Lignitubers may develop as protuberances on the inner opposite the site of penetration. Sometimes, the cell wall being penetrated by the infection peg swells and becomes suberized or lignified.

Biochemical Defense

There are several articles and books on biochemical defense mechanism in plants against infection. Some of the important ones are those of Asada *et al*. (1982), Bailey and Deverall (1983), Nicholson and Hammerschmidt (1992, 1999), Dixon *et al*. (1994), Bowels (1990), and Hutcheson (1998).

Pre-existing Biochemical Defense Mechanism

Inhibitors Released by the Plant in its Environment Walker and Link (1935) worked on onion smudge caused by *Colletotrichum circinans*. Varieties with red scales were resistant in contrast to

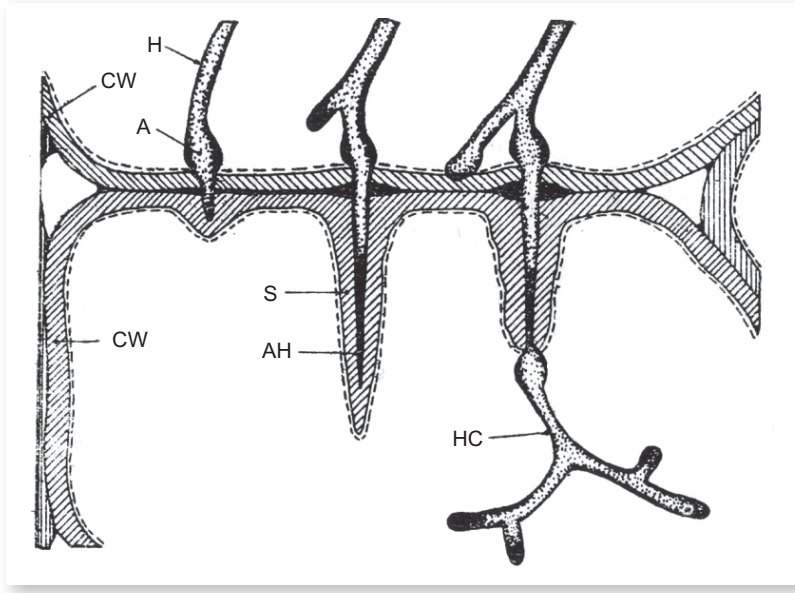
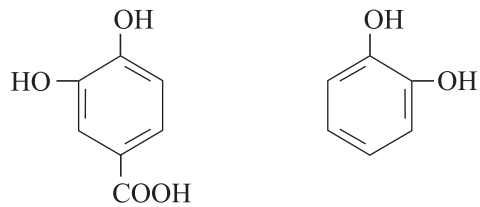


Figure 6.7 Formation of sheath around hypha penetrating cell wall. CW = cell wall, H = hyphae, A = appressorium, AH = advancing hypha still enclosed in sheath, HC = hypha in cytoplasm, S = Sheath.

varieties with white scales. This resistance is due to the fact that the onion varieties with pigmented outer scales contain catechol and protocatechuic acids which are phenolic compounds and which diffuse out from the dead red scales and prevent spore germination. According to Hafiz (1952), the blight resistant varieties of *Cicer arietinum* have more glandular hairs, which secrete malic acid on the leaves than the susceptible varieties. A higher concentration of malic acid inhibits spore germination and retards the hyphal growth of the fungus.



Protocatechuic acid Catechol

Fungitoxic substances have been detected in plant waxes, and cutin acids of citrus lime are toxic to *Gloeosporium limetticola*. Johnstone and Sproston (1965) have reported the resistance of *Ginkgo biloba* leaves with cuticular wax to disease.

Timonin (1941) maintains that root exudates also protect plants against diseases. It was reported that the root exudates from the resistant varieties of flax excrete a glucoside, which on hydrolysis produces hydrocyanic acid which is inhibitory to the soil inhabitants but is less toxic to *Trichoderma viride*. Resistant varieties have *T. viride*, which helps flax to resist wilt caused by *Fusarium oxysporum* f. sp. *lini*. *T. viride* was earlier reported to produce two antibiotic substances, gliotoxin and viridin but now it is known that the antagonistic effect of *T. viride* is not due to these two antibiotic substances which, in

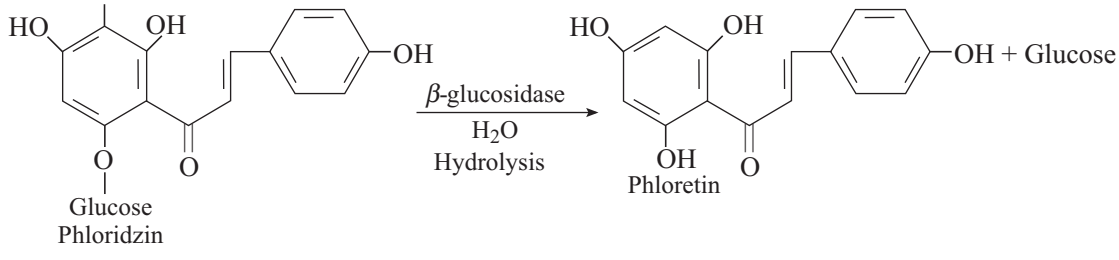
fact, it does not produce (Webster and Lomas, 1964). The antibiotic action of *T.viride* is due to the production of volatile antibiotic substance known as trichodermin. Buxton (1957) has done classical work on the resistance of pea cultivars due to root exudates. Exudates from the roots of one pea variety reduce the germination of the spores of a race of *Fusarium oxysporum* f. sp. *pisi*, non-pathogenic to this variety. This inhibitor of unknown composition showed a far weaker effect on the pathogenic race.

Inhibitory Substances Present in the Plant Cells before Infection In potato scab caused by *Streptomyces scabies*, higher concentrations of chlorogenic acid have been reported in potato tubers (Johnson and Sachaal, 1952) and in citrus lime leaves, and Martin, Baker and Byrde (1966) suggest that isopimpinellin (a furocoumarin) gives resistance to citrus lime leaves and protects them against *Gloeosporium limetticola*.

Tuner (1961) reported that oat leaves and roots contain a fluorescent glucoside (avenacin) which is inhibitory to the growth of several fungi. The growth of *O. graminis*, the cause of the take all disease of wheat, is also inhibited by the sap exuded from the oat roots, but the growth of *O. graminis* var. *avenae* is not. Yet, the purified inhibitor is equally toxic to both the strains. On the other hand, Turner (1961) has shown that a specific glucoside (avenacinase), produced by the strain var. *avenae*, pathogenic to oats while the wheat race completely inhibited by 5µg/ml avenacin, lacks this enzyme. This enzyme destroys the biological activity of the inhibitor and hence the fungus is able to infect the host. Thus, the pathogenic ability and specificity of *O. graminis* var. *avenae to* oat is dependent on the inactivation of the inhibitor. According to Bergman (1966), the infection of tulips by *F. oxysporum* f. sp. *tulipae* mainly takes place during the last week before harvesting. The phase of susceptibility of tulip bulbs to *F. oxysporum* coincides with the drop in the tuliposide content.

In the case of resistance of pears to fire blight caused by *Erwinia amylovora*, it was seen that resistance in certain tissues is related to the presence of phenolic glucoside arbutin. This compound is hydrolyzed by β-glucosidase of host origin to yield glucose and an antimicrobially active semiquinone. Arbutin is uniformly distributed in the tissues of the pear tree, yet these tissues vary in their susceptibility to *E. amylovora*. These investigators positively correlated those portions of the tree that were resistant (floral bracket) with the high levels of β-glucosidase activity. Likewise, those parts of the tree that were the most susceptible (nectaries of flowers) had decreased levels of β-glucosidase activity.

According to Kuc' (1963), phloridzin, the glucoside of phloretin, is present in the leaves of both resistant and susceptible apple varieties. There is no correlation between the contents of phloridzin and resistance to pathogens.



The hydrolysis of phloridzin to phloretin by β-glucosidase

When the pathogen penetrates the leaves of a resistant variety, the plant cells around the point of penetration collapse and die. Apparently, the non-toxic glucoside phloridzin is hydrolyzed by a

β-glucosidase to yield phloretin and glucose. The phloretin in turn is oxidized by phenoloxidases to yield highly fungitoxic compounds. In susceptible varieties, the fungus penetrates the leaves and grows extensively below the cuticle for 10–14 days without initiating the collapse of the host cells. Thus, phloridzin is not hydrolyzed and the pathogen is not contained.

Phenolic Substances Phenolic compounds and their role in disease resistance have been reviewed by Nicholson and Hammerschmidt (1992). Resistance to several fungal plant pathogens has been ascribed to higher concentrations of fungitoxic phenolic substances and their oxidation products and to increased polyphenol-oxidase (PPO) activity which generally, but not invariably, results from infection. In the case of *Puccinia coronata*, more that 60 years ago, Dufrenoy (1936) observed that when the progress of some pathogens is checked, phenolic compounds, mostly tannins of the gallic group, develop in abundance within the vacuoles of adjacent cells, whereas in specialized parasites tending to become systemic, tannin is formed very slowly, in small amounts and only in vacuoles close to the parasite.

The activity of polyphenol-oxidase (PPO) seems to be important because it can oxidize phenolics to quinones which may be more fungitoxic. Polymerization into dark-coloured melanins may then occur. PPO produced by the pathogen may oxidize the host polyphenols to more highly fungitoxic substances which may prevent further development of the pathogens. For example, a PPO inhibitor reduces the toxicity of catechol to *Cochliobolus miyabeanus* (leaf blight of rice) and prevents melanin development, presumably by preventing enzymic conversions of catechol into fungitoxic quinones. The melanin is apparently fungitoxic although in other cases insoluble pigments are relatively non-toxic to fungi. Germinated urediniospores of *Puccinia graminis* release phenols and PPO, and resistance of barley plants to *Blumeria* (*Erysiphe*) *graminis* appears to be associated partly with the collapse of the mesophyll cells with an accompanying release of a phenolic substance which accumulates around the haustoria and inhibits further development of the fungus. Chlorogenic acid is partly responsible for the resistance of potato tubers to scab (*Streptomyces scabies*), perhaps by stimulating protective cambium activity (Johnson and Schaal, 1952).

Hare (1966) pointed out that aromatic substances, such as polyphenols, phenolic glucosides, flavonoids, anthocyanins, aromatic amino acids and coumarin derivatives tend to accumulate in and around infected plant tissue and also in tissues adjacent to wounds where presumably they might exert a fungistatic effect. Phenolic compounds are produced in plants primarily via the shikimic and acetic acid pathways. In the latter, phenolics are produced by a head to tail condensation of acetate units derived from the breakdown of sugars during respiration. In the shikimic acid pathway, phosphoenol pyruvate from glycolysis reacts with erythrose produced during the pentose pathway, the activity of which is increased in diseased plants, and forms dehydroquinic and then shikimic acid. From the shikimic acid and various intermediate compounds, some of which are still unknown, phenolic compounds are produced. Some of the most important phenolic compounds involved in the defense of plants are caffeic acid, ferulic acid, chlorogenic acid, phloretin and various phytoalexins.

The high concentration of chlorogenic acid in the roots of certain potato varieties has been considered the main mechanism of defense against the *Verticillium* wilt pathogen. Resistant varieties contain more chlorogenic acid than the susceptible younger roots and are thus not attacked, but they become susceptible later on. Chlorogenic acid is a competitive inhibitor of IAA oxidase and may be involved in diseases where resistance is increased with high IAA content of the plant. Chakravarty and Srivastava (1967) had attributed the resistance of carrot roots to *Pythium aphanidermatum* to an unidentified, apparently pre-formed phenolic substance. It is said that the phenolic contents of rice are responsible for resistance in *Pyricularia grisea*. Polyphenols have been implicated resistance to *Venturia inaequalis*

(apple scab) and *Venturia pirina* (pear scab). Sproston (1957) holds that balsam (*Impatiens balsamina*) leaves are free from fungal diseases due to the abundance of phenolic substances or glycosides which are oxidized to fungitoxic quinones.

Allen and Kuc' (1968) presented evidence of the fact that sterols may be involved in defense mechanisms. The steroid glycoalkaloids, α-chaconine and α-solanin, were the major fungitoxic substances in potato peel, being present in concentration by at least eight times the amount necessary to inhibit 50% of the growth of *Helminthosporium carbonum*, a fungus which does not attack potato. *Phytophthora infestans* is not affected due to the lack of cell-membrane sterols, one of the two prerequisites for susceptibility to saponins. Tomatine, another steroid glycoalkaloids of solanaceous plants, was more toxic to non-pathogens than to pathogens of tomato and *Septoria lycopersici* (tomato leaf spot), was able to detoxify tomatine, whereas other *Septoria* spp., non-pathogenic to tomato, were unable to do so. These results provide some circumstantial evidence that tomatine may protect tomato leaves against certain pathogens and further investigation is needed. The mode of action of these fungitoxic steroid glycoalkaloids is not properly understood, but as suggested by Hendrix (1970) in his review on "Sterols in Growth and Reproduction in Fungi", this may be brought about by the disruption of permeability in fungi.

Absence of Nutrients Required by the Pathogen Unless the host tissue supplies all the nutrients necessary for the growth of a pathogen, the latter will not grow, and cause infection. Walker and Stahmann (1955) suggested that in such pathogens the availability of highly elaborate growth factors, such as vitamins, polypeptides, amino acids and proteins, and the presence of complex enzyme systems may be essential for successful parasitism, and that resistant plants may lack one or more of these factors. Some mutants of *Venturia inaequalis* which were deficient in choline and riboflavin were unable to cause scab unless these substances were supplied (Kline *et al.,* 1957). A total of 54 biochemical mutants including those in which synthesis of various vitamins, organic bases, and amino acids had been studied in *Venturia inaequalis*, and the effect of each mutation on the pathogenicity of the fungus had been determined. Of the vitamins requiring mutants, only those in which metabolic reactions involving choline or riboflavin have been affected, were found to have lost in pathogenicity. All mutants in which the metabolism of purines, pyrimidines, and amino acids had been affected, incited only the fleck reaction and were non-pathogenic (Keitt *et al.,* 1959).

Lukezic and De Vay (1964) have correlated the resistance of plum varieties to *Rhodosticta quercina* (canker) with the amount of myoinositol in the bark of trees. This fungus is naturally deficient in myoinositol, and more of this was present in the bark of the susceptible cultivar President than in the bark of the two resistant cultivars Beauty and Duarte; further, the resistance of Duarte was reduced by adding myoinositol to the bark tissue.

Resistance has also been attributed to the concentration of nutrients in plant tissues, as distinct from the absence of specific nutrients needed by the pathogen. Sugar content is perhaps the best known example of this. High sugar content of the tissues favours some pathogens, such as certain rusts and powdery mildews, whereas the reverse is true for other, including *Alternaria solani* (early blight of potato) and *Ophiostoma ulmi* (Dutch elm disease), as discussed by Horsfall and Dimond (1957). Plants presumably become resistant to high sugar pathogens when the sugar content of their tissues falls to a certain level and this may be brought about, for example, by treatment with certain growth regulating substances. Boron facilitates the translocation of sugars so that these tend to accumulate in the leaves of boron deficient plants and the latter are apparently resistant to *Cochliobolus sativus* (barley leaf spot), a low sugar pathogen, but susceptible to *Blumeria* (*Erysiphe*) *graminis*, a high sugar pathogen. *Erwinia carotovora* var. *atroseptica* which causes bacterial soft rot of potatoes is less severe on potatoes with low reducing sugar content than on potatoes with high in reducing sugars.

Beever (1970) found that the xylem sap of trees (peach, plum) susceptible to *Stereum purpureum* contained more nitrogen and carbohydrate and was more favourable for the growth of the fungus than was the sap of resistant trees (apple, pear). There were also qualitative differences in that the main sugar and sugar alcohol in the sap from resistant trees were sucrose and sorbitol, neither of which supported good growth of *S. purpureum*, whereas the sap of susceptible trees also contained glucose and fructose which were more favourable for growth of the fungus.

pH There are few convincing cases of disease resistance directly attributable to *p*H, although the resistance of ripe tomatoes to *Xanthomonas campestris* pv. *vesicatoria* (*Bacterium vesicatorium*) may be due to their high acidity (*p*H 4-4.5) as reported by Gardner and Kendrick (1921). The *p*H of the plant tissues influences many physiological processes in both plants and parasites, notably enzyme activities, and resistance to certain *p*H sensitive pathogens might be related to unsuitable *p*H of the plant tissue.

Osmotic Pressure and Permeability Effects The cells of parasitic fungi have a unusually higher osmotic pressure than that of the surrounding host cells, this being necessary for absorption of water from the latter. In the case of *Erysiphe cichoracearum* (lettuce mildew), cells of resistant tissues or varieties have a higher osmotic pressure than those of the susceptible ones. Further, resistance can be modified by altering the mineral nutrition of the plant and shaded leaves or those cultured on distilled water become susceptible, whereas young lettuce seedlings with high osmotic pressure are resistant.

Defense through Absence of Common Antigens The gene-for-gene concept of the relationship between the host and pathogen evolved from the work of Flor (1955) with flax rust. The idea that the antigens shared by the host and pathogen may determine the nature of disease reactions has been explored by Doubly *et al*. (1960). Tests of globulin antigens from four races of rust and four from near isogenic lines of flax suggested that susceptibility depended on the presence, in the host, of a protein serologically similar to the one possessed by the pathogen. Some support for the common antigen hypothesis has been obtained from work on non-obligate parasites. Pathogenic races of *Xanthomonas campestris* pv. *malvacearum* which causes the angular leaf spot of cotton had more antigens in common with those of cotton leaves than did the non-pathogenic races of bacteria and the black rot fungus *Ceratocystis fimbriata* which attacks sweet potato (De Vay *et al*., 1967).

Phytoalexins Lasting immunity against certain virus diseases in humans and animals has been known to science for many years. However, there has been no evidence that plants possess anything like an antibody-forming mechanism which makes for recovery and protects against subsequent infections as in animals. The virus diseased plants do not normally recover and in fact there were symptoms of recovery in tobacco ring-spot-virus-infected plants. Wingard's view that these infected plants acquired an immunity to the disease was vigorously supported by Price (1932, 1936a, b).

Later, workers opposed the use of the term immunity to this phenomenon of recovery, largely because the disease clearly had two phases—the acute and chronic. Moreover, the recovered plants contained the virus. Indeed, Wallace (1938, 1940) suggested that in the case of tobacco plants infected with the sugar beet curly top virus, recovery was due to the *in vivo* development of protective substances and considered that they could be transferred to other plants where they would confer a form of passive acquired immunity. This suggestion put forward by Wallace was followed soon after by a somewhat similar approach to a host reaction in the potato—*Phytophthora* disease, resulting in the discovery of substances called phytoalexins (Mùller and Börger, 1940). During the last 75 years many papers have described the structure synthesis and activity of such compounds, now called phytoalexins. The term

was introduced by Muller and Boerger (1940) to describe the substances that inhibit fungus development formed when living plant tissues are invaded by a fungal parasite. It has since been redefined and broadened to include substances formed by host tissue "… in response to injury, physiological stimuli, infectious agents or their products … [that] accumulate … to levels which inhibit the growth of microorganisms" (Kuc, 1972). While Day (1973) proposed to include substances that are formed in response to infection that are toxic to, or which repel insect or nematode parasites. Phytoalexins can be described as low-molecular-weight antimicrobial compounds that accumulate in plants as a result of infection or stress (Kuc, 1995). They may be associated with host cell necrosis (hypersensitivity) at the infection site but not necessarily so when induced by chemicals. It was envisaged that the factor, which induces the formation is released by the parasite into the host cell. The role of the fungus and the nature of the fungal metabolites involved in host parasite interactions have as yet received very little attention from research workers in this field. Phytoalexins are now being increasingly investigated into and have been detected in several diseases, but more investigation of their over all significance as a resistance mechanism is needed. Among other papers are those by Mὺller and Börger (1939), Mὺller (1956, 1961), Cruickshank (1963) and Kuc (1966, 1976). There is a comparatively recent article on Phytoalexins: "Stress Metabolism, and Disease Resistance in Plants", by Kuc (1995). Hammerschmidt (1999) has ably reviewed the subject "Phytoalexins: What Have We Learned After 60 Years?" There are two hypotheses put forward to explain the mechanism of the induction of phytoalexins:

1. *De novo* synthesis of enzymes, or
2. Activation or activity control of enzymes already formed.

In the field of phytoalexin research, only the end-products are known with certainty. The metabolic pathways and associated enzymes involved in the biosynthesis of these products are largely unexplored.

The initial work of Mὺller and Börger (1940) laid the foundation of the concept of phytoalexins. The conclusions of Mὺller and Börger (1940) are given below:

1. A principle designated as phytoalexin which inhibits the development of the fungus in hypersensitive tissues is formed or activated only when the host cells come into contact with the parasite.
2. The defense reaction occurs only in the living cells.
3. The inhibitory material is a chemical substance and may be regarded as a product of necrobiosis of the host cell.
4. The phytoalexin is non-specific in its toxicity towards fungi; however, fungal species may be differentially sensitive to it.
5. The basic response that occurs in resistant and susceptible hosts is similar. The basis of differentiation between resistant and susceptible hosts is the speed of formation of phytoalexin.
6. The defense reaction is confined to the tissue colonized by the fungus and its immediate neighbourhood.
7. The resistant state is not inherited.

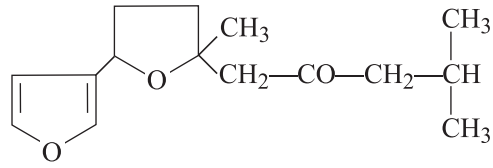
Phytoalexins are probably fungistatic rather than fungicidal but this no doubt depends partly on the quantity present.

According to Cruickshank (1963), the rate of formation of phytoalexin is probably critical in combinations of host variety parasite strain (as in the reaction of the different varieties of potato to different races of *Phytophthora infestans*) but probably less so when combinations of pathogens and non-pathogens of specific hosts are involved. Host specificity of phytoalexins occurs between genera but probably not within a species or even between related species. Gene-for-gene relationship might be interpreted by the phytoalexin characteristic of the pathogen, both being genetically determined; a range of

host–parasite interaction types might thus arise. Susceptibility could be related to the failure of the invading pathogen to stimulate the formation of phytoalexins or to its tolerance of the amounts produced, which in turn is related to its ability to degrade the phytoalexin.

There is increasing evidence that some of the fungi which attack a plant are able to do so because they have the capacity to degrade its phytoalexin, and the non-pathogens are not able to do this. Relatively few phytoalexins have been investigated for their contribution to varietal resistance. From the point of view of practical agriculture, the mechanism of varietal resistance and susceptibility are much more pertinent than a fundamental consideration of immunity.

Ipomeamarone This is an abnormal sesquiterpinoid (tetrahydrofuran, $C_{15}H_{22}O_{3;}$ mol. wt. 230) which seems to be induced in sweet potato (*Ipomoea batatas*) tissue infected with black rot fungus *Ceratocystis fimbriata* by a possible disturbance of carbohydrate and fatty acid metabolism of the host (Uritani and Akazawa, 1959). These authors considered that the biosynthesis of ipomeamarone may involve mevalonic acid as a precursor and could perhaps also be induced by poisonous chemicals and pectinase (Tomiyama, 1963). The presumption was that the carbon sources being utilized for respiration and biosynthesis of phenolic compounds may, by an inhibition being utilized for respiration and biosynthesis of phenolic compounds, may, by an inhibition of the TCA cycle and pathway of the biosynthesis of phenolics, be diverted to the biosynthesis of ipomeamarone. Ipomeamarone increases substantially in diseased tissues adjacent to infected tissues. Such tissues show terpenes which are also produced by slices of sweet potato in response to a chemical stimuli. The interesting part is that ipomeamarone is toxic to both non-pathogenic and pathogenic fungi and to some bacteria, and there seems to be no correlation between its inhibitory effect and pathogenicity.



Ipomeamarone

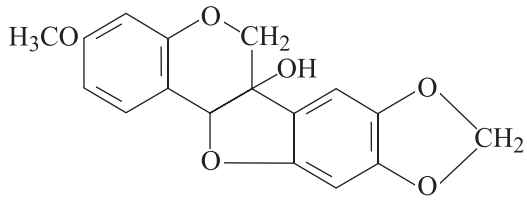
Pisatin Pisatin has the chromocoumarin ring system and is phenolic ether ($C_{17}H_{14}O_{6;}$ mol. wt. 314). Cruickshank and Perrin (1960) and Perrin and Bottomeley (1962) isolated pisatin from the pods of the garden pea inoculated with fungi, using the drop diffusate technique. The subsequent studies by Cruickshank and his colleagues are classic in phytoalexin research and provided the impetus for much of the research which followed thereafter.

This is produced by the exposed endocarp of detached pea in response to inoculation with many fungi or injury. When different fungi, pathogenic and non-pathogenic to pea, are used for inoculation, they induce the production of different concentrations of pisatin. The non-pathogenic fungi induce the formation of pisatin at concentration high enough to inhibit their growth. If pea pathogens induce the formation of pisatin, it is at concentrations much below these that are toxic to the pathogen. Production of pisatin by pea pods inoculated with *Monilinia fructicola*, a non-pathogen, is reduced or delayed at high temperature on anaerobic storage, and the pea pods become susceptible to this fungus, while storage at normal temperature conditions results in the accumulation of high levels of pisatin and resistance.

Pisatin can also be formed when dilute solutions of certain metallic salts (especially those of mercury, silver, and copper) are placed on pea pod endocarp tissues and it is possible that phytoalexins are

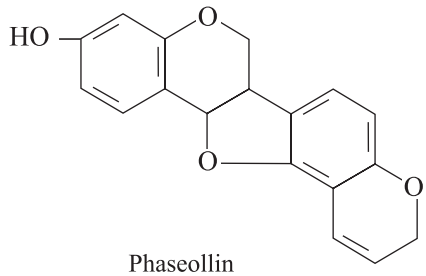
produced when metallic fungicides are applied to crops. Certain metabolic inhibitors, such as sodium fluoride and sodium monoiodoacetate, are reported to have similar effects. Other substances reported to induce pisatin formation by pea pods include ethylene, which at a concentration of 1 ppm induces a slight production. At certain concentrations, a number of antibiotics (actinomycin D, cycloheximide, chloramphenicol, and others) and ribonuclease stimulate pisatin formation. However, they fail to do so at higher concentrations (Schwochau and Hadwiger, 1969). Many of these substances interfere with protein synthesis in plants.

Species or races of fungal pathogens, pathogenic to a particular species seem to stimulate production of generally lower concentrations of phytoalexins than nonpathogens. In case of pea pods inoculated with the pathogen *Ascochyta pisi*, pea varieties produce concentrations of pisatin that are approximately proportional to the resistance of the variety to the pathogen.



Pisatin

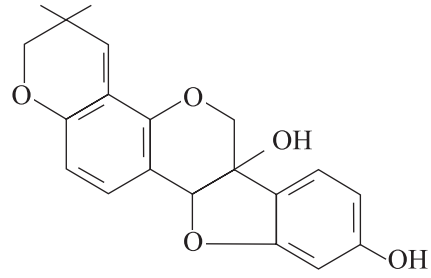
Phaseollin Phaseollin is similar to pisatin in chemistry and function. It was isolated from detached, open bean pods, following inoculation into the non-pathogen, *Monilinia fructicola*. More recently, a number of compounds structurally similar to phaseollin have been identified from diffusates or tissues inoculated with fungi, bacteria and viruses. These are phaseollidin and phaseollinisoflavan and kievitone. According to Rahe *et al*. (1969), phaseollin is important in determining varietal resistance to *Colletotrichum lindemuthianum*, the cause of anthracnose of beans. It accumulates most rapidly in bean hypocotyls inoculated with an avirulent race. Inoculation with a virulent race produces a much lower level of accumulation. Three races of the same organism examined by Bailey and Deverall (1971) were equally sensitive to phaseollin, suggesting that if phaseollin has a role in race specific resistance, it would depend on the amount produced and not on differential toxicity.



Phaseollin

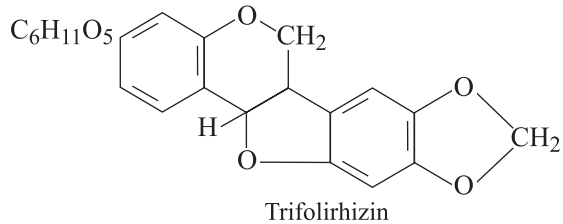
Glyceollin This is produced in soybean plants infected with the fungus *Phytophthora megasperma* f. sp. *glycinea*. Inoculation of fungal races on incompatible host cultivars resulted in higher concentrations of the phytoalexin glyceollin than in inoculations of fungal races on compatible cultivars. It has been

suggested that the higher concentrations of glyceollin in incompatible host–pathogen combination are the result of reduced biodegradation rather than increased biosynthesis.



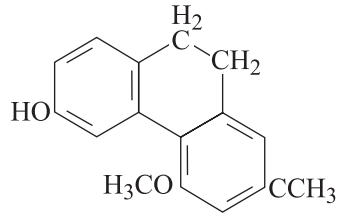
Glyceollin-I

Trifolirhizin This is a new glucoside which has been isolated from the roots of red clover. Its aglycone has the formula, $C_{16}H_{12}O_5$. Trifolirhizin is of interest here, as its structure indicates that it is chemically closely related to pisatin.



Trifolirhizin

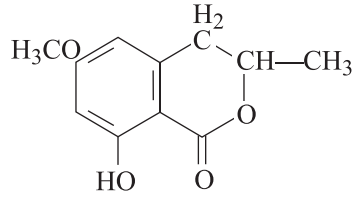
Orchinol and Hircinol Orchinol is a phenanthrene and one of the three compounds formed by the interaction of diffusible metabolities of *Rhizoctonia repens* with *Orchis militaris* tuber tissues (Gaumann *et al*., 1950). According to Gaumann, when there is a stimulus from outside, both *Orchis militaris* and *Loroglossum hircinum* synthesize concurrently two substances: orchinol and hircinol. *O. militaris* produces 50–100 times more orchinol than hircinol, whereas *L. hircinum* produces 100 times more hircinol than orchinol. Both substances are found in tissues of bulbs, roots, and stalks under aerobic conditions, only as a defense mechanism from specific fungal infection, especially mycorrhizae, and not against common saprophytic or semiparasitic soil fungi (Gaumann, 1964). Gaumann considers that the stimulus causing the synthesis of hircinol is a microbially induced antigenic effect resulting in antibody production or from lesions, a traumatic induction of antibody production. Antimicrobial spectrum of orchinol is broad and hardly specific.



Orchinol

Isocoumarin This has been isolated from carrot root tissues inoculated with a fungus non-pathogenic to carrot, *Ceratocystis fimbriata* (Condin and Kuc', 1960). This compound is of special interest as it can be produced in tissues in response to a number of non-pathogenic microorganisms like *Ceratocystis ulmi, Helminthosporium carbonum*, *Fusarium oxysporum* f. sp. *lycopersici* race 1 and *Thielaviopsis basicola*, whereas when *Stemphylium radicinum*, a pathogen of carrot, was inoculated into this tissue, no such response was noticed.

Klarman and Sanford (1968) obtained a crystalline compound with absorption maxima at 288, 293, 315 nm from soybean (Glycine max). They suggested that these compounds may be chemically related to the pterocarpan phaseollin.



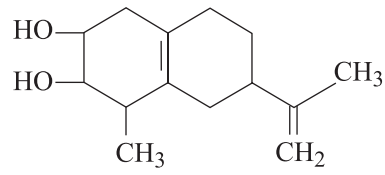
Isocoumarin

Medicarpin Alfalfa (*Medicago sativa*) inoculated with a series of pathogens and non-pathogens of this host have been studied by Higgins and Millar (1968). The antifungal compound was isolated from leaf diffusate solutions and has been identified as (-) demethyl-homopterocarpin and given the name, medicarpin.

Wyerone acid Purkayastha and Deverall (1965) and Deverall and Vessey (1969) had reported an ether soluble acid identified as wyerone acid, CH_3—CH_2—CH═CH. C═C.CC. C═CH. CH═C. CH=CHCOOH in broad bean. Infection of broad bean leaves by *Botrytis fabae* leads to an increase in wyerone acid and wyerone, its methyl ester.

Cicerin Kunzru and Sinha (1970) reported the occurrence of a new phytoalexin associated with *Ascochyta* blight of gram. The inhibitory principle is composed of two fluorescent phenols or phenolic compounds. Phytoalexin diffuses into inoculation droplets during the course of interaction between *Ascochyta rabie* and the pod tissues of *Cicer arietinum*. The name cicerin was tentatively suggested by them.

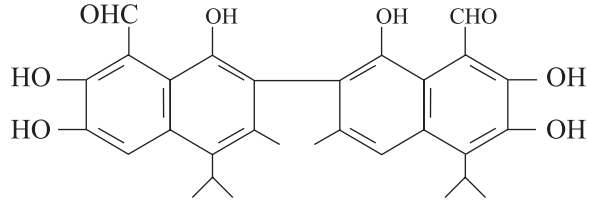
Rishitin Mὺller and Börger (1940) and Mὺller (1953) were the first to show that the potato tubers carrying the gene R1 for late blight resistance responded when inoculated with avirulent race (race 0) of *Phytophthora infestans* by producing a phytoalexin that inhibited the development of a virulent race (race 1) applied a short time later. A number of other examples of host parasite systems in which inoculation with avirulent strain protects the plant against subsequent infection were reviewed (Matta, 1971). Two non-pathogens of potato (*C. fimbriata* and *Helminthosporium sativum*) also stimulated accumulation of rishitin in both kinds of tuber tissues. In studies on potato tubers (*Solanum tuberosum*) infected with an incompatible race of *Phytophthora infestans*, isolated a new compound with phytoalexin-like properties. It is a bicyclic non-sesquiterpene alcohol (Katsui *et al*., 1968) and has been given the name, rishitin.



Rishitin

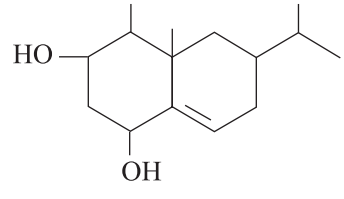
Gossypol Bell (1967) has reported the accumulation of several antifungal compounds as a result of the introduction of conidia of *Verticillium albo-atrum* and *Rhizopus nigricans*, heavy metal ions and various metabolic inhibitors into boll cavities of xylem vessels of the excised stems of two species of *Gossypium* and several cultivars of cotton (glandular and glandless). The compound has been identified as gossypol. Bell (1967) has identified it as an ether soluble phenol. In 1969, he related the accumulation of gossypol and gossypol-like compounds in stem sections and intact plants to host resistance and inversely to the virulence of the pathogen.

Other diseases in which there is evidence of the formation of phytoalexin-like substances include black spot of rose (*Diplocarpon rosae*), leaf spot of wheat caused by *Septoria tritici*, leaf disease of *Primula* caused by *Ramularia primulae* and *Cercosporella primulae*, leaf spot of *Brassica* species, and viciatin from broad bean, the structure of which does not appear to have been characterized.



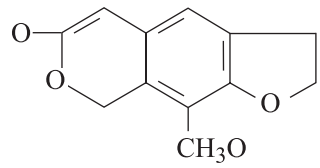
Gossypol

Capsidiol Diffusates from pepper (*Capsicum frutescens)* fruits inoculated with a number of non-pathogenic fungi accumulate a sesquiterpene phytoalexin capsidiol in concentrations that are sufficient to inhibit these fungi *in vitro* (Gordon *et al*., 1973).



Capsidiol

Xanthotoxin This phytoalexin was isolated from the top millimeter of parsnip root discs inoculated with *C. fimbriata*, a non-pathogen of parsnip (Johnson *et al*., 1973). Inoculation with other non-pathogens of parsnip also resulted into productions of Xanthotoxin.



Xanthotoxin

Camalexin is produced in *Arabidopsis* and Momilactone A phytoalexin has been reported in rice. However, it will be worthwhile quoting Hammerschmidt (1999): "Studies on phytoalexins have generated substantial useful information on secondary metabolism, gene regulation, and novel secondary metabolite chemistries. These results alone have provided valuable contribution to plant biochemistry and molecular biology. However the real question to be answered is whether phytoalexins are contributors to defense or are just the end product of pathogen disturbed metabolism that shunts carbon into compounds that are antimicrobial for serendipitous rather than defensive reasons."

Defense Through Induced Synthesis of Proteins and Enzymes

Molecular biology of pathogenesis and induced systemic resistance has been reviewed by Vidhyasekaran (1998). In addition to the phenolic substances produced in plant tissues in response to infection, induced synthesis of proteins and enzymes seems to play a role in disease resistance. In the case of the black rot of sweet potato caused by the fungus *Ceratocystis fimbriata*, sweet potato tissues inoculated with the pathogen and with a non-pathogen that induces immunity, showed marked changes in 9 of the 13 enzymes examined by Weber and Stahmann (1966). The influence of host–parasite interactions on proteins, enzymes, and resistance had been discussed by Stahmann (1967). Nucleic acid and protein changes in wheat leaf nuclei during rust infection were studied by Bhattacharya *et al*. (1965). There were decreases in histones and marked increases in RNA and acidic proteins as early as two days after inoculation in the nuclei of the mesophyll cells of susceptible wheat infected with *Puccinia graminis tritici*. Deese and Stahmann (1962a, 1962b) reported that pectic enzyme formation by *Fusarium* or *Verticillium* was greatly reduced when the pathogens were grown on tissues from resistant varieties as compared to that from susceptible varieties.

In numerous incompatible host–parasite combinations, peroxidase activity is often several times higher than in compatible ones (Fric and Fuchs, 1970). In other cases, however, peroxidase activity is greater in compatible reactions (Fric, 1969; Grzelinska, 1969; Wood and Barbara, 1971). It is, therefore, clear that the activation of peroxidase in the host and parasite complex need not always be accompanied by the incompatible reaction.

Plants defend themselves from pathogen infection through a variety of mechanisms that can be local or systemic, constitutive or inducible. In most cases, the extent to which these various systems affect disease progression is poorly understood, and is an area of extensive biochemical and genetic research (Kessman *et al*., 1994). One particular inducible systemic response, known as systemic acquired resistance (SAR) has become a subject of great importance and inquiry. SAR is a broad, physiological immunity that develops from infection with a necrogenic pathogen. In addition to this, certain natural and synthetic chemical compounds can trigger similar plant responses. The fresh, carefully controlled laboratory study of SAR was published in 1961 when Ross (1961a, 1961b) used tobacco mosaic virus (RMV) on a local lesion host (*Xanthi-nc* tobacco) and found that subsequent infections were reduced in

severity. Ross coined the term SAR for the resistance that developed in the distal, unheated portions of TMV-inoculated plants. Resistance that developed in the same leaf that had been TMV-inoculated, he called as local acquired resistance (LAR). Cruickshank and Mandryk (1960) showed high level of resistance in field grown tobacco against blue mold (*Peronospora tabacina*) when plants were injected in the stem with the spore suspension of the same fungus, Cohen and Kuc (1981) subsequently showed that resistance takes approximately 3 weeks to develop. Heat killed conidia and other pathogens of tobacco failed to induce SAR. Other workers have shown that inoculation of tobacco leaves with *Thielaviopsis basicola* leads to necrosis and subsequent systemic resistance to other pathogens, TMV, and tobacco necrosis virus (TNV). The SAR response in tobacco gives a broad spectrum disease resistance to fungal bacterial and viral pathogens but is ineffective against aphids and tobacco horn worm (*Manduca sexta*).

Some of the examples where SAR has been recorded are for cucumber, muskmelon and watermelon. Localized infections of cucumber with TNV, *Pseudomonas lachrymans or Colletotrichum lagenarium* lead to broad spectrum systemic resistance.

In monocotyledonous plants, there are well developed SAR systems established both in rice and wheat. In rice, Smith and Metraux (1991) reported that the inoculation of the first, fully developed leaf with *Pseudomonas syringae* pv. *syringae*, which causes a rapid hypersensitive necrosis results in systemic resistance against *Pyricularia grisea* (*P. oryzae*) the causal agent blast of rice. There are several other examples of SAR and LAR in various host–pathogen combinations.

Mechanism of SAR (Self Acquired Resistance)

In 1970, the groups of Van Loon (1970) and Gianinazzi (1970) showed that viral infection of tobacco induced the accumulation of a distinct set of problems called pathogenesis-related proteins (PR proteins).

PR-Proteins Pathogenesis related proteins often called PR proteins are generally a group of plant proteins that are toxic to invading fungal pathogens or other pathogens. Several groups of PR proteins have been classified according to their function, serological relationship, amino acid sequence, molecular weight, and certain other properties. PR proteins are either extremely acidophilic or extremely basic and therefore are highly soluble and reactive. Some better known PR proteins are PR1 proteins, β,1,3,glucanases, chitinases, lysozymes, PR4 proteins, thaumatin like proteins, osmotin like proteins, cysteine-rich proteins, glycine-rich proteins, proteinase inhibitors, proteinases, chitosanases, and peroxidases (Agrios, 1997).

Although healthy plants may contain trace amounts of several PR proteins; however, if it is attacked by plant pathogens, treatment with defense elicitors, wounding or stress induces transcription of a battery of genes that code for PR proteins. It has been shown that β-1,3 glucanase, and chitinase, diffuse towards and affect (the break down) the chitin supported structure cell walls of several but not all pathogenic fungi, whereas lysozyme degrade the glucosamine and muramic acid component of the cell wall of bacterial plant pathogens. In those plants which have been genetically engineered and in which chitinase gene has been introduced show good resistance against *Rhizoctonia solani.*

Some characteristics of systemic acquired resistance (SAR) are that:

1. It is induced by agents/pathogens causing necrosis, e.g., local lesions.
2. There is a delay of several days between induction and full expression.
3. Production is conferred on tissues not exposed to induced inoculation.
4. Is expressed as reduction in lesion number, size, spore production, etc.
5. Production is long lasting.

6. Production is non-specific.
7. Development of SAR is associated with expression of several gene families, e.g., PR proteins.
8. The signal for SAR is translocated and graft transmissible.
9. The protein is not passed on to next progeny; the transmission to clonal tissues is unresolved so far.

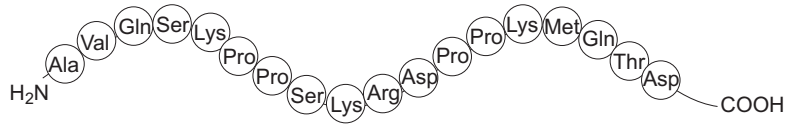
Most current interest in SAR concerns the possible identity of the translocable signal. It has been known for sometime that the signal is graft transmissible and presumably moves in the vascular system.

In addition to developing as a result of a primary infection, resistance can be induced by treatments of plants with such compounds such as viral coat proteins, bacterial or fungal proteins, lipoproteins, polysaccharides, and yeast RNA and by synthetic molecules mostly polyanions such as polyacrylic acid, salicylic acid (Aspirin) and 2-chloroethyl phosphonic acid, 2,6-dichloro-isonicotanic acid (CGA-41396) and its methylated ester (CGA-41397) both referred to as INA. In general, greater the concentration of the inducer, faster and more effective the induced resistance is seen in the host.

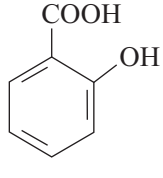
Localized induced resistance in inoculated (treated) leaves develops 2–3 days after primary infection (treatment) while systemic induced resistance, if present, usually develops 7 or more days after the primary infections and may persist for 3–5 weeks. It is interesting to note that induced resistance may be prevented if the plants are treated with Actinomycin D. Actinomycin D prevents transcriptions of the cell DNA to messenger RNA and therefore prevents productions of new proteins (enzymes).

Further evidence shows that salicylic acid, which plays an important part in the signal pathway, has come from the work on *Arabidopsis* mutants altered in SAR expression, and transgenic plants engineered to break down salicylic acid.

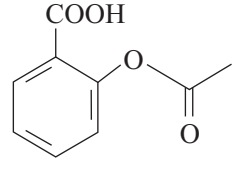
The signal molecules implicated in the induction of plant defense are given as follows: these are salicylic acid, Acetyl salicylic acid, Dichloro-iso-nicotinic acid Ethylene, xylanase, the polypeptide systemin, jasmonic acid and probably others.



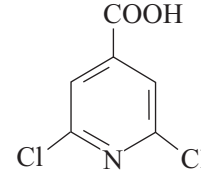
Systemin



Salicylic acid



Acetyl salicylic acid



DCINA
(Dichloro-iso-nicotinic acid)

A number of reports have claimed resistance inducing activity for certain chemicals or extracts, but have failed to demonstrate that the compound in question fulfills the criteria previously established for SAR activators.

Fosetyl-Al, Metalaxyl (Ward, 1984), and more recently triazoles are among these compounds (Hauthal, 1993). Fosetyl-Al shows very weak antifungal activity in traditional *in vitro* tests. Plants pretreated with these compounds accumulate phytoalexins faster, and the effectiveness of metabolic inhibitors like glyphosate decreases. These experiments led to speculations that Fosetyl-Al was a resistance inducing compound.

There are several other compounds which elicite the defense mechanism of plants such as Probenazole, a systemic compound used successfully to protect rice against *Pyricularia grisea (P. oryzae*) and weakly against *Xanthomonas oryzae* pv. *oryzae*.

An astonishing case of induced resistance was reported by Arimoto *et al*. (1991) in which they claimed that rice plants can be protected for generations when the seeds are soaked in 500 ppm solution of DL-alanine dodecylester HCl. However no further information of this has come from other quarters.

Cohen and co-workers (1993) reported that jasmonic acid and its methyl ester induces resistance in tomato towards *Phytophthora infestans.*

Defense Through Formation of Substrates Resisting the Enzymes of Pathogens

The resistance of green immature apple fruit to several fungi which can infect mature fruit has been attributed to the presence in the green fruit of a pectin-protein-polyvalent cation complex which is resistant to hydrolysis by fungal hydrolytic enzymes and suppresses the production of pectin methyl esterase and polygalacturonase by the fungi.

An induced mechanism of resistance through increased resistance of the substrate to enzymes by the accumulation of polyvalent cations has been reported for *Rhizoctonia* infected bean tissues. In the resistant variety, calcium ions accumulate in and immediately around developing *Rhizoctonia* lesions, inhibit further tissue maceration by polygalacturonase, and therefore, play a role in confining the pathogen to lesions of a limited size. Fungal PME demethylate pectic substances near and in advance of the pathogen and result in the formation of pectate salts with polyvalent cations, particularly calcium, which in turn are resistant to hydrolysis by fungal polygalacturonase.

Disease resistance introduced through artificial application of auxins to infected plants has been reported for *Fusarium* wilt of tomato and late blight of potato. The resistance to disease in auxin-treated plants is accompanied by higher levels of insoluble pectates and lower levels of water soluble pectins that are found in untreated, susceptible plants.

The resistance of cider apples to wound infection by the brown rot fungus, *Sclerotinia fructigena*, seems to be due to the ability of phenolic oxidation products in these apples to inactivate extracellular pectinolytic enzymes produced by the fungus. The phenolics produced in the juice of the resistant variety do not inhibit the growth of the fungus, but do inhibit the activity of its polygalacturonase.

Defense Through Detoxification of Pathogen Toxin

Detoxification may operate in resistance of vascular wilt diseases caused by *Fusarium oxysporum*. Its significance depends on the importance properly ascribed to toxins in these diseases. Lakshminarayanan (1955) reported that varieties of cotton resistant to *F. oxysporum* f. *vasinfectum* contain relatively high concentrations of cystine and that cystine reduces the toxicity of the complex formed when ferric iron is added to filtrates from the cultures of the parasite. When shoots are allowed to absorb cystine from solutions before the complex is transferred to the former, they are less damaged than are plants not pretreated with cystine.

In the case of the toxin, pyricularin, Tamari (1959) found that chlorogenic acid could detoxify the pyricularin when applied to rice seedlings in equimolar concentrations. Tamari *et al*. (1962) also showed that pyricularin was detoxified with ferulic acid, which was oxidized by peroxidase, and its oxidized products were also shown to detoxify the pyricularin. Those varieties of rice which have more chlorogenic or ferulic acid are more resistant to the blast disease.

The partially purified toxin produced by *Alternaria kikuchiana* is inhibited by various kinds of oxidizing substances, including dehydroascorbic acid (Mori, 1962). Mori (1962) showed that the resistant varieties of pear contain more dehydroascorbic acid and more ascorbic acid oxidase than the susceptible varieties.

Defense Through Altered Respiration

The relation of increased respiration to disease resistance and susceptibility is unclear. In some incompatible host pathogen combinations (resistance), the increase is initially greater than in compatible combinations (susceptibility), although the increase may be of greater total magnitude and longer in duration in susceptible reactions (Uritani, 1962).

From observations, however, indicating that substances (narcotics) inhibiting respiration also decrease resistance to disease in the host, it is suggested that increased respiration is often a necessary condition for the development of defense reaction in the host. A marked increase in oxygen uptake has been reported for the hypersensitive defense reactions. This is also accompanied by the accumulation and oxidation of phenolic compounds and by the activation of phenoloxidases.

Defense Through Hypersensitive Reaction

Hypersensitivity reaction or hypersensitivity functions as a defense reaction in plants against pathogens. It occurs only in incompatible host–parasite combinations. This is a significant and common form of reaction in plants. Usually, a small part of the plant tissue quickly dies after infection, resulting in a minor or local lesion, or one as extensive as to cause the death of the plant. Hypersensitive reaction is believed to be caused by the disturbance of the balance between the oxidative and reductive processes in the cells resulting in excessive oxidation of phenolic substances and leading to the breakdown of cellular structures (Fig. 6.8)

The hypersensitive response is the culmination of the plant defense response, indicated by the recognition by the plant of specific pathogen produced signal molecules (elicitors). Recognition of the

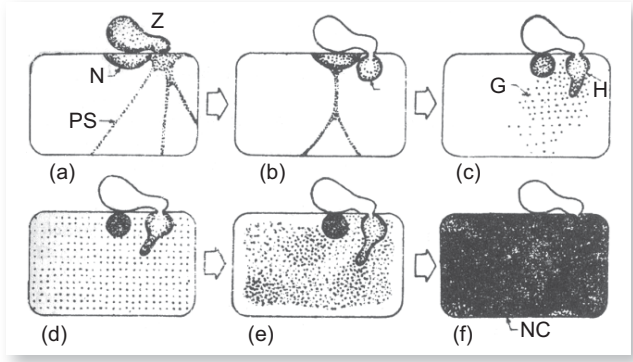


Figure 6.8 Different stages in the development of necrotic defense reaction (hypersensitivity) in a cell of a very resistant potato variety infected by *Phytophthora infestans*. N = nucleus, Z = zoospore, PS = protoplasmic strands, H = hypha, G = granular material, NC = necrotic cell. (Redrawn from K. Tomiyama [1956], *Ann. Phytopath. Soc.*, Japan, **21**: 54–62).

elicitors by the invaded host plant elicited a cascade of biochemical reactions in the plant attacked, and surrounding cells lead to altered cell function and new or greatly activated defense-related compounds.

The Concept of Phytoncides

In 1930, at the All-Union Conference of Zoologist in Kiev, USSR, Tokin reported the results of his preliminary observation on the antibiotic properties of plants. Counting this work, he published a monograph in 1942 on antibacterial substances formed by plants, which he called phytoncides. This term was later extended by other Russian worker to include the substances of plant origin active against other organisms, both animals and plants. Phytoncides, by definition, include phytoalexins, and pre-formed and post-infectionally formed substances which give resistance to plant pathogens, in addition to those which deter or destroy insect pests.

Rubin and Artsikovshaya (1963) discussed this aspect of phytoncides in detail. It is being felt by plant pathologists that the term is too all-embracing to be of much use and the individual categories of chemical substances known so far are adequately covered by more specific terms.

Defense Through Genes

It must be clear that whatever the kind of defense or resistance a host plant shows against infection, or against abiotic factors, it is finally controlled by genes or genetic material of the host plant and of pathogen.

It is easy for the plant to defend itself when it comes in contact with a pathogen to which the plant is not a host and it is called as non-host resistance. For example, apple trees are not infected by the pathogens of wheat, tomato, or others because genetic constitution of apple is different for such infection to the pathogens of wheat and tomato. All such unsuccessful infection is due to non-host resistance. It has been seen in some related pairing, e.g., when powdery mildew of wheat caused by *Blumeria graminis* f. sp. *tritici* is inoculated on barley, the fungus produces haustoria as a result of infection, the host plant in

response to infection produces Hydrogen Peroxide, and as a result of hypersensitive response, the cells die and further infection is controlled. This is due to again non-host resistance.

When a plant is attacked by its own pathogen, it may develop no disease, only mild disease or severe disease, depending on the genetic makeup (genes) of the plant and of the pathogen that attacks it. This difference of disease severity is mainly due to the large number of genes being involved in keeping a plant protected from pathogenic attack. Some of the genes provide protection to plant for general keep up and normal functioning of the plant. Some of the latter plant genes code for those chemicals that are toxic to pathogen or neutralize the toxin. Plant in addition to this has genes that slow down or inhibit the growth of the pathogen that cause disease. These structures can be present in a plant naturally or may be produced as result of host–pathogen interaction or by abiotic agent are important in the defense mechanism. When a pathogen attacks a host plant, the genes of the pathogen are activated and release all their weapons at their command, i.e., toxins and enzymes against the plant and infect it. As plants have some pre-existing chemical substances, plants defend themselves either partially or completely. Some of the plants that survive and produce yield, and this type of defense are known as polygenic. This type of resistance which is present at different levels against pathogens in plants is called as partial, horizontal, multigenic, or minor gene resistance. In some polygenic plant–pathogen combination like early blight of tomato caused by *Alternaria solani*, the resistant varieties accumulate more rapidly pathogen-induced pathogenesis related (PR) proteins than in susceptible varieties. These PR proteins include some antifungal isozymes of chitinase and β-1,3-glucanase. In resistant varieties, total enzymes able to release elicitors of the hypersensitive response (HR) whereas enzymes of susceptible varieties do not. This suggests that production of hydrolytic enzymes may act as a mechanism of elicitor release in tomato resistance to the early blight.

In many plant-pathogenic combinations like downy mildews, powdery mildews, many bacteria, nematodes and viruses, defense of a host plant against pathogens is governed by pair of genes for the disease in the host plant and the pathogen. The host plant carries one or few resistance genes (R) per pathogen while each pathogen carries matching genes of avirulence (A) for each of the R genes of the host plant. These avirulence genes of the pathogen trigger the host R genes, this in turn starts the defense mechanism of host plants that finally neutralize and eliminate the specific pathogen. During this process only a few cells die. This type of resistance or defense is called as race-specific, major gene, R gene or Vertical resistance or monogenic.

Lack of Recognition Between Host and Pathogen

How does a pathogen recognize that the plant with which it comes in contact is a host or non host? Plants may not become infected by a pathogen if their surface cells lack specific recognition factor that can recognized by the pathogen. If the pathogen does not recognize its host, it may not become attached to the host plant because the pathogen fails to produce certain enzymes or other structures like appressoria, penetration peg, etc., which are necessary for infection. For example, *Puccinia graminis tritici* will infect wheat plant and not tomato which is not a host for the pathogen. The exact mechanism that is involved in the recognition of host and pathogen is not known but it is thought that various types of oligosaccharides, polysaccharides and protein or glycoproteins are involved.

Defense Through Lack of Host Receptors and Sensitive Sites for Toxins

In host–pathogen interaction, the pathogen produces a host-specific toxin which is responsible for the symptoms is thought to attach and react with the specific/sensitive sites in the cell. Only sensitive sites become diseased. Plants that lack such receptor or sensitive sites remain free from infection as host-specific toxins are not produced which are responsible for infection.

Defense Through Recognition of Pathogen by the Host Plant

Early recognition of the pathogen by the host will enable the plant to mobilize the biochemical defense mechanism. Various pathogens release a variety of substances like toxins, glycoproteins, enzymes (proteases and pectic) after infection that act as non-specific elicitors of pathogen recognition by the host. In various host–pathogen interactions, certain substances secreted by the pathogen like, *avr* gene product, *hrp* gene product, and suppressor molecule act as specific pathogen elicitors. In some case either the host enzymes break down a portion of polysaccharides of pathogen or pathogen enzymes break down plants' polysaccharides; the released oligomers or monomers of the polysaccharides act as recognition elicitors for the plant.

The locations of host receptors that recognise pathogen elicitors are present outside or on the cell membrane or present intracellularly. In powdery mildew of cereals, a soluble carbohydrate that act as an elicitor from the wheat powdery mildew fungus *Blumeria graminis* f. sp. *tritici* is recognised by a broad range of cereals in which it induces the expression of all defense related genes tested and also induces resistance to subsequent attacks with the fungus. This elicitor alone, in absence of the powdery mildew fungus *B. graminis* did not induce a hypersensitive response but it did induce on accumulation of thaumatin like proteins in the various cereals.

Once a plant molecule recognises and reacts with the molecule (elicitor) released by the pathogen, that means the plant has recognised the pathogen. After that, a series of biochemical and morphological changes occur in the plant cell(s) to ward off its pathogen and its enzymes and toxins, etc. How quickly the plant recognises the presence of a pathogen and how quickly the host can send message(s), and mobilize its defense mechanism, or how much the pathogen will develop and show symptoms before the host defense finally stops further development of the pathogen depends upon resistance or susceptibility of the host plant. In resistant plant, it will mobilize its defense mechanism very quickly in comparison to susceptible plant.

Defense Through Transmission of the Alarm Signal to Host Defense System

Once the pathogen elicitors are recognised by the host plant, a series of alarm signals are sent to the host cell proteins and to nuclear genes, causing them to become activated, to produce inhibitory substances towards the point of cell attack by the pathogen. These alarm substances and signal transductions are only intracellular but in some cases either it is also transmitted to several adjacent cells or transmitted systemically to the plant. In intracellular signal transduction, several types of molecules such as protein kinases, calcium ions, ATPases, Hydrogen Peroxide, and ethylene and in systemic signal transduction salicylic acid, jasmonic acid, fatty acids, etc., have been reported. Such natural or synthetic chemicals also activate those signaling pathways that lead to systemic acquired resistance against many pathogens.

Induced Biochemical Defense in Hypersensitive Response (Race-Specific, Monogenic, R-Gene Or Vertical Resistance)

(a) The Hypersensitive Response (HR)

The HR is a localized induced cell defense, which is the result of quick mobilization of the defense responses by the affected and adjacent cells and the subsequent release of toxic substances that kill the host tissue and the pathogen. This phenomenon is at times not seen in resistant plants because single or few cells are involved. However, *in vitro* (artificial condition) the inoculation of virulent strain of plant pathogen into the tissue of non host plants or when avirulent strains are injected into susceptible plant results in the development of hypersensitive response. The hypersensitive response occurs in the plant in response to the pathogen produced signal molecules called elicitors. During the process of hypersensitive response, there is increase of oxidative reactions, increased K^+ and H^+ ion movement, disruption of membrane, and loss of cellular compartmentalization, production of antimicrobial substances like phenolics, pathogenesis related proteins such as chitinase, etc.

The hypersensitive response occurs where the host and the pathogen are incompatible. It occurs due to the presence of resistance gene (R) in the host plant which triggered the release of molecules of the pathogen which ultimately triggers the development of resistance in the host plant that makes the pathogen avirulent is called as avirulent gene. For example, in case of *Pseudomonas syringae* pv. g*lycinea* infecting soybean, the avirulent gene (*avr.* D) was a protein molecule. The avirulent gene synthesised a substance called syringolides which has the ability to elicit the hypersensitive response in soybean that carry the resistance gene D complementary to *avr* D of the bacterium.

More than 20 resistance (R) genes have been isolated from plants like corn, tomato, tobacco and *Arabidopsis.* For example in corn R gene (Hm1) for northern leaf spot caused by *Cochliobolus carbonum* codes for an enzyme that detoxifies the HC toxin whereas the tomato gene Pto gives resistance to tomato bacterium, *Pseudomonas syringae* pv. *tomato*, as this gene Pto codes for a protein kinase enzyme that inactivates the bacterium. The functions of the proteins encoded by R gene are not known but most of them contain domains, such as leucine-rich repeats, found in proteins involved in protein-protein interactions.

These proteins coded by R-gene are appeared to be present in the plant cell cytoplasm or plant cell membrane and, therefore, recognise pathogen elicitors that reach the cytoplasm or plant cell membrane.

Genes Induced During Early Infection With the help of recent plant molecular biology techniques (cDNA library, DNA sequencing, and DNA microarrays), it is now possible to detect and identify plant genes and secondly those genes that are induced during compatible or incompatible interaction between host and pathogens. For example, in case of a compatible interaction between *Phytophthora infestans* and potato, about 43 genes appeared to be induced, 10 of which showed increased activity as a result of infection. Some of them were homologous to genes already known to be activated during infection, e.g., for β-1,3 glucanase, some have homology to enzymes involved in detoxification and some code for proteins that had not been reported earlier to be induced during infection. In another example of incompatible interaction between *Arabidopsis* with *Alternaria brassicicola,* or after treatment with defense signaling compounds such as salicylic acid, methyl jasmonate or ethylene and about 2400 genes were involved. More than 700 genes showed transcriptional changes in response to one or more treatment. Treatment with salicylic acid and methyl jasmonate activated around 192 and 221 genes but they also

repressed the transcription of 131 and 96 genes respectively. These activated genes were involved in antimicrobial defense, phytoalexin production and defense signal transduction. So this clearly shows that in incompatible host fungal interaction, the activated genes account for resistance.

Functional Analysis of Plant Defense Genes Involvement of large number of genes for a particular physiological rate in host such as inoculation with a pathogen or with some treatment implies the involvement of some genes in that physiological rate. For this, the study of the function of each gene is carried out individually which is very time consuming because many genes are responsible for the same function. The identification of transcription factors and their binding sites in the promoter regions of defense-related genes is also critical for understanding how defense gene expression is controlled. It is now possible to study and identify novel regulatory elements in the promoter region of co regulated genes with bioinformatics.

Classes of R Gene Proteins The various plant R genes irrespective of the type of pathogen to which they provide resistance have many structural similarities. These R genes exist as clustered gene families. Based on structure and function, R genes have been divided into 6 classes:

Classes	Functions	Examples of R Gene
1.	Membrane-associated, transcription regulating, mediated broad spectrum resistance	PRW8
2.	Cytoplasmic signal transducing serine-threonine protein kinase	*Pto*
3.	Extracellular Leucine Rich Repeats (LRR) transmembrane anchor	Cf-2- Cf-9
4.	Extracellular LRRs, with a transmembrane receptor and cytoplasmic serine-threonine kinase	*Xa*21
5.	Cytoplasmic, membrane associated. Contain LRRs, NBS and TIR domains	RPP5, N^1, L^6
6.	Also cytoplasmic membrane associated. Contain LRRs, NBS, and coiled coil domain	RPM1, RPS2

The mechanisms by which R genes bring out disease resistance to a plant pathogen are not properly understood but it is believed that the elicitor molecule produced by the avr gene of the pathogen is recognised by a specific plant receptor encoded by an R gene. Following recognition of the elicitor by the receptor molecule, one or more kinase enzymes may become activated, which then amplify the signal by phosphorylating and thereby energizing other kinase enzymes. This leads to a chain of biochemical reaction result in the hypersensitive response and thereby localized host resistance at the point of attack by the pathogens. In many cases, the hypersensitive response is followed by the development of various levels of systemic acquired resistance (SAR), which is expressed in the vicinity of the attack as well as in distant parts of the plants.

Example of the Plant Defense Through R Genes and Their Matching Avr Genes For example, the Pto R gene of tomato, which provides resistance to the bacterium, *Pseudomonas syringae* pv. *tomato*, codes for a cytoplasmic protein kinase that directly interact with the bacterial avr Pto protein that is released by the bacterium into the host cytoplasm. The Pto kinase protein can interact with other proteins including kinase and some of that have homology to transcription factors. These transcription factors possess a DNA-binding domain that recognises a sequence present in the promoters of genes

that encode ethylene-induced defense related protein such as PR proteins. For example, when one of the transcription factor genes is over expressed in a Pto R gene plant, the avr Pto- mediated hypersensitive response is increased, which shows that the Pto protein can activate several distinct signaling pathways simultaneously. It has been observed that expression of Pto requires the presence and expression of another gene, *Prf* which is present within the pathogen cluster. Recent study shows that Pto is not the true R-gene but encodes the virulence target of Avr Pto. The Avr Pto-Pto complex is then recognised by the true R-protein of the *Prf* gene which is "guarding" the virulence target.

In other example of fungal avr proteins, when rice blast fungus (*Magnaporthe grisea*) carries the avirulence genes avr-Pi-ta are effective on rice cultivars carrying the resistance gene Pi-ta. Now Pi-ta encodes a cytoplasmic protein that contains NBS domain and a leucine-rich carboxyl terminus. Direct interaction has been seen between the Avr- Pi-ta protein and the leucine rich domain of Pi-ta. This is the first experimental proof that an AVR protein interacts directly with its R protein.

Defense Involving Bacterial Type III-Effector Proteins

Most pathogenic bacteria have three different types of secretion by which they secrete exoenzymes and other pathogenicity factors.

Type I Secretion System

It allows bacteria to secrete proteases and plays little role in pathogenicity.

Type II Secretion System

It makes it possible for bacteria to secrete pathogenicity determinants like pectinases and cellulases which are essential for pathogenicity.

Type III Secretion System (TTSS)

It consists of 15–20 proteins attached with the bacterial cell membrane which ultimately form secretion system/apparatus. This secretion system translocates host specific "effector proteins" from the bacteria into the host cell. By delivering these bacterial "effector proteins", the TTSS interferes with host cell signal transduction and other cellular processes thereby increasing the virulence of the bacteria in susceptible host plant cells. During delivery, a chaperone protein gets attached to "effector protein" that protects the effector protein from any premature interaction with other proteins. The type III secretion system occurs in all or most gram negative pathogenic bacteria like *Erwinia, Pseudomonas, Xanthomonas, Ralstonia* including those causing disease in animals and human beings.

But when these "effector proteins" were translocated in non host plants, these secretions elicit a hypersensitive response in host plant. That is why, TTSS is known as the hypersensitive response and pathogenicity (hrp) system. These type III effectors were identified as the product of typical avirulence (*avr*) genes. The induction of hypersensitive response (HR) depends on the presence and reaction of R-genes and matching pathogenicity (*hrp*) genes and it was later found that the product of *hrp* genes are secreted by the TTSS into the host cytoplasm containing R-gene and lead to the induction of a hypersensitive response, i.e., death of cells. As the secretion of "effectors" is essential for pathogenicity, so the bacteria secrete multiple effector proteins and therefore they contribute to virulence in an incremental or

partially redundant manner. *Pseudomonas* and *Xanthomonas* infect and colonize the intercellular spaces (apoplast) of leaf surfaces where nutrients are in abundance but water is a limiting factor. The bacteria would benefit from a susceptibility response involving leakage of water from the host cell (symplast) to the intercellular space (apoplast). However, the plant would benefit from a defense response such as thickening of the cell wall that would deprive the infection site from water.

Harpin protein, which is different from avr protein is produced by the bacterium *Pseudomonas syringae* pv. *syringae* when injected in the leaf apoplast can also induce hypersensitive-like response, i.e., cell death. This implies that harpin (hrp) might function on the outside of the plant cell. The harpin HR like response differs from HRs induced response by *Avr* genes in that it does not depend on a matching R gene. Harpin protein associate with liposomes and with bilayer plasma membrane where it forms pores, through these pores nutrients and water move out of the cell to the apoplast and these pores act as opening so that other types of "effectors" can be translocated into the cells.

Active Oxygen Species, Lipoxygenases and Disruption of Cell Membrane

The plant cell membrane consists of a phospholipid bilayer where different types of proteins and glycoprotein molecules are present. These protein molecules are arranged in such a manner on the membrane that it forms channels on the membrane and allow ions and metabolites to enter and move out of the cells. In the cell, endoplasmic reticulum and organelles compartmentalizes the cell in which specific compounds are separated and certain biochemical reaction occurs. The cell membrane also serves as the anchor of R-gene coded proteins that identify the elicitors released by the pathogen and subsequently trigger the defense mechanism especially hypersensitive response.

As a result of infection by pathogen or exposure to pathogens toxins and enzymes, some structural and permeability changes occurs on the cell membrane and some of the changes play a role in defense mechanism against the pathogen. These changes are generally thought to be an expression of susceptibility and disease development. The important membrane associated defense responses include:

1. Release of substances involved in signal transduction within and around the cell.
2. Release and accumulation of lipoxygenases and active oxygen species.
3. Loss of compartmentalization and release or activation of Phenol oxidases and polyphenol oxidases.

In many host–pathogen interaction or cells treated with fungal elicitors, there is rapid accumulation of activated oxygen species including superoxide, hydrogen peroxidase and hydrogen radical as a result of defense response. These reactive oxygen species are released by the multisubunit NADPH oxidase enzyme complex of the host cell plasma membrane with seconds or minutes from contact of the cell with fungus and reach a maximum activity within minutes to few hours. This activated oxygen species trigger the hydroperoxidation of plasma membrane phospholipids thereby producing lipid hydroperoxides which are toxic and impairs the plant cell membrane synthesis and involved in hypersensitive response induced cell collapse and even death. Active oxygen species through the process of oxidation of phenolic compounds produce more toxic quinones and lignin like compound. The production of reactive oxygen species in affected cell is controlled by several enzymes like catalase, superoxide dismutase and ascorbate peroxidases etc.

The oxygenation of plasma membrane lipids are involved in the production of lipoxygenases enzymes that catalyse the hydroperoxidation of unsaturated fatty acids such as linoleic acid and linolenic acid which are converted by the cell into several active molecules such as jasmonic acid that play an

important role in defense mechanism. For example, jasmonic acid which is the precursor of the wound hormone traumatin appears to induce many proteins changes and act as a signal transduction of the defense mechanism in plant–pathogen interaction.

Reinforcement of Host Cell Walls with Strengthening Molecules

The host cell wall also accounts for resistance as the cell wall of host that come in contact with fungus produce, modify or accumulate several substances that reinforce the resistance. Some of the substances produced or deposited in plant cell walls after infection are callose, glycoproteins (which are rich in amino acid hydroxyproline), phenolic compounds, lignin, suberin, silicon, and calcium. These substances form complex polymers thereby forming insoluble substances that restrict the invading fungus and inhibit the further development of disease. These substances are produced in more quantity and quickly in resistant plants while in susceptible plants produce them but too slowly to be effective.

Production of Antimicrobial Substances (Pathogenesis Related Proteins) in Infected Host Cell

Pathogenesis related proteins (PR proteins) are plant proteins that are toxic to invading fungal pathogen. They are generally present in small amount in plant intercellular and intracellular spaces but are produced in much greater concentration following pathogen attack or stress. Different types of PR proteins have been isolated from different plant organs.

Around 14 families of PR proteins have been classified according to their function, amino acid sequence, molecular weight, and serological relationship. These PR proteins are either acidic or basic in nature and are highly soluble. The better known PR proteins are PR1 proteins (anti-oomycetes and antifungal), PR2 (β-1,3 glucanases), PR3 (chitinases), PR4 (antifungal), PR6 (proteinase inhibitors) and so on. Some of the PR proteins inhibit spore germination as they are antifungal and antimicrobial in activity whereas some are associated with the strengthening of the host cell. For example, β-1,3 glucanase and chitinase diffuse towards and break down the chitin supported cell wall of fungi (except oomycetes) whereas lysozymes break down the glucosamine and muramic acid components of bacterial cell wall. Plants genetically engineered to express chitinase genes show resistance against soil borne fungus *Rhizoctonia solani*. The signal compounds like salicylic acid, ethylene, and jasmonic acid responsible for induction of PR protein like lipoxygenases and lipid peroxidases seem to be transported systematically to other parts of the plant and reduce disease intensity.

Defense Through Genetically Engineered Disease-Resistant Plants

A large number of resistance genes (R) have been isolated. In 1992, the first plant gene for resistance to be isolated was the Hml gene, which codes for an enzyme that inactivates the HC toxin produced by the leaf spot fungus *Cochliobolus carbonum*. The resistance genes are triggered into action by the corresponding avirulence genes of the pathogen, the product of which serves as signals that elicit the hypersensitive response in the host plant. In addition to these specific genes, several other plant genes encoding PR proteins and enzymes have been shown to impact resistance to transgenic plants. For

example, tobacco plants transformed with a chitinase gene from bean became resistant to infection by the soil-borne fungus, *Rhizoctonia solani*, but not to infection by *Pythium aphanidermatum* (oomycetous fungus) because cell wall of which lacks chitin. Similarly, transgenic tobacco plants expressing a PR1 protein gene were found to be resistant to *Peronospora tabacina* and plants expressing the SAR (systemic acquired resistance) genes SAR8.2 were also resistant to *Phytophthora parasitica*.

A mixture of PR proteins are more effective as antimicrobial than each of them tested separately, as it was shown that transgenic tobacco plant expressing both the chitinase and β-1,3 glucanase were more resistant to the fungi *Cercospora nicotianae* and *R.solani* as was tomato to *Fusarium oxysporum* f. sp. *lycopersici* than plants expressing their genes independently.

Resistance or immunity of a plant to viral attack can be seen when plant genes that govern the external or internal receptor to which the virus binds may be modified, which may result in an inability of virus to bind and replicate in the plant cell. So the induction of resistance in potato and tobacco transgenic plants with a mouse gene coding for an enzyme involved in the synthesis of an interferon like compound and with a mouse gene coding for an antibody called plantibody against the coat protein of a plant virus (artichoke mottled crinkle virus).

Engineering plants with constitutive genes that trigger or increase the accumulation of pathogenesis-related proteins, with genes such as stilbene synthase is an additional mechanism of enhancing the resistance of plant. This enzyme increases the production of certain phytoalexins that subsequently reduce infection of tobacco by *Botrytis cinerea* by 50% or engineering plants with less active genes that reduce the level of calmodulin and of activity of catalase, thereby increasing the production of active oxygen species and expression of PR proteins which both are toxic to the pathogen. Other types of plant genes engineered into plants for disease resistance include defensin genes that inhibit plant attacks by fungi and lectin genes which prevent plant infection by nematodes. It has also been seen that a particular gene that confer resistance in one host is isolated and transferred to another plant is not able to confer resistance to new plant.

Defense with Pathogen-Derived Genes

In 1986, it was reported for the first time that tobacco plants genetically engineered to express the coat proteins gene of TMV showed resistance to the same virus. Once the TMV coat protein genes was integrated in the tobacco genome it was carried to next generation and behaved like any other tobacco gene. Since then many other solanaceous crops, cereals, cucurbits and several other plants have been transformed with the coat protein gene of one or more of the viruses that infect them. In some cases, the transgenic plants were resistant to virus if they were inoculated mechanically or by their aphid or fungus vector but not if inoculated by the specific vector of the virus. Genetically Engineered plants transformed with viral genes other than the protein coat gene often exhibit higher levels of resistance to the virus. For example, highly resistant tobacco plants have been produced by transformation with modified virus replicase-coding genes of several viruses. Resistance to non-viral pathogens has also been increased through the engineering of plants with appropriate genes from pathogenic or non pathogenic fungi. For example, potato plants engineered to express the Hydrogen peroxide generating glucose oxidase gene from the fungus *Aspergillus niger* continually produce high levels of peroxide ions, thereby increasing the resistance to the potato plants to *Phytophthora infestans, Alternaria solani* and *Verticillium dahliae* .Similarly, potato and apple plants transformed with the chitinase gene obtained from the

fungus *Trichoderma harzianum* which is a biocontrol agent against many plant pathogenic fungi. The wall of fungus is hydrolysed with its chitinases and showed resistance to potato early blight (*Alternaria solani*) and to potato gray mould fungus (*Botrytis cinerea*) whereas the apple trees showed resistance to the apple scab fungus *Venturia inaequalis*.

Defense Through RNA Silencing by Pathogen Derived Genes

RNA Silencing (Post transcription gene silencing) is due to a process that occurs after transcription of RNA and it involves target mRNA degradation. It is a type of gene regulation that occurs in plants and serves as antiviral defense. RNA Silencing occurs in broad range of Eukaryotic organisms, including plants, fungi and animals. Plant uses RNA Silencing to defend themselves against viruses, which in turn encode proteins by which they attempt to suppress the silencing of their RNA. RNA Silencing was first observed in transgenic plants transformed with viral genes providing "Pathogen derived resistance". Vance and Vaucheret (2001) have discussed "RNA Silencing in Plants: Defense and Counter Defense".

RNA Silencing has an important role in defending cells against parasitic genes—viruses and transposons—but also in directing development as well as gene expression in general. RNA Silencing is found in many eukaryotes including animals and is initiated by the enzyme "Dicer", which cleaves long double-stranded RNA (dsRNA) molecules into short fragments of ~20 nucleotides that are called siRNAs. Each siRNA is unwound into two single-stranded (ss) ssRNAs, and then RNA Induced Silencing Complex (RISC) bound with single stranded RNA and stops the transcription. Then perfectly matching mRNA segments are cleaved into small mRNA segments too small to code for proteins and mismatched mRNA segments stick to RISC and cannot be read by ribosomes, hence no proteins can be coded.

RNA Silencing produces strong virus resistance in transgenic plants. These plants have neither accumulation of virus in their inoculated plants nor this resistance be overcome by high amount of inocula. Once RNA Silencing is established in transgenic plants, all RNAs homologues of the transgene as well as of infected viruses are degraded. If RNA Silencing is triggered locally it will spread throughout the plant via mobile silencing signal. Generally movement of RNA Silencing signal in plant is parallel with that of viruses, moving at first from cell to cell then moving into phloem and from there spreading out to parenchyma cells. When the viruses infect the RNA Silencing plant, there is a race between the two, if viruses moves faster than infection will occur or if due to resistance by the plant, i.e., lack of infection, RNA Silencing becomes established first and then degrading of viruses will occur. Virus induced gene silencing (VIGS) is mild, transient, and restricted to the veins. RNA Silencing has not yet been reported to occur in plant DNA viruses.

Suppressors of RNA Silencing

After the discovery of RNA Silencing, it was discovered that many plant viruses encode proteins that suppress mRNA silencing. Helper component—proteinase of potyviruses is so effective in suppressing viral RNA Silencing. Another suppressor, the potato virus X p25 protein, is much less effective in suppressing RNA Silencing and is apparently targets and interferes with Systemic silencing. In addition to these virus-encoded proteins, RNA Silencing can be suppressed by certain host genes. Some of these are expressed in transgenic plants and in transgenic plants carrying the potyvirus suppressor protein.

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Questions

1. The cuticle acts as a physical or chemical barrier to infection. Comment.
2. What are the different morphological defense mechanisms in plants? Give suitable examples.
3. What is biochemical defense mechanism?
4. Explain why in onion smudge caused by *Colletotrichum circinans*, varieties with red scales are resistant in contrast to varieties with white scales.
5. Give an example of a soil borne disease in which root exudates are implicated in the protective mechanism.
6. What is the role of phenolic substances in the resistance mechanism in plants?
7. Give an example of a host pathogen combination where:
   (a) Absence of nutrients brings about resistance to disease.
   (b) Resistance is attributable to *p*H.
   (c) Resistance is attributable to osmotic pressure.
8. Is the defense mechanism in plants akin to that in animals?
9. Who suggested the theory of gene-for-gene responsible for host specificity?
10. What are phytoalexins? Are phytoalexins responsible for host specificity?
11. What is hypersensitivity? How does it bring about resistance to pathogens?
12. How does altered respiration bring about resistance?
13. Give a summary of the potential ways in which metabolic pathways altered by infection play a role in disease and pathogen development.
14. Wheat is the concept of phytoncides? Who evolved this concept? Are phytoalexins phytoncides?
15. What is induced resistance? What is its possible mechanism and what are some of the induced chemical signals?
16. What are PR- proteins? What is their role in resistance mechanism?
17. Discuss in brief the defense in plant against infection through genetically engineered plants.
18. What is RNA silencing? How this has been utilized in bringing resistance in plants against infection?

Genetics of Plant Pathogen Interaction

7

Genetics of Host–Parasite Interaction

Each host–parasite interaction is a struggle for survival between two organisms. In nature, there exists a state of balance and surviving plants and their parasites/pathogens are capable of coexistence. The attack and defense mechanisms discussed earlier are determined genetically in the pathogen, some of which have different pathogenic abilities and may be able to attack hitherto resistant plants. In both organisms, the more effective races and varieties tend to survive; the less effective ones tend to disappear. Effective disease develops when a virulent race meets a susceptible variety but not when the pathogen is avirulent or the plant is resistant. The genetics of the host–parasite interaction have been discussed by Day (1974) and Ellingboe (1976).

The molecular genetics of pathogenicity of phytopathogenic bacteria was reviewed by Daniels *et al*. (1980). Advances in molecular genetics of plant–microbe interaction have been elucidated in an edited volume by Hennecke and Verma (1991). Genetics of plant–pathogen interaction specifying plant disease resistance and the concept of plant disease resistance proteins, and how biotechnological approaches can engineer durable resistance in agricultural crops had been critically discussed by Staskawicz (2001). The molecular characterization of the gene-for-gene system in plant–fungus interactions and the application of avirulence genes in control of plant pathogens have been reviewed by deWit (1992); the role of gene-for-gene interaction in the determination of host specificity has been reviewed by Heath (1991); molecular mechanisms of fungal pathogenicity in plants has been reviewed by Schaffer (1994); plant resistance genes in signal perception and transduction has been reviewed by Lamb (1994); molecular genetics of plant disease resistance has been reviewed by Staskawicz *et al.* (1995); and molecular approaches to manipulation of disease resistant genes has been reviewed by Michelmore (1995). Hahn (1996) has reviewed microbial elicitors and their receptors in plants. Chakraborty (2013) has discussed analytically the molecular events in recognition of fungal pathogens, activation of defense response and strategies for plant disease management.

Resistance and Susceptibility

A plant is either immune to a pathogen, that is, it is not attacked at all by the pathogen even under the most favourable conditions, or it may show varying degrees of resistance ranging from immunity to complete susceptibility. Resistance may be conditioned by a number of internal and external factors

which reduce the chance and degree of infection. There are several kinds of resistance: it may be physical or chemical, it may depend upon the lack of a nutrient or a substance that the parasite needs for development, or it may be due to a toxic or repellent substance that is either performed or formed only in response to infection. Irrespective of whether the morphological or biochemical defence mechanism is involved in the resistance of a particular host–parasite combination, it is ultimately the genetic makeup of the two that determines whether a particular host will be susceptible or resistant under given environmental conditions. The mechanisms by which genes control the physiological processes that lead to disease resistance or susceptibility are not yet clear, but they are presumably no different from the mechanisms controlling any other physiological process in a living organism. Under the influence of the genetic material (DNA), a specific protein, which may be a structural protein or an enzyme, may be produced. The produced enzyme, of course, under certain conditions will participate or initiate biochemical reaction related to one or other cellular process, and may result in the production of certain morphological characteristics or accumulation of a certain chemical substance. Most of the approximately 100,000 known species of fungi are strictly saprophytic and degrade dead organic material for their nutrients. Only 8000 can cause disease to one or more species and only 100 are pathogenic to humans and animals. It is the same for all other groups of plant pathogenic organisms such as bacteria, viruses, mycoplasmas. What then distinguishes this small minority of pathogenic fungi or other pathogens from the majority of saprophytic fungi or other microorganisms? The fungus *Fusarium oxysporum* f. sp *lycopersici* that causes tomato wilt, attacks tomato and has absolutely no effect on wheat or barley. Similarly the fungus *Phytophthora infestans* causing late blight of potato and tomato does not infect wheat or apple and the fungus *Puccinia graminis tritici* infects wheat only and not apple or potato.

Interactions between a host and pathogen are mediated by environmental conditions but are ultimately determined by genotype. Flor (1956) studied the genetics of flax cultivars and isolates of the linseed rust pathogen, *Melampsora lini* in field observations. He demonstrated a close genetic relationship between the host and pathogen and formulated the gene-for-gene concept, which is still regarded as an important hypothesis in Plant Pathology. The concept states that for each gene conditioning avirulence or virulence in the pathogen there is a corresponding gene conditioning resistance or susceptibility in the plant. Usually, although not always, genes for resistance in the host are dominant (R) and those for susceptibility (lack of resistance) are recessive (r). In the pathogen, genes for avirulence are dominant (A) and those for virulence are recessive (a). Where the gene-for-gene hypothesis operates therefore, four gene combinations are possible in host–pathogen interaction. These combinations are: R–a, r–A, r–a and R–A. Three combinations R–a, r–A and r–a give rise to compatible reaction (susceptible) and infections are successful. One combination, R–A results in an incompatible reaction and no infection occurs (resistant).

Table 7.1 Gene for gene combinations and disease reaction types in a host–pathogen system in which the gene for gene concept operates

Virulence or Avirulence Genes in the Pathogen	Resistance or Susceptibility Genes in the Plant	
	R (resistant) dominant	r (susceptible) recessive
A (avirulent) dominant	AR (-)	Ar (+)
a (virulent) recessive	aR (+)	ar(+)

+ = Compatible (susceptible) reaction
– = Incompatible (resistant) reaction

Genes for virulence appear in pathogens in response to new genes for resistance in the host. New resistance in the host leads to a situation which selects new virulence in the pathogen. Hosts and pathogens are in a continuing state of co-evolution. Exceptions to this hypothesis have been seen, particularly where virulence or resistance is controlled or modified by more than just a few genes. It is also possible that not all host–pathogen interactions are represented in this way. According to Staskawicz (2001), that in its most simple form, the gene-for-gene hypothesis states that plants contain single dominant resistance (R) genes that specifically recognise pathogens that contain complementary avirulence genes. Avirulence genes can be defined as genes in the pathogen that codes a protein product that is conditionally recognised directly or indirectly only by those plants that contain complementary R gene. According to Isaac (1992) the gene-for-gene concept remains largely theoretical and provides information, which is important in epidemiological terms but it does not consider the biochemical or molecular basis of resistance.

According to Isaac (1992) although a number of theories have been put forward to explain the specificity of host–pathogen interactions in biochemical terms, very little is known about the genes involved and their regulation. Few gene products have been analyzed but modern molecular biological techniques now provided means by which these factors may be investigated.

In an incompatible reaction, molecular recognition leads to the activation of a range of defense mechanisms. In a compatible reaction however, recognition does not occur, or is suppressed, and successful host penetration and colonization of the host may be achieved by this invading pathogen. In an incompatible reaction, structural and biochemical host defence mechanisms are initiated by the activation of the defence response genes. Crop plants are endowed with numerous defence genes which encode various proteins responsible for synthesis and accumulation of defence arsenals in plants. The defence genes are involved in the production of antimicrobial compounds such as phenolics, phytoalexins, pathogen-related (PR) proteins, and active oxygen-species or involved in the reinforcement of the cell wall by accumulating hydroxyproline-rich glycoproteins, callose, lignin, and wall-bounded phenolics (Vidhyasekaran, 1997). Some of them show antifungal action, some show elicitor-releasing activity, some increase the mechanical strength of host cell walls and some others are involved in the release of secondary messengers and systemic signal molecules. While defence genes are functional genes, resistant genes are regulatory genes which regulate the functions of the defence genes. Defence genes are quiescent in healthy plants, but activated when the pathogens come into contact with the plants, releasing signals. These signal molecules are transferred to the host plant nucleus through a signal transduction pathway activating defence genes. Several disease resistant genes have been cloned and characterized (Baker *et al.* 1997). Products of most of the resistance genes share striking structural similarities and they appear to be involved in the signal transduction system to activate defence genes. Analyses of several cloned resistance genes revealed the presence of leucine-rich repeats (LRR), leucine zippers and nucleotide-binding sites (NBS). Analogues of resistance genes with LRR, NBS, and protein kinase regions have been recently detected in barley, wheat, lettuce, soybean, pepper, chickpea, common bean, and *Brassica napus* (Vidhyasekaran, 1998). LRRs are contained in a variety of proteins and have been suggested to be involved in protein-protein interactions in a downstream step of the signal transduction pathway. Leucine zippers and LRRs serve as sites for protein-protein binding; leucine zippers may directly interact with DNA. The NBS may directly transport the signals to the plant nucleus. Protein kinases are involved in protein phosphorylation, and the transcriptional phosphorylation and the transcriptional activation of plant defence genes is modulated by phosphorylation.

It is now established that signals are required to activate defence genes which are commonly present in both susceptible and resistant plants. There is no plant in the world without defence genes. These signal molecules have been detected in fungi, bacteria, and viruses, irrespective of whether they are pathogens or non-pathogens and are released by host enzymes when the pathogens come into contact with plants. Endogenous elicitors of host origin, pectate fragments, are released by pathogen enzymes. These signals have to be transferred to the plant nucleus to generate mRNA for the synthesis of various defence-related proteins.

Although the area of host–pathogen specificity has been the subject of many previous investigations, the basic molecular mechanisms have yet to be discovered. Past strategies have often employed a comparative biochemical approach in which a pathogen is typically inoculated on near isogenic lines of the host plant and a difference in a biochemical process between the susceptible and resistant host plant is studied. This has led many researchers to hypothesize the role of compounds such as phytoalexins and lectins as determinants of host–pathogen specificity. Thus most researchers are looking for the gene products that determine specificity and have ignored the physical isolation of the genes that encode for these products.

The closest approaches to the identification of gene products which determine disease reactions and account for pathogen specificity come from the work of selective pathotoxins.

The role of cell wall–degrading enzymes has been implicated with host specificity in certain host–pathogen combinations. Initial results with the bean anthracnose system indicated that -galactosidase was produced in greater quantities when the pathogen was grown on cell walls from susceptible plants than when grown on those from resistant plants. However, further work with this and other systems failed to provide clear evidence for such selectivity.

Evidence for Specific Inducers of Resistance

Despite extensive research, there is little evidence for materials produced under the control of specific avirulence genes which, on the basis of Flor's concept, should have selective effects on resistant plants. Work in Holland which indicated that filtrates of avirulent cultures of a certain strain of *Venturia inaequalis* were selectively toxic to resistant plants was not confirmed in later work (Day, 1974).

The Common Antigen Hypothesis

The idea that plants and pathogens which interact to give susceptible (compatible) reactions have more antigens in common than those that give resistant (incompatible) reactions was explored first with the flax rust system. Further attempts to explore the hypothesis that antigens shared by the plant and pathogen may account for specificity have been carried out with several types of pathogens. Pathogenic races of *Xanthomonas campestris* pv. *malvacearum,* which cause angular leaf spot of cotton, had more antigens in common with those of cotton leaves than did nonpathogenic races of bacteria. However, in another system, Fusarium wilt of cotton, such a relationship was not observed. In this case loss of virulence in the pathogen did not result in a marked change in antigenic relationships between the plant and pathogen. Taken together, serological data suggest that common antigens may play a role in pathogen specificity.

The Phytoalexin-Induced Hypothesis

Phytoalexins are low molecular weight antimicrobial compounds produced *de novo* in plants as results of infection or abiotic stress. The degree of resistance of hosts has been correlated with the rate of production/accumulation of phytoalexin in the host or detoxification/suppression of phytoalexin by the pathogen. Although in a few cases (bean: *Colletotrichum lindemuthianum* and potato: *Phytophthora infestans*) there was no correlation between concentration of phytoalexin and resistance of the host (Agrios, 1997) but in most of the cases, a positive correlation did exist between the two.

Vertical and Horizontal Resistance

True host resistance is genetically controlled. It has been shown that resistance may be controlled by a single gene or very few genes (2–3) genes (oligogenic resistance) or many genes (polygenic). Van der Plank (1963) introduced the concept of vertical resistance to describe the condition in which plants showed a very high level of resistance to a particular physiological race of the pathogen but showed very little resistance to other races of the pathogen. Such hosts very often show a hypersensitive response usually very early in the infection, and the pathogen is not able to establish or multiply within host tissues. This is also known as race-specific resistance. Thus vertical resistance is complete but not permanent in nature as any change in the race flora will make the host plants susceptible if the host does not possess genes for resistance against the new races which may be more virulent than the other race or races of the pathogen present in that particular area.

Some plants have a general level of resistance to pathogens, which is race non-specific and controlled by a large number of genes (polygenic resistance). Equal resistance is shown to all pathogen races. Although this resistance called horizontal resistance, may not totally protect plants from infection. It does reduce susceptibility of the host plants and slows down the establishment of the pathogen. It is also known as field resistance. Thus horizontal resistance is incomplete but is of a permanent nature as some resistance in the host will always be present to all the races of the pathogen. Breeders try to bring in this resistance into the crop plants.

To understand and control plant disease, it is essential to know not only what a pathogenic organism looks like and what it is called, but also its behavior and that too not only in a single plant under one set of conditions, but on populations of different kinds of plants under all conditions in which plants grow.

The mechanisms which bring about variations in plants principally hybridization and mutation, will not be considered here. Those which bring about variations in fungi are perhaps more diverse and include mutation, hybridization, heterokaryosis, parasexual recombination and possible adaptation.

Mutation

Mutations are abrupt changes in the genetic material of a cell, which is then transmitted in a hereditary fashion to the progeny. Mutations occur spontaneously in nature in all living organisms which reproduce only sexually or only asexually and also in those which reproduce both sexually and asexually. Mutations, apparently, are the results of unavoidable but rather infrequent accidents that take place during cell division and result in irregularities in the replication or rearrangement of minute parts of the genetic

material of the cells. Mutations can also be induced at a high rate artificially by exposing the organisms to either physical agents, such as ultraviolet light, X-rays, gamma-rays, and extreme temperatures, or to highly reactive chemicals such as the nitrogen and sulphur mustards, epoxides, peroxides, phenols, alkaloids and alkylating agents (e.g., ethyl methane-sulphonate, methyl methane-sulphonate, diethyl sulphate, etc.). One highly potent mutagenic chemical, often styled as a magic bullet, is N-methyl-N nitro-N nitrosoguanidine.

The pathogenic abilities of parasites as well as their morphological and other characteristics can be changed in this way and these are important in the development of new strains. In a haploid fungus, such mutations should have immediate expression but this may be modified by epistatic or other effects. If the cell in which gene mutation occurs contains more than one nucleus, the expression will be modified by the other nuclei present and in such heterokaryotic fungi, pathogenicity and other characteristics will have a composite effect.

New races attributed to mutations have been reported in *Cladosporium fulvum* (tomato leaf mould) where step mutation (each mutation overcoming the protective effect of a single gene) is thought to have occurred and similar mutations have been achieved experimentally by ultraviolet irradiation. Pathogenic variation due to mutation has also been reported in *Phytophthora infestans*, *Puccinia graminis*, *Melampsora lini* and various smuts, to mention but a few. Many of these mutations are likely to be recessive and their expression will be dependent on sexual reproduction with an accompanying recombination of pathogenicity factors. Anastomosis between hyphae and germ-tubes may be followed by an exchange of nuclei and this may also permit the expression of recessive pathogenic mutations.

This could be of considerable significance in Fungi Imperfecti and in those Chytridiomycota, Oomycota, Ascomycota, and Basidiomycota in which the perfect stage is rarely formed as in some Erysiphaceae in which cleistothecia fail to develop under certain climatic conditions. Although the inadequate information available suggests that mutations affecting pathogenicity are fairly rare, they are doubtless an effective mechanism for variations when the enormous numbers of spores produced by many pathogens are taken into account. For example, Watson (1957) reported increased pathogenicity due to mutations in *P. graminis* when large numbers of urediniospores were involved. Mutation can also bring about reduced pathogenicity or other changes disadvantageous to the pathogen. The practical implications of mutation in the aggressiveness of pathogens are difficult to assess. The sudden appearance of new races of increased pathogenicity in a pathogen which has hitherto been of only moderate pathogenicity has been ascribed to mutation and this may well be true. The appearance of a new, highly destructive race of *Helminthosporium maydis* (southern leaf blight of maize) in the USA in 1970 may be cited as an example of this.

Mutations also provide the basic pathogenicity changes, which are subsequently brought out by hybridization or similar recombination mechanisms, and without mutation the latter would be of a more limited scope. Beadle and Tatum (1946) demonstrated the role of mutations in producing hereditary variants in fungi. Several workers have discovered mutations in plant pathogenic viruses, bacteria, and fungi.

Heterokaryosis

Heterokaryosis and variability in the plant pathogenic fungi had been ably summarized by Parmeter *et al*. (1963). Hansen and Smith (1932) defined heterokaryosis as a term which "…precisely describes the

condition of a cell containing two or more genetically different nuclei…". In a later classification of the use of the term heterokaryosis, Hansen (1942) pointed out that the term may be used whenever such a condition exists, not only in a single cell, but also when it exists in any or all cells of an individual thallus. This clearly implies that heterokaryosis involves the occurrence of dissimilar nuclei in a single cell, even if it is only one cell in a thallus.

Nelson *et al*. (1955) used the term "heterokaryosis" to designate the association of more than two genetically different kinds of nuclei in a vegetative cell or spore. Considerable information on the potential advantage of heterokaryosis is available in the existing literature. It is agreed by most workers that heterokaryosis provides a haploid organism with many of the advantages of heterozygosity enjoyed by diploid organisms. Nuclear changes could provide additional somatic flexibility with changing environments (Davis, 1966). However, the main advantage of heterokaryosis appears to be the fact that it is a requisite step for genetic recombination brought about by interchanges of whole chromosomes or through mitotic crossing over. Heterokaryosis may thus play an important role in homothallic and imperfect fungi. This aspect was reviewed by Caten and Jinks (1966).

Heterokaryosis can arise in several ways:

1. **Gene mutation:** These are not easily detected at a gross level since their frequency in nuclei is very small.
2. **By fusion of vegetative cells:** Anastomosis is undoubtedly important in the initiation of heterokaryosis. Anastomosis, as it is related to the establishment and development of heterokaryosis, involves fusion of hyphae, movement of one or more nuclei into one or the other of the fused cells, and the establishment of a compatible heterokaryotic state. Failure to establish heterokaryons may involve any one of these steps.
3. Heterokaryosis may take place at the time of spore formation in many fungi, such as inclusion of + and – nuclei in the same ascospore of *Neurospora tetrasperma* or *Podospora anserina*.

Apart from dikaryotics in smuts, rusts, and Hymenomycetes, heterokaryosis has been reported in pathogens belonging to the Fungi Imperfecti, Ascomycetes, and to a lesser extent, in lower fungi, but not all these reports are entirely convincing (Parmeter *et al*., 1963).

Heterokaryosis appears to be necessary for pathogenicity in heterothallic rusts, but not in infection by basidiospores as the latter are normally uninucleate and hence necessarily homokaryotic. There is considerable evidence that heterokaryosis not only conditions pathogenicity but also enables the effect of mutation on pathogenicity (often recessive) to be expressed. Buxton (1956) produced nearly avirulent race of *Fusarium oxysporum* f. sp. *pisi* by ultraviolet irradiation and showed that heterokaryons were recovered by culturing from the diseased plants. Buxton (1960) concluded that some of the increases in the virulence of pathogens in nature may be due to heterokaryosis between weakly virulent strains.

The plant pathogenic role of heterokaryosis in producing new strains with altered pathogenicity has been discussed by several workers.

Nutritional deficiencies can affect pathogenicity as shown by Hrushowvitz (1957), who demonstrated that repeated culturing of *H. sativum* on media supplemented with certain amino acids led to a progressive loss in pathogenicity in some cases. The mechanism involved is uncertain but such repeated culturing may have led to the selection of deficient nuclei, thalli containing such deficient nuclei being less pathogenic. The increased pathogenicity due to heterokaryosis in the mycelium would not be inherited by the spores unless the latter contained a full complement of the parental nuclei and would not be obtained with uninucleate conidia. Heterokaryosis is thus unlikely to bring about any permanent inheritance change in pathogenicity which would persist after sporulation, although parasexual

recombination in which heterokaryosis is the first step, could do so. Heterokaryosis had been discussed by Davis (1966).

Heterokaryosis is certainly a way in which avirulent strains may acquire virulence. One of the best documented cases is that of *Thanatephorus cucumeris*. Heterokaryosis between avirulent strains, both spontaneous and induced, or between avirulent and virulent strains were all highly pathogenic, in the latter case more so than either component.

Heterokaryosis may give rise to races or strains with novel characteristics. One of the best documented examples is *P. striiformis* (Little and Manners, 1969).

Parasexual Recombination

This process in which genetic recombination occurs in the vegetative thallus in the absence of a sexual stage has been investigated chiefly in species of *Aspergillus* and *Penicillium* in the laboratory of Pontecorvo and his co-workers (Pontecorvo *et al*., 1963; Pontecorvo and Sermonti, 1954; Pontecorvo, 1956; Bradley, 1962).

The sequence of events in a complete parasexual cycle is as follows:

1. Formation of heterokaryotic mycelium.
2. Fusion between two nuclei:
   (a) Fusion between like nuclei.
   (b) Fusion between unlike nuclei.
3. Multiplication of diploid nuclei alongside haploid nuclei.
4. Occasional mitotic crossing over during the multiplication of diploid nuclei.
5. Sorting out of diploid nuclei.
6. Occasional Haplodization of the diploid nuclei.
7. Sorting out of the new haploid strains.

The results are similar to those achieved by meiosis, a much more precise and regular process. The frequency of these events appears to be low and there is little information about the significance of this somatic recombination in nature, especially about fungi which attack plants. Parasexuality, however, produced new races of *F. oxysporum* f. sp. *pisi* and has also been reported in *Ascochyta imperfecti* responsible for black stem of alfalfa, *Verticillium albo atrum*, *F. oxysporum* f. sp. *cubense* responsible for banana wilt, *Cochliobolus sativus* and others. There is evidence that somatic recombination may occur in smuts and rusts as in *Ustilago maydis* (Rowell, 1955), *Puccinia graminis* and *Puccinia recondita* (Vakili and Caldwell, 1957).

Adaptation

Adaptation in fungi has been reviewed by Buxton (1960) and Person (1968). The possibility that pathogens adapt themselves to their host plants has been debated for many years. There seems to be no incontrovertible evidence that this occurs although the evidence for adaptation to chemicals is stronger. Apparent adaptation to host plants might be due to mutation or to the selection of pre-existing strains which are better able to attack the host. Changes in the enzymatic capabilities of a pathogen may be mediated by the presence of appropriate substrates (adaptive enzymes) and this might modify its pathogenicity. Many years ago, Ward (1903) suggested the concept of a bridging host by means of which a pathogen

is able to pass from a susceptible to a hitherto resistant host: for example, in *Puccinia dispersa*, the pathogen becomes adaptive to resistant species of *Bromus.* After propagation in some species of grass, somewhat similar changes were reported by Salmon (1904) in powdery mildews, and more recent work indicates that passage through some host plants can sometimes affect pathogenicity. Repeated passage through a resistant plant sometimes enhances the aggressiveness of a pathogen and vice versa, as has been demonstrated in several fungi and bacteria: for example, *Phytophthora infestans* and *Xanthomonas stewartii.*

Changes in the pathogenicity of *F. oxysporum* f. sp. *pisi* are brought about by exudates. Although there is increasing evidence in favour of parasitic adaptation, the mechanism involved remains obscure. Host selection of more aggressive biotypes from a mixture of biotypes, together with adaptation, mutation (perhaps by some mutagens in the host) and cytoplasmic variation may be involved in different cases.

Saltation

This phenomenon, also described as dissociation or sectoring, refers to the appearance of morphologically different sectors in fungal colonies. It occurs frequently in some fungi including some isolates of *Fusarium* and *Helminthosporium*, but infrequently in others. Some such sectors remain fairly stable when sub-cultured while others are unstable. Saltation may be influenced by the compaction and thickness of the culture medium and can affect such characteristics as colony colour and topography; rate of growth and sporulation; and size and shape of the spore. Pathogenicity might also be affected and this has been studied in *Fusarium* species by several investigators. The results have been somewhat conflicting; saltants tended to be less aggressive than the parent cultures, but occasional ones were more aggressive and these may be of significance where the saltation rate is high. No new pathogenic races with altered host ranges have been reported in saltants. The mechanisms involved in saltation are varied and not well understood.

Cytoplasmic Variation

There is a great deal of evidence for the existence in fungi of genetical determinants located in the cytoplasm outside the nucleus. Inheritance of such cytoplasmic determinants differs from that of nuclear genes. Cytoplasmic inheritance has been attributed to plasma genes. Cytoplasmic RNA (possibly some ribosomes) is the genetic material detached from the chromosome (episomes). There is little information as to the possible significance of cytoplasmic factors in bringing about variations in the pathogenicity of plant pathogens, although Johnson (1954) had described the cytoplasmic control of pathogenicity of *Puccinia graminis* f. sp. *tritici*; this is a subject which needs investigation. Cytoplasmic inheritance had been discussed by Jinks (1966) and Fincham and Day (1965).

Sexual Recombination

The origin of new physiological races through meiotic recombination is well attested in many pathogenic fungi, rusts, smuts, powdery mildews, and the potato blight fungus. Hybridization is likely to be

encouraged by incompatible mechanisms which prevent or reduce inbreeding, and many pathogens which are heterothallic.

Hybridization can be intraspecific, interspecific or even intergeneric and the resulting hybrids may have different pathogenic abilities than the parental races. Often the hybrids are intermediate in pathogenicity between the two parental races but some may be more pathogenic than others and similar considerations apply to other inherited characteristics.

Extensive recombination of genes occur in autoecious rusts such as *Melampsora lini* (flax rust) in which all the spore stages occur in the same plant. Interspecific hybridization had been discussed by Nelson (1963).

Transformation

In addition to sexual recombination, there are a number of possibilities for the production of new variants in microorganisms by asexual means. One of these, a sexual type of combinations of genetic characters, is bacterial transformation. The observation of Avery *et al.* (1944) called attention to this process. They were successful in converting a non-pathogenic non-capsule-forming strain of *Diplococcus pneumoniae* into a pathogenic strain which forms a carbohydrate capsule. This was done by culturing the non-pathogenic strain in the presence of the biologically-active fraction of the capsule forming a pathogenic strain. It was later demonstrated by these workers that this transformation was brought about by the introduction of purified deoxyribonucleic acid (DNA of the donor). Thus transformation is a specific form of recombination, involving the naked genes of DNA.

Several workers have demonstrated the production of new strains of plant pathogenic bacteria by means of transformation. Corey and Starr (1957) were able to change the type of colonies and resistance of *Xanthomonas campestris* pv. *phaseoli* (a pathogen of beans) to streptomycin by transformation. Similarly, Klein and Klein (1953) have shown that the tumour-inducing ability of *Agrobacterium tumefaciens* can be transferred from virulent donor strains to avirulent strains. In this case, the material transforming the avirulent cells was also deoxyribonucleic acid (DNA).

Transduction

Another process of recombination, different from sexual recombination is transduction. This process has been found in a variety of bacteria where DNA is transferred from cell to cell through the agency of temperate viruses. Bacteria need not necessarily be destroyed by their infective viruses (bacteriophages). In certain cases after infection of the bacterium with a bacteriophage, the phage (temperate) may produce in the host an immunity that prevents multiplication of the phage and lysis of the bacterial cell. Such an association is called the lysogenic state. Under the influence of different chemicals and physical treatment (e.g., ultraviolet irradiation), lysogenic bacteria liberate their latent phages by lysis. These are infective viruses with the ability to enter the new bacterial cells. During symbiosis, the phage becomes the genetic material of the host, with page DNA coming into close contact with the host DNA. This makes it possible for the DNA molecule, carrying the genetic factor of the donor bacterium to be transformed to a recipient bacterial cell through the vehicle of a phage particle. Pathogenicity can also be transduced by bacteriophages. Therefore, transduction is an important mechanism for genetic recombination in bacteria. This interesting mechanism of genetic exchange was discovered by Zinder and

Lederberg (1952) in *Salmonella typhimurium*. Its importance was also demonstrated in a plant pathogenic bacterium (*Ralstonia solanacearum*) by Okabe and Goto (1955). One can suppose that transduction has a role in producing new biotypes of plant pathogenic bacteria in nature; however, this remains to be proved.

Viruses also show considerable variability in several characteristics, such as infectivity, longevity, symptoms produced on the host plant, host range, and specificity to vectors. These variations are possibly due to some sort of mutation process and are inherited in that they are perpetuated during the transfer of the virus from one plant to another. The mechanism involved in these changes is not quite well understood. In virus-resistant plants, the relationship is, in effect, between the nucleic acids of the resistant genes of the host plant and RNA of the virus particle.

Physiological Specialization

Many factors in host–parasite interactions present the fundamental problems of plant disease. The major factor towards the elucidation of host–parasite interactions is the genetic aspect.

The rediscovery of Mendel's laws of heredity in 1900 and the subsequent rapid development of the basic science of genetics gave a strong impetus to the study of plant pathology. The genetic basis of disease resistance was analyzed in many cases. Although the genetics of the host received most attention initially, advances in the knowledge of the sexuality of fungi led to the development of materials and methods for genetic studies of pathogenic fungi. There is now positive evidence that the general patterns of the laws of inheritance are the same in fungi as in other groups of plants. The fungi undergo heritable variations brought about by segregation and recombination of genes, mutations, heterokaryosis, cytoplasmic inheritance, etc. Not only this, because of their great variety in sexual and asexual reproduction and their unique suitability for experimental manipulation, microorganisms now afford the best available material for many types of fundamental studies in genetics, biochemistry, pathology, and other fields. Despite all these advances, however, knowledge of the actual physico-chemical mechanisms that determine parasitism, pathogenicity or disease resistance is still very meager. In a nutshell, we can say that disease resistance is a genetic character as regularly inherited as any other character. However, the genetic behavior of resistance usually differs in different cases.

Resistance to one disease does not necessarily imply resistance to any other disease. Thus, Thatcher wheat, while resistant to stem rust is highly susceptible to leaf rust (*P. recondita, P. triticina*).Various kinds of genetic behavior have been observed with respect to disease resistance. For example, simple monohybrid inheritance of resistance is seen, in cabbages to yellows (*Fusarium conglutinans*), and lettuce to downy mildews (*Bremia lactucae*) and peas to wilt (*Fusarium oxysporum sp. pisi*). In these cases, resistance is a dominant characteristic.

Resistance as a recessive characteristic in simple monohybrid inheritance is seen in powdery mildew of barley (*E. graminis*), smut of sorghum (*Sphacelotheca sorghi*), and speckled leaf blotch of wheat (*Septoria tritici*).Two gene segregations appear to be involved in resistance to anthracnose of beans (*Colletotrichum lindemuthianum*) and some cereals rusts. Many genes which are more difficult to analyze are known to be involved in a large number of diseases. And finally, there may be a number of alleles (instead of two) for one gene, giving a complex series of resistance relationship when several parents are studied.

Physiological Specialization in Fungi

Complexity of organisms Like all other organisms, fungi are also complex and variable. This complexity is governed by the genetic material contained in the chromosomes. The crop plants are similarly complex, for example, there are 14,000 varieties of wheat; similarly there are innumerable varieties of apples, beans, grams, roses, corn, and so on.

Gene Actions When fungal species parasitize a number of host varieties they often develop physiological specialization, which is the development of certain character in the fungus which are not morphological but physiological, and can be detected only when the effect is noted on the host. A special species of fungus such as the black stem rust, *Puccinia graminis*, may include a number of "physiological races" which are similar in morphological structure but may be different in their capacity to produce disease. So the difference between them is physiological and not morphological. Such races are commonly encountered in many groups of parasites such as rusts, smuts, powdery and downy mildews, and so on.

Gene action expresses itself in various morphological features and it also always governs the various physiological processes of the organism. The interaction between the host and parasite is actually the interaction between the enzyme systems of the two (proved by Dodge and others in *Neurospora*) and the result is the effect produced.

The details of this phenomenon can be easily explained by taking the example of the black stem rust, *Puccinia graminis*. The discovery of pathogenic specialization is fundamental to studies on pathogenic variability. Eriksson (1894) showed that *P. graminis* on cereals and grasses is composed of several pathogenically different strains which he described as specialized forms (*formae speciales*). These are now known as varieties. There are at least six varieties of *P. graminis* which can be recognized by the size of the spores and also by their pathogenicity on different hosts (Table 7.2):

Table 7.2

Variety	Urediniospores	Host attacked
P. graminis tritici	32 × 20 µm	Wheat, barley, and many wild grasses
P. graminis secalis	27 × 17 µm	Rye, barley, and many wild grasses
P. graminis avenae	28 × 20 µm	Oats, and wild grasses (*Phleum pratense*)
P. graminis phleipratensis	24 × 17 µm	Timothy, and other wild grasses
P. graminis agrostidis	22 × 16 µm	*Argrostis* spp.
P. graminis poae	19 × 16 µm	*Poa pratensis*, and other grasses

The general characteristics of all these varieties of *P. graminis* are so alike that it will not be possible to differentiate between them unless microscopic examination is conducted and measurements are taken.

Each variety, for example, *P. graminis tritici*, is further subdivided into pathogenic units called physiological races. These races cannot be distinguished by morphological examinations but by noting the different effects they produce on various varieties of wheat. The only method by which the nature (identity) of physiological races can be known is to grow them on a number of host varieties and note the symptoms which will be different if the isolates differ in physiological capacity. In order to have a

standard method for the identification of a physiological race, some host varieties are fixed, which are called "standard differentials," and the record of each race is taken. These differentials, now used to distinguish *Puccinia graminis tritici* and *Puccinia graminis avenae,* were selected about seventy years ago. Whenever the necessity arises new ones are added. For identifying the races, three varietal reaction classes are used: these are resistant (R), susceptible (S), and mesothetic (M). Further degrees are organized within these three classes, for example, immune, practically immune, extremely resistant, moderately resistant, moderately susceptible, completely susceptible, and heterogeneous mixed reaction (x). In addition to these infection types, a plus (+) or a minus (-) sign or two plus (+ +) or two minus (- -) signs are included to indicate variations of a slightly greater or lesser extent.

Infection Types According to the degree of spotting produced, each type of infection is indicated by one of the six given symbols:

Type 0 Very faint, light flecks; host therefore considered immune
Type 1 Uredosori very minute; host strongly resistant
Type 2 Small to medium; host resistant
Type 3 Medium size; host moderately susceptible
Type 4 Large and confluent; host very susceptible
Type x A mixed reaction; uredosori of variable size, mostly those of types 1 and 4

To identify a race of *Puccinia graminis*, the isolate is inoculated on 12-standard differentials and the symptoms produced are noted and classified into infection types. These are then compared with the existing records and the race is identified. Key numbers are given each on the basis of its identity.

Production of New Races

All cereals rusts are heteroecious. An aecidial stage is never produced on a cereal host. As long as the fungus produces itself by means of urediniospores, it breeds true except for the occasional appearance of a mutation. On the alternative hosts (barberry, buckthorn and so forth), the sexual stage of the fungus is developed (Cragie, 1927) and this gives an opportunity for hybridization. This could result in the formation of heterozygous characteristics with a difference in physiological specialization. When such a heterozygous race forms the basidiospores it segregates again and different recombination may result again. In this way the appearance of new races are recorded more in those regions where *Berberis* spp. plays an active role, as in the USA and Europe. On the other hand, in countries where *Berberis* spp. plays no part or little part in the life cycle, the number of races is much less, as in Australia, India and Kenya. In *P. graminis tritici* alone there are over 250 races in the USA, and 32 races and biotypes in India.

In other species of *Puccinia*, the number of physiological races is lesser. In *Puccinia recondita* there are 100 in the USA and about 30 in India. In *Puccinia striiformis* there are 30 in the USA and 23 in India.

Biotypes A biotype is a population of individuals that are genetically identical. The descendants of a single non-sexually produced stem rust spore constitute a biotype. The biotype will remain strictly pure unless a mutation occurs. Most species of pathogenic fungi are now known to have many genotypes. The most closely related races are similarly grouped into varieties and finally the varieties into species. Actually, a reverse procedure is followed. The larger groups (varieties) are recognized first and then smaller groups (races and biotypes) are easily distinguished by more refined methods. Examples of the

occurrence of biotypes can be given in the case of race 59 in which several biotypes are known to exist. Biotypes show enough similarities to be placed within one race; but when different biotypes are studied very closely, they show minor but consistent differences in their effects on some differentials, proving that they are genetically pure, though with different identities. Such races are, therefore, known to have biotypes designated as 59, 59A, 59B, 59C.

Another instance may be given. Race 15 of wheat stem rust was discovered in 1918. It was fairly homogenous rust for a long time and was identified as such. Later, some dissimilarity in isolates from different regions was noted. These differences were consistent enough and 15A and 15B were recognized as biotypes. Race 15B which was known to occur occasionally near barberry bushes became widespread in 1950 and attacked the well-known hitherto resistant varieties. Besides rusts, specialization of parasitism is found in other fungi also. In smuts, as in rusts, races and biotypes are known to occur in different species. As a matter of fact, smuts offer better material for the study of genetics. In smuts (*Ustilago zeae*) the chlamydospores undergo reduction division and the sporidia produced are haploid structures which can be grown into haploid mycelium in culture media and matings done as per plan. The colonies from haploid sporidia are genetically pure and differ from one another in characters such as size, color and surface pattern. It is now known that in *Ustilago zeae* there are thousands of haploid biotypes. Biotypes may differ from one another in one or more of all known characters, including pathogenicity. Similar, though less elaborate, work has been done on other smuts, for example, *Ustilago segetum* var. *tritici*, *Ustilago nuda*, *Sphacelotheca sorghi*, *Ustilago avenae* and *Ustilago hordei*.

Investigations in rusts and smuts are complementary. In rusts only the binucleate uredinial stage can be propagated clonally, while in smuts it is only the haplophase which can be propagated in this way. In both smuts and rusts three distinct phases correspond with the nuclear condition, the haplophase, the dikaryophase and the diplophase. In rusts, both dikaryophase and haplophase are parasitic in life, but in smuts, the haplophase is generally saprophytic. The dikaryophase, both smuts as well as rusts, can be hybrid in character. This character is multiplied (in an equational manner) vegetatively through the urediniospore stage. In many cases, rusts (in India) can go on repeating indefinitely from year to year. In smuts, such multiplication does not occur and sexuality is an indispensable process. Variability in smuts is, therefore, more common, and hence there is a very large number of races, but these races have to be expressed in terms of haploid strains and not in the dikaryotic phase, as in rusts. No sooner is the chlamydospore formed in smuts, than the dikaryotic phase comes to an end and is not propagated clonally (by urediniospore, like conidia) as in rusts.

Blumeria (*Erysiphe*) *graminis* is also strongly specialized into physiological races.

Adaptation of Fungi to Different Hosts

The Gene-for-Gene Theory of Host–Parasite Relations

Flor (1956) in his studies on rust disease of Flax (*Melampsora lini*), came to the conclusion that for each gene that governs resistance in the host, there is a specific and related gene in the pathogen that governs its virulence (pathogenicity). In other words, there is a genetic inter-relationship between the host and pathogen. This is the complementary gene-for-gene theory. The mechanism for the evolution of this relationship in a host–parasite system is an automatic consequence of the distribution of new resistance genes by mutation and selection in the host, and virulence genes in the parasite population. The genetic definition of specificity embodies the so-called "quadratic model" in which gene in the host are clearly

defined in terms of resistance or susceptibility and the complementary genes in the pathogen in terms of virulence or avirulence. The relationship between the host and parasite induces a genetic polymorphism in both partners (Person, 1959, 1968). This genetic variability, exhibiting a self regulation automatic mechanism, is the reason for the vicious circle that consists of the alternative prevalence of the resistant host and the virulent pathogen.

The gene-for-gene hypothesis was proposed by Flor (1942, 1955, and 1956) as the simplest explanation of the results of studies on the inheritance of pathogenicity in the flax rust fungus (*Melampsora lini*). In the varieties of Flax (*Linum usitatissimum*) that have a gene to resist the avirulent parent race, F_2 cultures of the fungus segregate into monofactorial ratios. In the varieties that possess 2, 3 or 4 genes for resistance to the avirulent parent race, the segregation in the F_2cultures is in bi, tri or tetrafactorial ratios.

Flor (1955, 1956) summarized the evidence for flax rust in the gene-for-gene hypothesis. This states that during their evolution the host and parasite develop complementary gene system so that "for each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite" (Flor, 1956). The gene-for-gene concept has been reviewed by Flor (1971). This hypothesis is applicable to most host–parasite system in which resistance is conditioned by major (vertical resistance) genes and virulence increase in a step-wise manner. Hatchett and Gallun (1970) showed that the ability of specific races of Hessian fly to survive on certain varieties of wheat was controlled by genetic systems in the host and insect that was complementary.

There is considerable literature on the inheritance of pathogenicity and resistance, including smuts, *Venturia inaequalis* and *Phytophthora infestans*. In the case of *Phytophthora infestans*, Black (1952) in Scotland identified four major dominant genes, R1, R2, R3 and R4. At least nine major genes are now known to control resistance. This polygenic resistance is apparently conditioned by different factors in the case of tuber rot compared with foliage blight. The reactions of host plants in the different pathogenic races are controlled by many genes, and within them, an even large number of alleles exist to enable resistance.

Resistance in plants is often dominant over susceptibility to disease. It is generally found that resistance of plant cultivars to disease is not a permanent character. The reason for this phenomenon is to be found in the self-regulating nature of genetic variability in host–parasite relations. The evolution of new genes for virulence in the pathogens is the result of the selective action of resistance cultivars on populations of the pathogens which are genetically plastic. Similarly, the gene for virulence in parasites promotes the selection of genes for resistance in the host population. This automatic regulation of population of the host and the parasites explains the fact that in many cases the utilization of resistant cultivars is very limited. For example as a rule, rust resistance is lost in 3–7 years. This is valid, first of all, for pure line cultivars of a self- pollinated plant species that is, for wheat which suffers from attack by new rust race much more heavily than the cross-pollinated plants (e.g., cultivars of corn). Cultivars of cross pollinated plants are composed of a mixture of different lines (biotypes). This means that some biotype will be resistant host population to selection effect (attack) of virulent gene of the parasite. However, they are very few, if any, resistant biotype in the susceptible population of pure line cultivars, since they are largely eliminated during the breeding process which produces a high degree of homozygosity. Thus pure lines will be highly vulnerable to attack by the parasite containing genes for virulence. On the other hand in cross-pollinated plants, the buffering capacity of the genetic pool is relatively high compared to the self-pollinated ones. In recent years, plant breeders have tried to incorporate greater variability into self- pollinated plants such as wheat, by developing multiline cultivars. The various lines in the multiline cultivars have different gene-for-gene system against the virulent genes of the rust fungi. By

continually manipulating intra-varietal heterogeneity within available resistance genes, it should be possible to provide a suitable control of widespread pathogen inoculum potential.

The advantage of using multiline cultivars, according to Sadasivan (1975), is that they enable quick synthesizing of well buffered "horizontal resistance" cultivars (HR). They are capable of utilizing, unlike pure line cultivars, several resistance genes at the same locus. The useful life of a resistant gene can be extended indefinitely and the breeding programme can be cut to size by this method.

There is an even chance for the cultivars to stabilize themselves in a given environment. This method helps to distribute developed varieties on a wide geographical scale without any risk of homogenizing the pathogen population. The multiple approaches are thought to be expensive, agronomically conservative and a breeding ground for new races, possibly even a super race. Another drawback is that because their variability is synthetic it is polarized.

The resistant cultivars of cross-pollinated plants remain resistant for considerable time. One such example, according to Borlaug (1965), is the relative stability of resistance in corn cultivars to the rusts, *Puccinia sorghi* and *Puccinia polysora*. These two rusts of corn are much less important in limiting corn production than the rust of wheat. The cross-pollinated corn usually possesses sufficient variability, that is, sufficient resistant lines are present in the population of the cultivar to resist the selective action of virulent genes of new rust races. This means that the development of rust epidemics in cross-pollinated corn is almost impossible.

Apparent Resistance

Limited or widespread plant disease epidemics occur on various crop plants in some area and almost every year. Under certain conditions, some susceptible plants may remain free from disease and thus appear resistant. The apparent resistance to disease of a susceptible plant is generally due to disease escape or tolerance to disease.

Disease escape occurs whenever genetically susceptible plants are not infected because the three factors necessary for disease: susceptible host, virulent pathogen and congenial environment do not coincide and interact at the proper time for disease development. For example, in case of soil borne pathogens, the seeds germinate faster before the temperature becomes favourable for the pathogen to attack them. Some plants escape disease because the plants are susceptible to a pathogen either at young stage or at fruiting stage, therefore if the pathogen is absent at that particular time, such plants are disease free. For example, *Pythium*, powdery mildews, and some bacteria and viruses infect the plants when they are young; however, *Alternaria* and *Botrytis* infect the fully grown mature plants. In many cases, plants escape disease if grown under mixed cropping rather than monocultural conditions. Some more factors like poor or absence of growth of the pathogen, weakening of the pathogen by antagonistic microflora at the place of production, lack of pathogen dissemination such as wind, rain also allow some plants to escape disease.

Several environmental factors like high temperature for *Phytophthora* and *Pythium*, while low temperature for *Fusarium* and *Rhizoctonia*, low moisture content are the most common cause of disease escape in plants. So many plant diseases are present some years in some areas, and absent on the same kinds of plants in others years or in nearby places. This suggests that in these areas or years the plants remain free of disease not because they are resistant but because they escape disease.

Genetics of Virulence in Pathogens and of Resistance in Host Plants

Infectious plant diseases as a result of interaction between host and the pathogen and properties of these two organisms are governed by their genetic material. The degree of susceptibility or resistance of host to various pathogens is an inherited characteristic. It has been known that every pathogen is having races, each different from others in its ability to attack certain varieties of a plant species but not other or all varieties. So when a variety is inoculated with two races of a pathogen, the variety is resistant to one race and susceptible to the other. This shows that one race possesses a genetic characteristic that enables it to attack the variety of a plant while the other race does not. When one race of the pathogen is inoculated on two different varieties, one is susceptible while the other is resistant. This shows that one variety possesses a genetic characteristic that enables it to defend itself against the pathogen so that it remains resistant while the other variety does not. Studies of the inheritance of resistance versus susceptibility in plants show that single gene control resistance and their absence allows susceptibility.

Similarly, studies further prove that single gene control avirulence and their absence allows virulence in pathogens. The number of genes determining resistance or susceptibility varies from plant to plant as the number of genes determining virulence and avirulence varies from pathogen to pathogen. Here we shall be discussing various processes leading towards disease development and how genes are responsible towards resistance or susceptibility of the plants.

Nature of resistance to disease

A microorganism is pathogenic or a host is resistant because it has the genetic ability to infect or immune to a pathogen even under most favourable conditions. The mechanism by which genes control the various physiological processes that lead to disease resistance or susceptibility are not yet very clear but they are presumably, no different from the mechanisms controlling any other physiological process in living organisms.

It is thought that for the production of enzymes, or fungitoxic substances required for defense, a stimulant secreted by the pathogen reacts with a receptor molecule of a host cell. This then transmits signals to other host cell molecules, activating plant defenses. However if a pathogen does not secrete the particular elicitor that activates the defense reaction, infection will occur and further disease development. The success of a fungal pathogen depends to a high degree of its ability to perceive and to respond to signals generated by the plant, especially at the early stages of infection (recognition), but also in later stages involving different cell types/tissues. Despite the rapidly increasing number of cloned signal component genes, the initial events of sensing of extracellular signals and transduction into an intracellular signal are still poorly understood. Components of signal chain have been studied in several pathogenic fungi, focusing of MAP kinase cascades, the classical cAMP pathway (heterotrimeric G proteins, adenylate cyclase, cAMP-dependent protein kinase A), and the crosstalk between them.

Genes Involved in Pathogenesis

"Pathogenicity genes" (pat) and "disease-specific genes" (dsp) are those involved in steps crucial for the establishment of disease. Such genes include those essential for recognition of host by pathogen, further infection, penetration, and colonization of the host tissue. Further pathogens utilize various genes that enable them to break down host wall macromolecules, produce toxins, neutralize host defenses, move through the host, multiply in the host, disseminate from host to host and so on.

Genes Controlling Production of Infection Structures

Depending on how fungi enter either through stomata or any other opening or through direct penetration, they need to degrade cuticle and the cell wall, for which they may need to form specialized structures called appressoria. Appressoria contain glycerol for creating a high turgour pressure that allows the penetration peg of appressoria to break or puncture the plant epidermal cells. A large number of genes have been isolated that affect appressoria differentiation and function. Most of these genes have been isolated from the large appressorium forming species of *Magnaporthe grisea* and *Colletotrichum* sp. Appressorium-related genes can be divided into three sub-groups: (1) Genes that operate prior to and are necessary for appressorium formation. Genes included are the *M. grisea* MPG1 and Pth11 and the *Colletotrichum gloeosporioides* CHIP6. An additional gene that affects appressorium formation in *M. grisea* and may be involved in recognition of physical factors on solid surfaces has been recently reported. This gene, named CBP1, encodes a chitin-binding protein and is specifically expressed in germ tubes before appressoria formation. (2) Genes that are uniquely expressed in appressoria or contribute significantly to specific features of appressoria structure and may be regarded as appressorium- specific. (3) Genes that control and affect appressorium germination, i.e., formation of the penetration peg and host penetration. Genes of group 2 and 3 include melanin biosynthesis genes, which are needed for functional appressoria and appressorium-specific genes that are expressed only in the appressorium. Melanin biosynthesis genes have been isolated from *Colletotrichum lagenarium* (PKS1, SCD1, and THR1) and from *M. grisea* (RSY, BUF). Mutant strains in either species are albino and unable to infect the host plants. There are not many known genes that be classified under group 3, which are genes that affect appressorium germination and penetration peg formation. The two *M. grisea* appressorium-specific genes, GAS1 and GAS2, are expressed exclusively in appressoria and are localized to the cytoplasm. Deletion mutant of either gene had normal growth and conidiation and formed normal appressoria, but were reduced in appressorial penetration and lesion formation. Exogenous cAMP suppressed defects associated with *pth*11 mutants, suggesting that *Pth*11 mediates cellular response through the cAMP pathway.

Genes Controlling Degradation of Cell Wall

It is known that enzymes that degrade cell wall and other physical structures essential for pathogenicity are encoded by more than one gene that too is not related. As a result, absence of one such gene does not eliminate pathogenicity of the pathogen because the other genes that encode the same enzyme fill in the need for the enzyme. The enzyme cutinase that degrade cutins and the enzyme pectinases (PG, PMG and PME) that degrade pectins are encoded by multigene families, and proof of their significance as essential pathogenicity factors is difficult. The disruption of a pectin lyase gene in *Cryphonectria parasitica*, *Alternaria* sp. and *Glomerella* sp. had no effect on its pathogenicity, whereas disruption of a pectinase gene is *Botrytis* reduced the pathogenicity of the fungus on apple and tomato. Another example is an endo-polygalacturonase (PG) gene from *B. cinerea*, the deletion of which reduced virulence on tomato, though altogether at least 5 endo PG genes are present. In *Claviceps purpurea*, replacement of two closely linked PG genes resulted in drastic reduction of pathogenicity on rye, this drastic effect was unexpected, since in this system the disruption of other cell wall–degrading enzymes (CWDE) genes (celluloses, xylanases) did not affect virulence significantly since the pectin content in cereal tissues are extremely low. On the other hand, a double mutant of *Cochliobolus carbonum* lacking the two major extracellular polygalacturonases (having less than 1% of total wild type PG activity) displayed normal virulence on maize.

Genes Controlling Secondary Metabolites

Apart from the needs that are required for producing infection structures (appressorium) and for degrading cutin and various cell wall components, pathogen require additional genes that will help them overcome the many secondary metabolites produced by the plant in order to defend themselves. The genes of pathogen respond to chemical defenses of the host plant that help pathogen to degrade secondary metabolites produced by the host through their action. Plants produce a vast variety of secondary metabolites, many of which have *in vitro* antimicrobial activity. Antimicrobial compounds include preformed substances (phytoanticipins) and compounds that are synthesized upon a microbial challenge (phytoalexins). Phytoanticipins are secondary metabolites that include saponins—avenacin and tomatine. Presence or absence of saponins has been found in correlation with disease development in several plant–fungus interactions. Infection of oats and wheat by the take-all fungu,s *Gaeumannomyces graminis*, is correlated with production of the triterpene saponin avenacin A in the host and the avenacin A degrading enzyme avenacinase (Ava 1) in the fungus. Ava 1 mutant strains are unable to detoxify avenacin; they are non-pathogenic on avenacin A producing oats but are fully pathogenic on wheat which does not naturally produce saponins and on oat mutants defective in saponin production. One saponin, α-tomatine is produced in tomato and has antimicrobial activity against many fungi. The fungus *Septoria lycopersici* encodes the enzyme tomatinase which degrade the saponin tomatine. Disruption of the tomatinase gene did not reduce the pathogenicity of *Septoria* on tomato, because the fungus has other enzymes that can degrade the saponin. Pisatin demethylase is an enzyme produced by the fungus *Nectria haematococca* and degrades the pea phytoalexin pisatin. Pisatin demethylase enzyme is encoded by one to six such genes of the fungus but disruption of the gene caused only a slight change/reduction in pathogenicity. However, disruption of few genes that detoxify another phytoalexin from chickpea resulted in reduction of pathogenicity whereas the insertion of additional copies of the same gene in the pathogen isolates resulted in greater disease severity. Another class of plant protecting molecules is antifungal proteins, also known as PR (Pathogenesis related) proteins. As for the secondary metabolites, these are both preformed and induced antifungal proteins that are assumed to play a role in plant protection by direct toxicity to the fungus and by release of fungal elicitors that may activate plant defenses. Recently a class of glucanase inhibition protein (GIPs) has been characterized in the soybean oomycete pathogen *Phytophthora sojae*. The GIPs are homologous with the trypsin class of serine proteases, but lack proteolytic activity. Structural motifs of protein-protein interaction found in GIPs and high affinity binding of the soybean endo-glucanases EGase A with GIP1 suggest that they may act as glucanase specific inhibitors. Thus the GIPs may represent a novel class of fungal counter defensive proteins that suppress plant defense responses.

Genes Controlling Fungal Toxins

There are several genes, often clustered involved in the synthesis of toxin. For example, disruption of toxin genes in *Cochliobolus* shows that a fungus with an altered toxin profile can still be pathogenic. Similarly disruption of genes involved in the biosynthesis of *Alternaria alternata* host-specific toxin resulted in reduced pathogenicity. Trichothecin—a mycotoxin metabolites are produced by several species of the fungus *Fusarium* (*Gibberella*) and *Myrothecium roridum*. Disruption of the gene that controls the first step of Trichothecin biosynthesis resulted in reduced pathogenicity on most of the hosts. It has been found that up to 11 genes have been found in trichothecin biosynthesis and not all the steps have been fully studied.

Signaling Genes used by Pathogenic Fungi

Fungal signaling genes include the mitogen-activated protein (MAP) kinase gene, cyclic AMP- dependent protein kinase genes and G-protein-coding genes. If these fungal signaling genes are disrupted by mutation, the fungus loses its pathogenicity and as a result of which growth of the fungus, multiplication, and toxin produced is lost or reduced. For example, in case of *Magnaporthe grisea*, three G-protein genes and three MAP kinase genes have been cloned and tested through disruption. Further disruption of the PMK-1 gene of *M. grisea* reduced appressoria formation and lost the ability to infect but had no effect on growth and multiplication of fungus. The CMK 1 gene from *Colletotrichum lagenarium* could complement a PMK mutant of *M. grisea* and could restore its pathogenicity. Disruption of the CMK1 gene also reduced the appressorial formation and pathogenicity when inoculated through injury or wound. The fungi, *Ustilago maydis* and *U. hordei*, are pathogenic only in a dikaryotic state when the two complementary strains mate. The gene loci a and b that control recognition and mating also control pathogenicity. Since Ca^{+2} has been shown to have severe impact on polar growth and differentiation in fungi, it is not surprising that Ca signaling is also involved in pathogenesis. In *M. grisea* recently a cyclophilin gene (CYP1) was identified during a screening for genes with high *in planta* expression level; deletion of CYP1 led to impaired penetration peg formation and appressorium turgour generation. The CYP1 encoded cyclophilin was shown to be the target of the immunosuppressive drug cyclosporine in *M. grisea*; since cyclosporine acts mainly on calcineurin (dependent on a complex formed with cyclophilin), these data strongly suggest that Ca-signaling is involved in early infection processes in *M. grisea.*

Genes Controlling Pathogenicity in Plant Pathogenic Bacteria

Most bacteria do not require adhesion mechanism except when they move through phloem and xylem. For adhesion on the plant surface, the crown gall bacterium, *Agrobacterium* requires genes for the synthesis of cellulose of host surface, and the *att* region of the bacterial genome that contains several genes for attachment and several other genes with homology to genes of mammalian pathogens for adhesion and for pilus biosynthesis.

After the attachment, the bacteria require number of genes for the production of pectinases, cellulases, proteases, and xylanases to degrade plant cell wall. All enzymes are present in forms and each encoded by independent genes. Such large number of enzymes are involved, disruption of the gene encoding anyone of the enzyme is not sufficient to stop cell maceration. For example, *Xanthomonas campestris* pv. *campestris*, the cause of black rot of crucifers have genes for two pectin esterases and polygalacturonases, genes for four pectate lyases, nine cellulases and five xylanases. *X. campestris* pv. *citri* responsible for citrus canker has no genes for pectin esterases, one gene less for pectate lyase and three genes less for cellulases. Since pectin esterases are important in tissue maceration, their absence in citrus canker bacterium and presence in the crucifer rot bacterium may explain the symptoms of the two diseases. Similarly toxins play an important role in parasitism and pathogenesis of plants by several plant pathogenic bacteria. A phytotoxin coronatine produced by *Pseudomonas syringae* functions by suppressing the induction of defense-related genes but it does not seem to be important for pathogenicity by all strains of *Pseudomona*s.

Extracellular polysaccharides (EPS) have been known to play important role in pathogenesis of many pathogenic bacteria by both direct intervention with host cells and by providing resistance to oxidative stress. EPS 1 is main cause of bacterial wilt of solanaceous crops caused by *Ralstonia solanacearum*.

EPS 1 is a polymer consists of a trimeric repeat unit having N-acetyl galactosamine, deoxy-L-galacturonic acid and trideoxy-D-glucose and in its biosynthesis about 12 genes is involved. In *Erwinia amylovora*, the main component of EPS is amylovoran which is regulated by several genes. Disturbance of production of amylovoran eliminates pathogenicity in the mutant bacterium.

Virulence and pathogenicity of some plant pathogenic bacteria, such as *Ralstonia solanacearum*, the cause of wilt and soft rot diseases of solanaceous plants, are regulated by a complex network of which the core is the phenotype conversion (Phc) system. The system consists of gene PhcA, a lysine-rich type transcriptional regulator and the products of the operon phcBRSQ, which control levels of active PhcA depending on cell density or crowding. Cells that have high levels of PhcA produce large amounts of major virulence factors such as extracellular polysaccharides (EPS1) and some exoenzymes and are very virulent and vice versa. So the PhcA gene acts as a switch mechanism that sometimes promotes the expression of one set of genes while repressing another set and other times do the opposite. The levels of PhcA gene is finally controlled by 3-OH palmitic acid methyl ester. The more dispersed the cells, the lower concentration of 3-OH PAME in the cells, the less the activation of PhcA, and the more the activation of genes for siderophores, swimming motility etc.

Example of Molecular Genetics

Magnaporthe grisea, the cause of rice blast has seven chromosomes and a genome size of 40 Mb, with about 9000 genes. The conidium of the fungus contains an adhesive glycoprotein that helps for attachment on the host. The conidium germinates with appressorium formation which penetrates the cuticle and enters the cell. There are large number of genes involved from contact, adhesion and penetration through appressorium.

A hydrophobin protein (MPG1) produced during appressorium formation helps it to recognize hydrophobic surfaces of host cell. Disruption of gene during this process reduces appressorial formation but the application of cAMP to such mutants overcomes this problem, indicating that appressoria formation is regulated through cAMP. Another mechanism of transmission is through receptor Pth11, a membrane protein. If a mutant is missing Pth11, do not form appressoria and hence cannot infect plants. Another gene, magB encodes an inhibitory Gα protein that also affects appressorium development as mutants fail to produce appressorium and infection. Mitogen-activated protein kinases also affect appressorium morphogenesis. After this, appressorium develops internal turgour pressure due to the glycerol in it. In addition, spores contain trehalose which also develops turgour pressure, which seems to be important for infection further. The exact mechanism by which turgour pressure is developed is not known fully yet.

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Questions

1. What are the various mechanisms which bring about variations in fungi? Give examples of diseases in which mutations have been instrumental in increasing pathogenicity.
2. What are heterokaryosis and somatic hybridization?
3. How does the parasexual cycle differ from the normal sexual cycle?
4. Fungi Imperfecti do not have a normal sexual cycle and yet they are quite abundant and successful in nature. Why is this so?
5. What are transformation and transduction?
6. What is physiological specialization?
7. How will you identify the biotypes in *Puccinia graminis tritici*?
8. What is the gene-for-gene concept?
9. Write in brief what you know of host–pathogen specificity. What are the various hypotheses in this regard?

Effect of Environmental Factors and Nutrition on Disease Development

8

The weather and soil conditions influence the seasonal development and geographical distribution of plant diseases. About 90 years ago, Jones (1924) directed increased attention towards the relation of the environment to the inception and development of disease. The effects of environmental factors on plant diseases have been reviewed by Colhoun (1973).The connection between nutrition and susceptibility to parasitic and non-parasitic diseases in plants has been discussed by Karl Boning (1976). The influence of environment on the development and the control of disease has been reviewed by McCartney (1997). Walters and Bingham (2007) have critically discussed the influence of nutrition on disease development caused by fungal pathogens and implications for plant disease control.

The term "environment" connotes all the external conditions affecting the life and development of an organism, which includes temperature, light, and moisture, may also include living factors, such as competing microorganisms or vectors.

The environment can affect the development of plant disease in different ways. It can affect the perpetuation or over wintering of the pathogen from one growing season to the next, the build-up of both primary and secondary inoculum, the dissemination of inoculum, germination, and host penetration. The environment can affect growth development of the host prior to being infected in such a way as to affect its susceptibility. Finally, it can influence the actual development of disease after the host has become infected. The development of all parasitic diseases also obviously varies with the pathogen, host, interactions between the pathogen and host, and environmental factors in the air and soil.

When we take into account the environment, we have to distinguish between macroclimate and microclimate and between weather in general and the particular weather under which specific diseases develop. For example, temperature and humidity in the general atmosphere may be quite different than in the foliar canopy of dense stands of growing plants.

Effect of Temperature on the Development of a Disease

Temperature is undoubtedly one of the most important factors, which influences the occurrence and development of many diseases. The late blight of potato is usually considered a disease which occurs in the northern latitudes, but it can become destructively epidemic when potatoes are grown during winter in the sub-tropics. Some pathogens develop best in low temperature regions. *Taphrina pruni* and *T. deformans* are confined to the cool, wet parts of the globe. Similarly, *Spongospora subterranea*, the powdery

scab, is limited to areas of cold temperature. *Puccinia striiformis* also thrives in cold temperature and in India it over summers at heights of 7000 feet above sea level while black rust survives at 5000 feet above sea level. *Cercospora beticola*, the cause of sugar beet leaf spot, needs a temperature of 15°C for sporulation and for the occurrence of germination and infection. *Urocystis cepulae,* the cause of onion smut, is spreading throughout the USA on infected onion sets. It is absent in the extensive southern onion growing regions, but is an established disease in the cooler, northern onion growing areas of the USA and Europe. Walker and Wellman (1926) had explained this in terms of temperature effects on the pathogen.

On the other hand, there are diseases which occur in the warm belts of the globe, for example, *Fusarium oxysporum* f.sp. *lini, F. oxysporum* f. sp *lycopersici, F. oxysporum* f. sp. *conglutinans* causing wilts of flax, tomato and cabbage yellows, *Erwinia amylovora*, the fire blight bacterium, *Ralstonia solanacearum* causing bacterial wilt of potato, tomato, tobacco, brinjal, pepper, etc. *Sclerotium rolfsii*, various rusts*, Macrophomina phaseoli, Phymatotrichum omnivorum* causing Texas root rot of cotton.

Ralstonia solanacearum which causes bacterial wilt and brown rot of potato is most severe in warm regions. Potato crop raised in winters in the plains is not affected by this disease but the wilt may occur when the crop is planted in January/February. A variety of this bacterial pathogen causes wilt of egg-plants (brinjal) at high temperature. The best development of the disease occurs at 37°C.

The stages in infection are also affected by change in temperature. Temperature can also affect the rate of penetration of the pathogen into the host. In epidemiology this is often quantified as incubation period. Both infection and latent periods are influenced by temperature. Latent periods tend to decrease with increasing temperature, providing other factors are favourable. Latent period for *Blumeria* (*Erysiphe*) *graminis* decreases from about 14 days at 5°C to 3days at 18–25°C. In the case of black stem rust of wheat, the incubation period is 22 days at 4.5°C, 15 days at 10°C, 9 days at 19°C and only 5days at 24°C.

In the sunhemp wilt (*F. oxysporum* f. sp. *crotolariae*), the optimum growth is accomplished at 25–28° C. This coincides with the maximum development of the disease when the soil temperature is between 25°C and 28°C. Above and below this temperature, the disease diminishes.

The seedling blight of maize and wheat are caused by *Gibberella zeae*, but the optimum temperature for the disease is different in the two crops. Blight of wheat is caused at a comparatively higher temperature that is 20–28°C. In the case of maize, severe disease is caused when soil temperature is between 16°C and 20°C. It should be mentioned here that wheat normally grows at lower temperature than maize, and the temperatures which produce the maximum amount of disease of these two crops are actually temperatures (too high for wheat and too low for maize) which are unfavourable for the growth of these two crops. Histological and biochemical studies of roots have shown that at these unfavourable temperatures, the cell walls of the roots remain for a considerable time in the primary pectic condition and are easily penetrated by the fungal hyphae. This is a case in which the influence of temperature on the disease acts mainly through the host.

The incidence of cereal rusts depends mainly on humidity and temperature. The incubation period and the germination of urediniospores of *Puccinia graminis tritici* is favoured by a temperature of 20° C. In the Indian plains this usually occurs in the period between February and March when the weather just begins to warm up. If the weather becomes cloudy and moist during this period, the rust incidence is immediately high, causing huge damage. According to Stakman and Harrar (1957), the time required for the production of urediniospores of *Puccinia graminis tritici* on a susceptible wheat cultivar, can range from 85 days at 0°, to 5 days at 24°C. The incubation period of *Pseudoperonospora humuli* can vary from 3 days at 21–25°C to 23 days at 5°C or 11days at 29°C.

Thielaviopsis basicola which causes black root rot of tobacco is highly damaging when the soil temperature is between 17°C and 23°C but causes little damage when the temperature is between 21°C and 30°C. This has been attributed to the formation of a phellogen layer at the higher temperature, which arrests the spread of the fungus.

Many viral diseases are more virulent when the temperature is between 20°C and 25°C. The most favourable temperature for disease is between 20°C and 25°C; these include the tobacco mosaic virus, potato mosaic virus, cabbage virus, potato yellow dwarf and others.

Late blight of potatoes generally does not occur in the plains of India, where potatoes are grown as a winter crop. At this time, the conditions necessary for the growth of fungi do not exist in plains. But in the hills where the crop is grown in the rainy season, late blight is very severe.

Musk melons are relatively resistant to seedling blight and wilt caused by *Fusarium oxysporum* f. sp. *niveum* when soil temperature is at the optimum of 30°C or higher for the growth of melons. However, the disease is abundant and destructive at temperatures below 27°C which is optimum for the growth of the pathogen. The resistance at high temperatures appears to be due to the greater ability of the host to exclude the pathogen from the vascular bundles by the formation of periderm.

Sunhemp wilt, due to *Fusarium oxysporum* var. *crotolariae* occurs in soils in India when the soil temperature is low—between 19°C and 29°C.

Humidity and Moisture

Atmospheric humidity and precipitation in the form of rain, fog, dew, etc., determine disease incidence to a great extent. Likewise, the amount of soil moisture determines the severity of the disease in soil. The speed of germination of inoculum, its entry into the host and the period of incubation are very much affected by the amount of moisture available. In case of soil diseases the amount of moisture will also affect the soil aeration and the concentration of various salts in the soil.

Downy mildew of grapes is very much favoured by moist weather and high temperature. Similarly, late blight of potato is favourably affected by high moisture content. A large number of diseases are favoured by increased humidity, such as the late blight of potatoes, Koleroga of areca nuts, downy mildew of cereals, blast of rice, early blight of potatoes, scab of apples and circular leaf spot of coffee. Precipitation and dew play their own part in increasing atmospheric humidity and cannot be considered in isolation. Heavy dewfall favours the germination of the spores of the pathogen and infections in diseases, such as downy mildews and rusts.

Soil moisture appears to have a decisive effect on the amount of the big vein disease of lettuce caused by a virus that persists in the soil. In experiments performed, 85% of the plants became infected in soil containing 95% of its moisture holding capacity, and there was a progressive decrease in infection with decreasing moisture, until only 10% of the plants became infected at 35% moisture.

Diseases such as the root rot of pear caused by *Aphanomyces euteiches*, the damping off of seedlings caused by *Pythium ultimun, Pythium debaryanum* and others, the club root disease of crucifers (*Plasmodiophora brassicae*) are greatly increased by an increase in the moisture content of the soil. But on the other hand, diseases like common scab of potatoes (*Streptomyces scabies*) and white rot of onion (*Sclerotium cepivorum*) usually spread under dry condition. According to Beach (1949), the severity of damping off caused by *Rhizoctonia solani* increases with increasing moisture up to 65% of saturation and then decreases abruptly. Moderate soil moisture is most favourable for head smut of Sorghum caused by *Sphacelotheca reiliana* and pea foot rot due to *Fusarium solani* f. *pisi*.

Soil moisture appears to have a decisive effect on the amount of big vein disease of lettuce, caused by a virus which persists in the soil. The disease is favoured with light moisture content up to 85%.

Moist weather (coupled with suitable temperature) favours the spread of rusts and downy mildew in general. The powdery mildews (Erysiphales), such as powdery mildew of pea caused by *Erysiphe polygoni* and mildew of *Dalbergia sissoo* caused by *Phyllactinia corylea*, are not so dependent on moisture and they are known to flourish even in conditions of drought.

Karnal bunt of wheat caused by *Tilletia indica* (formerly known as *Neovossia indica*), which is air borne, occurs as an epiphytotic only when there is heavy rain and air and soil temperature is around 15–20°C two to three weeks before flowering time. The rain water soaks the spores thoroughly and the low temperature promotes germination. The disease is rare in the absence of both these conditions.

Colocasia blight due to *Phytophthora colocasiae* occurs only in the rainy season, especially when there is a continuous spell of moist and cloudy weather. Plants recover as soon as dry condition return. Soil moisture also materially affects the development of disease in Phytophthora foot rot of *Piper betle* and gummosis of *Citrus*.

The moisture content of the air is usually referred to as relative humidity. This is defined as the ratio of the actual amount of moisture in the air to the amount of moisture the air could hold if the latter were saturated at such a temperature. However, since the amount of water the air can hold varies with temperature (warm air can hold more moisture than cold air), relative humidity is not the best measure of moisture in the air when we are concerned with its effect on the pathogen. A better measure is vapour pressure deficit (VPD). The VPD is measured in terms of pressure of atmosphere and is the difference between the actual vapour pressure of water and the vapour pressure of water in a saturated atmosphere.

Lack of atmospheric humidity is also the reason for the relative absence of certain geographical areas of foliage diseases, particularly those of beans, such as anthracnose (*Colletotrichum lindemuthianum*), bacterial blight (*Xanthomonas campestris* pv. *phaseoli)*. Beans are grown for seed in the arid portions of the USA to avoid seed infections by those organisms which require free moisture on plant parts. Late blight of potato seldom occurs in drier areas, except sometimes in those under irrigation.

The powdery mildew fungi, a group of pathogens, are affected by moisture in an unusual manner. Germination of conidia of the lettuce powdery mildew fungus (*Erysiphe cichoracearum*) greatly decreases at 100% relative humidity (VPD = 0 mm Hg), germinates best at 93% RH (VPD = 2 mm Hg), and germinates to some extent at 0.1% relative humidity (VPD = 25 mm Hg). Thus it is not hard to see why powdery mildews develop in the driest of climates. It is believed that the reason these conidia germinate under such dry condition is that they contain about 70% water and they carry their own water supply with them.

Soil moisture does not appear to have the same controlling effect on the development of disease as soil temperature. In fact, in judging the effect of soil moisture, it is difficult to consider this factor in isolation from other soil factors, such as physical condition, water holding capacity, drainage, retentively, and so on. All these factors tend to make up a microclimate which influences the course of disease collectively and not individually.

Diseases favoured by wet soil or high relative humidity are damping off of papaya and vegetable seedlings, foot rot of *Piper betle* caused *Phytophthora nicotianae* var. p*arasitica* or other *Phytophthora* species, collar rot of citrus caused by *Phytophthora citrophthora,* late blight of potato, cereal rusts, downy mildew of bajra (pearl millet), sorghum or maize bacterial blight and streak of rice and many others. Diseases favoured by dry soil or water deficient soils are common scab of potato (*Streptomyces scabies*), seedling blight of cereals caused by *Fusarium* species. Fusarium wilt of tomato, peas and

cotton are more severe in wet soils in comparison to dry soils. There is very close relationship between plant pathogenic nematodes and the amount of soil moisture in the soil.

Soil pH

The effect of alkalinity and acidity of the soil is so pronounced in many cases as to level the incidence and distribution of certain diseases and to be the most important factor in their control.

Alkaline soils are conducive to the spread of the scab of potatoes (*Streptomyces scabies*). It usually develops between *p*H 5.2 and 8.0. Its incidence fails sharply when the soil *p*H falls below 5.2. Successful attempts have been made to control this disease by acidifying the soil with sulphur or sulphuric acid.

The club root disease of crucifers (*P. brassicae*) is favoured by acidic soil. It develops most at about *p*H 5.7 and drops sharply if *p*H is raised so that it totally fails to develop at 7.8. Liming, therefore, helps to control the disease. The influence of *p*H in this case is on the swarming myxamoebae.

Other pathogens which are favoured by the alkaline conditions of soil are Texas root rot pathogen, *Phymatotrichum omnivorum*, which attacks more than 1000 spp. of hosts, *Gaeumannomyces* (*Ophiobolus) graminis*, the take all wheat and *Verticillium albo-atrum* vascular parasite of many kinds of plants. Other diseases which are associated with acidic soils are *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. *vasinfectum* which cause tomato and cotton wilts, *Synchytrium endobioticum* which causes potato wart and *Spongospora subterranea,* the cause of powdery scab of potatoes.

High soil acidity is inimical to the development of tobacco root rot caused by *Thielavia basicola*, while such a condition is highly favourable to wilt of tomatoes. The behaviour of *F. oxysporum* f. *vasinfectum*, which causes the cotton wilt in the USA and India, was studied by Mundkur (1936), who found that the American strain was favoured under acidic condition, while the Indian strain developed best in alkaline conditions. Root rot of cotton caused by *Rhizoctonia destruens* is favoured by light soils with acid reactions; while in India, Fusarium wilt of cotton develops best in heavy alkaline soils. Covered smut of oats develops at a *p*H value of 7.8 and falls sharply with either a decrease or increase in the soil *p*H; it is moderate at 6.5 *p*H.

Although the *p*H of the soil affects the growth and reproduction of some pathogens directly and this determines disease development, it sometimes is only an indicator of certain other conditions or it acts indirectly by affecting certain biological and chemical processes in the soil. Just as plant pathogens are affected differently by soil *p*H, so many saprophytic soil organisms are also affected. Thus, the *p*H may sometimes influence disease development through its effect on soil microflora. A still more common indirect effect probably is its role in determining the availability of nutrient elements. It is well known, for example, that phosphates, calcium, iron and manganese may be present but in insoluble form and therefore, may be unavailable.

Soil Texture

In the black cotton soils which are clayey and heavy, cotton wilt (*F.oxysporum* f.sp. *vasinfectum*) occurs abundantly and root rot due to *Rhizoctonia bataticola* does not occur.

On the other hand, in regions of lighter soils (Gujarat) the case is reversed, that is, only root rot occurs and there is no wit disease. These types of soils are alkaline and the determining influence in this case appears to be texture (clayey or sandy); *Rhizoctonia* cannot survive poor aeration. It is very sensitive to oxygen tension.

Effect of Light

Although light can influence the development of some diseases, it is rarely a limiting factor in their seasonal or regional occurrence. It probably is not usually a determining factor neither in the development of epidemics nor in the geographical distribution of diseases.

Light intensity and length of day, however, may affect the survival of inoculum, prepenetration processes, possibly the entrance of some pathogens' length of the incubation period, abundance of sporulation and sometimes the type of pathogenic effect. Light intensity and day length also affect the development of many host plants and can be the determining factors in their viability as media for the growth of pathogens and their relative resistance or tolerance to pathogenic effects.

Although wheat stem rust develops well at light intensities ranging from 500 to 10,000 foot candles, the incubation period is likely to be shorter and sporulation to be more abundant with increasing light intensity. In experiments on *Melampsora lini*, a flax rust, uredinia appeared 9 days after inoculation when plants were kept under normal light and after 14 days under reduced light, It has been found that high light is most favourable for leaf rust of rye, *Puccinia rubigovera* var. *secalis.*

Light is necessary for photosynthesis and thus the general well-being of the plant is affected by both the quantity and quality of light. Several virus diseases are known to be influenced by light intensity. Reduced light intensities generally increase the susceptibility of plant to virus infection. For example, tomato plants will get infected by sugar beet curly top virus when they are grown under conditions of low light intensity, that is, shaded areas, whereas in full sunlight they will die. Apparently, the concentration of carbohydrate in the leaf affects virus symptom expressions. The intensity of light also affects several fungal diseases. Powdery mildews often develop better under shaded conditions. Although it is difficult to separate the response of the host from that of the fungus to light, it has been shown by Masri and Ellingboe (1966) that low light intensities (25–50 foot candles) favour maturation of appressoria of *Blumeria* (*Erysiphe*) *graminis* while high intensities (7200 foot candles) and darkness inhibit appressorial maturation.

The quality of light may also affect plant pathogenic fungi. Leach (1962, 1967) has shown that the ultraviolet portion of light spectrum initiates and stimulates the sporulation of several plant pathogens for example, *Botrytis cinerea* and *Helminthosporium avenae*.

Sclerotia of *Sclerotinia sclerotiorum*, when placed in moist saw dust and in the dark, only produce apothecial initials and not apothecia. They develop apothecia as soon as they are exposed to light

Ultraviolet light and direct sunlight inhibit the germination of the teliospores of *Puccinia graminis*. If the dishes are covered with green light filters, germination normally occurs. *Colletotrichum lagenarium* (anthracnose of cucurbits) produces perfect stages of the culture if exposed to ultraviolet light. Prolonged exposure to ultraviolet light is harmful to fungus for they have fungicidal properties.

Effect of Oxygen and Carbon Dioxide Concentration

The ratio of oxygen to carbon dioxide in the atmosphere is a almost fixed and generally there is no marked affect of these gases on the foliar pathogens. However, in the soil borne diseases, the soil aeration O_2 and CO_2 are the two gases which, are of importance. The oxygen present in the soil also influences the root growth of host plants and carbon dioxide in the soil also influences the general microbiota and also the growth of roots in the soils of the host plants. The role of available oxygen in the soils also

appears most important in determining the population limit reached by the nematode species. Increased oxygen concentration stimulates activity of larvae of nematodes while reverse is seen when carbon dioxide concentration is increased.

Amount of oxygen is not that important as the concentration of carbon dioxide. *Rhizoctonia solani* infecting various crops is inhibited if the concentration, of CO_2 in soil is more. *Fusarium culmorum* and *Gibberella zeae* cause root rot and seedling blight of wheat. These fungi can tolerate higher CO_2 concentration, plants become weak and increased activity of fungal pathogens causes more diseases. Many species of *Phytophthora* require some oxygen for their growth and zoospore production. The availability of oxygen determines the vertical distribution of fungi in soils which has marked relevance to the occurrence of disease.

Effect of Wind/Air

Most plant diseases are likely to assume large epidemic proportions that are spread either directly by wind or indirectly by insect vectors that can be carried over long distances by wind. Some conidia and sporangia and zoospores are delicate and do not survive long distances transport in the wind but urediniospores and teliospores can be transported by the wind. Wind dispersal is important in disease development if it is accompanied by rain. Windblown rain release spores/bacteria from infected tissue and then carries them through air current and deposits them on wet surfaces of plant and can cause infection immediately. Wind also influences the number of wounds on host plant by rubbing against one another and this facilitates infection in some mechanically transmitted viruses. Winds to some extent prevent infection by increasing the drying of wet surfaces of plants. For example, the inoculum for black rust of wheat builds up in the south India and it is disseminated towards North India through the air currents.

Effect of Herbicides/Fungicides

Use of herbicides/fungicides is common and widespread in agriculture as they not only result in environmental and safety hazards but also leads to development of new strains of pathogens. In many cases, herbicides have been shown to increase of some diseases on crop plants like *Fusarium* wilt of cotton and tomatoes or decrease diseases for example, *Phytophthora* collar rot of many crops. The direct effects of these chemicals may include stimulation or inhibition of the growth of the pathogen or increase/decrease in the population of beneficial microorganisms specially arbuscular mycorrhizal fungi. The higher dozes of these chemicals generally suppress the activity of soil microflora or alteration of microclimate of the crop plant canopy by changing humidity.

Effect of Air Pollution

As pathogens and infected parts of the plant are both exposed to the air pollution, it is not clear whether a particular air pollutant causes an increase or a decrease of a disease caused by the pathogen. For example, the rust of wheat and oats, ozone reduces hyphal growth, uredinial growth and number of urediniospores on the infected leaves whereas with the powdery mildew of barley the rate of infection is increased if exposure is early. With non-obligate parasites, ozone may increase the disease percentage on

wheat by *Drechslera* fungus. Similarly, the bacterium *Pseudomonas glycinea* infecting soybean caused a smaller number of lesions on plants exposed to ozone in comparison to unexposed ones.

Influence of Nutrition on Disease Development

Mineral nutrition can exert a profound effect on disease development. With fertilizer application increasing or decreasing, effect on disease development has been encountered. The outcome of this interaction is dependent on many factors like species of plant, plant growth stage, type of pathogen and the effect of environment on plant and fungus.

Nitrogen Nutrition and Disease Development

Nitrogen is important for plant growth and development and crop plants obtain it from the soil as nitrate or ammonium and legumes by symbiotic nitrogen fixation (Lea *et al.,* 2007). Depending on the plant species, nitrate reduction can take place in shoot, root, or both. Therefore, an invading pathogen will encounter different forms of nitrogen in plant tissue ranging from inorganic nitrogen like nitrate ranging through organic nitrogen like glutamine. Once the pathogen has entered the host, the nitrogen sources available to it will depend on tissues being colonized. For example, a pathogen infecting ears of cereals may have different nitrogen sources compared to those infecting the roots, stem and leaves of the same plant. Application of nitrogen fertilizers above the recommended dozes can lead to significantly greater disease incidence for biotrophic pathogens like powdery mildews, rusts (*Puccinia recondita*) and for necrotrophic pathogens like rice blast fungus (*Magnaporthe grisea*). On wheat, leaf spot caused by *Septoria tritici* and leaf blotch caused by *Stagonospora nodorum* have been found to increase with increasing nitrogen application (Simon *et al*., 2003). Nitrogen has also been shown to increase the severity of *Fusarium* Head Blight (FHB) in wheat caused by *Fusarium graminearum* and F. *culmorum*. Lemmens *et al.* (2004) showed that increasing nitrogen supply increased the intensity of FHB and also increased the concentration of mycotoxin deoxynevalenol in wheat grains. Recent work on yellow rust of wheat suggested that early N application lead to larger shoot number and leaf area and rust was more severe than receiving less nitrogen. Applying nitrogen later has had no effect on vegetative growth but did increase symptoms compared to control (Neumann *et al.*, 2004). The influences of nitrogen supply on plant disease are inconsistent, for example, several studies have reported no effect of nitrogen on the severity of leaf spot caused by *Septoria* (Oleson *et al*., 2000). There was no effect of nitrogen on susceptibility to the vascular wilt pathogen (*Fusarium oxysporum* f.sp. *lycopersici*) while susceptibility to powdery mildew fungus *Oidium lycopersicum* and bacterial pathogen *Pseudomonas syringae* pv. *tomato* increased with increasing nitrogen concentration (Hoffland *et al*., 2000).

Sulphur Nutrition and Disease Development

The most important source of sulphur (S) for plants is sulphate taken up by roots and then distributed mostly unmetabolised throughout the plants. The compounds synthesized as a result of S metabolism include glutathione, phytoalexins, glucosionolates and H_2S (Hydrogen Sulphide).

Sulphur is also important for fungi like *Neurospora crassa* which prefer the sulphur containing amino acid, methionine, and cysteine but when these are not available they can use inorganic sulphur, proteins, or aromatic sulphates. Some obligate biotrophs can be cultured, like rusts can be cultured axenicaly

when sulphur containing amino acids are supplemented. It has been known that foliar applied elemental sulphur has fungicidal effect. Indeed, sulphur was recommended for control of diseases in 19th century and by the early 1900s. Bearchell *et al.* (2005) found that abundance of wheat pathogens *Phaeosphaeria nodorum* and *Mycosphaerella graminicola* were correlated with changes in SO_2 emissions. The abundance of *P. nodorum* increased with increasing SO_2 emissions while the opposite was true for *M. graminicola.* If inorganic sulphur is assimilated into organic sulphur (cysteine), a number of sulphur compounds associated with plant defense like glucosionolates, phytoalexins and H_2S can be formed. The cysteine concentration has been shown to increase in resistant plants in response to attempted infection (Vidhyashekhran, 2000). Further cysteine accumulation can be catabolised into H_2S, which are involved in plant defense.

Recently, Bloem *et al.* (2004) found that Sulphur fertilization increased total sulphur, organic sulphur and cysteine in oilseed rape grown in field while infection with *Pyrenopeziza brassicae* increased.

Phosphorus (P) Nutrition and Plant Disease

The orthophosphate ion ($PO4^{-3}$) is critical for all the cells as it is incorporated into many important molecules like nucleic acids phosphoproteins, phospholipids, and energy currency molecules like ATP. In general P tended to improve plant health, reducing plant disease in majority of the cases but in some cases increased pest infection or disease has also been reported. Brennan (1995) found that severity of take-all on wheat caused by *Gaeumannomyces graminis* decreased with increasing P application while Sweeney *et al*. (2000) reported that P has moderately suppressive effect on wheat leaf rust. The effect of P on plant disease may be due to the following mechanisms (Perrenoud, 1990):

1. Direct effect of pathogen development, survival and multiplication.
2. Direct effect on plant metabolism which in turn will affect the pathogen food supply.
3. Effect of plant defenses and stomatal function, thus affecting pathogen establishment to the host and spread in the plant.

Gottstein and Kuk (1989) reported that dibasic and tribasic phosphate salts could induce systemic protection against Anthracnose in cucumber caused by *Colletotrichum lagenarium.* Foliar application of phosphate salts has been found to induce disease resistance in cucumber (Descalzo *et al*., 1990), maize (Reuveni, 1995) and barley (Mitchel and Walters, 2004). Orober *et al*. (2002) reported that phosphate mediated resistance induction in cucumber was associated with localized cell death, preceded by rapid generation of superoxide and hydrogen peroxide while in barley resistance was associated with increased activities of phenylalanine ammonia lyase (PAL), peroxidase and lipoxygenase (Mitchel and Walters, 2004).

As for phosphate induced resistance, it has been suggested that foliar applied phosphate could be used as part of an integrated disease control programme in future.

Potassium (K) Nutrition and Disease Development

Potassium uptake by the plants is highly selective and is closely coupled to metabolic activity. Potassium is widely reported to decrease disease severity in many plants. In many cases, it has been seen that potassium deficient plants tend to be more susceptible to the pathogens than the plants receiving a proper and adequate supply, for example, in rice the severity of infection by *Helminthosporium sigmodeum* was maximum at 0 potassium supply while decreased significantly as potassium application was increased (Ismunadji 1976) and similar results were reported with powdery mildew fungus *Blumeria*

(*Erysiphe*) *graminis*. Scientists have shown that effect of Potassium applied as KCl fertilizer may be due to chloride (Cl^-) rather than (K^+). Sweeny *et al*. (2000) reported that the reduced disease severity and increased yield with potassium fertilizer might have been because of chloride in KCl fertilizer.

Silicon (Si) Nutrition and Disease Development

Silicon is one of the most abundant elements in lithosphere and most soils contain considerable amount of it. According to Datnoff *et al.* (1997), continuing cropping pattern can deplete Si available in the crop making application of Si fertilizers necessary in order to maintain crop productivity. Siliceous fertilizers have been reported to increase wheat yield by 9% (Liang *et al*., 1994). Silicon application has been reported to suppress diseases in cucurbits caused by foliar and soil-borne pathogens and reduces susceptibility of rice to large number of pathogens. Recently Rodgers-Gray and Shaw (2004) showed that soil amendment with silicon reduced powdery mildew infection in wheat. Large numbers of workers have suggested that effect of Si in providing disease control because of the formation of mechanical barriers to penetration (Kim *et al.,* 2002). A number of studies have suggested that silicon activates plant defense mechanisms. Silicon mediated resistance in rice to *Magnaporthe grisea* was reported to be associated with accumulation of antimicrobial compounds at infection site including phytoalexins (Rodrigues *et al*., 2004).

The relation between nutrition and susceptibility to parasitic and non-parasitic diseases in plants has been discussed by Karl Boning (1976). We can distinguish three main types of reactions in the behaviour of the host plants towards the parasite. These are susceptibility, resistance and reduced receptivity.

These different kinds of reactions to parasite attack are correlated in the following way with the different forms of nutrition. Susceptibility is usually most evident where there is potash deficiency, resistance where there is nitrogen deficiency and reduced receptivity where there is phosphoric acid deficiency. When one nutrient is given in excess there is also a relative deficiency of the two other main nutrients. Usually, however, only one deficiency factor predominates and it behaves in line with the excess factor as regards the kind of reaction involved. Thus, one-sided nitrogen excess usually reacts in a susceptible way as in the case of potash deficiency, although, of course, where nitrogen feeding is very one-sided behaviour may also resemble that associated with phosphoric acid deficiency. The relative phosphorus deficiency component acts in a similar way, as resistance and reduced susceptibility frequently have similar effects. Excess phosphorus tends to behave more or less like nitrogen deficiency although the potash deficiency component may also show itself. Generally, we find that nitrogen and potash determine the reaction of the plant to the disease organism more than phosphoric acid does. This may have something to do with the fact that phosphoric acid deficiency represents a combination of two excess factors that act in a contrary manner. The effect of different levels of application of lime is considerably less than that of different levels of nitrogen and potash.

Nitrogen abundance results in the production of more vegetative and succulent growth of the plants. It is known that fertilizer application with large amounts of nitrogen increases the susceptibility of the pear to *Erwinia amylovora*, cause of fire blight, rusts of wheat caused by *Puccinia* species and powdery mildew. While reduced availability of nitrogen increases the susceptibility of tomatoes to Fusarium wilt, early blight of potato and tomato caused by *Alternaria solani,* wilt of solanaceous plans caused by *Ralstonia* (*Pseudomonas) solanacearum* and damping off of seedlings due to *Pythium* species.

Not only the amount of nitrogen but the form of nitrogen has its bearing on certain diseases. For example *Plasmodiophora brassicae, Sclerotium rolfsii* and *Pyrenocheata lycopersici* are more serious

when ammonium nitrogen fertilizer is applied; on the other hand *Gaeumannomyces graminis, Phymatotrichum omnivorum* and *Streptomyces scabies* are more serious when nitrate nitrogen is supplied in the soil. The effect of each nitrogen form appears to be related to soil *p*H influences (Agrios, 1997). Phosphorus has been shown to reduce the severity of take-all disease of barley caused by *G. graminis* and potato scab caused by *Streptomyces scabies*. Potassium reduces the severity of several diseases. Stem rust of wheat, stalk rot of corn are reduced if more of Potassium is available to the plants. However blast disease of rice caused by *Pyricularia grisea* and root knot of nematodes are increased due to higher amount of Potassium.

Calcium reduces the severity of several diseases such as diseases caused by *Rhizoctonia, Sclerotium* and *Fusarium* species. But black shank of tobacco and common scab of potato are enhanced by Calcium. Micronutrient applications have tremendous impact on the occurrence of several diseases such as copper application enhances take-all, and ergot diseases caused by *G. graminis* and *Claviceps purpurea*. Addition of silicon in the nutrient solutions has been shown to reduce the blast of rice and brown leaf spot of rice.

Role of Biotic Environment

For the foliar pathogens, the phyllosphere is the region which influences the disease in the aerial parts and for the root diseases it is the rhizosphere which greatly influences the activity and growth of several pathogens. Phylloplane is natural habitat on the leaf surface which supports a heterogenous population comprising of both pathogens and non-pathogens. Last (1995) termed the niche as phyllosphere (now known as phylloplane). The phylloplane microbes are of special interest from various points of view. For example, some of them have antagonistic action against fungal pathogens, degrade plant surface wax and cuticles, produce plant hormones, decompose plant material, activate plants to produce phytoalexins, and influence growth behaviour and root exudates of plants. Thus, phylloplane exerts a great influence on the diseases caused by aerial plant pathogens. Andrews (1990) has reviewed the work on biological control in the phyllosphere.

The microbiota in the soil influences the growth of plant roots as well microbial population in the rhizosphere or rhizoplane. A rich flora and fauna of soil microbiota lessens the danger of epiphytotic outbreaks of diseases caused by soil-borne pathogens. There are antagonistic and associative effects on the plant pathogens in the soil. This aspect has been discussed at great length in the chapter on root diseases (Chapter 20).

Role of Environmental Factors in Epiphytotics

A plant disease is called as epidemic or epiphytotic when the amount of disease present increases rapidly from a low level to a high level. In an endemic disease the disease level remains fairly constant. The science dealing with this area is called epidemiology. Environmental factors which predispose the plants to infection and leading to the occurrence of epiphytotics are temperature, light, relative humidity and moisture, wind and the presence of inoculum or introduction of a new pathogen species or race. Thus, a combination of environmental factors both at macro and micro level has great relevance in the causation of plant disease epiphytotics. These have been discussed at the appropriate places in the chapter 10.

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Questions

1. Give examples of plant diseases which are influenced by soil temperature.
2. Why does late blight of potato generally not occur in the plains of northern India?
3. How does *p*H of soil influence the occurrence of a disease? Give examples of diseases which occur:
   (i) in alkaline soils
   (ii) in acidic soils.
4. Name the environmental factors necessary for the development of Karnal bunt of wheat.
5. Explain why wilt of cotton occurs in clayey soil while root rot generally occurs in sandy or loamy soils of India?
6. Give examples of diseases which are influenced by light condition.
7. How does nutrition affect the incidence of diseases?
8. Name some plant pathogens which require high relative humidity throughout their development.

9. Name some plant pathogens which can cause infection at low relative humidity.
10. Give an example of a disease in which high levels of phosphorus bring about resistance in the plants.
11. How do phylloplane and rhizosphere organisms influence diseases in plants?
12. How do the following affect the incidence of disease?
    (i) Effect of wind/air
    (ii) Effect of Herbicides/Fungicides
    (iii) Effect of air pollution

Survival and Dispersal of Plant Pathogens

9

There are two important events of any plant disease cycle, namely (i) the ways in which the pathogen survives between cropping seasons or when the environmental conditions are unfavourable, and (ii) the means by which it spreads from diseased plant to healthy plant. If survival or spread of the pathogen is prevented, the disease will not occur. Thus, a fair knowledge of survival and dispersal mechanisms often reveals a weak link in the disease cycle that can be utilized in managing the disease.

Pathogens of perennial plants may be permanently present in or on plant tissue but not necessarily in active state; but in annual crops, pathogen must be capable of surviving during the intercrop season when host plants are absent. If the pathogen does not possess mechanism that enables it to survive during intercrop periods or during periods when the environment is unfavourable, the disease cycle will be broken and the pathogen will not survive.

Survival of Plant Pathogens

Most plant pathogens can survive in nature by repeatedly reinfecting host plants. This process of repeated infection is called as an infection chain. If the infection chain is broken the pathogen dies out. The Swiss plant pathologist, Ernst Gaumann (1950), in his book *Principles of Plant Infection* divided infection chain into two types depending on how the inoculum of the pathogen survives between leaving the infected host and arriving on the host to be infected, i.e., from the distributor host to the receiver host. The infection chain is divided into two categories:

1. **Continuous infection type**: It is further divided into two types
   (i) Alternative host
   (ii) Alternate host
2. **Discontinuous infection type**: It is further divided into three types
   (i) Epiphytic phase
   (ii) Saprophytic phase
   (iii) Resting phase

Continuous Infection Chain Involving Same or Alternate Host

In this process, the pathogen survives by continually infecting plants of the same host or plant species other than the main crop species. Such plants are called alternate hosts and are generally related to the

crop species. If the alternative host, which is often a weed species, does not show symptoms of disease, it called a disease carrier. The dependence of pathogen on the alternate host is important because the pathogen does not form any resting structures nor does it survive saprophytically in nature. In tropical regions, where round the year growing of plant species such as rice and root crops like cassava, sweet potato, taro, etc, is a common practice, the continuous presence of host plants enables pathogens to survive by continuously infecting new plants/hosts. In temperate regions, self sown volunteer crops plants (weeds) which are growing along roadside, irrigation channels enables pathogen to survive during intercrop period and serves as source of inoculum when the main crop is grown. For example, in case black rust of wheat caused by *Puccinia graminis tritici*, barberry is the alternate host while wheat is the main host. In case of black rot bacterium of Brassicas caused by *Xanthomonas campestris* pv. *campestris*, the pathogen can survive on related weed species as wild reddish. Some plant pathogens can not be transmitted from an infected plant to another plant without vector. The vector therefore acts as an alternate host and the infection chain alternates between the two unrelated species, i.e., plant–insect vector–plant. If the alternate host (the vector) is absent, the infection chain is broken and disease will not occur.

Discontinuous Infection Chain Involving an Epiphytic Phase

Many plant pathogenic fungi and bacteria survive on the surface of their host and other host/plants as epiphytes. For example, the peach leaf curl caused by *Taphrina deformans* develops yellow to reddish areas, causing the leaf to curl and drop prematurely. Naked asci containing ascospores are produced on the infected curled leaves. These ascospores germinate to form budding that can only infect young leaves which are unavailable at the time when the budding colonies are formed. The fungus survives during the summer months and through the winter months as an epiphyte on the bark. In the following spring season, when the leaf buds swell and young leaves begin to emerge, the budding cells infect the young leaves and form mycelium in undifferentiated host tissue. The fungus will produce asci in the early summer and life cycle of the pathogen is completed. Collection and burning of fallen leaves is recommended for elimination of the source of primary inoculum.

Discontinuous Infection Chain Involving Saprophytic Phase

Several plant pathogenic fungi and bacteria survive during intercrop period as saprophytes on diseased plant, debris, or on soil. For example, the apple scab caused by *Venturia inaequalis* infects apple leaves and fruits during spring and summer. The fungus overwinters as pseudothecial initials in infected leaves and fruits that fall to the ground during autumn. The saprophytic stage is considered the major source of survival of the pathogen during dormancy of the host in winter and provides the primary source of inoculum in the form of ascospores for primary infection of new leaves and flower buds in the spring season. This initiates the active parasitic stage by forming lesions on the leaves in which large number of conidia are produced. Fallen leaves bearing stroma of ascocarp development are the main source of survival of the pathogen. Attempts should be made to destroy this source in the orchards.

Other plant pathogens like *Agrobacterium tumefaciens* causing crown gall, *Streptomyces scabies* causing common scab of potato and take all disease of wheat incited by *Gaeumannomyces graminis* var. *tritici* and several species of *Fusarium, Pythium and Rhizoctonia* can complete very effectively as saprophytes with the normal soil microflora.

Discontinuous Infection Chain Involving a Resting phase

Plant pathogenic bacteria, viruses, and phytoplasmas don't form resting structure and can survive by means of continuous infection chains, but fungi and nematodes often form resting structure that enables them to survive for long periods in the absence of suitable host or when the environmental conditions are unfavourable. The resting spores of *Spongospora subterranean* (the cause of powdery mildew of potato) and *Plasmodiophora brassicae* (the cause of club rot of Brassica) can survive in soil for many years. Moreover, the spores of both species are stimulated to germinate only by exudates secreted from the roots of certain plant species reducing the probability of spores germinating in a "no win" situation when hosts are absent. The downy mildew produce oospores, the rusts and smuts form teliospores, many fungi produce sclerotia (e.g., *Sclerotium rolfsii, Sclerotinia sclerotiorum*) which can survive in soil or in infected plant debris for periods ranging from months to years, depending on the species.

In case of loose smut of wheat caused by *Ustilago segatum*, infect developing wheat embryos during flowering enter into a dormant state and resume activity at a later stage of host development. The fungus survives as dormant mycelium within the embryos of the seed. When infected seed germinates, the pathogen is activated and with the passage of time the inflorescence is replaced by masses of smut teliospores. Drying of seeds in summer when temperature is 40˚C or more is the best method to kill the dormant mycelium present in the wheat seeds. Alternatively, treating the seeds with a systemic fungicide before sowing will eliminate seed borne infection.

Distribution implies the spread of plant pathogens into new geographical areas and its establishment there. The spread of a plant pathogen within the general area in which it is established is termed dissemination or dispersal. Contamination—the movement of inoculum (even if only a few inches) to susceptible plant—is a kind of dissemination. Knowledge of the methods by which pathogens are dispersed is usually essential before effective control measures can be devised. There are essentially two types of dissemination of plant pathogens:

1. Direct transmission
2. Indirect transmission

In direct transmission, the dispersal takes place along with the seeds and vegetative parts, while indirect transmission may be autonomous, that is, by wind, water, animals or human beings.

Autonomous dispersal by plant pathogens had been discussed by Muskett (1960), dispersal of inoculum by insects and other animals including man had been discussed by Broadbent (1960) and liberation and dispersal of fungal spores have been ably discussed by Ingold (1971) in his book, *Fungal Spores*.

1. **Direct Transmission**
    a. Germinative Transmission
    b. Vegetative Transmission
    c. Adherent Transmission
2. **Indirect Transmission**
    a. Autonomous Transmission
    b. Wind dispersal of pathogens-Anemochory
    c. Water dispersal of pathogens-Hydrochory
    d. Animal dispersal of pathogens-Zoochory
    e. Human dispersal of pathogens-Anthropochory

On more classification is that into continuous and discontinuous transmission

Continuous Transmission
Transmission of disease is termed as continuous when it occurs naturally by way of growth, multiplication and spread of the pathogen in an area or country where the disease is established.

Discontinuous Transmission
Sometimes the disease gets introduced into a new area, locality or country, where the disease has never occurred by the agency of man. Such transmission is unnatural and regarded as discontinuous transmission.

Direct Transmission

It occurs when the pathogen is carried externally or internally in the seed or planting material like tubers, bulbs, cuttings etc.

Germinative Transmission

This takes place along with the seeds or propagules. Bean mosaic is transmitted through pollen grains and carried in the seeds. In the case of loose smut of wheat and barley caused by *Ustilago segetum* (*U.tritici*) and U. *nuda* respectively the seeds get infected without producing any symptoms in the parent carrier. The seeds carry the intraseminal mycelium. When such apparently healthy looking seeds are sown, the symptoms appear when the ears emerge. The pathogen develops systemically inside the plants. Alternaria leaf blight of wheat is both internally as well as externally seed borne (Prabhu and Prasada, 1966). The Helminthosporium blight of wheat and barley is seed borne. The seed-borne infection is present in the form of mycelium within the seed including the endosperm and embryo. The fungus has been found to remain viable up to seven years on barley seed (Christensen, 1922). Bacterial blight of paddy caused by *Xanthomonas oryzae* pv. *oryzae* is believed to be seed borne.

Vegetative Transmission

Transmission of a large number of plant pathogens takes place through the vegetative parts of plants used as seed, such as tubers, cutting, runners, grafts, etc.

Ring rot of potato caused by *Corynebacterium sepidonicum* is carried through infected potato tubers to newer areas as in the case of the pathogen *Ralstonia* (*Pseudomonas*) *solanacearum* responsible for brown rot of potatoes. A large number of viruses are transmitted through vegetatively propagated parts.

Dormant mycelium of *Phytophthora infestans* in carried through the potato tubers. Seed setts of sugar cane carry the pathogens and when such apparently healthy looking vegetative parts are sown, the pathogen develops systemically. Whip smut of sugar cane caused by *Ustilago scitaminea* and red rot of sugar cane caused by *Colletotrichum falcatum* are "seed"-borne (i.e., through the diseased cane setts).

Adherent Transmission

In this case, the propagules of the pathogens are carried over the surface of the seed or vegetatively propagated parts. Many pathogens are present on or in seeds, transplants, hard woods or nursery stock and are disseminated by them as the latter are transported to other fields or areas or are sold and transported

to other areas far and wide. Bunt of wheat caused by *Tilletia foetida* and *T. caries* is carried through the seeds externally, as in the case of covered smut of barley, where the smut spores attach themselves to the surface of the seeds at the time of threshing. There are many other fungi whose spores remain adherent, such as *Synchytrium endobioticum* responsible for potato wart and species of *Helminthosporium, Rhizoctonia solani* (black scurf) sclerotia on the tuber surface of potato and *Alternaria* which causes various diseases.

Indirect Transmission

It occurs when the pathogen is transmitted by natural agencies like wind, water, insects, nematodes, mites, birds, animals etc.

Autonomous Transmission

Autonomous dispersal has been discussed in great detail by Muskett (1960). Plant pathogens can be spread to short distances by this method. Autonomous transmission takes place by active growth of hyphae or hyphal strands. It is characteristic of wood rotting fungi, such as *Armillaria, Fomes,* and *Ganoderma, Polyporus* spp. belonging to the Hymenomycetes which migrate independently through the soil from plant to plant or even from field to field by the active growth of their hyphal strands.

Armillaria mellea rhizomorphs spread from one root to the other in forest trees. Hyphal mats or strands grow out to short distances in the soil from the sclerotia of *Rhizoctonia solani* and *Sclerotium rolfsii*. The rate of such spread in *Phymatotrichum omnivorum,* responsible for Texas root rot of cotton crop is estimated at 5–30 feet per season and 2–8 feet per month in alfalfa crop. It may be emphasized that in *Armillaria mellea*, the cause of the tree root disease, *Gaeumannomyces* (*Ophiobolus) graminis*, the cause of the take all disease of wheat, *Phymatotrichum omnivorum*, the cause of root rot of cotton, the pathogens do not grow and ramify through the soil as do the non specialized types, but require a food base such as the roots or crop debris of the host plants which they attack.

Wind Dispersal of Pathogens

This type of spread is termed by Gaumann (1950) as “Anemochory”. Some plant pathogenic bacteria are carried to short distances by the wind. The seeds of some angiospermic plant parasites are effectively carried by the wind. All fungal pathogens which produce spores or conidia on the surface are disseminated by the wind, such as downy mildews, powdery mildews, ruts smuts, leaf spot causing pathogens, sooty molds, etc. The pathogen must produce numerous spores which are successfully liberated, dispersed, and deposited in a viable condition on susceptible plants under condition conducive to infection; in other words production liberation, dispersal, and deposition of spores are involved.

Many fungal pathogens are remarkably well adapted to wind dissemination as they produce and liberate into the air almost countless numbers of small and very light spores that are carried to long distances.

Propagative spores are produced in many different ways in sporangia on simple or complex conidiophores in or on ascocarps and basidiocarps, by budding or fusion, within pycnidia, perithecia or sori of various types, and in other ways as described in books on mycology and plant pathology (Stakman and Harrar, 1957; Hughes 1953; Hawker, 1960). Fungi show a great diversity in their methods of producing propagules and many different ways of more efficient production and liberation of spores seem to have

been explored. Spores production per unit area of infected leaf tissue may be increased by close aggregation of sporophores, branched or whorled sporophores, production of spores in chains or clusters, by a more or less continuous production of spores within the sorus or by successive crops of spores. The number of spores produced can be astronomical. Powdery mildews may produce several thousand conidia per square centimeter of infected leaf surface, a fairly, modest output compared with 1,00,000 or more in some downy mildews. A single smut sorus may contain millions of spores, a heavily infected barberry bush is said to produce up to about 70,000 million aeciospores of *Puccinia graminis* at one time (and there may be several crops of spores in the spring), and a relatively small apothecium of *Sclerotinia* can produce about of 30 million ascospores. The spores produced by large polypores, such as *Fomes* are even more numerous. Heavily rusted wheat fields produce so many spores that spore clouds may be seen rising during harvesting and implements too may be layered with a fine reddish deposit of spores.

The duration and periodicity of sporulation is as important as the number of spores. Some pathogens produce a more or less simultaneous crop of propagules followed by other crops, as in the case of sporangiophores of *Peronospora.* The sporophores of others may bear several successive propagules, for examples, those of *Phytophthora*, *Cercospora*, etc. The capacity to produce a steady stream of infective propagules over a prolonged period of time is likely to be advantageous to the pathogen. Some fungi show a diurnal periodicity in sporulation, as nocturnal production of sporangia by some species of *Sclerospora*. This is probably chiefly due to temperature moisture effects, but might in some cases be related to periodicity in susceptibility of the host plant. Similar periodicity in spore discharge (Hirst, 1953), and in the germination and formation of appressoria (Yarwood, 1936) has been reported

Apart from spores, bits of mycelium and nematode cysts are also sometimes disseminated by wind. In India the molya disease of wheat and barley (*Heterodera major*) is believed to have been introduced into Haryana from Rajasthan.

According to Schrodter (1960), the problem of dissemination of plant diseases where propagules cannot move themselves is more amenable to theoretical treatment than any other problem in phytopathology. Fungal spores in air behave as inert particles, perhaps similar to those in smoke, with terminal velocities ranging from about .05 to about 2.5 cm per second, the larger spores falling more rapidly than small ones.

Although fungus spores are generally more abundant in the air near the earth than at high altitudes, several investigators have encountered clouds of spores, numerous bacteria, and other minute objects several thousand feet above the earth. Urediniospores of *Puccinia graminis tritici* have been caught as high as 14000 feet above infected grain fields, living spores of various fungi were caught from aeroplanes above the Carribean sea 600 miles from their nearest possible source and living spores of several common molds were caught in a spore trap released from the balloon Explorer II at 72,500 feet and set to close at 36,000 feet.

Long distance dissemination of rusts is a well established phenomenon. In the Indian subcontinent, rust spores are known to makes big jumps from the source areas to the plains (Mehta, 1940; Nagarajan and Singh, 1974, 1975).

Studies by Nagarajan and Singh (1973, 1974, and 1975) have shown that for south and central India the spores are wind-borne, rain deposited, and are transported from an altitude of around 700 mb (300 m). The urediniospores get transported by upper winds from the Nilgiri and Palney hills and are then washed down over central India by rain. Such a spread occurs to a distance of 600 km or more without infecting the fields in between.

Turbulence redistributes spores and affects the progressive dilution of spores with increasing distance

from their source. There is ample evidence that with normal wind and turbulence conditions, spores especially small ones can travel considerable distance. Those of *Phytophthora infestans* have been reported by various observers to travel distance of 200 m to more than 60 km depending upon the wind degree of turbulence.

An interesting example of wind dispersal is in case of fire blight bacterium (causing diseases in apples and pears). The pathogen produces fine strands of dried bacterial exudate containing bacteria, and these strands may be broken off and disseminated by wind.

Water Dispersal of Pathogens (Hydrochory)

Water as an agent of dissemination appears to be relatively less important as compared to wind. Although it sometimes facilitates dissemination in various ways, does not furnish the motive power for long distance dissemination, except as streams and rivers. Flood and irrigation water may carry inoculum, especially that in or near the soil. It is clear that water separates and distributes spores in a microenvironment. Spores that are extruded in gelatinous tendrils that may harden when dry can be separated from each other and washed down trees and other kinds of plants during rain or heavy dews. The splashing and spattering of water during heavy rains may result in distributing inoculum to plant parts near the soil and may distribute bacteria and spores to different parts of the same plant or neighbouring plants.

Bacteria, nematodes and spores and mycelial fragments of fungi present in the soil are disseminated by rain or irrigation water that moves on the surface or through soil.

In some places, rain seems to be more important than insects in disseminating the fire blight bacteria, *Erwinia amylovora*, by its washing or spattering effect. The secondary spread from initial infection of *Xanthomonas campestris* causing black rot of cabbage and other crucifers, of *X. translucens* which attacks cereals and grasses and of bacterial blight of tomato, *Corynebacterium michiganense*, results largely from splashing during rains.

Xanthomonas campestris pv. *malvacearum*, the bacterium that causes angular leaf spot of cotton, can be spread by wind blown rain.

Surface water resulting from rains or irrigation is known to disseminate some pathogens and undoubtedly a large number of spores. The bacterium causing angular leaf spot or black arm of cotton is spread largely by irrigation water or surface water in Arizona, USA, and Uganda. *Plasmodiophora brassicae*, the organism causing club root of crucifers, is spread by surface water, and *Phytophthora infestans*, causing late blight of potatoes, has been observed to spread from plant to plant in dry areas by means of irrigation waters.

Colletotrichum coffeanum which produce spores within the acervuli in the maturing bark of coffee twigs and on diseased berries is disseminated by water.

Water, then is an important agent of local dissemination, but it is not as important as wind for long distance dissemination.

Large drops of water in the form of rain or overhead irrigation, failing at their terminal velocity, are probably most active in splash dispersal, much more so than small drops. In splash dispersal, the spores are carried and often deposited on the plant surface in a droplet of water which could provide the moisture required for their especially in humid weather. Splash dispersal, although usually considered a mechanism for liberating and dispersing slimy spores, may also play a part in the dispersal of roughened spores which adhere strongly to each other or the plant surfaces. Examples of this provided by *Hemileia vastatrix* (coffee rust) in which, according to Nutman *et al.* (1960), the roughened urediniospores cling

together and are broken up by water. They then float singly to the surface of the water drop, from which they are splash dispersed by further rain drops. The cells of many plant pathogenic bacteria and the slimy spores of such important genera of fungal plant pathogens as *Septoria, Fusarium* and *Colletotrichum* are splash dispersed and it is surprising that relatively little information on splash dispersal is available.

The eelworm diseases of potato and *Piper longum* have been known to be carried through surface water from field to field. The spread of infection in the red rot of sugar cane caused by *Colletotrichum falcatum* and the dissemination of the sclerotial bodies of *Sclerotium rolfsii* through soil are chiefly brought about by irrigation water. Water is also an important agency of dissemination for the seeds of flowering parasites such as *Orobanche,* dodder (*Cuscuta*) and *Striga* through the soil. Short distance transmission of semi-aquatic rains accompanied by wind such as for *Phytophthora arecae, P. parasitica, P. infestans* and other downy the formation of deciduous conidia capable of germinating ether by zoospores or germ tubes and thus develops into dangerous pathogens capable of widespread damage within a short period.

Dissemination by Insects, Mites, Nematodes and Birds

Insects Since the discovery by Waite (1891) that bees and wasps can transmit bacteria causing fire blight of apple and pear, *Erwinia amylovora*, much information has been accumulated on the role of insects and other small animals in the dissemination of plant pathogenic viruses, bacteria, and fungi. Many small animals help in the dissemination of some pathogens occasionally and incidentally but insects fall into a special category.

Insects not only transmit and disseminate many plant pathogens but are sometimes agents of inoculation also. Moreover, some pathogens multiply within insects and some are present in them during periods when host plants are dead or dormant.

The various interesting relationships between microorganisms including plant pathogens and insects have been discussed in detail by Leach (1940) and Carter (1962). Shorter accounts have been given by Broadbent (1960), Gäumann (1950) and Austwick (1957) on fungi, by Bawden (1964) on viruses and by Madelin (1966, 1968) on entomogenous fungi.

The largest number of insect vectors have sucking mouth parts which belong to the Homoptera. Within this order the largest number of species which are known to transmit some viruses or the other is contained within two families, Aphididae (aphids) and Cicadellidae (leaf hoppers). A few belong to the Aleyrodidae (white flies), the Cercopidae (Spittle insects), the Coccidae (mealy bugs) and the Membracidae (tree hoppers) families. Thrips transmit the spotted-wilt virus. Some viruses are transmitted by both chewing and sucking insects, while a few are transmitted only by the former. Squash mosaic, cowpea mosaic and turnip yellow mosaic viruses are transmitted by beetles (Coleoptera) while the last is transmitted also by grass hoppers (Orthoptera) and earwigs (Dermaptera).

It is of interest that practically all vectors of the so-called yellows group of viruses are leaf hoppers while most vectors of the mosaic group are aphids. In other words, those viruses which are more readily transmitted mechanically are aphid borne and those which do not yield to such transfers are leaf hopper borne.

More than fifty species of aphids are known to transmit the viruses of plants. Some species can transmit only a few kinds and others transmit many; *Myzus persicae*, the green peach aphid, alone is the vector of fifty different viruses.

Some of the diseases transmitted by aphids are Katte or Marble disease of cardamom, mosaic and grassy shoot of sugarcane, mosaic streak of wheat, maize mosaic, barley mosaic, bean mosaic, cowpea

mosaic, bunchy top of bananas, banana mosaic, papaya mosaic, potato necrosis (viral), potato leaf roll, tomato mosaic, and chilli mosaic.

Some of the diseases transmitted by white fly are tobacco leaf curl, mung (*Phaseolus aureus*) yellow mosaic, double bean (*Phaseolus lunatus*) yellow mosaic and yellow mosaic of *Dolichos lablab*. Tungro of rice is transmitted by leaf hopper vectors, while bottlegourd mosaic is transmitted by red pumpkin beetle.

Three important questions arise with respect to the transmission of viruses by aphids:

1. How long must the insect feed on infected plants before it can transmit the virus to healthy plants?
2. Is an incubation period in the insect necessary before the virus can be transmitted?
3. How long do the insects remain infective after having acquired the virus?

Some viruses are quickly acquired and lost; others are acquired more slowly and are quite persistent. Some require an incubation period and others do not. In general, the viruses that require no latent or incubation period in the insects are relatively nonpersistent and those that require incubation period are persistent. Insects are important in the dissemination, inoculation, and persistence of certain bacterial pathogens of plants.

Erwinia tracheiphila, the cucurbit wilt organism is completely dependent on cucumber beetles for its spread. *Erwinia* (*Xanthomonas*) *stewartii*, the corn wilt pathogen, overwinters inside the corn flea beetle (*Chaetocnema pulicaria*). The cucumber beetles, *Diabrotica vittata* and *D. duodecimpunctata,* chew the plant parts affected by *E. tracheiphila* and carry the bacteria inside the body where they overwinter, passing on the host in the next season. It is believed that the relationship between the insects is symbiotic. Beetle transmission of viruses has been reviewed by Fulton *et al*. (1987).

Insects are important agents of dissemination and inoculation of *Erwinia carotovora*, causing black leg of potato. The pathogen survives in the seed tubers, which can account for primary infection in the spring. It was originally thought that the organism did not survive in the soil and there was no secondary spread in the field. Circumstantial evidence later indicated that there must be a secondary spread, but the agents remained unknown until Leach in 1926 proved that the seed corn maggot, *Hylemya cilicrura*, is a very important agent of effective dissemination and inoculation.

The seed corn maggot and closely related species are the pests of many kinds of plants, such as corn, cabbage, beets, beans, pear, radish, onion, tomato, etc. These insects and certain others contribute to the development of soft rot caused by *Erwinia carotovora* in some of these crops in much the same way as has been described for black leg.

Erwinia amylovora, the cause of fire bright of apples, pears and certain other member of the Rosaceae, was the first plant disease known to bacteria and transmitted by flies and ants. *Xanthomonas campestris pv. citri* which causes the well known citrus canker is carried by leaf miner from diseased to healthy plans.

Erwinia (*Xanthomonas*) *stewartii* and *X. campestris pv. vasculorum* which cause wilt of corn and gummosis of sugarcane respectively are transmitted by insects and can persist inside the vectors. *Erwinia stewartii is* also transmitted on seed and *X .campestris pv. vasculorum* on sugarcane setts used for planting. The corn wilt organism, however, is carried from plant to plant in the fields by flea beetles. *Chaetocnema pulicaria* and *C. denticulata* by *Diabrotica duodecimpunctata* mentioned in the discussion on cucurbit wilt and probably by other insects as well. Moreover, the bacteria can live during the winter inside the corn flea beetle*, C. pulicaria,* and can live for some time in the adult *Diabrotica* beetle

The big-vein disease of lettuce has been known since the 1930s. The disease causing agent was not identified at that time but was known to be soil borne. In the 1950s the chyridiomycetous fungus, *Olpidium brassicae* was found to be intimately associated with this disease. Finally, in 1964, Campbell and Grogan showed that the causal agent could be transmitted by grafting in the absence of *Olpidium.* Thus, this disease is now known to be caused by a virus. Later work by Campbell and his co-workers has shown that this virus is transmitted by the zoospores of *Olpidium* of and survives within the resting spores of this fungus. *Olpidium* also transmits other viruses that are soil borne. They are also known to be transmitted by various chytridiomycetes.

According to Austwick (1957), about 66 species of fungi causing plant diseases are transmitted by more than a 100 species of insects belonging to at least six orders. Transmission occurs in various ways with or without injury to the plant.

Spores of *Ustilago violaceae* (anther smut of Caryophyllaceae) and *Botrytis anthophila* (anther mold of clover) are thus carried from the infected anthers to uninfected flowers by pollinating insects. The conidia of *Claviceps* are spread from infected ovaries to healthy ones by insects which feed on the sugary honey dew on which the conidia are produced. Other fungi infecting the shoots and roots of plants are likely to be fortuitously disseminated by insects which move or are carried from plant to plant. Richardson and Saunders (1968), for example, attribute the partial control of potato blight by aphicide sprays to the destruction of aphids which carry mycelial fragments and sporangia of *Phytophthora infestans* on their bodies and leg bristles, and so spread the pathogen over short distances.

More frequently, however, transportation is accompanied by injury to the plant the insect carries the fungus and places it in the wounds, thus facilitating infection. The injury may be caused by mechanical breaking, by other animals or by the insect itself. The spores of *Ceratocystis fagacearum* (oak wilt) are carried by various insects, chiefly nitulid and scolytid beetles, to existing wounds caused by breaking, wood peckers, squirrels and other causes. Mycelial mat of the fungus develop beneath the bark, the crack is open and gives off a characteristic odour which apparently attracts insects. These feed on the fungus and also carry small spores which fertilize the receptive hyphae and so lead to the development of perithecia of the fungus. In some cases the insect or the fungus is adapted to insect transmission. Female Siricid (*Sirex*) wood wasps carry hyphae and conidia of *Stereum sanguinolentum* (heart rot of conifers) within the intersegmental sacs at the anterior end of the long ovipositor. The eggs are contaminated with the fungus during laying and are deposited within the wood of the tree and the fungus is reported to precede the larva as the latter bores through the wood. The fungus is contained in the hypoplural organ modifications of the larval integument. *Ceratostomella ulmi* (Dutch elm disease) grows within the egg galleries of bark beetles (*Scolytus*) and contaminates emerging adults, which also inject the slimy spores and thus spread the fungus to adjacent healthy trees. More specific are the diseases (stigmatomycoses) in which the fungus is injected into plant tissue by the insect vector. Several parasitic yeasts, notably *Nematospora*, are transmitted in this way, the acicular ascospores being sucked up the feeding stylet of stainer bugs (*Dysdercus*, *Nezara*) whence they lodge in the stylet pouches and are injected into young cotton bolls causing internal boll disease due to *Nematospora gossypii* (Frazer, 1944), bean pods (*N. phaseoli*, yeast spots) or coffee berries (*N. coryli, N. gossypii*). It is thought that *Nematospora* is almost entirely dependent on plant bugs for dissemination and entry into the plant. In the case of chestnut blight caused by *Endothia parasitica*, the insects responsible for the disease are European bark beetle (*Scolytus multistriatus*) and the native elm bark beetle (*Hylurgopinus rufipes*).

Many insects attack the fruits of plants, and fruit rotting fungi often enter through the wounds. The spores of *Aspergillus niger*, which causes smut of fig fruits, are reported to be introduced by certain beetles and fruit flies, and *Sclerotinia* sp. (brown rot of various fruits) can enter through feeding and oviposition wounds made by insects. Some fungi causing stem rot (e.g., *Physalospora tucumanensis*, red rot of sugarcane) also invade through insect wounds and it is suspected that some leaf attacking fungi behave in a similar way. *Pycnostysanus azaleae* (bud blast of *Rhododendrons*) is associated with the leaf hopper *Graphocephala coccinea* and the disease is very slight in the absence of the insect (Howell and wood, 1962). Little is known of dissemination of the root pathogens by insects but it seems likely that this occurs in the soil and those insects feeding on roots also introduce fungi into them. The original insect damage may thus be greatly aggravated by subsequent fungal invasion. Insects which feed on cotton plants have been found to contain *F. oxysporum* f. sp. *vasinfectum* (cotton wilt), the fungus having been recovered in a viable condition from faecal pellets of various grass hoppers. It seems possible that such insects disseminate this normally slowly spreading fungus which might explain the occasional appearance of the disease in hitherto disease free fields or parts of fields, but other explanations are possible, and the relationship between the soil-borne pathogens and insects needs further investigation.

Insects which act as diploidizing agents for some heterothallic fungi, transfer fertilizing spores (spermatia, oidia) between compatible strains and so bring about the formation of the perfect state. The best-known example of this diploidization is that of receptive hyphae of rusts by insect-borne spermatia, but similar fertilization occurs in some Ascomycetes and other Basidiomycetes. This phenomenon has obvious similarities with insect pollination in higher plants.

Mites These, although wingless, transmit some viruses (reversion disease of currants) which are perhaps carried by winged insects or wind. Transmission of fungal pathogens by mites has not been extensively studied.

Nematodes Some viruses as well as some bacterial and fungal plant pathogens are transmitted by nematodes. *Corynebacterium fascians*, leafy gall of various herbaceous plants, is carried by ectoparasitic nematodes (*Aphelenchoides*) and typical cauliflower symptoms develop only when both nematode and bacterium are inoculated into strawberry plants (Cross and Pitcher, 1952).

Yellow ear rot of wheat caused by *Corynebacterium tritici* is dispersed by the ear cockle nematode (*Anguina tritici*)

There is also evidence that some root infecting pathogens, particularly fungi, may enter roots through wounds made by nematodes and this may perhaps be of more frequent occurrence then is at present realized. Cotton wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* is an example of this, the pathogen being associated with root knot nematodes and probably others (Smith 1953). The nematodes may also affect the physiology of the root, making it a more suitable medium for the fungus (Powell and Nusbaum, 1960; Powell, 1963). The reverse has also been reported; the fungus (*Pyrenochaeta lycopersici*) causing tomato brown root rot reduced the invasion of tomato roots by the potato root eelworm *Heterodera rostochiensis* (James, 1968). Bacterial wilt of carnations, caused by *Pseudomonas caryophylli*, is reported to be more severe in the presence of root knot nematodes which probably facilitate the entry of the bacteria (Stewart and Schindler, 1956). Pitcher (1965), Fielding (1959), Christie and Perry (1959), Leach (1940) have discussed this.

Hewitt *et al*. (1958) demonstrated that the grapevine fan leaf virus is disseminated by the nematode *Xiphinema index.* Both polyhedral and tubular viruses have since been shown to be nematode transmitted, the former by species of *Xiphinema* and *Longidorus* and the latter by *Trichodorus* spp. Transmission of viruses by plant nematodes has been reviewed by Brown *et al*. (1995).

Animals Grazing animals could easily transmit the disease mechanically from an infected plant to a healthy one. Domestic herbivores like goat, sheep, cows and some other plant eating animals including deer, feral pigs, rabbits, etc., tend to feed on different plant parts and thus transmit diseases, which is a mechanical mode of transmission. Sometimes as a result of their grazing, there is an increase in the population of insects like aphids due to change in the habit from long grasses to small grasses. These aphids are in turn responsible for the transmission of plant pathogens from one area to another.

Biological Transmission

Plant viruses could be transmitted from an infected to a healthy plant through a bridging dodder, parasitizing both plants. An association of dodder and host cell is formed and transmission occurs through directional movement of nutrients.

Cochran (1946) has also found transmission of tobacco mosaic virus that occurs through dodder plants can be controlled by pruning the dodder and shading healthy host while keeping the infected host in bright light.

Human Dispersal (Anthropochorus) Man is, to a large extent, responsible for the dissemination of plant pathogens which he does in two ways: through his person, and through the objects he transports from one place to another.

Local dissemination of pathogens on man's person is illustrated by the angular leaf spot disease of cucumber caused by the bacterium *Pseudomonas syringae* pv. *lachrymans*. When cucumbers are grown for pickles, the fruits are picked. When a person comes in contact with or picks an infected plant, his hands become contaminated, particularly when the plants are wet with dew. The pathogen is thus spread down the row of plants.

Another pathogen spread by man is *Clavibacter* (*Corynebacterium*) *michiganense*, the cause of bacterial canker on tomatoes. This pathogen is seed borne, and when it develops on tomato seedlings to be used as transplants, the workers handling the transplants may contact the bacterium on their hands. Since the handling of transplants often causes small injuries to the plant, the worker placing the transplants into the ground can easily inoculate healthy plants. In California, USA, this disease has become a rarity since most tomato fields are no longer planted with transplants but are direct seeded.

The viruses that are spread by animals can also be spread by man. Tobacco mosaic virus is known to survive in dry tobacco leaves. Hence, workers who handle tobacco plants in the field are advised not to smoke while they are working and to thoroughly wash their hands before entering the field. The long distance dissemination of plant pathogen by man is usually accomplished by the transport of infected plant parts, such as seeds, nursery stock or timber products. The cause of some of the worst plant disease epidemics is the introduction of a pathogen into a new area. In many cases the plants growing in these areas are very susceptible since they do not develop resistance through evolution in the presence of the pathogen. Table 9.1 gives a bird's eye view of the diseases which have been introduced into India from other countries of the world.

Table 9.1 Examples of plant diseases introduced in India from other countries

Disease	Original Home	Year of introduction
Leaf rust of coffee caused by *Hemileia vastatrix*	Sri Lanka	1879
Late blight of potato caused by *Phytophthora infestans*	England	1883
Flag smut of wheat caused by *Urocystis tritici*	Australia	1906
Powdery mildew of grapes caused by *Plasmopara viticola*	Europe	1910
Downy mildew of cucurbits caused by *Erysiphe cichoracearum*	Sri Lanka	1910
Downy mildew of maize caused by *Sclerospora phillipinensis*	Jawa	1912
Black rot of crucifers caused by *Xanthomonas campestris*	Jawa	1929
Powdery mildew of rubber caused by *Oidium heveae*	Malaya	1938
Black shank of tobacco caused by *Phytophthora nicotianae*	Holland	1938
Crown gall of apples and pears caused by *Agrobacterium tumefaciens*	—	1943
Bunchy top of banana (viral)	Sri Lanka	1940
Hairy root of apple (viral)	England	1940
Onion smut caused by *Urocystis cepulae*	Europe	1958
Bacterial blight of paddy caused by *Xanthomonas oryzae* pv. *oryzae*	Philippines	1959
Golden nematode of potato caused by *Heterodera rostochiensis*	Europe	1961

Table 9.2 gives examples of plant diseases which have been introduced into some other countries from different parts of the world.

Table 9.2 Examples of plant diseases introduced in some other countries

Disease	Introduced Home	Original Home	Year of Introduction
Late blight of potato caused by *Phytophthora infestans*	Europe	S. America	1830
Powdery mildew of grapes caused by *Uncinula necator*	England	N. America	1845
Blister rust of pine caused by *Cronartium ribicola*	USA	Europe	1910
Downy mildew of grapevine caused by *Plasmopara viticola*	France	USA	1878
American Gooseberry mildew caused by *Sphaerotheca morsuvae*	England	N. America	1899
Chestnut blight caused by *Cryphonectria (Endothia) parasitica*	USA	Asia	1904
Cittus canker	USA	Asia	1907
Paddy blast caused by *Pyricularia grisea*	India	Sri Lanka	1918
Striga asiatica	N. America	Africa or Asia	
Rust of coffee caused by *Hemileia vastatrix*	Brazil	Africa and Asia	1970

(Contd.)

(Contd.)

Disease	Introduced Home	Original Home	Year of Introduction
Dutch elm disease caused by *Ceratostomella ulmi*	USA	Holland	1928–30
Fire blight caused by *Erwinia amylovora*	New Zealand	N. America	1919
Golden nematode caused by *Heterodera rostochiensis*	USA, Mexico, Peru, India, USA	Europe	1881

Agarwal *et al*. (1990) have listed major plant diseases introduced and established in India from other countries and have also listed destructive plant pathogens of quarantine importance not yet established in India.

Man has repeatedly carried pathogens to areas which otherwise would have remained free of them. Almost all countries have suffered to such an extent from pathogens introduced by man that the Food and Agricultural Organization of the USA and several international committees of plant pathologists are promoting studies and regulations designed to reduce the danger.

Man is an agent of transportation of pathogens from continent to continent, from country to country and even from plant to plant on his own farm. Eternal vigilance is necessary to protect economic plants against the pathogens distributed by man.

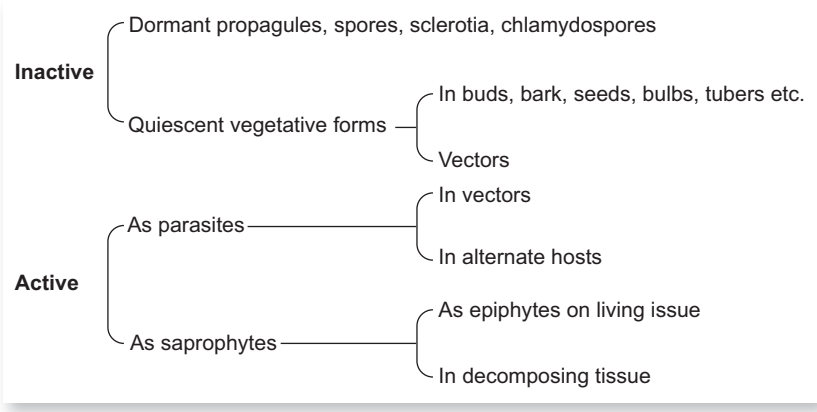
Overwintering of Pathogens and Their Role in Epidemics

Overwintering is the ability of a pathogen to survive from one growing season to the next. For a plant disease to occur, a susceptible host, a pathogen (causal agent), and favourable environmental conditions must be present and interact with one another. If any one of these requirements is not met, a plant disease will not occur.

Most of the fungal strains have developed the mechanisms to survive in perennial as well as in annual crops. Severe disease outbreaks can be prevented by manipulating the host through the use of resistant cultivars and through cultural practices and fungicidal or bactericidal sprays. On perennial plants, the fungi survive as mycelium in the diseased parts of the plants, as in cankers, directly as spores near the infected area or as mycelium or spores on the fallen infected leaves/fruits/buds. In annual plants the pathogen survives as mycelium, resting spore or as sclerotia in the infected plant debris, in or on the seeds, tubers, bulbs etc. The leaf curl fungi, *Taphrina*, survive in the crevices in the bark of peach and almond. Similarly, powdery mildew pathogen of perennial hosts such as apple or roses overwinters in dormant bud which forms in autumn.

The bacteria overwinter or survive unfavourable environmental periods or the absence of a susceptible host in the same way as fungi do by either going dormant in infected tissue, plant debris, seeds, tubers, buds, infested soil, or water and sometimes within the body of their insect vectors. But the viruses, viroids, mollicutes, fastidious bacteria and protozoa overwinter on living plant tissue only. A small portion of the virus can be transmitted through seeds and some are transmitted through vegetative propagation by tubers, bulbs and also in the roots of infected perennial plants. Tobacco rattle virus (TRV) remains inactive on dormant nematodes for at least a year before causing disease. Another example is that of barley yellow mosaic virus (BaYMV) which persist for at least ten years in resting spores of its vector *Polymyxa graminis.*

Survival Strategies Adopted by Plant Pathogens



For a pathogen to cause disease, it actually depends on the duration of time between available hosts and on the relative hostility of environment. Sometimes the spores or propagules overwinter for a very brief period away from their hosts and sometimes pathogen survives between annual crops planted in successive season and cause disease on the arrival of favourable host and environmental conditions. In the intervening period, an environment extreme actually reduces the chances of pathogen survival like draught, water logging and extreme temperature that reduces the chances of pathogen survival.

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Question

1. What is the essential difference between distribution and dissemination?
2. Give some examples of viral diseases which are carried in the seeds.
3. Give examples of plant diseases in which dissemination of the plant pathogens is dependent upon special types of insects.
4. Which insects act as diploidizing agents for some heterothallic fungi?
5. Name the diseases which have been introduced in India from other countries.

Plant Disease Epidemiology and Plant Disease Forecasting

10

Introduction

The forecasting of plant diseases has become considerably important in the present day situation in India and other agriculturally advanced countries of the world due to large-scale adoption of new agricultural strategy for increasing food production. The new approach which involves use of fertilizers, intensive cropping, change in cultural practices as well as in cropping pattern, cultivation of high yielding varieties has no doubt paid rich dividends by increasing the production, but has also disturbed the natural agrosystem. This has resulted in the increased incidence of diseases which sometimes results in heavy losses or even near failure of crops. The modern agriculture being cost oriented requires more vigilance than before for ensuring stable and good quality yields and for reducing expenditure on all types of inputs including chemicals for disease management. This is possible only if reliable disease-forecasting systems are developed at least, for some of the destructive diseases of major crops (Bedi, 1986).

Forecasting the future has been one of most rewarding professions in ancient times. A forecaster was considered a prophet, a saint and often referred to as "jyotishi", and prophet has his troubles. Prediction, forecasting, prognosis, forewarning, or foretelling has been used in the literature of plant pathology. The word prognosis is used mainly to describe the expected progress of disease in humans. Taken literally it means knowing beforehand. Forecasting means to foresee or to calculate beforehand. Thus, the calculation of probabilities is implicit in the meaning of any word related to forecasting. Gaumann (1950) defines epidemic as "the massive occurrence of a disease in a limited time period" as influenced by the large number of factors that interact with this increase. Vanderplank (1963) defines epidemiology as the science of disease in population. But according to Kranz (1973), epidemiology is the science of population of host disease resulting therefore under the influence of environment and human interference. Kranz (1973) further defined it as the science that deals with the increase or decrease of plant disease in a host population in time and space.

The ultimate aim of the science of plant pathology is to have a complete understanding of plant diseases—host, pathogen, and environment interactions—so that it becomes possible to control diseases economically. The most efficient and most economical way to control diseases is usually to obtain a thorough knowledge of the disease epidemiology. We can not effectively control certain diseases without some prior indication as to how they will behave in the coming period, i.e., without some knowledge as to where and when they will occur, which will enable preventive action to be taken. Unless we know

where and when the sprays or dusts, etc., are to be applied, we are likely to waste them. For certain diseases, no effective or practicable chemical control measures are available. Forecasting gives us an opportunity to revise our plans in order to avoid or reduce losses.

The practical implications of forecasting have been incorporated in the definition proposed by Miller and O'Brien (1952) who stated: "Forecasting involves all the activity in ascertaining and notifying the growers of a community that conditions are sufficiently favourable for certain diseases, that application of control measures will result in economic gain, or on the other hand, and just as important, that the amount of disease expected is unlikely to be enough to justify the expenditure of time, energy, and money for control." Miller and O'Brien (1952) thus included a warning service for plant disease prevention in their definition of forecasting.

There are excellent reviews and books covering this aspect and related area, such as those of Bourke (1953), Cox and Large (1960) concerned with the forecasting, from weather data, of potato blight and other plant diseases and pests. Bourke (1970) has dissertated on the "use of weather information in the prediction of plant disease epiphytotics" , Miller (1967) on plant disease epidemics—their analysis and forecasting", Miller and O'Brien (1957) on "prediction of plant disease epidemics", Waggoner (1960) on "forecasting epidemics" and "ground level climate and disease forecasting", Krause and Massie (1975) on "predictive systems—modern approaches to disease control", Shrum (1978) on "forecasting of epidemics". During the last two decades of the last century are those of Zadocks and Stein (1979) *Epidemiology and Plant Disease Management*, Zadocks (1984) *A Quarter Century of Disease Warning*, Fry and Fohner (1985) *Construction of Predictive Models*, Leonard and Fry (1986) *Plant Disease Epidemiology* Vol. I and II, Campbell *and Madden (1990) Introduction to Plant Disease Epidemiology*, Madden and Hughes (1995), *Plant Diseases Incidence: Distribution, Heterogenicity and Temporal Analysis*, McCartney (1996), *The Influence of Environment on the Development and Control of Diseases*.

In our country, Nagarajan (1983) has critically discussed the epidemiology of plant diseases in a comprehensive book entitled, *Plant Disease Epidemiology*. Bedi (1986) had reviewed the work on important plant diseases and their forecasting while Mayee and Datar (1988) have reviewed the work on forecasting crop diseases in India till then. Aggarwal and Mehrotra (1991) have compiled information on *Phytophthora* diseases in India in relation to environmental factors.

Forecasting the incidence of plant disease must take into account not only the disease itself but the weather also, which is more or less predictable. Disease forecasting is made more reliable if the reasons for a particular disease developing and certain conditions and not under others are known.

Importance of Disease Forecasting Services

Plant pathologists are interested in forecasting for three major seasons:

1. Disease forecasts are useful to farmers in the field in the practical management of diseases of their crop,
2. Research aimed at accurate forecasting helps us to identify or pinpoint the gaps existing in our knowledge and,
3. Modern research on modeling on the complexities of epidemics is stimulating and rewarding in the long run.

The ultimate goal of plant pathology is to minimize losses due to plant diseases. As pointed out by Main (1977), the farmer must balance three factors in disease management: risk, cost and benefit. Here, forecasting can be useful, if the forecaster suggests that the risk of disease is great, there is big advantage

in spraying or using resistant varieties, may be of moderate yield. Forecasting provides a complex tool for determining as to when and where a given management practice should be applied.

Some Important Epiphytotics of the Past and Present

The Bible, ancient Hebrew, and Sanskrit literatures refer to plant diseases. During each spring in the Roman Empire, the elaborate ceremony of Robigalia was done to satisfy the "Rust God" from causing cereal rust epidemics. Disease epidemics are known to mankind since time immemorial.

The great potato famine in Ireland that followed the late blight epiphytotic of 1845 is undoubtedly one of the landmarks of the 19th century. The crucial role played by *Phytophthora infestans* in that episode is well known to each and every mycologist and plant pathologist.

Epidemics of plant diseases have influenced man's food, health, social customs, economies, and even his ability to wage war. Horsfall and Cowling (1978) in one of their reviews analyzed the impact of plant disease epidemics such as ergotism of rye, late blight of potato, Dutch elm disease, coffee rust, helminthosporiose of rice, peach yellow, chestnut blight, wheat rust and maize leaf blight.

The coffee rust problem of Sri Lanka that made her switch over to tea, the banana wilt of central America, downy mildew of grapes of France and parts of Europe, rice tungro outbreak in Indonesia are first a few random samples from the hundreds of plant disease epiphytotics (epidemics) of the world. In most of the cases, there was lack of epidemiological information to give any protection oriented recommendation at that time.

Nagarajan (1983) had a close look at some of the epidemics that swept parts of India and resultant socio-economic problems.

The first example of an important plant disease epidemic in India is the stem rust of wheat. Quoting Major Sleeman (1839), "Old and respectable landlords in the district of Jabalpur state, that is Sambut 1843 (A.D.1786), wheat crops throughout the district were destroyed by the same calamity, and that the Saugor Government (MP) obliged not only to remit the revenue, but to provide a supply of wheat grains for almost every village by advances from the public treasury." So wrote Major Sleeman (1839) about the oldest known stem rust epidemic, i.e., *P. graminis tritici.*

The second one is of Helminthosporium blight of paddy. In 1942, there was a fall in rice production in Bengal due to the widespread epidemic created by *Helminthosporium oryzae.* India, which was exporting wheat prior to World War I, was no more on the list of exporters, her production falling short of demand. The administration as usual, ignored the notes and files. The easily avoidable human suffering got no attention, as life and property every where was unsafe due to the war. The acute shortage drove many to starvation and death—the Great Bengal Famine swept across Bengal and Bangladesh (Padmanabhan, 1973). Turning the pages of history reveals that there was an isolated epidemic of *H. oryzae* during 1918–19 in the Krishna delta of Andhra Pradesh (Sundararaman, 1922). The weather conditions recorded during that epidemic year seems to be similar to the 1942 epidemic.

There are many other epidemics that India witnessed, such as red rot of sugarcane (*Colletotrichum falcatum*), downy mildew of pearl millet (*Sclerospora graminicola*), the apple scab (*Venturia inaequalis*) epidemic in Himachal Pradesh (H.P.) and Kashmir (J.K.) and bacterial blight of paddy (*Xanthomonas oryzae* pv. *oryzae*) in the rice belts.

Epiphytotic Growth and Analysis

The interactions of the structural elements of epidemics as influenced over time by factors of the environment and by human interference are expressed in patterns and rates. The pattern of an epidemic in terms of the numbers of lesions, the emergent of disease tissue, or the number of diseased plants is given by a curve that is called the disease-progress curve. The point of origin and the shape of disease-progress curve reveals information about the time of appearance and amount of inoculum, changes in host susceptibility during the growing period, recurrent weather events, and the effectiveness of cultural and control measures. If the epidemic growth curve is plotted it resembles the English alphabet "S" and is called a "sigmoid curve". The shape of the curve depends upon the level of initial inoculum and the growth rate of the disease. For example, a saturation-type curve is characteristic for monocyclic diseases, a sigmoid curve is characteristic for polycyclic diseases, and bimodal curve is characteristic for diseases affecting organs (blossoms, fruit) of the plant. Knowledge of disease progress curves also allows disease forecasting and selection of the best control strategy for the particular disease and time.

Vanderplank (1963) broadly classified the types of diseases into two categories: simple interest and compound interest types. Aerial borne pathogens like cereal rust starts from few lesions and by virtue of their heavy sporulation, disease growth is similar to compound interest paid by bank. In the case of simple interest type of disease, growth is discontinuous, fluctuating both on the upper and lower sides. The simple interest type of disease spends one generation on that crop and the second cycle is completed on the next season crop. Meanwhile, the environment and host factors interact and decide the disease level on the succeeding crop. Seldom have such diseases followed the sigmoid growth curve to reach epidemic proportions. Wheat smut (*Ustilago segetum tritici*) and Karnal bunt caused by *Tilletia indica* (formerly known as *Neovossia indica*) are good examples of simple interest type of disease growth.

Computer Simulation of Epidemics

The availability of computer has allowed plant pathologists to write programmes that allow simulation of epidemics of several plant diseases. In 1969, first computer simulation programs called EPIDEM was developed and resulted from modeling each stage of the life cycle of a pathogen as a function of the environment. This (EPIDEM) was designed to simulate early blight of tomato and potato caused by *Alternaria solani*. Later on, computer simulators were written for *Mycospherella* blight of Chrysanthemums (MYCOS), for apple scab caused by *Venturia inaequalis* (EPIVEN) and for southern corn leaf blight infected by *Helminthosporium maydis* (EPICORN).

BLIGHTCAST (Krause *et al*., 1975) for late blight of potato caused by *P. infestans* is a computer programme written in Fortan IV developed in the USA. This was a synthesis of the Hyre and Wallin fungicide scheduling systems developed for north-eastern and mid- western US. BLIGHTCAST is based on predicting periods when infection is likely to occur. Spray recommendations were issued in two different triggers: after 10 consecutive days when the cumulative rainfall exceeded 30 mm and the 5 day average temperature did not go beyond 25.5 °C, or after an accumulation of 18–20 "severity units". Each day was scored on a 0–4 "severity" scale according to temperature and hours with relative humidity more than 90%.

TOM-CAST (Pitbaldo, 1992)—Modern *et al*. (1978) developed a computer-based forecasting system for tomato early blight (FAST). This was modified by Pitbaldo (1992) in Southern Ontario in Canada

for use in that region. TOM-CAST, identifies periods when environmental conditions are favourable for disease development and recommends fungicidal application schedule. TOM-CAST required direct measurement of wetness duration in the field which was read by wetness sensors and data collecting systems such as those used with BLIGHTCAST.

PLASMO (Rosa *et al*., 1993, 1995)—This is a forecasting model developed in Northern Italy to control downy mildew of grapes caused by *Plasmopara viticola*. It is a very sophisticated computer programme which simulates the critical phases in the life cycle of the pathogen using infection proportions (rates) determined by environmental parameters (temperature, relative humidity, rainfall, and leaf-wetness duration).Conventional control of the disease is done by regular spray of fungicides at 7 to 10 days intervals.

In addition to the above some recent disease forecasting/warning simulation models have been developed which include *Pyricularia grisea* on rice (Calvero *et al*., 1996), *Blumeria* (*Erysiphe*) *graminis* f. sp. *tritici* on wheat (Friedrich, 1995).

In a computer simulation of an epidemic, the computer is given data describing the various subcomponents of the epidemic and control practices. The computer then provides continuous information regarding not only the spread and severity of the disease over time, but also with regard to the final crop and economic losses likely to be caused by the disease under the condition of the epidemic as given to the computer. Computer simulation of epidemics is extremely useful as an educational exercise for students of plant pathology and also for farmers so that they can better understand and appreciate the effect of each epidemic subcomponent on the final size of their crop loss. The computer evaluates not only the current status of the disease but also the effectiveness of the applied management measures in controlling the epidemic. Computer simulation of epidemics is used fairly in plant disease forecasting systems. Such systems allow farmers to take appropriate control measures against a disease as soon as the conditions that are likely to lead to disease development are present or are likely to be present soon.

System Approach in Epidemiology

We need to develop forecasting procedure for most plant diseases which can handle all significant components of an epidemic—the pathogen, the host, the weather, the time and perhaps the space. The first job of a system's analyst at the conceptual stage is to recognise the problem itself, its limit and the objectives. Conceptual models are the first step in model building and aims at sensibly arranging available information or processing thoughts for a critical analysis. The conceptual models show the sequence of operations and the decision-making level. It also gives an imperical idea on the system and the factors that interact influencing the system's behavior. Joshi *et al.* (1974) attempted to correlate disease, weather, time, and space relationship for rusts of wheat. In field studies to establish the reasons for the non-functional nature of wheat stem rust over north-west India, Joshi and Palmer (1973) took to geophytopathology. Spread of wheat black, brown and yellow rusts from a point source was mapped over a period of time, at Karnal, Haryana. Their primary aim was to demonstrate that even if stray stem rust urediniospores survive in the Himalayas, which is otherwise definite, disease will not spread. Meanwhile, the continued cold weather of North India favour the brown and yellow rusts of wheat. Disease gradient from point source clearly showed that stem rust spread is the poorest between January and March. A well planned and laid out geophytopathological study can therefore reveal many facts, that are apparently unclear.

As explained earlier, weather is an important factor that influences disease development. Where the host and pathogen coincide, weather is the only variable that influences epidemic development. Therefore, by monitoring the relevant weather parameter and relating them to disease development, empirical prediction models can be developed.

Everdigen's Method

Van Everdigen (1926) in the Netherlands apparently was the first to base his forecasting system for late blight of potato on scientific principles. His forecast was based on what became known as the "Four Dutch Rules". These rules were based on four weather parameters:

1. Minimum temperature (night temperature must not be lower than 10 °C [50 °F]).
2. Dew (leaves must remain wet for at least 4 hours the day following a night dew).
3. Cloudiness (the day following the night dew must be cloudy or with no less than 0.8 of the sky covered by clouds).
4. Rainfall (during such periods described above there must be at least 0.1 mm of rainfall).

When all these conditions are satisfied under field conditions continuously for a few days, then in Netherlands control measures are recommended.

Beaumont and Stainland Method

Beaumont and Stainland (1934, 1938) modified the Dutch rules for England and evolved Beaumont's rules based on:

(i) A minimum temperature of 10 °C (50 °F) or more.
(ii) Relative humidity of 75% or above for 2 consecutive days.

The blight could be expected approximately 10 days afterwards. These periods are called as Beaumont periods and are the basis of blight forecasting system in England and Wales.

Cook's Method

Cook (1947, 1949) in the United States used 7 day moving average total rainfall and average mean temperature charts for blight forecasts. Cook (1949) studied the relationship of temperature and rainfall during the preceding 17 years to blight on potatoes and tomatoes in eastern Virginia. He found that the critical period for blight developed in that area started on 8th May. The cumulative mean rainfall line starting on 8th May was plotted for blight and non-blight years and a median line was designated the critical rainfall line. The critical temperature was taken as 75 °F. Fewer epidemics were likely to follow a two week period when rainfall was continuously above the line and temperature below 75 °F. Later Cook included the analyzes for 31 years (1917 to 1947).

Hyre's Method

Hyre (1954) developed criteria for north-eastern region of the USA which were essentially the same as that of Cook excepting that 10 day moving graph method was followed and which according to him was more accurate than 7 days moving graph method (Cook, 1949).

Wallin and Hoyman's Method

The forecasting system very much successful in eastern United States for prediction of blight in advance and development did not seem to be applicable in midwest USA. Hyre and Horsfall (1951) attributed the difference to variation in occurrence of primary inoculum which varied a great deal from year to year in the reasonably blight free areas of the Midwest. According to Wallin and Hoyman (1954) a late blight favourable period consists of 10 or more consecutive hours of temperature of 75 °C or less and RH 90% or more and if during a given 7 day interval there was least one such period with the following days, maximum temperature 90 °F or less. The more frequent occurrence of the favourable periods, the greater was the expected blight development.

Hyre and Bonde's (1955) Method

This is modification of Cook's (1949) and Hyre's (1954) method of 7 day moving graph in which a 10 day total rainfall and a moving 10 day average mean temperatures are considered suitable. In this case also the critical rainfall line is calculated on the basis of total crop season as above.

In India Choudhuri and Pal (1959) compared Cook's (1949) and Hyre's (1954) moving graphs of temperature and relative humidity at Darjeeling and observed that 7 day moving graph was more accurate. Later correlations were worked out by different methods between weather parameters and the disease appearance for Shimla, Shillong and Ootacamund by Bhattacharya *et al.* (1982). This forecasting system so developed for the hills is still working well for the country. The system developed by Bhattacharya *et al*. is given in somewhat more details later in this chapter.

The need for a warning service for the late blight of potato in the USA was evident long before a cooperative project was established. Moreover, a considerable amount of experience of various groups had already shown that the appearance and spread of late blight and some related fungi could be successfully anticipated, provided that means for watching and reporting progress were available. The event that finally led to the establishment of the regional forecasting project was the severe, widespread, and unexpected epidemic of late blight of tomato in 1946. The project was organized to include a warning service and contributory research for three downy mildew diseases, late blight of potato and tomato, blue mold (*Peronospora tabacina*) of tobacco, and downy mildew (*Pseudoperonospora cubensis*) of cucurbits. In the whole of North America all the way into Canada, the seasonal programme of these crops is particularly well adapted to observations and predictions on a widespread regional basis. Canadian pathologists cooperate in the warning service.

The appearance and spread of late blight of tomato and potato, blue mold, and downy mildew of cucurbits are promptly reported to the plant Disease Epidemics Unit at Beltsville, Maryland, USA. The reports are compiled into a warning letter sent twice a week during the growing season to the pathologists in the various states, Canadian provinces, and other authorized recipients. By watching the occurrence and severity of disease in areas with an earlier growing season and applying their own previous experience, together with the weather forecasts, the key pathologists are able to judge rather accurately whether or not the local crops are likely to be affected, and if so, how soon and how seriously; and can make their forecasts accordingly. Manufactures of control chemicals and equipment also use the warning letters as a guide to allocate their products.

Forecasting and warning systems involve considerable expense and should be economically justifiable, as in the following cases:

1. For destructive diseases of important case or food crops.

2. For diseases which can be reliably predicted early enough for effective and economical control measures to be applied.
3. For diseases against which economical control measures are available.

Methods Used in Plant Disease Forecasting

Disease forecasting allows the prediction of probable outbreaks or increases in intensity of disease and, therefore, allows us to determine whether, when, and where a particular management practice should be applied. It is also useful to farmers in the practical management of crop disease. Most frequently, farmers need forecasts of plant disease development to decide whether to spray a crop right away or to wait for several more days before they spray, since, if they can wait, they can reduce the amounts of chemicals and labor used without increasing the risk of losing their crop. In developing a plant disease forecast, one must take into account several factors of the particular pathogen, host and environment.

The methods used in disease forecasting mostly fall into 5 main groups based on the following:

1. Weather condition during the intercrop months particularly those affecting the survival of inoculum
2. Weather condition during the crop.
3. The amount of disease in the young crop.
4. The number of propagules of the pathogen in the air, soil or planting material
5. Change in the host physiology.

Weather-Based Predictions

When the importance of weather conditions in determining the host–pathogen interaction was realized, prediction based on weather conditions were frequently used in forecasting diseases. According to Shanta (1960), the severity and subsequent spread of tikka disease of groundnut caused by *Cercospora personata* (now *Cercosporidium personatum*) and *C. arachidicola* depend upon the prevalence of high humidity and low temperature during crop season. According to Jensen and Boyle (1966), the chances of rapid development of epidemics of Cercospora leaf spot of peanut are optimum when foliage remain wet for a period greater than 10 hours or above, and minimum temperature is 21 °C or higher for the next 24 hours.

Padmanabhan (1965) examined the meteorological factors associated with the epiphytotics of *Helminthosporium oryzae* involved in the "Great Bengal Famine" of 1942. It was revealed that heavy rainfall in September accompanied by favourable temperature of 25–30 °C, followed by cloudy weather in subsequent months resulting in low solar radiation were the critical conditions for outbreak of brown leaf spot of rice. Padmanabhan (1965) used weather analysis for predicting outbreak of blast of rice (earlier *Pyricularia oryzae* now *P. grisea)*. Nycto-temperature ranging from 20–24 °C in association with 90% relative humidity or above lasting for a week during any susceptible phase of crop brought epidemic of blast.

Nagarajan *et al.* (1980) correlated the epidemics of wheat rusts of Indian with the prevalence of certain environmental conditions at certain places in the country during crop season. According to them, epidemic of leaf rust (*Puccinia triticina*) of wheat is likely to develop if:

1. Rust infections are observed in Uttar Pradesh and Bihar between 15th and 20th January at 5–6 different locations separated from each other by a minimum distance of 25 ± 5 km

2. The number of rainy days during January to mid April over north-west India is at least twice the normal number. i.e. 12 days, and
3. The weekly mean maximum temperature during March to mid-April over north-west India is with 1 °C of the normal temperature (26 °C).

If the first and the second condition exist fully, then a severe epidemic of leaf rust is likely to occur. If they exist partially, isolated outbreaks of the disease are possible. If the mean maximum, temperatures are usually low, stripe rust is likely to become a serious problem throughout north-west India. Similarly Payak *et al.* (1970) found that the brown stripe downy mildew of maize caused by *Selerophthora rayssiae* var. z*eae* was severe in areas where mean precipitation, was between 100–200cm. In area of low rainfall, the disease would develop under conditions of continuous rains, cloudiness and low temperatures of 24 °C or less for a few days. Saxena and Lal (1984) developed a linear equation based on sunshine hours and rainfall for prediction of *Erwinia* stalk rot of maize. Saharan and Singh (1980) found that the latent period of *Melampsora lini* varied greatly and the variation can be predicted by relative humidity. Mayee (1986) developed a forecasting system based on temperature for groundnut rust epidemics in central India. Mayee and Ekbote (1983) noted that the inoculum of rust was available throughout the year as also the suspect. However, highest infection rates occurred only during rainy season. Factors such as mean temperature, relative humidity, and rainy days can predict not only the sub-phases of infection chains but also the disease development.

Gupta (1992, 1995) listed various forecasting procedures for apple scab, a disease where sufficient work has been done on forecasting. In many countries, electronic scab warning instruments have been developed for monitoring the ambient temperature, duration of leaf wetness and relative humidity above 90 percent for predicting infection periods.

Thakur and Khosla (1999) have investigated the relevance of Mill's scab infection criteria for improving efficiency in the monitoring of apple scab (caused by *Venturia inaequalis*) control programme in Himachal Pradesh. Observations on potted and orchard plants showed that the light infection period required 3 more days for development of scab symptoms at a leaf stage. Wetness duration of 9–13 hours and average temperature range of 8.5–21.4 °C where as moderate and severe infection periods (> 15 hours of leaf wetness) required 1 more day to exhibit symptoms. Rescheduling of fungicide application on the occurrence of infection periods could save at least 3 sprays. They concluded that forecasting and monitored spray programme may be devised in accordance with prevailing weather conditions of the region. Diseases and pest forecasting for integrated apple crop management has been critically reviewed by Thakur and Vaidya (2006).

Inoculum-Based Prediction

Forecasting of many diseases is based on the estimates of amount of initial inoculum likely to infect the host plant at the beginning of a crop season. In many cases there is a positive correlation between the amount of primary inoculum in vicinity of host plant and severity of infection. To quote Shrum (1978) again, "the forecast based on inoculum potential contain a hidden assumption that the weather will continue to be favourable for disease". If weather changes, the forecast may be inappropriate and the degree of error will be proportional to the degree of change. Plant disease forecasting can be based on the amount of inoculum available in air, soil, or planting material. Utility of inoculum potential based forecasting can however be found for a number of soil-borne diseases.

The severity of apple scab caused by *Venturia inaequalis* is related to the amount of perithecia surviving in dead leaves and the subsequent amount of ascospores liberated from them. The forecasting of

apple scab epidemic is mostly based on temperature and moisture conditions, affecting the discharge of ascospores from perithecia at full-bloom stage of the crop. The discharge of ascospores takes place when perithecia are thoroughly wetted and the infection takes place 30 hours after leaf wetness at 5 °C, 14 hours at 10 °C or 9.5 hours 15 °C.

A system for predicting blister blight of tea caused by *Exobasidium vexans* has been developed which is based on the number of spores in the tea garden in the air and the duration of surface wetness on the leaves. It was found empirically, by observation that the number of basidiospores of the pathogen in the air at a given time was related to the percentage of shoots which were infected. The duration of sunshine is negatively correlated with the duration of surface wetness of the leaves. Therefore, the duration of sunshine was used as a measure of surface wetness indirectly. A prediction equation for the development of epiphytotic has been developed.

In India, very limited efforts have been made to develop prediction based on inoculum. Murlidharan and Rao (1981) developed rice blast warning with the help of trap plots. Shukla and Anjaneyulu (1992) attempted to correlate the rice tungro development with vector population and initial infection. Bedi and Saini (1979) showed that the severity of early blight of tomato caused by *Alternaria solani* was positively correlated with the concentration of inoculum sprayed on the crop.

In India the plant disease forecasting service is in its incipient stage. For forecasting rust epidemics, a number of rain samplers have been set in stations where facilities exist for their handling. The rain analysis gives precise data on the advent of the inoculum in a particular locality. Based, on this information as well as data collected by survey teams from time to time, a large number of wind trajectories have been prepared. The air-borne inoculum and its deposition from the southern hills has been traced by wind trajectories and weather satellite-cloud images. If ground level conditions are favourable for infection, the early arrival of inoculum can lead to severe rust epidemics (Nagarajan and Singh, 1975).

In the case of brown stripe downy mildew caused by *Sclerophthora rayssiae* var. *zeae*, Payak *et al.* (1970) have observed that the disease is severe in areas in India where average rainfall is 100–200 cm or more. In areas of low–average rainfall, the increase in disease severity may occur only if there is a continuous spell of heavy showers and cloudiness for a few days accompanied by lowered temperature (25^0C or less).

Brown leaf spot of rice is caused by *Helminthosporium* or*yzae*. Certain abnormal trends have always been associated with a severe outbreak of *Helminthosporium* disease. For instance, according to Sundararaman (1922), usually heavy rains at the time of ear formation followed by flooding and ill drained conditions had favoured the epiphytotic of 1918–19 in the deltaic tracts of the Krishna and Godavari.

Padmanabhan (1963) studied the meteorological factors associated with the epiphytotic of 1942 in Bengal. A comparative examination of the meteorological data of 1942 with those of the non-epiphytotic years of 1943 and 1944 revealed that in 1942 there was heavy rainfall in September accompanied by a favourable temperature variation of 25–30 °C, followed by continuous cloudy weather during October and November, a higher minima than usual in November as well as low solar radiation in this month. Aeroscope studies carried out by Chandwani *et al.* (1963) had shown that it was precisely under these conditions that an abnormally large number of spores were reported on the slides.

In the case of bacterial blight of paddy, our knowledge of the relationship of the environment to the disease appears to be based on field observations rather than on critical studies under controlled conditions (Srivastava, 1972). The bacterial blight of paddy develops readily above 25 °C and symptoms may not appear below 20 °C. Goto *et al*. (1955) have reported that a combination of rainy weather, strong winds and a temperature of 22–26 °C favour the outbreak of the disease.

Ramalingam (1970) has discussed the results obtained in Andhra Pradesh in the development of rice blast. He mentioned that a temperature range of 25–27 °C, relative humidity of 86–90%, mild winds and darkness (midnight to sunrise) were critical for the development of epiphytotic. Rice crop is most susceptible to blast at three stages of crop growth, viz., seedling stage beyond 21 days, post transplanting stage, that is, 25–50th day after transplanting and at flower emergence. When these three stages coincide with favourable environmental factors, blast outbreaks occur. It has been determined as a result of a study made at the Central Rice Research Institute, Cuttack, Orissa, that a minimum temperature of 20–24 °C and a relative humidity above 90% occurring in the morning hours are very favourable for the outbreak. When such conditions continue to prevail for two days or more during the susceptible stages of the crop, as indicated above, blast outbreak is expected. Thus with the help of meteorological data, the outbreak of blast can be forecast (Padmanabhan, 1950). Govindswamy (1970) stressed the need for recording the number of low temperature days (20 °C and less) in forecasting blast of rice.

Type of Reproduction of the Pathogen

Some of the microbes such as most fungi, bacteria, and viruses produce many offspring while a few fungi, all nematodes, and all parasitic plants produce comparatively small number of offsprings. Some plant pathogenic fungi, bacteria and viruses are polycyclic, i.e., they produce many generations of offspring in a single growing season. Most of the rusts, mildews, and leaf spots are polycyclic and are responsible for most of the plant disease epidemics. Some of the soil fungi like *Fusarium*, *Verticillium*, and nematodes have one to four reproductive cycles per growing season. Such type of pathogens often cause localized and slower developing epidemics. Several monocyclic pathogens such as smuts require whole year to complete a life cycle. In such cases (monocyclic diseases) the epidemics usually develop over several years and lead to long term epidemics.

Ecology of the Pathogen

Some pathogens produce their inoculums on the surface of the aerial parts of the host and these can be dispersed easily over a range of long distances and can cause wide spread epidemics while in some pathogens such as bacteria, viruses, protozoa, and vascular fungi reproduce inside the host and spread of such pathogens are rare or impossible without the help of vector. Therefore, such pathogens can cause epidemics only when vectors are in large number and active. Still some soil-borne bacteria, fungi and nematodes produce the inoculums on infected plant parts in the soil and here the inoculums disperses slowly and chances of sudden and wide spread epidemics are little or minimum.

Mode of Spread of the Pathogen

The spores of rusts, mildews, and leaf spots are released into the air and can be dispersed by strong winds over long distances. Such types of fungi are responsible for widespread epidemics. The next most important group of pathogens includes those whose inoculum is carried by air borne vectors. Some fungi (Dutch elm disease), bacteria (bacterial wilt of cucurbits), many of the viruses, mollicutes, and even nematodes are transmitted primarily by beetles, aphids, whiteflies, and leaf hoppers. Some of the pathogens such as anthracnose, and apple scab are transmitted by wind blown rain and are responsible for serve but localized epidemics. Generally soil borne fungi often cause local disease but when such soil borne fungi like *Sclerotinia sclerotiorum* on lettuce produce wind-disseminated ascospores can spread over a long distance and can cause epidemics on lettuce plant.

Host Physiology/Factors based Prediction

According to Agrios (2005), the physiology of the host keeps changing in response to environmental conditions to which it is exposed during its growth. The study of physiology of plant is important for forecasting plant disease epidemics.

Resistance or susceptibility of the host Host plants carrying vertical resistance will not allow a pathogen to become established and thus no epidemic can develop while plants carrying horizontal resistance allow a pathogen to become established but the degree at which the disease and epidemics will depends on the environmental factors and the levels of resistance. So, susceptible host plants lacking genes of resistance against the pathogen provide the ideal substrate for establishment and development of new infections.

Genetic uniformity of host plants When genetically uniform host plants with regard to the genes associated with disease resistance are grown over longer areas, there is every possibility that a new pathogen race will appear that can attack the genome and result in an epidemic. The highest rates of epidemics development generally occur in vegetatively propagated crops in comparison to self pollinated or cross pollinated plants.

Type of crop In diseases of annual crops such as vegetables, rice and cotton epidemics generally develop usually in a few weeks in comparison to perennial woody plants. Some epidemics of fruit and forest trees take years to develop.

Age of the Host plant

1. Pathogens attack their host plant at certain period of its growth. For example, *Phytophthora infestans,* may cause severe infection of either sprouts on tubers or crop at its later stage of growth. Potato plants during intermediate stage of their growth do not show much susceptibility to the pathogen.
2. The host plant growing under normal conditions usually has sufficient nitrogen and mineral matter for requirement of the attacking fungus, but at certain stages of its growth there is not sufficient spare carbohydrates for the fungal pathogens needed for their growth and reproduction. At this stage of its growth, host plant generally shows less susceptibility to the attacking fungus.
3. Plants are more susceptible at that time when they have plenty of spare carbohydrates.
4. Leachates from various plant tissues are known to exert influence on growth, reproduction, and other activities of microorganisms in the environment of a plant. If the quality of leachates from roots and other plant tissues can be determined or regulated in relation to prevailing weather condition, it may be possible to forecast the occurrence of many diseases caused by soil-borne and air-borne pathogens.

Effect of Human Cultural Practices

Human intervention has a direct or indirect effect on plant disease epidemics as some of them favouring and some reducing the rate of epidemics. Like low lying and poorly drained areas if near the infected fields tend to favour the development of epidemics. Similarly the use of infected seeds, nursery stock and propagating material increases the initial inoculum within the crop and favours the development of epidemics. Another practice like monoculture, addition of large amount of fertilizers, dense planting, excess irrigation and poor sanitation conditions increase the development of epidemics in a particular

area. Generally fungicides, cultural practices, and biological control reduce the possibility of epidemics but continuous use of certain fungicides may lead to the development of resistant strains of the pathogen that lead to epidemics. The movement/transportation of infected seeds, tubers, and nursery stock from one place to another increases the possibility of introducing pathogen invite new areas where the hosts are not resistant to these pathogens.

Molecular Tools based Prediction

The most important molecular tools are the use of genetic (DNA) probes that allow the detection and identification of a particular plant pathogen in a mixture of other microorganisms in the vicinity of host plant. The detection and identification of a particular pathogen by its genetic (DNA) probe are made through the use of PCR (polymerase chain reaction) technique which amplifies a specific fragment of DNA present on a probe and produces many copies of it.

RAPD (random amplified polymorphic DNA) markers is another technique, that are used to detect genetic similarities among pathogenic strain known to show genetic heterogeneity can also be used for designing SCAR (sequence characterized amplified region) markers for detecting the pathogen in diseased plant tissue. The significance of these techniques lie in the fact that they detect the pathogen arrival much earlier thereby allowing farmers to get ready and use whatever control measures are most effective against the pathogen. Even new mutant pathogens that could attack plant varieties or could not attack earlier or tolerate the fungicide and produce a new resistant pathogen can be detected. Such information is important in epidemics/epidemiology because such changes make revision of any previous predictions about the development of epidemics and recommendations for control of the disease.

GIS (Geographical Information System)

The GIS is a computer system that can be installed to computer and is capable of collecting, storing and displaying data that are referenced by geographic coordinators. This system can be used from a single field to a large agricultural region. It is used in better understanding of environment and management of plant disease epidemics which often go unnoticed without GIS. GIS techniques are also incorporated in disease forecasting systems.

Global Positioning System (GPS)

GPS consists of a hand hold device that is coordinated with man-made satellite and enables one to identify a particular tree or a specific area of a field that are infected by a pathogen. So this particular area or tree could be treated with a particular fungicide without visiting the entire area. GPS can also be used to apply fungicide, plant nutrients, etc., in only areas that are infected with the pathogen or in areas deficient in a particular micro or macro nutrients. Elimination of a particular pathogen by early detection with the help of GPS is quite effective in not allowing the pathogen to cause an epidemic in a particular area.

Remote Sensing

It is an instrument used for measuring electromagnetic radiation reflected or emitted in the ultraviolet, infrared, or visible part of the spectrum from an object. The instruments used for remote sensing may be digital camera, video systems, ground based camera, and radiometers that may be carried on bal-

loons, aircraft and satellites. With the help of this system, an epidemic of particular tree or in a particular locality can be seen by aerial photography and that too without going to this particular field. The various remote sensing instruments store data, analyze directly or transfer to a computer for further studies. Generally infrared light or light of other wave lengths is used for the detection and progress of a plant disease in plants. Plants when infected with various pathogens change their colour like yellow or brown and have different reflectance from the healthy plant which is green. These different colors become more distinct when photographed with the different wavelengths which can be examined with specific equipments to distinguish disease colored plants and will provide information of newly diseased plants and rate of progress of a particular disease.

Examples of Plant Disease Forecasting Systems

Here we shall discuss some Indian attempts regarding the plant disease forecasting.

Predicting Rust Epidemic in India

Nagarajan and Singh (1975) formulated "Indian Stem Rust Rules" for *Puccinia graminis tritici*. These rules explain satisfactorily if the primary inoculum from South India would travel tens of hundreds of kilometers before its deposition in central India or not. The stem rust prediction methods proposed by them consist of the following steps:

(a) Check if there were any rains during November in central India coupled with southerly winds.
(b) Check the urediniospore (uredospore) content of the rain.
(c) Check if the Indian stem rust rules are satisfied. If a tropical cyclone occurred during November (Rule "**a"** of the Indian Stem Rust) disease would appear near the area where cyclone dissipated. If the inoculum arrives early, disease can develop into severe proportions. If rainfall satisfying the weather condition of rule "**b"** or **"c"** occurred during January or February, only traces of the disease would appear; that is too late in the crop season to be of serious threat.
(d) Check the weather over the area of urediniospore (uredospore) deposition.
(e) Check the viability of the transported spores, i.e., if the trajectory for 700/850 m bar passed near the south foci within 120 hours, spores would be viable.
(f) Check if the ground level conditions following the deposition by rain are favourable for infection or not.

If all the conditions are satisfied, the first detectable infection could occur in 25–30 days at locations close to where the cyclone dissipated. Once infections have occurred in central India, their further spread can be predicted following the same rules with the deletion of step "a" and "c". Further spread occurs to the east and north of India from these established foci. More the number of foci, greater are the chances of epidemic development.

Based on this, Nagarajan *et al.* (1978) developed an empirical model, given below to predict of brown rust of wheat can become severe or not.

1. At least five to six infection sites should be observed at a minimum distance of 25 ± 5 km around 15–20 January during the survey of wheat crop in Uttar Pradesh and North Bihar.
2. Amount and number of rainy days during January to mid-April over north-west India should be two times the normal.

3. Over north-west India weekly mean maximum temperature during March and mid- April should be normal ± 1 °C. If the first and second steps are satisfied, then severe epidemic would occur. But it all of them are partly satisfied then the chances are that isolated epidemic can occur. On the other hand, if the temperature is low then in addition to brown rust, yellow rust also would appear aggravating the situation.

Predicting Late Blight of Potato in India

Forecasts of Potato blight have been made ever since the disaster of 1845. Bhattacharyya *et al.* (1982) had developed methods/rules for late blight of potato under India conditions. According to Bhattacharyya *et al.* (1982), under Indian hill conditions, rainfall is the primary factor for the generation of conducive condition for late blight. Bhattacharyya *et al.* (1982) worked out a correlation between the weather data and the actual data of appearance of the disease under Shimla (15 years), Shillong (7 years), and Ootacamund (5 years) conditions. It was established that:

1. If the day moving precipitation (30 mm for Shimla, 28.9 mm for Ootacamund, and 38.5 mm for Shillong which were observed to be the critical rainfall lines) associated with mean temperature of 23.9 °C or less continues for 7 days consecutive days the potato late blight would appear within 3 weeks and,
2. Hourly temperature ranges within 18–20 °C and associated with high RH (80% or more) for continuous 18 hours for 2 consecutive days, the potato late blight would appear within a week. Accordingly a warning service for spraying fungicides on potato crop against late blight has been initiated in the northern India hills and Nilgiris.

Establishment of disease forecasting services in India is a must for important diseases of vegetables, fruits, and food crops. In India, priorities have to be decided on type of diseases that need to be considered for forecasting. For the development of reliable forecasting systems, extensive laboratory and field studies are required for understanding the behavior of the pathogen and the host populations under varying environmental conditions. Forecasting of disease epidemics is still in its infant stage in India. There is a great scope and necessity to develop this branch which is, as pointed out in text, not only useful to farmers but also intellectually stimulating. No forecasting system in use at present is perfect. With the information of various environmental variables, forecasting has become more accurate. The problem remains of significant factors which are not measured. Both macro-and micro-climate is important and adjustment has to be made to monitor and predict the disease or epiphytotic depending on the disease and the host–pathogen combination

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Questions

1. How does plant disease forecasting help to control disease?
2. What are the environmental conditions necessary for the development of the epiphytotics of the following in India?
   (i) Potato blight
   (ii) Blast of rice
   (iii) Downy mildew of bajra
   (iv) Rusts of wheat
3. Give at least two examples of forecasts based on
   (i) amount of initial inoculum
   (ii) weather conditions
   (iii) host physiology
4. What are BLIGHTCAST, CERCOS, MYCOS, EPICORN, EPIVEN, and EPIDEMIC?
5. Discuss in brief the contributions of the following towards the epidemiology of the diseases.
   (i) K.C. Mehta
   (ii) S. Nagarajan
   (iii) Van Everdingen
   (iv) A. Beaumont
   (v) H.T. Cook

(vi) S.K. Bhattacharya
(vii) S.Y. Padmanabhan
(viii) V.S. Thakur
(ix) C.D. Mayee
(x) L.M. Joshi

6. What are GIS and GPS? How do they help in plant disease forecasting?
7. How molecular tools can be utilized in plant disease forecasting?

Management of Plant Diseases

11

Although there are several reviews, articles, and books on management of plant diseases, an article entitled, "Advances in Plant Health Management in the Twentieth Century" by Cook (2000) needs special mention as it discusses the advancement in plant disease management in a comprehensive manner including biological control.

The principal methods of controlling plant diseases are: (i) Avoidance, (ii) Exclusion (iii) Eradication, (iv) Protection, and (v) Immunization.

In order to eliminate all the impediments to maximum production of food, it is imperative to control the spread of plant pathogens by cultural methods, chemical methods, and by breeding disease-resistant varieties.

Cultural Methods

Stevens (1960) had discussed the cultural methods of disease control. According to him, these measures involve agricultural cropping, harvesting, and storage, tillage, crop rotation, soil management, growing of resistant varieties, planning of land use, and other related practices. In this book, the breeding of resistant varieties had been dealt with separately.

Avoidance of the Pathogen

Many plant diseases can be prevented by a proper selection of the land or field, choice of time of sowing, selection of varieties, seed and planting stock, and by modifications of cultural practices. The aim of these measures is to enable the host to avoid contact with the pathogen or to ensure that the susceptible stage of the plant and favourable conditions for the pathogen should not coincide.

Proper Selection of Geographical Area

Many fungal and bacterial diseases are more severe in wet areas than in dry areas. Crops which are susceptible to these diseases, if grown in wet areas, are likely to be affected by plant pathogens. *Moesziomyces penicillariae* (formerly *Tolyposporium penicillariae*), the pathogen responsible for the smut disease of bajra and the ergot of bajra caused by *Claviceps fusiformis* (*C. microcephala*), are more severe in wet areas, in regions where rain occurs for long durations during the flowering stage of the crop.

Selection of Field The selection of a suitable area or field for cultivation is very important from the point of view of better yields as well as protection of the crop from the ravages of pathogens. In the case of many soil-borne pathogens, if the same field is chosen for cultivating a specific crop, there is very likelihood of the disease appearing in severe form due to the build-up of the inoculum potential of the pathogen. Hence, it is advisable not to grow the crop in the same field, where in the previous year it was infected with a pathogen capable of surviving in the soil. *Colletotrichum falcatum* can persist in the soil for a couple of months. Even this short duration is sufficient for the development of disease in the case of sugarcane, if it is successively cultivated in the same field. It follows that a number of soil-borne diseases can be prevented if the same crop is not grown in the same field year after year. The examples are wilt of arhar (*Cajanus cajan)*, green ear disease of bajra (*Pennisetum typhoides,* the pearl millet), late blight of potato, stem gall of coriander, powdery mildew of wheat, smut of bajra, ergot of bajra, ear cockle of wheat, root knot of vegetable crops, and bacterial wilt of solanaceous plants.

The drainage conditions of the field are also important; low lying, waterlogged fields favour such diseases as red rot of sugarcane and downy mildew of bajra. In the case of fruit orchards, the selection of a suitable site is very important. If apple trees are planted in areas where oak trees were previously grown, there are chances of collar rot due to *Rosellinia* sp. becoming severe.

Choice of Time of Sowing Pathogens are able to infect susceptible plants only under certain environmental conditions. For example, downy mildews require prolonged high moisture for infection. If the crop is grown in such a way that it is not attacked by pathogens during its most susceptible period, disease incidence can be considerably reduced. *Rhizoctonia* root rot of gram is severe if gram is sown immediately after the rains. This is due to the fact that the pathogen develops rapidly under high temperature and moisture conditions. A slight delay in sowing is helpful in this case. In the same way, *Puccinia graminis tritici* does more damage to a wheat crop sown late. Early seeding also induces losses due to bunt of wheat.

Disease-Escaping Varieties Certain varieties of crops escape damage because of their growth characters. These varieties escape the onslaught of the pathogen and resist the attack due to their inherent characteristics, for example, early maturing varieties of wheat or pea escape damage due to *Puccinia graminis tritici* and *Erysiphe polygoni*, respectively.

Selection of Seed and Planting Stock In India, seed potatoes are invariably imported in the southern states from the Shimla hills for control of viral and bacterial disease. In the USA, seed growing areas have been shifted to the dry Pacific regions for crops such as cabbage, turnip, beans, and peas for obtaining disease-free seeds and indirectly controlling such diseases as black leg and black rot of cabbage and turnip, and anthracnose of beans, peas, etc. In the case of foot rot and leaf rot of *Piper betle* caused by *Phytophthora parasitica* var. *piperina* and rhizome rot of ginger (*Zingiber officinale*) caused by *Pythium myriotylum*, it is advisable to obtain seeds (cuttings or rhizomes) from disease-free areas where the disease has not occurred due to certain environmental factors. Since many plants propagate by vegetative parts, the selection of disease-free planting material forms a very important control measure. The planting of disease-free fields is often an important control measure for certain diseases such as red rot of sugarcane, black scurf of potato, or various viral diseases of potato. Tuber indexing has been resorted to for obtaining virus-free seeds and protecting crops against the attack of various viral diseases of potato. Loose smut of wheat, bunchy top and Panama disease of banana, and whip smut of sugarcane can be effectively kept under check if seeds or cuttings are properly selected before sowing or planting.

Modification of Cultural Practices The distance between plants, time and amount of irrigation, quantity and quality of fertilizers or organic manures, time and method of planting, mixed cropping method, depth of sowing, and so on are some cultural practices which influence the incidence and severity of certain diseases. The necessary changes in these practices can help to reduce disease incidence and loss of the crop.

Tillage is an effective method of control of pathogens harboured in crop residue, but it leads to loss of soil organic matter and increased soil erosion. Tillage provides a temporary improvement in aeration, accelerates drying, and redistributes substrates. These environmental and physical changes can predispose pathogens to antagonistic effects of the microbiota. Early and deep ploughing kills cotton root pathogen and favours saprophytes rather that *Phymatotrichopsis (Phymatotrichum) omnivorua*. If the plant debris is buried deeper by ploughing, the pathogen is at a disadvantage as compared to other saprophytes in soil. Brooks and Dawson (1968) observed less take all disease due to *Gaeumannomyces (Ophiobolus) graminis* in England when wheat was grown in a no-till system.

Faulty mechanical sowing makes plant susceptible to infection. This may also result in an unequal and uneven distribution of seeds. Overcrowding may result in damping-off of seedlings. The depth of sowing has an important effect on some pathogens, notably those which attack seedlings. By delaying the emergence of seedlings, deep sowing may help to increase the resistance of a susceptible crop to a pathogen. This is true in many diseases caused by *Fusarium* and *Rhizoctonia* species, and in the smut diseases in which seedlings are attacked. Good control of blind seed disease (*Gloeotinia temulenta*) is obtained by planting infected seeds at least 1.25 cm deep and with complete soil coverage. Burying the infected seeds prevents the emergence (above the soil line) of the apothecia of the fungus *Gloeotinia temulenta* formed on them which is responsible for bringing seed disease of perennial rye grass. The dissemination of the ascospores is prevented by the procedure.

The incidence of wheat bunt caused by *Tilletia foetida* and *T. caries* and flag smut *Urocystis agropyri* (formerly *U. tritici*) of wheat is higher in crops sown deep. Mixed cropping is of special significance in Asian countries. This practice helps to effectively check the spread of infectious diseases.

Ploughing under the surface refuse before sowing controls *Sclerotium rolfsii* on peanuts. This deprives the fungus of a food base since it can grow only on near the soil surface. The effect, therefore, is very much like that of crop rotation. Deep ploughing of soils that contain diseased potato tubers can effectively reduce the amount of inoculum of *Phytophthora infestans*.

The root rot of cotton caused by *Rhizoctonia bataticola* and the blight of pulse crops caused by *Phyllosticta phaseolina* have been successfully controlled in Punjab by mixed cropping. Mixed cultivation of cotton and "moth" (*Phaseolus aconitifolius*) reduces the incidence of root rot of the former crop (caused by *Rhizoctonia solani* and *R. bataticola*). Mixed crops, such as wheat and barley, wheat and gram, arhar and jowar, cotton and moth reduce the economic losses from diseases. Management of soil water offers a means to manage the associated microbiota—directly, through water potential, and indirectly, through gas exchange of the soil.

Common scab of potato can be controlled by keeping the soil water potential near the tubers higher than -0.4 bar during tuber formation. The dry land foot rot of wheat caused by *Fusarium roseum* "Culmorum" can be controlled by irrigation, partly because the water reduces stress in the host and also because the germlings of *Fusarium* lyse or convert into new chlamydospores at water potentials above -10 to -15 bars. Flooding helps to rid the soil of *F. oxysporum* f. sp. *cubense* (Stover, 1962), responsible for Panama disease of banana and sclerotia of *Sclerotinia sclerotiorum* (Stover and Moore, 1953).

In Israel, the diseases of tomato caused by *Stemphylium botryosum* (*Pleospora herbarum*) and *Xanthomonas campestris* pv. *vesicatoria* were increased by overhead sprinkling, whereas *Leveillula taurica*, a powdery mildew of tomato which thrived in dry conditions, was considerably reduced. In the presence of excess free water, bacterial cells and fungal spores are disseminated easily. Sprinkler irrigation spreads bacterial cells and fungal spores in the same manner as raindrop splashes. In diseases where there is heavy sporulation of the pathogen or exudation of bacterial cells on the leaf surface, sprinkler irrigation should be avoided. Pathogens, such as *Pythium* and *Phytophthora* species, are dependent on high soil moisture content. Cereal rusts are more common in heavily irrigated crops or those grown in very wet soil. Dhingra and Sinclair (1975) observed a rapid deterioration of sclerotia of *Macrophomina phaseolina* in warm wet soil and proposed that disease could be controlled by keeping the soil wet for 2–3 weeks when the temperature was about 30˚C. Naturally, a proper control of irrigation practices helps to control these diseases.

Exclusion of Inoculums

These methods aim at preventing new pathogens and diseases from reaching an uninfected area and avoiding contact between the pathogen and the crop of field. Seed certification, crop inspection, growing crops in regions unfavourable for the pathogen, and quarantine measures are some means of preventing the spread of pathogens.

Seed treatment Seeds, tubers, grafts, bulbs, and other propagating material can be given heat, gas, or chemical treatment to keep them free of pathogens.

Inspection and Certification The crops grown exclusively for seeds are inspected periodically for the presence of diseases that are disseminated by seeds, and necessary precautions are taken to remove the diseased plants. The crop is then certified as disease-free. This practice helps to prevent inter and intra-regional spread of seed-borne diseases.

Quarantine regulation Quarantine can be defined as a legal restriction on the movement of agricultural commodities for the purpose of exclusion, prevention, or delay in the spread of plant pests and diseases in uninfected areas. In most agriculturally advanced countries, plant quarantine legislation has been placed on the statute book to restrict the movement of diseased plant material or of fungi, bacteria, mycoplasma, viruses, or nematodes that cause diseases in plants. Quarantine measures are of three types: (i) Domestic, (ii) International, and (iii) Total Embargoes. The quarantine laws were first enacted in the USA in 1912, and are known as Federal Quarantine Acts. In India, the Destructive Insect and Pest Act was passed in 1914 and subsequently supplemented by other provisions to enforce regulatory measures on import of biologically active material. It has been followed by Seed Act (1966), New Policy on Seed Development (1988), National Seed Policy (2002), Insecticides Act (1968), Environment Protection Act (1986), Protection and Plant Varieties and Farmers Right (PPVFR) (2001), and Biodiversity Act (2002). Such quarantine laws were first enacted in France in 1660, in Denmark in 1903. They aimed at a rapid destruction or eradication of barberry, which has been known since early times to harbour black rust. Some of the examples of diseases, which have been introduced into other countries, are listed in Table 11.1. Chahal (2010) has discussed legislative measures pertaining plant protection till 2010.

Table 11.1 Some examples of diseases introduced into other countries

Disease	Year	Introduced in	Introduced from
Late blight of potato caused by *Phytophthora infestans*	1830	Europe	S. America
Powdery mildew of grapes caused by *Uncinula necator*	1845	England	USA
Blister rust of pine caused by *Cronartium ribicola*	1910	USA	Europe
Downy mildew of grapevine caused by *Plasmopara viticola*	1878	France	USA
Chestnut blight caused by *Cryphonectria* (*Endothia*) *parasitica*	1904	USA	Asia (Mediterranean region)
Citrus canker caused by *Xanthomonas campestris* pv. *citri*	1907	USA	Asia
Bunchy top of banana (viral)	1940	India	Sri Lanka
Paddy blast caused by *Pyricularia grisea* (*oryzae*)	1918	India	South East Asia

Examples of plant diseases introduced in India before and after the enforcement of quarantine are given in Table 11.2.

Table 11.2 Some of examples of plant diseases introduced in India before and after the enforcement of quarantine

Disease	Year	Introduced from
Leaf rust of coffee caused by *Hemileia vestatrix*	1879	Sri Lanka
Late blight of potato caused by *Phytophthora infestans*	1883	Europe
Flag smut of wheat caused by *Urocystis tritici*	1906	Australia
Downy mildew of grapes caused by *Plasmopara viticola*	1910	Europe
Downy mildew of cucurbits caused by *Erysiphe cichoracearum*	1910	Sri Lanka
Downy mildew of maize caused by *Sclerospora phillipinensis*	1912	Java
Paddy blast caused by *Pyricularia grisea* (oryzae)	1918	Southeast Asia
Powdery mildew of rubber caused by *Oidium heveae*	1938	Malaya
Black shank of tobacco caused by *Phytophthora nicotianae*	1938	Dutch east
Crown gall of apples and pears (*Agrobacterium tumefaciens*)	1940	England
Bunchy top of bananas (viral)	1940	Sri Lanka
Wart disease of potato caused by *Synchytrium endobioticum*	1953	Netherlands
Onion smut caused by *Urocystis cepulae*	1958	Europe
Golden nematode of potato caused by *Heterodera rostochiensis*	1961	Europe

The DIP Act in India was passed in 1914 and has been revised several times from 1930 to present time, but it still does not fulfill the needs under the prevailing circumstances; of late, with the World Trade Organization (WTO) coming in place, the country has a challenge to appropriately comply with governing rules of agreements under WTO. In India, there are 53 quarantine stations operating under the Directorate of Plant Protection and Quarantine: nine at airport (Amritsar, Mumbai, Kolkalta, Chennai, New Delhi, Patna, Tiruchirapalli, Trivandrum and Varanasi) and seven on land frontiers (Amritsar

railway station, Attari-Wagah Border, Attari Railway station, Bongaon, Gede Road, Kalimpong and Sukhia Pokaria) and eight at sea ports (Bhavnagar, Mumbai, Kolkatta, Cochin, Chennai, Rameshwaram, Tuticorinm and Vishkhapatnam). The enforcement of plant quarantine regulations is carried out by the technical officers of the Directorate of Plant Protection and Quarantine, Ministry of Agriculture, Government of India, under the overall supervision of Plant protection Advisor. The research material is examined by these agencies: The National Bureau of Plant Genetic Resources, New Delhi, for agricultural and horticultural crops; the Forest Research Institute, Dehradun, for forest plants; and Botanical Survey of India, Calcutta, for all other plants of general economic significance. NBPGR has established two field stations at Pantnagar (for tropical plants) and Ranichauri (for temperate plants) for post-entry quarantine checking of imported plant material (Chaube and Pundir, 2005). There are a number of lacunae in the DIP Act, and as a result many serious plant pathogens have already been introduced in the country. These are now established and are taking a heavy toll on crops in certain areas. Golden nematode (*Heterodera rostochiensis*) and wart (*Synchytrium endobioticum*) diseases of potato have been introduced in India from the European countries and are now well established in the Nilgiri Hills and Darjeeling. Fortunately, timely action had prevented the spread of wart disease to other parts of India. *Synchytrium endobioticum* was first described by Ganguly and Paul in 1953 and golden nematode of potato (*Heterodera rostochiensis*) was reported by Jones for the first time in India in 1961.

Bunchy top disease of banana was introduced into India around 1940 (Verghese, 1945). By 1943 it had spread to a few areas in Kottayam and it has now affected an area of about 3000 square miles in Kerala. The disease has also spread to Tamil Nadu, Orissa, West Bengal, and Assam. In India, domestic quarantine measures exist for two insect pests: Fluted Scale (*Icerya purchasi*), and San Jose scale (*Quadraspidiotus perniciosus*); and for three diseases: wart (*Synchytrium endobioticum*) of potato, bunchy top (virus), and mosaic (virus) of banana with a view to preventing the spread of these pests.

Raychaudhuri (1973) had discussed seed pathology in relation to plant quarantines and the seed industry in India. Joshi (1974) had dissertated on the regulatory control of plant disease weeds and pests in India. Plant quarantine measures have also been discussed by Lambat and Ram Nath (1974). Ram Nath and Lambat (1971) had listed fungi recorded on imported seeds and other plant materials in India.

The problem of plant diseases is global and not national. Hence, in 1951 in Rome, an international Plant Protection Convention was drawn up which at present has large number of nations.

Management of the pathogens under quarantine regulations fall under the following heads: (1) Seed Certification, (2) Notification of Plant Diseases, (3) Prevention and Removing or Roguing of Diseased Plants or Seeds Containing the Pathogen, and (4) Plant and Field Sanitation for Reduction of Inoculum.

Eradication of the Pathogen

These measures primarily aim at breaking the "infection chain" by removing the foci of infection and starving the pathogen. Knowledge of the life cycles of the pathogens, method of perennation, host range, and habit of growth are essential prerequisites for devising suitable measures to prevent the disease. Eradication involves eliminating the pathogen from infested areas, and the magnitude of the operation involved may vary considerably. Eradication is attempted through roguing, sanitation, crop rotation, eradication of alternate and collateral hosts, heat, and chemical treatment of diseased plants, soil treatments, biological control, and so on.

Roguing This practice entails careful removal and destruction of infected plants or plant parts from the fields at an early stage by removing the "foci" of infection and preventing wide dissemination of

the pathogen. Roguing is employed in such diseases as loose smut of wheat, loose, and covered smut of barley, sorghum, and maize, red rot of sugarcane, green ear disease of bajra, ergot of bajra, wilt of arhar, and many viral diseases, such as yellow vein mosaic of lady's finger. "Katte" disease of cardamom, Panama disease of banana, *Loranthus* growing on trees, etc.

Eradication of Alternate or Collateral Hosts Many plant pathogens complete their life cycles on two hosts; one of them is generally a wild plant. The destruction of such a wild host plant breaks the chain in the completion of the life cycle of the pathogen. Barberry eradication in temperate countries helps to control the black stem rust of wheat. Many plant pathogens have several alternate/collateral hosts on which they pass their life cycles. *Pyricularia grisea* (*P. oryzae*) can infect and survive on several wild species of rice and other grasses, such as *Panicum repens*, *Digitaria marginata* and *Setaria intermedia*. The destruction and removal of collateral hosts is an important control measure of the disease. The yellow vein mosaic of lady's finger (*Abelmoschus esculentus*) persists on wild hosts (*Hibiscus tetraphyllus*) in nature, and the systematic removal of the wild plants helps to control the disease. Similarly removal of *Panicum antidotale* or *Cenchrus ciliaris* for pearl millet fields contributes to lowering of ergot disease.

Crop Rotation Very often, continuous cultivation of the same crop or related crops leads to the perpetuation of a pathogenic soil-borne fungus and the gradual increase in the intensity of the disease. Soils generally suffer from what we call soil sickness. When the same crop is raised year after year in the same field, the soil-borne pathogens of that crop easily perennate in the soil and increase their population. After a certain period of time, the soil becomes so heavily infested with the pathogen that it no longer supports the growth of the crop. Such soils are called "sick soils". The interposition of 2 or 3 non-susceptible crops between the cultivation of the susceptible crops in the same field is effective in reducing the soil sickness and populations of soil-borne plant pathogens. Crop rotation helps to control many soil-borne diseases, such as wheat mosaic, wilt of arhar, wilt of linseed, finger and toe diseases of crucifers, wilt of pea, wilt of gram, red rot and wilt of sugarcane, ergot and smut of bajra, bunt of wheat, leaf smut and bunt of rice, flag smut of wheat, molya disease (a nematode disease) of wheat, and barley ad root rot of vegetable crops.

Crop rotation method is also important from other aspects. If the same crop is sown in the same area year after year, there are chances that a particular essential nutrient in the soil will be exhausted. There can also be the accumulation of toxic substances and organic acids if a specific crop is continually cultivated in the same area. Crop rotation is generally recommended with botanically unrelated crops as these will have different nutritional requirements and tend to be affected by different pathogens and pests.

Sanitation Like plant sanitation, field sanitation is also essential for the control of many plant diseases. Pathogens survive or perennate through dormant structures in plant organs in the soil. The collection and destruction of these structures form an important control measure. It is a common practice to burn diseased stubble for plant disease control. The effectiveness of fire for controlling plant disease has been reviewed by Hardison (1976). In fact, sanitary conditions are helpful in almost all infectious diseases. Powdery mildew of wheat, barley, peas, downy mildew of peas and maize, red rot of sugarcane, Panama disease of banana, *Verticillium* wilt of cotton and wilt of arhar, Koleroga of Areca palms, foot rot and leaf rot of *Piper betle*, brown rot of stone fruits, late blight of potato, angular leaf spot of cotton, etc., are some examples where sanitary precautions help in minimizing losses. The spread of certain diseases, for example, tobacco mosaic virus, would not be rapid if the workers who smoke were to wash their hands before handling the plants.

Heat or Chemical Treatment of Diseased Plants The pathogen present in the plant can be killed or inactivated by applying heat or chemicals. Hot water treatments have generally been most successful and are used to eliminate *Ustilago segetum* (*nuda*) from wheat and barley seed, *Alternaria brassicae* from cabbage seeds, *Puccinia menthae* from mint runners, the nematode *Dictylenchus dipsaci* from Narcissus bulbs and ratoon stunt and other diseases from sugarcane setts. The most important application of hot air treatments is to eliminate viruses from vegetatively propagated material. Kunkel (1936) started this practice in the USA and Kassanis (1949, 1954) in England.

Thermotherapy is a time old practice that is still in vogue in the control of several crop diseases. Thermal inactivation points of sugarcane and its pathogens are taken advantage of freeing infected stocks of many shoot, ratoon stunting smut diseases and red rot of sugarcane. Four types of heat treatment are used for administering thermotherapy to sugarcane setts: (i) Hot air treatment (HAT) at 54ºC for 8 hours, (ii) Moist hot air treatment (MHAT) at 54ºC for 4 hours, (iii) Hot water treatment (HWT) at 50º C for 2 hours, and (iv) Aerated steam treatment (AST) at 50ºC for 1 hour.

Treating the seeds with fungicides, especially organomercurials, was a common practice to remove the superficially-borne inoculum, and there are now systemic fungicides available which can eliminate deep-seated fungal infections. Tyner (1953) found that soaking barley seeds for 48 hours in a 0.2% Chloranil (Spergon) solution at 22.2–25º C satisfactorily controls the loose smut fungus *Ustilago segetum hordei* (*Ustilago nuda*), on barley. Thiram soak has been successfully utilized to eliminate *Septoria appiicola* from celery seed, and *Mycosphaerella pinodes* and *Ascochyta pisi* from pea seed. The systemic fungicides, Vitavax and Plantavax, have been successfully used as seed dressing for the control loose smut (*U. segetum* var. *tritici*) and rust (*Puccinia graminis tritici*) of wheat respectively.

Soil Treatments Soil treatment involves the use of chemicals, heat energy, flooding, fallowing, and so on. Plant pathogens can be eradicated from the soil by dry heat, steam or hot water, flooding and fumigation with chemicals. Heat and chemical treatments have been successfully used for the eradication of plant pathogens from green house soils as well as soils in plant beds. The treatment of soils in large fields has not been so effective. Nematodes can be eradicated from the soil by fumigating it with Dichloropropene and Dichloropropene (DD) mixture or with other nematicide. Pentachloronitrobenzene (PCNB) has been found to be effective in eradicating sclerotia producing soil pathogens, carbon disulphide in eliminating *Armillaria mellea* and formaldehyde in controlling onion smut disease. Newhall (1955) had reviewed the literature on the subject of soil disinfestation by heat, flooding, and fumigation. Munnecke and Van Gundy (1979) had reviewed movements of fumigants in soil, dosage responses, and differential effects.

Physical Agents that Reduce or Eradicate the Inoculum

Many different types of control methods aim at eradicating or reducing the amount of pathogen present in an area or plant part.

Soil Sterilization by heat Soil can be sterilized by the heat carried in live or aerated steam or hot water. Soil sterilization is completed when the temperature of the soil has remained 82˚C for at least 30 minutes, at which temperature most of the pathogens present in soil are killed. At about 50˚C, some Oomycetes, nematodes are killed, whereas most plant pathogenic fungi and bacteria are killed at temperature around 60–72˚C. At about 82˚C, some weeds, the remaining plant pathogenic bacteria, viruses and insects are killed. It is important to see that very high temperatures not only kill all microflora in the soil but also result in the release of toxic levels of some salts like manganese. In addition to this, by

killing the nitrifying bacteria, there is accumulation of toxic level of ammonia which may kill the plants afterwards.

Soil Solarization Solarization is just to capture the solar radiations/energy to heat up the soil. The soil is covered airtight with polyethylene mulches/sheeting during the period of intense solar radiation.

Katan *et al.* (1976) from Israel suggested for the first time, the actual technology of soil solarization, i.e., use of transparent polyethylene film to increase soil temperature to the levels to eliminate the propagules of *Fusarium oxysporum* f. sp. *vasinfectum* and *Verticillium dahliae* in soil. Soil solarization involves trapping of solar heat through polyethylene covering during sunny summer days, to raise the soil temperature at the top 5 cm of soil as high as 52°C where it becomes lethal to temperature-sensitive soil microorganisms. The physical and chemical balance of soil is also altered by the action of trapped solar energy. Solarization is a complex process involving direct thermal inactivation/destruction of pathogen propagules, shifting in microbial balance populations and activity towards creating soil suppressiveness with simultaneous changes in physico-chemical properties of the soil. Many plant viruses are transmitted by aphids, leafhoppers, white flies, beetles, and thrips etc. When vertical, sticky yellow polyethylene sheets are erected along the edges of susceptible crops. A considerable number of insects are attracted to and stick to the plastic sheet. This is done mainly to trap and monitor insects. It also helps in reducing the amount of virus inoculum reaching the crop.

Hot Water Treatment Hot water treatment is used to kill any pathogen which may be present inside or outsides of seeds, bulbs, and nursery stock. This treatment is quite effective for some diseases like loose smut of cereals in which the pathogen resides as mycelium inside the seed where it could not be treated by contact fungicides. Similarly for nematodes that may be present in bulbs and nursery stock such as *Ditylenchus dipsaci* in bulbs of various ornamentals and *Radopholus similis* in citrus root stocks, hot water treatment frees them from nematodes. In the loose smut of wheat, the seeds are kept in hot water at 52°C for 11 minutes, whereas bulbs treated for *D. dipsaci* are kept at 43°C for 3 hours because the dormant plant organ can tolerate higher temperature than their respective pathogens.

Disease Control by Radiation Various radiations such as UV light, X rays, gamma rays, and β particles have the ability to control post harvest diseases of fruits and vegetables. Some pathogenic fungi like *Alternaria*, *Botrytis* and *Stemphylium* sporulate only when they receive UV light below 360 nm. This can be done by constructing the green house with a special UV absorbing vinyl film that blocks the transmission of light wavelength below 390 nm. Post harvest diseases of strawberries, peaches and tomato were controlled by gamma radiations (Agrios, 2005).

Disease Control by Drying Stored Grains and Fruits All grains and legumes carry a large number of microorganisms that can cause decay and production of mycotoxins in the presence of sufficient moisture. Such decay can be controlled if such seeds are dried until the moisture content is reduced to about 12%. Similarly, many fruits like grapes, plums, dates and figs can be stored dry for a long time and free of fungal infection if they are dried properly before storage and moisture is kept below a certain level during storage that will not allow fungal infestation.

Disease control by Refrigeration It is the most widely used and the most effective method of controlling post harvest diseases of perishable fruits and vegetables. Low temperature inhibits the growth, and inactivates the pathogens thereby reducing the spread and initiation of new infection. The magnitude of disease control through this method and its value to consumers is immense.

Biological Eradication of Plant Pathogens More than ninety years ago, a paper by Sanford (1926) on potato scab caused by the actinomycete *Streptomyces scabies*, brought the rather sudden realization that other soil microorganisms must exert a natural biological control even on root disease pathogens. The term biological control clearly implies control of a disease through some biological agency, and the term "biological agency", means a living microorganism or macroorganism other than the diseased or damaged plant acting as host and the pathogen or pest causing the disease or damage. According to Garrett (1965): "Biological control of plant disease may be defined as any condition or practice whereby survival or activity of a pathogen is reduced through the agency of any other organism (except man himself), with the result that there is a reduction in the incidence of the disease caused by the pathogen."

There is an enormous amount of literature on biological control of plant pathogens. Some recent books and review articles are those of Adams (1990), Hornby (1990), Nelson (1991), Stirling (1991), Tjamos *et al.* (1992), Mukhopadhyay (1994, 1996), Dwivedi (1993), Agarwal (1991), and Bagyaraj and Govindan (1996), Mehrotra *et al.* (1996), and Mehrotra (1997). Gnanamanickam (2002) has edited a book entitled *Biological Control of Crop Diseases*. Several examples of the biological control of plant pathogens can be given. Millard and Taylor (1927) reported the control of scab in potatoes grown in sterilized soil and inoculated with *Streptomyces scabies*, through simultaneous inoculation of the soil with *S. praecox*, a vigorous saprophytic species.

It was in as early as 1932 that Weindling showed that *Trichoderma viride*, a common, saprophytic fungus, is able to parasitize the mycelia of other fungi. Later, he showed that the lethal action of *T. viride* is due to the secretion of an antibiotic substance which is called "gliotoxin". Still later, Brian and McGowan (1945) isolated another antibiotic substance from *T. viride* which they named "viridin". These discoveries raised hopes of controlling plant diseases by biological means.

In 1951, Bliss, in the USA, who was working on root diseases of citrus caused by *Armillaria mellea* opened a new approach. He suggested that if the soil is fumigated with carbon disulphide, the disease inciting fungus is killed not directly by the fumigant but indirectly by the dominance of *T. viride*, the population of which increases after fumigation. Garrett (1958) tested the effect of fumigation and the antibiotic effect of *T. viride* and assessed the relative parts played by these two components. He found that 30% of *Armillaria mellea* was killed due to the dominance of *T. viride*, while the rest was directly killed by the fumigant. He also pointed out that in the thicker plant roots, *T. viride* may be instrumental in the process of killing than the fumigant which might not be able to reach easily.

The most conspicuously successful biological control is by Rishbeth in England. Rishbeth has been studying a root disease of pines (*Pinus sylvestris* and *P. nigra*) caused by basidiomycetous fungus *Heterobasidion* (*Fomes*) *annosus.* The disease spreads through infection by basidiospores produced in the large fructifications. The spores infect the left-over stumps when the trees are felled. Rishbeth at first tried to control the disease by painting these stumps with fungicidal paints, such as creosote. This brought only partial control because the stump tissue cracked with progressive drying; causing fissures through which the fungal spores of *H.* (*Fomes*) *annosus* could enter and cause infections. Rishbeth's research associate, Meredith (1960), made an important observation that along with *H.* (*Fomes*) *annosus* there were a few other fungi which were of the nature of weak parasites that colonized the cut surfaces of stumps. Rishbeth (1963) had pointed out that he originally selected *Phleviopsis* (*Peniophora*) *gigantea* for this purpose because it is the most common and effective competitor of *H.* (*Fomes*) *annosus* in the natural colonization of pine stump surfaces. It decomposes lignin and cellulose vigorously and the fungus establishes itself in the stump quickly and from there into the stump roots. From these stump

root infections, the pathogen invades the roots of other healthy trees. Rishbeth showed that if paste of oidia of *P. gigantea* is applied on the stumps. It parasitizes it rapidly and kills the tissues, making it easy for saprophytes to colonize and decompose the stump and making tissues unsuitable for an attack by *H.* (*Fomes*) *annosus* which is a specialized parasite. Rishbeth determined that a dose of $1x10^4$ oidia of *Phleviopsis* (*Peniophora) gigantea* allowed a satisfactory margin of safety for effective biological control of the largest spore inoculum of *H. annosus* for a pine stump of 16 cm wood diameter. Dehydrated tablets of oidia have been produced for distribution and use in controlling *H. annosus.*

Although a large number of microorganisms have been shown to be antagonistic to the growth of a large number of plant pathogens in the laboratory, only a few have been found effective and practicable in field conditions. According to Agrios (2005) relatively few microorganisms have been registered so far. These are *Gliocladium virens* sold as GlioGard for control of seedling disease. *Trichoderma harzianum* sold as F-stop for control of several soil-borne fungi. *T. harzianum/T. polysporum* sold as BINAB-T for the control of wood decays, *Agrobacterium radiobacter* K-84, sold as Gallex or Galltrol for controlling crown gall disease caused by *Agrobacterium tumefaciens*, *Pseudomonas fluorescens* sold as Dagger G for use against *Pythium* and *Rhizoctonia* species, and *Bacillus subtilis* sold as Kodiak and utilized as seed treatment. Pseudomonas species primarily of *P. fluorescens*, *P. putida*, *P. cepacia* and *P. aureofaciens* applied to seeds, seed pieces, and roots of plants have resulted in less damping off, less soft rot, and an increase in the growth of the crop. According to Thind (2015) in India, so far only 12 biopesticides are registered as compared to more than 175 globally. Some of the more commonly used biopesticides in India include *Bacillus thuringiensis* against lepidopterous pests, *Trichoderma viride* and *T. harzianum* against root rot, collar rot, stem rot and wilt diseases in several crops particularly relevant for dry land crops, fungal insect pathogens such as *Metarhizium anisopliae* and *Beauveria bassiana* against several insects, nuclear polyhedrosis viruses against lepidopteran pests of cotton, rice and vegetables, *Trichograma chilonis* as egg parasitoid for control of stem borer in sugarcane and neem-based product azadirachtin (technically azadirachtin may not be called as biopesticide, it would better termed as botanical pesticide) against sucking pests and fruit borers in many crops.

Different mechanisms have been proposed to explain disease suppression by *Pseudomonas* spp. They include:

1. Production of siderophores (iron chelating compounds) which remove iron from the soil environment and make it unavailable to pathogens.
2. Antibiosis—various antibiotics have been reported to be produced by members of genus *Pseudomonas*.
3. Induction of resistance, and
4. Competitive root colonization.

Biological control of chestnut blight caused by *Cryphonectria* (*Endothia*) *parasitica* is controlled naturally in Italy and artificially in France, through inoculation of cankers, caused by the normal pathogenic strains of the fungus, with hypovirulent strains. The hypovirulent strains carry viruses like double standard RNAs (ds RNAs). The disease was first encountered in the USA in 1904 and despite attempts to eradicate it, spread to destroy most of the native chestnuts in the eastern states. When in 1938 the disease was first recorded in Italy, it was feared that European chestnut trees would suffer a similar fate. The initial outbreak was severe but later it was found that many infected trees would begin to show spontaneous signs of recovery with healing. When the strains of the pathogen were isolated from such healing cankers, it was found that these strains were less virulent than the original pathogen. Furthermore, if these hypovirulent strains were coinoculated with highly virulent strains, the resulting cankers also

healed. Most strains could be converted to the hypovirulent culture. Some transmissible factor moved from the hypovirulent strains to the virulent strains to make it hypovirulent strains.

The agents responsible were shown to be cytoplasmic and subsequently identified as double-standard RNA molecules. It seems that hypovirulent strains are due to a type of viral infection. The natural transfer of hypovirulents of *C. parasitica* suggests that the disease might be managed by introducing hypovirulent isolates into the pathogen population. In Europe this has happened to some extent naturally. But the situation is quite different in USA. MacDonald and Fulbright (1991) have discussed aspects of the use and limitations of hypovirulence. In the USA due to larger genetic variations between *Cryphonectria* in that region, for the transfer to occur, hyphae must fuse and this often is limited by natural compatibility barriers. To overcome this difficulty there is now interest in engineering replicating forms of the ds RNA into a range of *C. parasitica* strains representing different compatibility types. In this way more efficient spread of the hypovirulence should occur and the disease epidemic could be restricted.

Mycorrhizal Fungi as Biocontrol Agents Mycorrhizal fungi have received considerable attention in recent years. Mycorrhizal fungi groups better in infertile soils. The role of mycorrhizal fungi in the biological control of fungal root pathogens is well known (Reid, 1990, Chandra 1992). Several mechanisms have been postulated. These are: (i) Creating a mechanical barrier for the pathogen to penetrate, (ii) Inducing thickening of cell walls through lignification and production of other polysaccharides, (iii) Stimulation of host roots to accumulate metabolites, (iv) Stimulating flavanolic wall infusions, (v) Increasing concentrations of dihydroxy phenols in roots, (vi) Producing antibacterial and antifungal antibiotics, and (vii) Stimulating microbial activity in the root zone.

Implementation of biological control is fraught with constraints, and successful application depends on a thorough knowledge of the ecology and biology of both the target pathogen and the biological control agent. Paramount to efficacious employment of biological control is knowledge of the specific mechanism of microbial antagonism involved. Documented biological control mechanisms for plant pathogens include competition, hyperparasitism, predation, and the production of extracellular metabolites such as antibiotics, hydrogen cyanide (HCN), and siderophores. Miller *et al.* (1996) discovered a novel antimicrobial metabolite, a biosurfactant. Stanghellini and Miller (1997) have discussed the biosurfactants, their identity, and potential applicability in the biological control of zoosporic plant pathogens. The intentional use of synthetic surfactants for the control of zoosporic pathogens was pioneered by Tomlinson and Faithfull (1980). During their studies on the control of big vein of hydroponically grown lettuce, which is caused by a virus vectored by zoospores of *Olpidium brassicae*, they observed that disease control was achieved following the addition of a Benzimidazole fungicide (Bavistin, BASF) to the recirculating medium. It was subsequently discovered that the inert ingredients in the fungicide formulation exhibited lytic activity against zoospores of *Olpidium*. It was suspected that the inert ingredients contained a synthetic surfactant. Further studies demonstrated the lytic effects of various surfactants. Biosurfactants of diverse chemical nature are produced by a variety of microorganism. For example, some isolated from the following genera are reported to produce surfactants: *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Rhodococcus*, *Actinobacter* and *Corynebacterium*. Several fungal genera including *Candida* and *Torulopsis* are reported to produce surfactants. Perhaps the best studies of the biosurfactants is a class of glycolipids, the rhamnolipids which are produced and secreted by *Pseudomonas* spp. Rhamnolipids as biocides have been demonstrated by Stanghellini and Miller (1997). Biosurfactants produced by *Pseudomonas aeruginosa* were tested against a range of zoospores of *Pythium aphanidermatum*, *Phytophthora capsici*, and *Plasmopara lactucae-radicis*. At concentrations ranging from 5–30 ug/ml they caused cessation on motility and lysis of the entire zoospore population in less

than a minute. Thus the possibility of utilizing bacteria or fungal species producing biosurfactants for biological control of plant pathogens on the aerial parts is a distinct possibility.

Soil Amendments Many root diseases such as potato scab have been controlled by ploughing organic material into the soil, which started the chain of these investigations. Several others have been successfully controlled in this way, such as *Phymatotrichum* root rot of cotton, "take all" (*Gaeumannomyces graminis*), several *Rhizoctonia* and *Fusarium* diseases. In the case of *Phymatotrichum* root rot of cotton, it was shown by Mitchell *et al.* (1941), Clark (1942), and King *et al.* (1934) that fresh organic materials stimulate the germination of sclerotia and this exhausts the inoculum.

In the case of *Gaeumannomyces* (*Ophiobolus*) *graminis*, it has been suggested that organic amendments, rich in carbon and deficient in nitrogen, control the "take all" disease of wheat. This is explained as follows: as a result of organic amendments with a high C/N ratio, there is a tremendous increase in the microbial number and activity. There is considerable liberation of CO_2 by soil saprophytes, which suppresses the pathogenic activity of this fungus. In the process of survival also, low nitrogen content in the soil reduces the longevity of the fungus.

The organic amendments increase the activity of saprophytic organisms in a flush which results in the abundant liberation of CO_2 as a result of the respiratory activity of the microorganisms. The pathogens sensitive to CO_2 are inhibited. Due to the rapid multiplication of microorganisms in the soil, the available nitrogen in the soil is rapidly utilized by the fast-growing saprophytes. This results in acute nitrogen scarcity which adversely affects the growth of the pathogens. In their studies on the control of bean root rot with barley amendments, Snyder *et al.* (1959) established that additions of nitrogen nullified control. It is summarized that *Fusarium solani* cannot develop without nitrogen which is required for germination and penetration (Cook and Schroth, 1965; Toussoun *et al*., 1960). Further studies by Maurer and Baker (1965) showed that C/N ratios greater than 25/1 significantly suppress symptoms of bean root rot. The germination of the chlamydospores is poor and the hyphae produced are also depauperate. This is perhaps due to the locking up of available soil nitrogen in the cells of microorganisms, which decompose the organic material. This is thought to deprive the chlamydospores of the pathogen of even the minimal amount of soluble nitrogen required for germination and host infection. The pathogen and the disease develop rapidly, if nitrogen is increased. It has been shown that the inability of the conidia to germinate in aqueous extracts of soil solution with cellulose (C/N ratio higher than 25) and barley (C/N ratio 55) can be overcome by as small a quantity of nitrogen as 5 ppm. All these factors show that nitrogen limits pathogenesis. Extensive data on the behavior of *Rhizoctonia solani* have been provided by Papavizas and Davey (1960,1961), Davey and Papavizas (1960) for the control of *Rhizoctonia* disease of snap bean (*Phaseolus vulgaris*) by the use of organic amendments. Blair (1943), in studies with Rossy Chlodny slides demonstrated that the growth of *Rhizoctonia solani* in unsterilized soil is checked by the incorporation of 1% finely divided organic matter, such as dried grass, alfalfa, or wheat straw. Concerning the possible mechanism of this biological control, Davey and Papavizas (1963) have produced data that largely confirm the explanation originally given by Blair (1943) based upon the carbon dioxide content of the soil atmosphere and the scarcity of available nitrogen in the soil solution. Both effects are produced by rapid decomposition of nitrogen-deficient organic matter in the soil. Davey and Papavizas (1963) showed that maximum inhibition of competitive saprophytic colonization of buck wheat stem segments by *Rhizoctonia solani* occurred in soils receiving amendments adjusted to C/N ratio from 100–400. A lower degree of inhibition was provided by amendments with either higher (400–200) or lower (20, 10, and 5) C/N ratios. Thus result can be explained by postulating that nitrogen exerts two distinct and opposite effects upon the activity of *R. solani*, the first being predominant down to C/N ratio 40 and the second coming into action at C/N rations of 20 and below.

The first or inhibitory effect can be attributed, as Davey and Papavizas (1960) have pointed out, to the action of nitrogen in increasing the rate of decomposition of the organic material, and hence promoting a more rapid output of carbon dioxide. This increase in carbon dioxide output can be inspected to closely follow the trend in total microbial number from C/N ratios 400 down to 5. At C/N ratio 20, however, the second or stimulatory effect (with respect to *Rhizoctonia solani*) of nitrogen comes into force and the total depressing effect of the organic amendment upon the fungus is suddenly replaced sharply. This can be ascribed to the presence of the available nitrogen that can be directly absorbed by the mycelium of *Rhizoctonia solani*, thus removing the disability of nitrogen starvation suffered by fungus in the presence of organic amendment adjusted to higher C/N ratios. This change is precisely where we might have predicted it to have occurred, that is, in the region of C/N ratio of 20. The C/N ratios of fallow soils and of microbial substances are generally around 20.

Mitchell and Alexander (1962) reported the control of root rot of bean by manuring soils naturally infested with *F. solani f. phaseoli* with chitin at the rate of 1020 kg/hectare. The number actinomycetes was greatly increased following the addition of chitin, but not of bacteria, while in the number of fungi was somewhat depressed. Pursuing the hypothesis that the addition of chitin and related substances to the soil promotes the heterolysis of chitin-walled fungi, Mitchell and Alexander (1963) reported the presence of a polysaccharide laminarin in the hyphae walls of *F. solani f. phaseoli* but not in *Pythium debaryanum*. Mitchell (1963) reported that soil amendment with either chitin or laminarin at 1020kg/hectare reduces incidence of bean root rot and also of radish wilt due to *Fusarium oxysporum f. conglutinans*. But none of these amendments can control diseases caused by pathogens, which do not contain chitin in their walls, such as *Pythium debaryanum* and *Agrobacterium tumefaciens*.

Phytophthora root rot of avocado was controlled by Zentmyer (1963) by amending the soils with alfalfa meal—a material of low C/N ratio. Similarly, Mehrotra and Tiwari (1976) could successfully control the root rot of *Piper betle* by organic amendments, and antagonists growing on organic amendment in the soil. Amendment with corn straw gave best control. Corn straw supplemented with nitrogen (NH_4NO_3) gave still better control. The effect of different antagonists growing on corn straw and till *Sesamum indicum* oil cake amendments showed that *Trichoderma viride* gave best control. Mehrotra and Claudius (1972) tried to control the root rot and wilt diseases of *Lens culinaris* by organic amendments of the soil and also by introducing antagonists in the soil along with the organic amendments. The wilt of lentil caused by *Fusarium oxysporum f. lentis* was checked with amendments of wheat straw, wheat straw plus ammonium nitrate, corn straw and sugarcane straw. The successful antagonists were *T. viride* and *Streptomyces gougeroti*.

Biological control in potato diseases of underground parts has been studied by Shrivastava and Saksena (1968) at Sagar. In soil amendments, the *Rhizoctonia* disease was controlled by nitrogen-rich amendments, such as the tops of bean plants. Carbon-rich amendments such as cellulose and sugarcane straw did not control the disease at all. This could be explained on the basis of enhanced activity of *Rhizoctonia solani* which readily decomposes cellulose. The wilt disease due to *Fusarium solani* could not be controlled in these experiments by organic amendments. One noteworthy example of biological control with green manuring is that of potato scab with a soybean cover crop in southern California (Weinhold and Bowman, 1968). *B. subtilis* multiplied equally well in pots of soil amended with either green soybean or green barley tissue, although the bacterium multiplies in much greater amounts on soybean than barley. There are many instances where attempts have been made to infest the seeds or propagules with organisms with a view to reducing root disease. The Russian workers are specially advanced in this line of work. Some workers have however, emphasized the inconsistent effects of seed inoculation with respect to biological control.

Hyperparasitism Certain fungi and bacteria are parasitic on plant pathogens. The genus *Cinccinobolus* includes species of fungi, which attack members of the family, Erysiphaceae. *Cinccinobolus* seldom reduces the attacks of the powdery mildews in nature because it rarely occurs in large quantities. *Cinccinobolus* can be cultivated on artificial media, however, and some good results have been obtained by artificial inoculation. Several workers have tried to utilize *Eudarluca australis* (pycnidial stage known as *Darluca filum*) for controlling rusts. The fungus does control the disease under certain natural climatic conditions over which man has little control. Extra inoculum of the hyperparasites will not be of much use as the weather, unless very favourable to the development of hyperparasite, is unlikely to permit any marked control of the disease. There are several other fungal species which attack rusts, such as *Tubercularia* spp. attacks aecia and spermogonia of several rusts and *Verticillium hemileiae* on *Hemileia vastatrix*.

Many fungi are known to parasitize phytopathogenic nematodes. In 1934, Rozsypal and Schmidt reported a *Protomycopsis chytridiale* which attacks *Heterodera schachtii.* In 1933, H. Goffart recognized that the eggs and embryos of *Heterodera avenae* could be parasitized by an ascomycete, *Cylindrocarpon radicicola*, which is known to attack the underground parts of various cultivated plants. Hyphomycetes as nematode predators are numerous, and are frequently met with in nature. Drechsler (1937) described 40 species of fungi which attack nematodes. The important ones are *Dactylaria*, *Acrostalgamus*, *Dactylella* and *Arthrobotrys*. Several attempts have been made to utilize fungi for the control of nematodes, such as meloidogynes which attacks pineapple in Hawaii, for nematodes parasitic on animals in France, and for controlling the golden nematode of the potato in England. Some encouraging results were obtained but practical utilization of these fungi in agriculture is still a remote possibility.

Bacteriophages In 1917, d'Herelle presented evidence of a transmissible lytic principle that acted on the Shiga bacillus, showing that bacteria have their infective diseases too. A number of phages have now been discovered for many phytopathogenic bacteria such as *Agrobacterium tumefaciens*, *Erwinia aroidea*, *E. carotovora*, *E. atroseptica, Pseudomonas angulata*, *P. coronafaciens*, *P. glycinea*, *P. lachrymans*, *P. phaseolicola*, *P. pisi, P. syringae*, *P. tabaci*, *P. xanthoclona*, *Xanthomonas campestris pv. citri*, *X. campestris pv. malvacearum*, *X. campestris pv. phaseoli*, *X. campestris pv. pruni*, *P. campestris pv. solanacearum*, *X. oryzae pv. oryzae*, etc., but not much success has been obtained in controlling these diseases in nature with the help of their respective phages. Also, no plant disease carried by a bacterium has been controlled yet by treatment with phage after the disease has developed.

Entomogenous Fungi There are several fungi which are capable of growing in the bodies of insects thus destroying them. Scientists are looking for ways of protecting crops against insect pests. Late in 1940, with the introduction of DDT, it was felt that a method had been found to destroy insects affecting crops. DDT and scores of other insecticides that were introduced exterminated not only all the insects but also the predators, including fish in water (the insect enemies), with the result that in some cases more damage than any tangible benefit has occurred. Plant pathologists can use microorganisms, which are immune or at least not affected by pesticides for biologically controlling insects. One important fungus which has been utilized is *Entomophthora*. About 100 species are known, mostly as insect parasites. While some appear to be rather specific in their host range, others are capable of attacking a wide range of hosts. Some species have been grown in nature. One of them, *Entomophthora coronata*, a fungus which has been referred to by various names, for example, *Delocroixia coronata*, *Conidiobolus villosus*, *C. coronatus* (Sreenivasan and Thirumalachar, 1964; Sreenivasan *et al.,* 1964). This fungus is a parasite of aphids and termites but is also a common saprophyte of plant detritus.

Chemical Methods

Plant diseases play an important role in determining the amount and cost of food. Plant pathology must alleviate problem by devising new control measures and improving the older ones. The annual loss to world crops as a result of disease has been estimated at 25, 000 million dollars; of this a major part is due to fungal pathogens. Surface deposits of fungicides can control many fungal diseases if properly applied, but it appears that more effective control can be achieved with systemic fungicides which can penetrate and move into the plant.

There is an enormous amount of literature available on chemicals used for plant disease control. Some important Indian literature is that of Bhagwagar and Patel (1999) "Review of Work on Fungicides in India up to 1940", Vyas (1983) *Systemic Fungicides*, Grover (1986) "Chemical Control of Plant Diseases in India—An Overview", and Nene and Thapaliyal (1993) *Fungicides in Plant Disease Contro,* "Role of Fungicides in Modernizing Agriculture in India" by Mehta (1971); *Chemical Control of Plant Disease: An Exciting Future* by Sbragia (1975); and "Acquired Resistance to Fungicides" by Dekker (1976). A comprehensive reference source available in the field of fungicides is *Antifungal Compounds*, in two volumes edited by Siegel and Sisler (1977), Thompson (1993) *Antifungal Chemicals, Book IV: Fungicides*. "Crop Disease Management Practice in Ancient Medieval and Pre-Modern India" is ably discussed by Nene (2002). Thind (2012, 2015a, 2015b) has discussed in depth the role of fungicides in crop health security, the relevance of fungicides in the present day crop protection and the overall perspectives on crop protection in India.

The use of chemical sprays, dusts, or seed treatment for protecting plants from the ravages of pathogens is not an innovation of the 20th century. The early agriculturists did try to control plant diseases by utilizing chemicals and fungicides. The first recorded mention of plant disease control is in the writings of the Greek poet, Homer (1000 B.C.), who mentions sulphur, which is still in use. Also, the Roman patriot, Cato (200 B.C.), mentioned the fumigation of trees with bitumen and sulphur.

The first landmark in the control of fungal diseases of plants was the discovery by Anton de Bary that the causal agents of many plant diseases are fungi. The development of fungicides rapidly followed this discovery. It is about this time that Louis Pasteur discovered the bacterial parasite of the silk worm. He conclusively proved the real nature of fermentation and put an end forever to the doctrine of spontaneous generation. It is after this period that Pierre Alexis Millardet (1885) showed that the downy mildew of grapes could be controlled by mixtures of copper sulphate and lime. Copper sulphate had been in use even before that but it was only in 1885 that its toxicity was reduced by mixing it with lime. The Bordeaux mixture was used both in Europe and the USA for the control of many diseases. Substances used as late as the middle of the 19th century had offensive or caustic properties, acid or bitter taste and a pungent odor. These were regarded as good qualities in a fungicide. The Bordeaux mixture was followed by lime sulphurs in the late 19th century, and formalin, copper carbonate dusts, and organomercurials as seed treatments (1913). These were the first generation of fungicides. This gave way to an era of organic fungicides (second generation of fungicides). These included Thiram, Chloranil, Dichlone, which were developed from the mid-1930 onwards, and Captan, one of the most successful ones, was developed only in 1952. The emphasis then shifted to the oxides of copper. Many groups of chemical compounds are now available with the plant pathologists for plant disease control. These include heterocyclic nitrogen compounds, quinines, phenols and antibiotics. There is increasing interest in the chemotherapy of plants, and systemic fungicides are of current interests to workers in this field. The third generation of fungicides were also organic but could penetrate the plant tissue, i.e., systemic and control established

infections. These included 2-aminopyrimidenes (Ethirimol, Dimethirimol), Dicarboxyimides (Iprodione, Vinclozolin, Procymidone), organophosphorus compounds (Iprobenphos, Edifenphos), Triazoles (Triadimefon, Propiconazole, Flusilazole, Myclobutanil), Piperazines (Triforine), Imidazoles (Imazalil, Prochloraz), Phenylamides (Metalaxyl, Ofurace, Benalaxyl, Oxadixyl, Mefenoxam), alkylphosphanates (Fosetyl-Al), Cyanoacetamideoximes (Cymoxanil), cinnamic acid amides (Dimethomorph) and Morpholines (Tridemorph, Fenpropimorph), Piperidines (Fenpropidine). The fourth generation of fungicides consists of compounds that are ecologically safe, have still new modes of action, somewhat broad spectrum and applied at quite low dose rates. Examples of these are Strobilurins (Azoxystrobin), phenoxyquinolines (Quinoxyfen), Oxazolidinediones (Famoxadone), Anilinopyrimidines (Cyprodinil, Pyrimethanil), Phenylpyrroles (Fenpilonil, Fludioxonil), Spiroketalamines (Spiroxamine), Benzamides (Fluopicolide, Zoxamide), Valinamides (Iprovalicarb, Benthiocarb), Mandelamides (Mandipropamid), Cyanoimidazoles (Cyazofamid), Thiocarbamates (Ethaboxam), representing different chemistries and mode of action. Majority of these have been developed for use against oomycete pathogens thus indicating the necessity to manage these pathogens.

Before we discuss the various chemicals for plants disease control it becomes essential to define a fungicide. A fungicide is a chemical agent that kills or inhibits the development of the fungus spore or mycelium. The fungicides used on plants may be classified as protectants, eradicants and therapeutants on the basis of their uptake by and mobility within plant.

Protectants

As the name suggests, protectant fungicides are prophylactic in their behavior. Protectant fungicides, which may be applied to seeds, plant surfaces or the soil, cannot penetrate plant tissues in efficient amounts. Therefore, they act outside the plant prior to infection by the pathogen.

Eradicants

These chemicals eradicate the dormant or active pathogen from the host. They can remain effective on or in the host for some time and function as protectants also. Chemicals can be protectants as well as eradicants.

Therapeutants

A therapeutant is an agent that inhibits the development of a disease syndrome in a plant when applied subsequent to invasion by a pathogen. Therapy can be by physical means, such as solar energy treatment or hot water treatment of smutted wheat grains, but more often it is by chemical means and is then called chemotherapy. Usually the chemotherapeutants are systemic in their action, i.e., they enter the plants and affect deep-seated infection.

To be successful, fungicidal sprays must have some constant characteristics. To be effective against rain and dew they must be nearly insoluble in water, but at the same time, they must be soluble enough to function as fungicides. Tenacity or adhesiveness is another quality needed to enable them to stick well and last for a longer period. Further, they should not be toxic to the host parts (phytotoxic) so that they can be used safely. To meet all these requirements, various modifiers are mixed with the fungicides; these are known as stickers, spreaders and safeners.

The fungicides are dealt with below under the following categories:

1. Inorganic Copper Compounds
2. Inorganic Mercury Compounds
3. Sulphur Sprays
4. Organic Sulphur Compounds
5. Quinone and Phenolic Fungicides
6. Heterocyclic Nitrogen Compounds
7. Benzene Compounds
8. Organomercurials
9. Systemic Fungicides
10. Organophosphate Fungicide
11. Antibiotics
12. Soil fumigants
13. Oils

Inorganic Copper Compounds

Bordeaux Mixture

Discovery of Bordeaux Mixture How to deter schoolboys from stealing their grapes had been a problem in the Modoc region for a long time. A poisonous looking mixture of lime and copper sulphate that struck tenaciously to the leaves when sprinkled on vines near road sides was the answer. Prof P. M. A. Millardet of the University of Bordeaux was deputed to study the disease introduced from America. Once in 1882, while walking through the Medoc countryside, he observed significantly less mildew symptoms on the sprinkled vines. It looked as if the cure of pilferage was the cure for mildew too. Prof Millardet and his chemist colleague, U. Gayon, in 1885, after several spraying experiment concluded that the mixture of copper sulphate and hydrated lime could effectively control the downy mildew of grapes caused by *Plasmopara viticola.*

In 1882, Millardet, investigating downy mildew of the vine (*Plasmopara viticola*), a disease then recently introduced in France from the USA, observed that along the roadside at Medoc in Gironde, certain vines bore leaves while others had been defoliated by the disease. Millardet believed that the foliage of some of the vines had persisted because of the dabbing of the leaves with verdigris, or with a mixture of lime and copper sulphate. Following this slender clue, Millardet in collaboration with Gayon was able to announce in 1885 the successful use of a lime–copper sulphate mixture as a fungicide against *Plasmopara*. This discovery has doubtless been the greatest boon to plant protection since about 50 million kg of copper sulphate is commonly used in the USA for this purpose every year.

The formula for Bordeaux mixture varies. The original mixture contained 15% copper sulphate and 8% lime (CaO). The proportion of these now varies according to the crop and the disease. Higher concentrations are useful in case of woody and tough tissues, while lower concentrations are used for succulent and softer parts. The following formula (5:5:50) is regarded as a good standard type:

Copper sulphate (blue stone)	5 lb (2.268 kg)
Stone or hydrated lime	5 lb (2.268 kg)
Water	50 gallons

The mixture is prepared by mixing $CuSO_4$ solution with limestone solution. The two solutions are prepared separately in wooden or earthen vessels and the diluted solution of $CuSO_4$ is slowly poured into a concentrated lime solution in a tub with constant stirring. The resulting mixture is neutral or

slightly alkaline and is not injurious. The mixture is used almost immediately after preparation and deteriorates if left for some time. However, the mixture may be stabilized by adding sugar or jaggery at the rate of 0.5 lb in 50 gallons of the mixture. The mixture stabilized in this manner will not deteriorate for several days under normal conditions. Alternatively, a stock solution of the two ingredients can be prepared separately and stored in wooden vessels. These can be mixed when actually required.

The chemistry of Bordeaux mixture is complex and is not fully understood. The expected reaction is:

$$CuSO_4 + Ca(OH)_2 = Cu(OH)_2 + CaSO_4$$

Cupric hydroxide is the active principle which is soluble and is toxic to spores and sporelings. The Bordeaux mixture is a suspension, which is fairly tenacious, nearly insoluble, and spreads quite well. It may be detrimental or beneficial to different hosts. Beneficial effects have been observed on grape, potato, and other crops. Greener foliage is often the outstanding result and it also has insect repellent properties. The Bordeaux mixture is sometimes phytotoxic to tender leaves and other parts and burns them as well as fruits and other aerial parts. Sometimes blemishes occur, as on apple fruit. The fungicidal action is due to the formation of soluble copper, which is toxic to spores or sporelings in drops atmospheric water.

The Bordeaux mixture is specific against downy mildews, such as late blight of potatoes, koleroga of areca nuts, coffee rust, various leaf spots diseases, blights, anthracnoses, and so on. It has remained the standard fungicide against downy mildews of grapes and other hosts since its inception in 1882.

Bordeaux paste Bordeaux paste consists of the same ingredients as those of Bordeaux mixture but it is in the form of a paste. It is mainly used for tree wound dressings to prevent fungal pathogen attacks such as in the control of stem bleeding disease of coconut. It is generally prepared by adding 1 lb of copper sulphate and 1 lb of lime in 1 gallon of water.

Burgundy Mixture

Burgundy mixture, which is a modification of the Bordeaux mixture, is often used for tender foliage, since it is less phytotoxic. In this, lime is substituted by sodium carbonate, and hence, is sometimes called 'soda Bordeaux'.

Copper sulphate	10 lb (4.536 kg)
Sodium carbonate	12.2 lb (5.666 kg)
Water	50 gallons

Burgundy mixture does not produce blemishes. It should be mentioned that neither burgundy mixture nor other substitutes of Bordeaux mixture have been widely accepted.

Cheshunt Compound

Bewley suggested this compound in 1921. It contains 2 parts of copper sulphate and 11 parts of ammonium carbonate. The two substances are well powdered and thoroughly mixed and the dry mixture is stored in an airtight receptacle for 24 fours before being used. If it is left in an exposed condition it gradually loses ammonia and becomes less effective. When the spray solution is to be prepared, one ounce of the mixture is dissolved in a little hot water and two gallons of the solution is made by adding cold water. Cheshunt compound is recommended for the control of "damping off" diseases as a soil drench in nursery beds.

Chaubattia Paste

This paste was developed by Singh (1942, 1943) at the Government Fruit Research Station, Chaubattia, in the Almora district of Uttar Pradesh (now Uttaranchal) in India. The paste is prepared by mixing

copper carbonate (800 g) and red lead (800 g) in 1 liter of lanolin or raw linseed oil. This paste was developed as a wound-dressing fungicide to be applied to pruned parts of pears, apple and peaches for the control of diseases, such as stem-black (*Coniothecium chomatosporum*), stem-brown (*Botryosphaeria ribis*), pink disease (*Corticium salmonicolar*), stem canker (*Monochaetia mali*) of apples and pears, and collar rot apples, peaches, apricots and plums (*Rosellinia* sp.).

Inorganic Mercury Compounds

Many inorganic mercury compounds are highly effective as fungicides and bactericides. Mercuric chloride ($HgCl_2$) and mercurous chloride (Hg_2Cl_2) are used as 1:1000 dilutions for soaking the seed, rhizome, and corms of vegetables and flowering plants to control certain bacterial diseases. Club root disease of crucifers can be controlled by treatment of *Brassica* seedlings against the pathogen. The use of inorganic mercury compounds is limited because they are highly toxic to animals and humans.

Inorganic Sulphur Compounds

The use of sulphur as a fungicide dates back to the Greeks but its effective use has been discovered only in 19th century. Sulphur is effective against powdery mildews on many plants, but is also effective against certain rusts, leaf blights and fruit rots.

Self-Boiled Lime Sulphur

This is made by using 8 lb of sulphur flour and 8 lb of burnt lime with 50 gallons of water. A small amount of water is added to the lime and when well shaken, sulphur is added and the ingredients are mixed vigorously. After boiling has ceased, the remainder of the water is added. This material is less toxic than the lime sulphur described above. Even this fungicide is now sparingly used and is being replaced by wettable table sulphurs. Lime sulphur has some eradicant properties also and is active against apple scab and powdery mildew.

Wettable Sulphurs

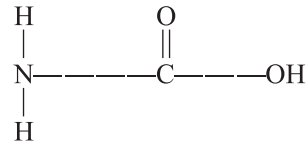
These are very finely divided (micronized) elemental sulphurs also called floatation sulphurs, which are made from hydrogen sulphide gas from the coke industry. These have come into extensive use because they cause less foliage injury. Wettable sulphurs are used against apple scabs, powdery mildews of grape and other crops, ornamentals, and vegetables. In green houses it is used against rusts also.

Strangely enough, the mode of action of sulphur as a fungicide is still unclear. Originally it was assumed that sulphur acted directly on the protoplasm of the fungus spore or mycelium and destroyed it. Later it was suggested that sulphur acted in the form of H_2S. Still others thought that it acted as sulphur dioxide. The most recent evidence suggests that sulphur acts as a hydrogen acceptor and thus interferes with the normal hydrogenation and dehydrogenation reactions of the cell.

Organic Sulphur Compounds

During the Second World War there was a dearth of metallic compounds of Cu and Hg which gave impetus to the development of organic compounds. Tisdale and Flenner (1942) first demonstrated the fungicidal possibilities of carbamates in 1931 in the laboratories of E. I. Du Pont Company, USA, but commercial production started a decade later. It was in 1934 that the patenting of dithiocarbamates was announced by Tisdale and Williams who patented "Thiram" and the use of this group of materials as insecticides and fungicides. Since then volumes of work have been done on the chemistry of thiocarbamates.

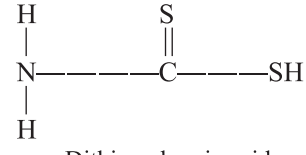
Carbamic acid is the starting point. It is derived from carbonic acid by the replacement of a hydroxyl group by an amino group.



Carbamic acid

Dithiocarbamic acid is derived by the replacement of two oxygen atoms by sulphur, giving the hypothetical dithiocarbamic acid.

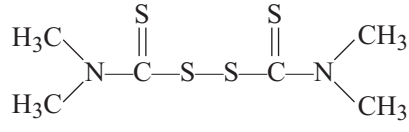
Form dithiocarbamic acid the following important fungicides have been derived.



Dithiocarbamic acid

Thiram (Tetramethyl thiuram disulphide)

TMTD is a condensation product of dithiocarbamic acid (2 molecules in which the H atoms have been substituted by methyl groups). This was used as an activator in the production of synthetic rubber.



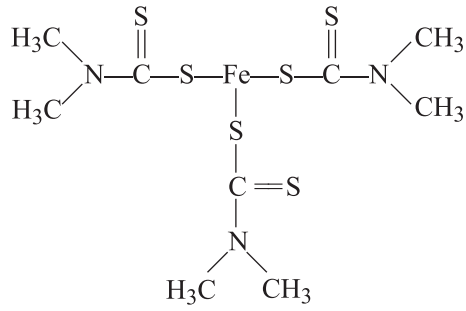
Thiram

Thiram is being sold under a variety of other trade names, such as "Arasan", "Tundas", "Terson", "Tulisan" and so on.

It is a leading seed treatment for wheat, rice, gram, peas, mustard, linseed, sorghum, and onion. Thiram is also used in the control of foliage diseases and acts as an insecticide. Among the soil-borne pathogens controlled by it are *Pythium*, *Rhizoctonia*, *Fusarium* and *Protomyces*.

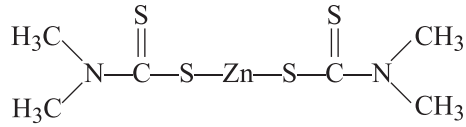
As a foliage spray, Thiram was not as successful as its metallic compounds. For this reason, several compounds have been developed which are as follows:

Ferbam (Ferric dimethyl dithiocarbamate) In Ferbam, three molecules of dithiocarbamic acid react with one atom of iron. Its trade names are Fermate, Ferbam, Karbam or Coromet. Ferbam is compatible with most pesticides, but its use with copper, lime or mercury compounds tends to reduce fungicidal efficiency. Ferbam has been successfully used as a protectant fungicide against a wide variety of fungus diseases of fruits and vegetables. As a foliage spray it is also used to correct iron deficiency.



Ferbam

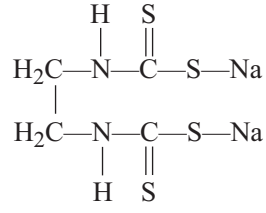
Ziram (Zinc dimethyl dithiocarbamate) The trade names are Cuman, Zerlate, Karbam white, Corozate, and so on. In India, this fungicide is available under the trade name of Cuman. It is used against many foliar diseases in field and orchard crops.



Ziram

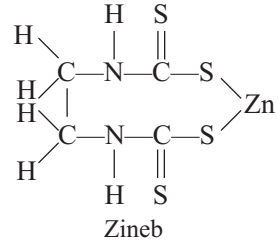
Nabam (Disodium ethylene bisdithiocarbamate) This is sold as Dithane D-14, Parzate liquid or Dithane A-40. Nabam has been found effective against many leaf blights, such as early and late blight of potatoes and seedling blights caused by *Pythium*, *Rhizoctonia* and *Fusarium* species.

A similar compound of zinc, Zineb was later developed.



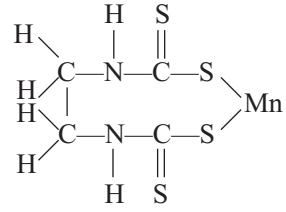
Nabam

Zineb (Zinc ethylene bisdithiocarbamate) Zineb is derived by the substitution of sodium in Nabam with Zinc. It is called Dithane Z-78, Parzate C or Lonacol. Zineb is mainly used for foliar sprays against diseases, such as early and late blight of potatoes and tomatoes, blast of rice, leaf blight of rice, ripe rot of chilies, and downy mildew of maize.



Zineb

Maneb (Manganese ethylene bisdithiocarbamate) This is called Dithane M-45. Maneb is excellent for the control of foliage and fruit diseases of many vegetables, flowers, trees, turf and some fruits. In many diseases Maneb has been found more effective than Zineb. It is especially used in vegetable crops as a spray material. Some of the diseases controlled by Zineb are bean anthracnose caused by *Colletotrichum lindemuthianum*, downy mildew and anthracnose of cucurbits, fruit rot of chillies, citrus greasy spot caused by *Cercospora* spp., lettuce downy mildew, maize leaf blight, blight of tea caused by *Exobasidium vexans*, and leaf spots and blights caused by *Alternaria*, *Phytophthora* and *Cercospora* on crops, such as potato, tomato, cabbage, carrot, beet, and cauliflower. In all these dithiocarbamates, the usual dose is 1 kg in 500 liters of water. These carbamate compounds are very widely used both as dust and sprays for a very wide variety of crop diseases, such as *Alternaria* blights of potato and tomato, *Colletotrichum* anthracnose of tomato, cucurbit, bean, several rusts of *Uromyces* spp., several downy mildews of vegetables by *Peronospora*, *Plasmopara* and many others like brown rot and scabs.

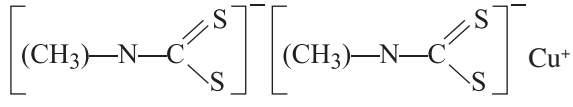


Maneb

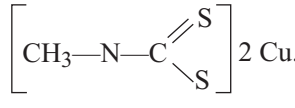
Two important clues to the toxic action of the dialkyldithiocarbamates and thiuram disulphides are their formation of metal chelates and their polymodal dosage response.

The first property is well known and is commonly used in analytical chemistry. The second property was first reported by Dimond *et al.* (1941) for Thiram. This latter effect is also called "inversion" and quenching is described in detail and explained by Goksoyr (1955).

The zone of inversion where increasing dosage actually depresses toxicity has been particularly hard to explain. The dimethyldithiocarbamte ion combines with minute traces in the medium to give the highly toxic 1:1 or half chelate.



This is the toxic species. But as the amount of copper in the medium is constant, the addition of more dithiocarbamate forms the less toxic 1:2 or full chelate, and toxicity falls.



As more dithiocarbamate is added, the intrinsic toxicity of the dimethyl dithiocarbamyl ion exerts itself, acting perhaps by adding essential metallic micronutrients. Thus, toxicity increases again.

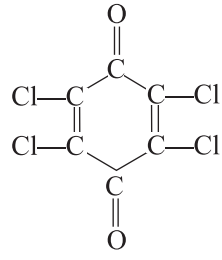
The toxicity of ethylene bisdithiocarbamates is believed to be due to the production of isothiocynate which reacts with SH groups.

Quinone and Phenolic Fungicides

Though not as important as the carbamates, quinone and phenolic compounds are quite important as fungicides in certain respects.

Chloranil or Spergon (Tetrachloro-p-benzoquinone)

It was reported in 1940 by Cunningham and Sharvelle to give protection to lima bean seedlings from damping off. Chloranil is mainly used in seed treatment. It is sold as Spergon and is used in seed and bulb treatment of legume flowers and vegetables. It is also used as soil drench.

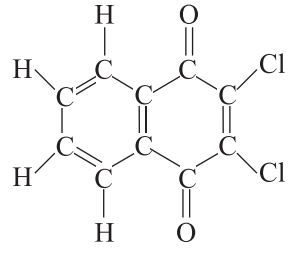


Chloranil

Dichlone or Phygon (2,3 dichloro-1,4 naphthaquinone)

This is a later development. Though it is used for seed treatment, it is also useful as a foliage spray against many plant diseases and is apt to cause dermatitis.

It has been reported four to eight times more effective than chloranil in the protection of legume and cotton seed.



Dichlone

There are two possible mechanisms by which Chloranil and Dichlone act—bringing the quinone nucleus to—SH and NH_2 groups in the fungus cell, and disturbances in the electron transport system.

Phenols

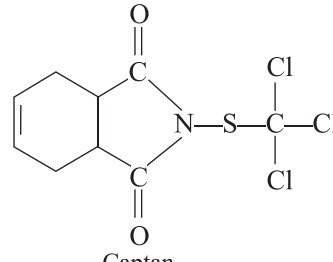
Phenols have shown exceptional qualities as bactericides, but as fungicides their use has been limited. Phenols have been chiefly applied to textiles and woods as preservative fungicides, but have also been used as soil fungicides and in seed treatments. Derivatives of cresol and O-phenyl phenol have been mainly used as preservatives. The bisphenols are a particularly interesting group of phenolic fungicides.

Heterocyclic Nitrogen Compounds

These compounds have a number of valuable fungicidal properties. Their principal uses are as foliage protectants and eradicants of fruits and vegetables. Glyodin (2-heptadecyl-2 imidazoline acetate) and Captan are the two important fungicides of this group.

Captan

Captan is the common name adopted for N-trichloromethyl thio-4-cyclohexene-1,2 dicarboximide. Kittleson (1952) prepared this compound and so it is sometimes called Kittleson's killer.



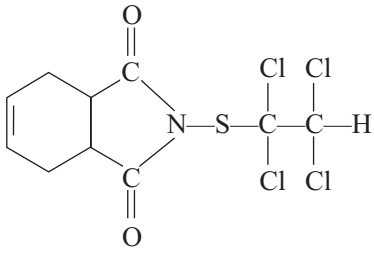
Captan

Captan is commercially sold under different names, such as Captan 50 W and Esso fungicide 406, but it is also claimed that it acts systemically. Hochstein and Cox (1956) suggested that Captan competes with co-carboxylase (thiamine pyrophosphate) for sites on co-enzyme–free carboxylase in the decarboxylation of pyruvate, and thereby interferes with the process of decarboxylation. Another theory of Captans action was presented by Lukens and Sisler (1958). They found that Captan interacts with -SH compounds, such as cysteine and glutathione, to give thiophosgene. Although a fairly broad spectrum fungicide, it is not generally effective against rusts, powdery mildews, and downy mildews. Owens and Blaak (1960 a, b) showed that Captan inhibits many oxidative enzymes and considered that its basic toxic action was an interaction of the intact Captan molecule with free sulphydryl groups. Captan is used as a spray, dust or for seed dressing.

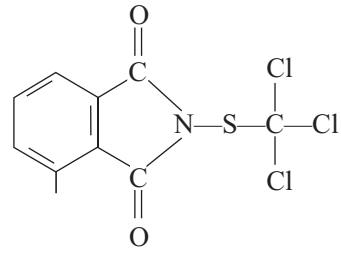
It has been used against diseases of different types of crops, but its use in fruit disease control and as a seed-dressing fungicide has found popularity all over the world.

Captafol or Difolatan (N-[1, 1, 2, 2-tetrachloro ethyl sulfenyl]-cis 4-cyclohexene 1, 2-dicarboximide)

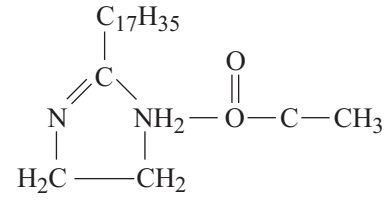
Captafol or Difolatan has properties similar to Captan. It is good fungicide for the control of early and late blight of potatoes and tomatoes.



Difolatan



Folpet



Glyodin

Folpet (N-trichloromethylthio phthalimide)

It is an analogue of Captan. Folpet is effective against *Sphaerotheca pannosa*. It is sold under the trade names of Phaltan, Orthophaltan, and so on.

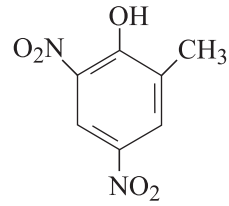
Glyodin (2-heptadecyl-2-imidazoline acetate)

West and Wolf (1955) suggested that the biological activity of Glyodin depended on the interference, in

the biosynthesis, of those purines which are components of nucleic acids. It is effective against *Venturia inaequalis*, *Coccomyces hiemelis*, *Diplocarpon rosae*, and *Podosphaera leucotricha*.

Benzene Compounds

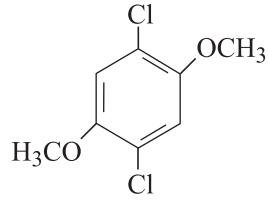
Many benzene compounds are toxic to microorganisms and several have been developed as fungicides. These have been discussed by Corden (1969). Dinitro-O-cresol is contained in the formulation called Kerenite or Elgetol. It is used as a dormant spray for the control of many diseases of fruit trees and ornamental plants and for the treatment of wounds in trees.



Dinitro-O-cresol

Chloroneb

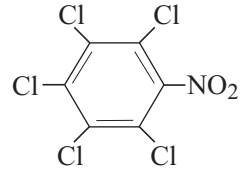
The fungicide, Chloroneb, 1,4-dichloro-2,5-dimethoxy benzene, was introduced by Ryker (1965) for the control of seedling diseases of cotton. It has been found active against *R. solani*, *S. rolfsii* and *Phytophthora cinnamomi*. This is a systemic fungicide and is unusual in that its activity does not follow clear-cut taxonomic divisions. This fungicide has been used for the most part as a seed or as in-furrow soil treatment at planting time for systemic control of treatment of seedlings diseases of cotton, beans, sugarbeets and soybeans.



Chloroneb

Pentachloronitrobenzene (PCNB)

This is sold as Quintozene, PCNB, Terrachlor, Brassicol, and so on. The chlorinated nitrobenzenes were introduced as fungicides in 1935 by Brown. They control many soil-borne diseases. It is used primarily against *Rhizoctonia* and *Plasmodiophora***.** PCNB also has nematicidal properties.



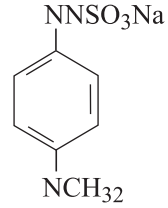
Pentachloronitrobenzene

Diseases caused by *Rhizoctonia*, *Sclerotium* and others are controlled by PCNB. It is not effective against Pythiaceous fungi.

Dexon

This is p-(dimethylamino)-benzene–diazosodium sulfonate and is used against soil-borne phycomycetous fungi causing root rot and damping off diseases of plants.

Zentmyer and Gilpatrick (1960) found that Dexon had a curative effect on avocado seedlings afflicted with *Phytophthora cinnamomi* when applied in the root region.



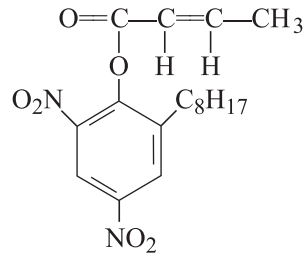
Dexon

Chlorothalonil (tetrachloro-isophthalonitrile)

Sold as Daconil, Bravo and Termil, this is a popular fungicide. It is a broad-spectrum contact fungicide belonging to the phthalimide group. It is used for the control of early and late blights, leaf spots, powdery mildews, downy mildews, apple scab, coffee berry disease, rusts (yellow and black), sigatoka of banana, and leaf spots of groundnuts.

Dinocap (Karathane)

Dinocap or Karathane is a mixture of isomers of 2-(1-methyl-heptyl), 4-6 dinitrophenyl crotonate. Dinocap is an excellent substitute for sulphur for the control of powdery mildews and is highly specific against them. It is also effective against mites. Actually it was developed as a miticide (insecticide for mites and leaf miners) and is now extensively used against powdery mildews.



Dinocap

Non-Aromatic Organic compounds

This group includes Dodine (n-dodecylguanidine acetate) used for the control of apple scab and several other diseases caused chiefly by Ascomycetes and Fungi imperfecti. Due to its toxicity to some crop plants, its use is limited. The mechanism of action of Dodine is due to its binding with sterols in the cell membrane, with the result that the membrane structure is altered.

Organomercurials

Organomercurials developed due to efforts directed towards finding less toxic substitutes for inorganic mercurials. The first important commercial product was "Uspulum" of the Bayer Company of Germany in 1915. Organomercurials are very widely used for seed treatment. They are derivatives in which the mercury atom is attached directly by one or both valency bonds to carbon atoms. The general structure of these derivatives is R.Hg.X where R represents a hydrocarbon with or without substituted groups; X represents an acid radical, such as chloride, nitrate, acetate, benzoate, gluconate. R is usually an ethyl or phenyl group. Some of the important compounds are Ceresan M (ethyl mercury p-toluene sulphanamide), Panogen (methyl mercury dicyandiamide), new improved Ceresan (ethyl mercury phosphate), Ceresan wet, Agallol or Aretan (methoxy ethyl mercury chloride), Ceresan dry (phenyl mercury acetate) and Agrosan G.N. (phenyl mercury acetate and ethyl mercury chloride mixture). Many other trade names are used for compounds which are in wide use. The chemicals can be used either as dips or dusts. Organomercurials fungicides are mainly used to control externally seed-borne diseases since these compounds are able to eradicate the inoculum from the seed. Their use in the control of externally seed-borne cereal smuts has been widespread.

Tubers and bulbs are also similarly treated with dusts against adhering organisms, such as potato scab, and black scurf. The hazards which organomercurials pose are too well known. Because of their persistence they get into the food chain. Several countries have now banned the use of these fungicides. India has also banned the use of organomercurials (Nene and Thapaliyal, 1993).

Systemic Fungicides

A new aspect was added to the control of fungal pathogens of plants when the oxathiin group of chemicals was developed in 1963. These compounds are systemic in their action and are receiving the active attention of pathologist. Most systemic compounds are absorbed by the roots and transported acropetally to the xylem. They are usually not transported downwards through the phloem tissue.

Major advances in plant disease control will now be made from systemic fungicide-compounds, which penetrate and move from their site of application to other parts of the plant. A systemic fungicide could eradicate established infection and protect the new parts of the plant as they are produced, and it would not be subject to loss by weathering. The idea of controlling disease by systemic fungicides is not new. Much of the earlier work has been done with antibiotics in view of their selective action against bacterial pathogens in animal tissues; sulfonalinides were investigated for the same reason. Systemic insecticides have been in use for several years, and after much effort, effective systemic fungicides are now becoming available. Vyas (1984) has ably dealt with systemic fungicides. They can be applied in solution as spray on the foliage is less likely to be effective against the pathogens responsible for root diseases. Several systemic fungicides have been tested as seed-dressing to eliminate seed infection. Systemic fungicides have not been very successful in the case of trees and bushes. From the practical point of view, the systemic fungicides offer unique possibilities in the tropics, since the active spray residues are not subjected to tropical weathering, thus permitting fewer applications.

Action of Systemic Fungicides

Very little is known about the mechanism of systemic fungicide. The following are the possibilities:

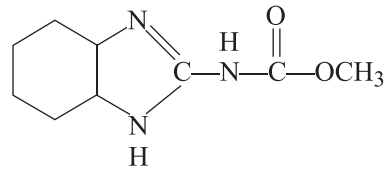
1. Inactivation of the enzymes and toxins of the pathogen.
2. Selective accumulation of the fungicide due to greater permeability of the fungus cell wall.
3. Damage to the membranes of the fungal hyphae and inhibition of structures, such as appressoria, cushion formation, emergence of germ tubes, and formation of haustoria.

4. Inhibition of fungal enzymes or their destruction. Systemic fungicides are more specific in their action than non-systemic fungicides.

The study by Horsfall and Zentmyer at the Connecticut Agricultural Research Station of 8-hydroquinoline as a systemic chemotherapeutant against Dutch elm disease was the first of its kind. Since 1964, however, a perplexing variety of successful systemic fungicides has appeared in quick succession on the agricultural scene.

The following are some of the systemic fungicides used in plant disease control.

Benomyl This is also marked as Benlate. This is methyl-N (butyl carbamoyl)-2 benzimidazole carbamate. Benlate was discovered by E. I. du Pont de Nemours and Co. Inc., the USA, in 1968. The benzimidazoles have been very effective in the control of *Mycosphaerella* on banana and peanut. The systemic activity of Benomyl, the most striking fungicide at present, was first reported by Delp and Klopping (1968). Benomyl hydrolyses in acidic solution to methyl benzimidazole carbamate (MBC). MBC has been shown to be the principal fungitoxicant of Benomyl, which is systemic in plant tissue.



Methyl Benzimidazole Carbamate (MBC)

Benomyl has been found effective against a wide range of pathogens. It has a high degree of miteovicide activity. It is effective against *Cercospora* leaf spot of sugar beet, rice blast, apple scab, powdery mildew on cucurbits, cereals and legumes. It is ineffective against Oomycetous pathogens and dark-colored fungi such as *Alternaria* and *Helminthosporium,* some basidiomycetes and bacteria. Application of Benomyl induced a remission of symptoms of spike disease of sandal caused by MLOs. Many soil-borne pathogens can be controlled, provided the roots are confined to the Benomyl-treated soil.

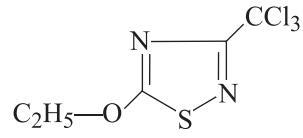
Another benzimidazole derivative is Thiabendazole, a thiazolyl compound originally developed an antihelminthic drug by E. Merck and Co. Inc., USA. In 1964, Starol and Allard described its systemic fungitoxicity for the first time. Another derivative is Bavistin. This is 2- (methoxy-carbamoyl)-benzimidazole which is effective against Ascomycetes and Fungi imperfecti which are considered to be hard to control. It is ineffective against oomycetous fungi.

Bavistin is translocated within the plant and has both prophylactic and curative actions.

The action of Benomyl and MBC are usually the same with most fungi. DNA synthesis is severely affected before other systems are affected (Clemons and Sisler, 1971). A study of synchronized cells shows that the inhibition of DNA synthesis occurs after a disruption of mitosis. Doublets formed contain only a single compact nucleus (Hammerschlag and Sisler, 1973). Benomyl can affect respiration, RNA and protein synthesis. Secondary actions can be attributed to butyl isocynate a co-product of MBC in the hydrolysis of Benomyl.

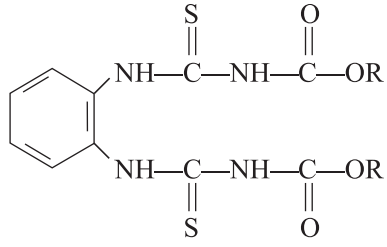
Terrazole Terrazole-5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole is toxic to oomycetous fungi, such as *Pythium* and *Phytophthora*. It has a systemic action in plants. Little is known about its fungitoxic action.

Like most other systemic fungicides, the serious disadvantage of the commercially-used present-day fungicides is that their repeated use results in the selection of resistant fungal strains.



Terrazole

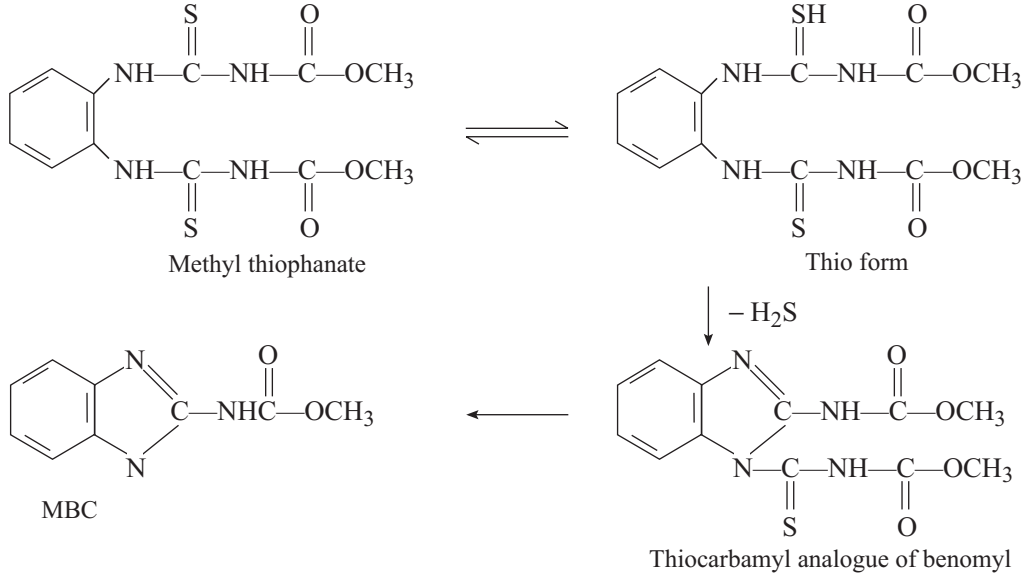
Thiophanates Thiophanates seem to occupy an exceptional position since they are not heterocyclic compounds, but instead disubstitued benzene derivatives. In 1962, Aelbers (1971) related the development of these bisthioureido benzene derivatives to the discovery of Sijpestein and Pluijgers of the systemic protectant action of phenylthiourea. It was found that both methyl and ethyl thiophanate are broad spectra fungicides which have each the same antifungal spectrum as Benomyl, though it is at a somewhat lower level of activity. Thiophanate (methoxy carbonyl thioureido benzene) is sold as Topsin M or Cercobin M.



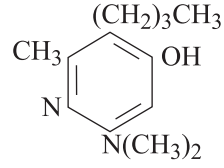
Where R = CH_3 or C_2H_5

Thiophanate

The fungitoxicity as well as systemic action of methyl and ethyl thiophanate may at least in part be attributed to their easy transformation into MBC and its ethyl analogue, respectively.

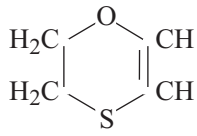


'pp' 675 A pyrimidine derivative coded 'pp' 675 was discovered by the Jealott's Hill Research station of Imperial Chemical Industries Ltd., and found to give outstanding results as a soil treatment for the control of powdery mildew (*Sphaerotheca fuliginea*) on the leaves and stems of cucurbits. Chemically it is 5 butyl 2 dimethylamino-4 hydroxy-6 methylpyrimidine.

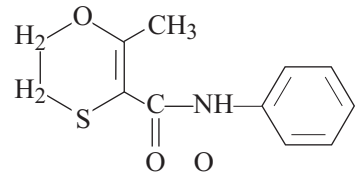


'pp' 675

Oxathiins In 1966, Von Schmelling and Kulka Uniroyal announced the development of carboxin, 2,3-dihydro-5 carboxy anilido-6 methyl-1,4, oxathiin, the effects of which were discussed by Edgington *et al*. in the same year. The oxathiins are systemic fungicides which are effective only against Basidiomycetous fungi, such as rusts, smuts, *Rhizoctonia solani* (*Corticium solani*). Vitavax (2,3 dihydro-5-carboxyanilido-6-methyl 1-1,4, oxathiin) has been used to free barley seed from *Ustilago nuda* (loose smut) and *Ustilago segetum* var. *tritici* (*U. tritici*) from infected wheat seeds. Plantavax, the sulphone derivative of Vitavax, is effective against rusts. Seed or soil treatment has given good control. The oxathiins are taken up by sensitive and insensitive fungi alike, with most of the fungicides accumulating in the ribosomal and soluble fractions of disrupted cells. Although synthesis of protein and nucleic acids is strongly interrupted, the site of action is the inhibition of succinate dehydrogenase in the mitochondrial fraction. Oxathiin compounds adversely affect nitrogen metabolism of the pathogens (Grover and Chopra, 1972). The hexose monophosphate shunt appears to be an important respiration pathway in sensitive fungi.

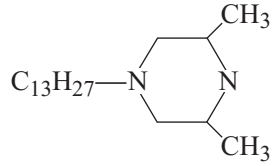


Oxathiin



Carboxin (Vitavax)

Calixin Among the recently-developed systemic fungicides is Calixin with excellent prophylactic and curative action against powdery mildew of cereals (*Erysiphe graminis*), Sigatoka disease of banana (*Mycosphaerella musicola*), and other ascomycetous pathogens. Its chemical name is N-tridecyl-2,6-dimethyl morpholine, commonly known as Tridemorph.



Calixin (Tridemorph)

Acylalanines In 1973, Ciba Geigy in Basle, Switzerland, discovered a new class of chemicals which showed promise with regard to curative activity and systemicity against Oomycetous pathogens. The most important acylalanine fungicides are Metalaxyl. It is sold as Ridomil for use in the soil. It is also sold as Apron for use as seed dressing.

Studies on the mode of action of Metalaxyl on fungi have been reported by Staub *et al.* (1980). Metalaxyl interferes with the development of the pathogens only after these pathogens penetrate their hosts. Metalaxyl interferes with RNA synthesis. It has also been reported that Metalaxyl fungicide elicits the production of phytoalexin in the plants which imparts resistance.

Organophosphate Fungicides

Historically, organophosphorus compounds are important because the first practically applied systemic fungicide Triamiphos is a member of the group. The fungicides in this group are Kitazin, Ediphenphos (Hinosan), Pyrazophos (Aufgon, Euramil) and Triamiphos (Wepsin).

Kitazin

Kitazin, a systemic fungicide developed by Kumiai chemical Industry Tokyo, Japan. The first fungicide of this group was S-benzyl diethyl phosphorothioate (Kitazin). Later another compound Kitazin P was developed which is now used for the control of blast disease of rice. Kitazin inhibits the incorporation of C-glucosamine into the cell wall fraction of *Pyricularia grisea*. Chitin biosynthesis is interfered with this systemic fungicide which was earlier developed as an insecticide.

Triazoles

Triazoles include several excellent fungicides such as RH-124 (INDAR), Triadimenol (BAYTON), Triadimefon (BAYLETON), Biloxazole (BAYCOR), Diclobutrazole (VIGIL), Tricyclazole and several others. These fungicides show great protective and curative activity against a broad range of foliar, root and seeding diseases such as leaf spots, blights, powdery mildews, rusts, smuts, and others.

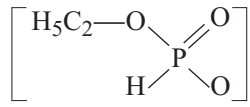
Morpholines

Morpholines are heterocyclic ring compounds, which have yielded derivatives showing systemic movements in plants. Two compounds have reached commercial use. Morpholines interfere with the function of cell membranes and also in some cases with respiration. Tridemorph (Calixin), a morpholine derivative, was developed under the code name BAS 220 05F (formerly BAS 220 F) by BASF A6, West Germany. It is marketed under the trade name Calixin. Tridemorph is active against representatives of all taxonomic groups of fungi except Peronosporales.

Phosphites

Two phosphate compounds, Aluminium-tris (aluminium ethyl phosphite), and LS 73 1038 (sodium ethyl phosphite) have been developed by May and Baker Ltd., UK, as systemic fungicides based on organometallic compound effective against Oomycetous fungi. However, only Aluminium tris has reached commercial production. Aluminium tris is not toxic to fungi *in vitro* but give excellent prevention and has curative activity in fields for the control of diseases caused by Oomycetous fungi.

Aluminium-tris (Aliette) This has been introduced under the code name LS 74-783 by Societe Rhone-Poulene, France, and is being marketed by May and Baker Ltd., UK, under the trade name Aliette. It possesses the following structural formula.



Aluminium-tris

There are quite a number of other systemic fungicides such as fungicides belonging to Piperazines (heterocyclic nitrogenous compounds) such as Triforine (Saprol). Triforine controls cereal, vegetable, fruit, and flower diseases such as powdery mildews, rusts, scabs, rots and post-harvest rots by foliar and soil applications.

Pyrimidine and Pyridine Derivatives such as Triarimol, Pyroxychlor

Triarimol is a broad spectrum fungicide which is particularly effective for the control of scab and powdery mildew of apple and pear. Triarimol inhibits sterol synthesis in fungi. Pyroxychlor is a fungicide based on the pyridine nucleus. The compound is of great interest because it was the first compound effective against phycomycetous fungi and capable of controlling root diseases by foliar application because of its downward translocation. Then there are the Acetamides (acetic acid amides) such as DPX-3217 (Curzate) effective against Oomycetes, aliphatics such as prothiocarbs (Previcur) effectively inhibiting Oomycetes such as Pythium and Phytophthora, Peronospora and Bremia spp.

Strobilurins or Quinone 'outer' Inhibitor (QoI) Fungicides

It is one of the most important classes of agricultural fungicides. Strobilurins represented a major development in fungas-based fungicides. The first strobilurins fungicide was isolated from wood-rotting mushroom fungus called *Strobilurus tenacellus*. The discovery of strobilurins fungicide was inspired by a group of natural b-methoxycrylate, the simplest of which are stroblurin A and Oudemansin A. BASF announced the first development of strobilurins, Azoxystrobin and kresoxim-methyl respectively. Since that time, other products have been announced, namely Trifloxystrobin, Metominostrobin, Pyraclostrobin, Picooxystrobin, Fluoxastrobin, and Dimoxystrobin. Strobilurins are mostly contact fungicides as they absorb into the cuticle and not transported any further. The strobilurins act by inhibiting mitochondrial respiration of fungi. Apart from this, they have suppressive effect, reducing competition for nutrients, disrupting metabolism and preventing growth of target fungi. Target diseases for nearly all strobilurin fungicides include downy mildews, rusts, powdery mildews, and many leaf spots. One of the key reasons for the outstanding commercial success of Azoxystrobin is that it gives control of fungi from all four groups of plant pathogens, namely the Ascomycota, Basidiomycota, Deuteromycota, and Oomycota.

Antibiotics

Antibiotics are substances which are produced by microorganisms and which act against microorganisms. Most antibiotics known until now are products of actinomycetes and some are from fungi and bacteria. The chemical nature of antibiotics is complex and they are not as a rule related to each other. A large number of antibiotics have been tried for plant disease control but only a few have been successfully utilized. The important antibiotics for the control of plant diseases are Streptomycin, Tetracyclines, Griseofulvin, Cycloheximide, and Aureofungin. The use of antibiotics is a comparatively new method of plant disease control, but antibiosis or antagonistic phenomena among microorganisms and their relation to plant diseases have been recognized for more than 80 years. Gliotoxin, the first antibiotic to be used for plant disease control, was isolated and purified by a plant pathologist even before the discovery of Penicillin.

Zaumeyer (1958), and Bajaj and Ghosh (1975) reviewed the antibiotics used for controlling plant diseases. Misato (1977) discussed in detail the development of agricultural antibiotics. McManus *et al*. (2002) have reviewed the work on the use of antibiotics in plant agriculture.

Streptomycin

It is produced by the actinomycetes *Streptomyces griseus.* Streptomycin controls both Gram positive and Gram negative plant pathogenic bacteria. Streptomycin or streptomycin sulphate is sold as Agrimycin, Phytomycin, ortho-streptomycin, and so on, and when used as a spray, shows activity against a broad range of bacterial pathogens, such as *Xanthomonas campestris pv. citri*, the cause of citrus canker, *Xanthomonas campestris pv. juglandis* of walnut blight, *Xanthomonas campestris pv. vesicatoria* of bacterial leaf spot of tomato and pepper, *Pseudomonas syringae* pv. *phaseolicola* of hollow blight of French bean, and *Erwinia amylovora* of orchard trees. This was first reported by Murneek (1952) of Missouri. In 1953, Goodman obtained 100% control of fire blight of apples by spraying streptomycin either in combination with oxytetracycline (Terramycin) or alone at concentrations of 100 to 500 ppm.

Streptomycin is also used as a dip for potato seed pieces against various bacterial rots of tubers and as a seed disinfectant in bacterial pathogens of beans, cotton, crucifers, cereals, and so on. Although streptomycin is an antibacterial antibiotic, certain fungal diseases (caused by Oomycetes) are also suppressed by it.

Saksena (1977) has reported that *Phytophthora parasitica var. piperina*, responsible for foot rot and leaf rot of *Piper betle*, can be controlled when cuttings are dipped in a solution of streptomycin sulphate. Streptomycin has been used to control early infection of hop downy mildew (caused by *Pseudoperonospora humuli*). It has been found effective to some extent against late blight of tomatoes and potatoes; even as little as 4ug/ml of nutrient solution is sufficient to check *P. infestans* completely on tomatoes. In greenhouse and field tests, it has been found that the seed piece decays caused by *Erwinia atroseptica* and *Pseudomonas fluorescens* could frequently be controlled by immersing infected, freshly cut potato seed pieces in water solution of this antibiotic or a mixture of this antibiotic and oxytetracycline hydrochloride. The activity of streptomycin against bacterial plant diseases may be explained as being due to a direct action of the antibiotic on the parasite. It is more difficult to understand its effect against diseases caused by fungi, mainly Oomycetes such as late blight of tomatoes and potatoes and hop downy mildew (*Pseudoperonospora humuli*). A mixture of streptomycin and 8-hydroxyquinoline sulphate as a combination has been successfully used as chemotherapeutic agents for the control of black leg disease of geraniums.

Cycloheximide

This is commonly referred to as "Actidione"— the trade name of Upjohn Co. and is obtained as a by-product in streptomycin manufacture. Cycloheximide (Actidione), a glutarimide antibiotic, is produced by different species of *Streptomyces*, including *S. griseus* and *S. nouresi*. A demonstration by Kerridge (1958) showed that the cycloheximide is a potent inhibitor of protein synthesis in *Saccharomyces carlsbergensis*. It is active against a wide range of fungi and yeast but is inactive against bacteria. Cycloheximide is the common name for β-(2-[3,5 dimethyl-2-oxcylohexyl]-2-hydroxyethyl) glutarimide. It has been reported to control cherry leaf spot caused by *Coccomyces hiemalis* at 2 ppm concentration. It is also used to control powdery mildew of beans caused by *Erysiphe polygoni*. This has also been utilized for the control of covered smut of oats caused by *Ustilago hordei,* bunt of wheat caused by *Tilletia* spp., brown rot of peach caused by *Sclerotinia fructicola,* and the post-harvest rot caused by *Rhizopus* and *Botrytis* spp. However, its use is limited, as it is extremely phytotoxic. Cycloheximide is sold as Actidione, Actispray, Actidione PM, Actidione RZ, etc.

Griseofulvin

This is produced by *Penicillium griseofulvum*, *P. patulum*, *P. nigricans* and *P. janczewski*, is toxic to several plant pathogenic fungi, such as members of powdery mildews, *Botrytis fabae*, *Alternaria solani*, and some rusts. It is active against chitinous-walled fungi. Griseofulvin has been successfully used for

the control of tulip fire caused by *Botrytis tulipae.* Root application of griseofulvin was found to be quite effective against certain powdery mildews. *Botrytis* diseases and bean rust (*Uromyces appendiculatus*). The systematic control of several powdery mildews, which are rather resistant to most fungicides, is interesting. Brian (1960) concluded that systematic control is not very practical since it is usually found to be no better or worse than other fungicides already in general use. In 1960, Brian postulated a hypothesis that Griseofulvin interfered with the biosynthesis of chitinous elements of fungal cell walls, which could also explain the relative non-toxicity of this chemical to animals and higher plants. Subsequent studies have shown that this antibiotic does not inhibit the synthesis of protein, chitin and lipids, nor does it alter the rates of respiration, glycolysis or cell wall synthesis in fungal cells. Byrde (1959) reported that Griseofulvin (0.1%) is effective as a foliar spray for the control of apple rot caused by *Sclerotinia fructigena. Monilia* disease of apples caused by *S. mali* was successfully controlled in Japan by Griseofulvin.

Blasticidins

It is produced by *Streptomyces griseochromogenes*. In Japan, it has been utilized for the control of blast disease of rice (*Pyricularia grisea*). It is active against both bacteria and fungi but is selective in its activity against the latter. The antibiotic inhibits the rice blast fungus at concentrations of 5 to 10 µg/ml. This antibiotic interferes with protein synthesis.

Aureofungin

Aureofungin is a new broad-spectrum antifungal antibiotic produced in submerged culture by *Streptoverticillium cinnamomeum* var. *terricola* (Thirumalachar *et al*., 1964). Chemical characterization indicated that it belongs to new aromatic group heptanes and is, therefore, distinct. Its remarkable property is that it is absorbed when applied as a spray or given to the roots as a solution. Thus it is translocated and can be detected in other parts of the plant. This has been detected both by bio-assay methods as well as by spectrophotometry.

The most useful property of the antibiotic is its high activity against a large number of phytopathogens and its absorption and translocation in living plants. Some of the examples of effective control of plant diseases incited by pathogenic fungi are: Citrus gummosis incited by *Phytophthora* species, identical with *P. citrophthora*, which is a devastating disease in several parts of India and which has been controlled by Aureofungin and powdery mildew of apples incited by *Podospharea leucotricha* which is completely by spraying 100 ppm antibiotic spray solution (Agarwal *et al*., 1970).

Systemic translocation and the arresting of Dutch elm disease have been reported by Dr. Benedict University of Windsor, Canada.

Spraying Aureofungin controls downy mildew, powdery mildew, and anthracnose of grapes. Seed treatment of rice effectively controls *Helminthosporium oryzae*. Similarly, *Pyricularia grisea* of rice and ragi are effectively controlled by Aureofungin. *Diplodia* rot of mango and *Alternaria* rot of tomato, *Sclerotinia* rot of peach, *Pythium* rot of cucurbits (Sharma and Wahab, 1970-71) have been controlled by Aureofungin. *Penicillium* rot of apples has been controlled by Aureofungin (Naik and Joshi, 1973-74). Stripe disease of barley caused by *H. gramineum* has been controlled by Aureofungin (Dharamvir and Raychaudhuri, 1968). Interestingly Aureofungin has also been reported to possess plant growth stimulatory activity.

Tetracyclines

Tetracyclines are antibiotics produced by a number of species of *Streptomyces*. Terramycin (oxytetracycline), Aureomycin (chlorotetracycline) and Actinomycin (tetracycline) have been used for plant disease control. Oxytetracycline has been used as a soil-drench or as a root dip for controlling crown gall and has been utilized for the control of fire blight of apples and pears in conjugation with Streptomycin.

Tetracycline has been found to be effective against plant diseases caused by mycoplasmas. An injection of concentrated solution of Tetracycline or oxytetracycline into tree trunks offers possibilities of curing peach-x, pear decline, coconut palm yellows and citrus greening. Tetracyclines when injected into trees infected with mollicutes or fastidious prokaryotes stop the development of the disease and induce remission of symptoms.

Agrimycin-100

This is 15% streptomycin sulphate and 1–5% Terramycin. Agrimycin-500 is 1.755% Streptomycin sulphate, 0.176% Terramycin, and 42.4% metallic copper. It has been utilized for the control of bacterial diseases, such as *Erwinia amylovora*, halo-blight caused by *Pseudomonas syringae* pv. *phaseolicola*, citrus canker caused by *Xanthomonas campestris* pv. *citri*, seedling blight, leaf spot and black arm disease of cotton caused by *Xanthomonas campestris* pv. *malvacearum* and soft rot, and black leg of potato caused by *Erwinia carotovora.*

Nystatin

Nystatin is also known as Mycostatin or Fungicidin. It is a polyene (tetraene) antifungal antibiotic produced by *Streptomyces nouresi.* It is manufactured for clinical use but has also been used to a limited extent for plant disease control. This has been found efficacious in controlling anthracnose of beans and downy mildew of cucumber, and as seed treatment against stripe disease of barley (Dharamvir and Raychaudhuri, 1968). Ananthnaryana and Seshadri (1965) reported that it can be utilized as post-harvest dip against peach brown rot and anthracnose of banana.

Bulbiformin

Bulbiformin is an antifungal polypeptide antibiotic produced by *Bacillus subtilis* and was reported by Vasudeva *et al.* in the year 1958. The production of this antibiotic was demonstrated in sterilized soil amended with molasses, groundnut cake, sweet clover roots, and so on. Inoculation of amended soil with *B. subtilis* controlled the wilt disease caused by *F. udum* on pigeon pea. Singh *et al.* (1965) demonstrated the production of bulbiformin on seed coats, spermatosphere and rhizosphere by bacterized pigeon pea seeds sown in sterilized and unsterilized soil. The antibiotic was absorbed, and because it is systemic, considerably reduced the incidence of wilt of pigeon pea.

Soil Fumigants

The most promising method of controlling nematodes in the field has been through the use of chemicals called nematicides. Some of these, including chloropicrin, methyl bromide, mylone, vapam and vorlex, give off gases after being applied to the soil. They are general purpose preplant fumigants and are effective against a wide range of soil microorganisms, including, in addition to nematodes, many fungi, insects and weeds. These are also highly volatile fumigants such as formalin and carbon-disulphide.

Formalin

Various chemical treatments of the soil have been developed, one of the oldest being the formaldehyde drench. Formaldehyde corrodes the hands and is somewhat erratic in effectiveness. For the control of damping off and seedling blights, a 37–40% solution in water is used. Excellent control of onion smut has been obtained in the USA by a solution of formaldehyde (1-128 parts) applied at seeding time at a rate of 200 gallons per acre with the help of machines especially devised for the purpose. It is an effective fungicide in seed beds to prevent damping off fungi.

Carbon disulphide (CS2)

Carbon disulphide is perhaps the earliest chemical used as a soil fumigant for checking nematodes. Carbon disulphide is used in citrus orchards against *Armillaria mellea*. It is used like formalin. This

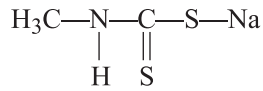
substance was originally employed in 1872 by Thenard against phylloxera of the vine (*Phylloxera vitifoliae*). Carbon disulphide is cheap, volatile and is an insecticide also.

Chloropicrin

This is useful both as a fungicide and a larvicide. It is injected into the soil at a depth of 3–6 inch in holes 9–12 inches apart. The soil is covered with impervious plastic sheeting for 48 hours.

Vapam

Other fumigants produced in recent years include methamsodium (Vapam: sodium N-methyldithiocarbamate), a colourless liquid which decomposes rapidly in moist soil to release a fumigating gas.



Vapam (SDMC)

Vapam is not volatile but it decomposes in soil to yield methyl isothiocyanate. In addition to methyl isothiocyanate, Vapam also yields carbon disulphide, hydrogen sulphide, methylamine and other products when it is mixed with the soil. Vapam has been used as a nematicide and selective weedicide. But it has been found to be quite effective in the control of wilt of cotton, damping off of papaya, root rot of beet (*Sclerotium rolfsii*).

Methyl Bromide (CH3Br)

Methyl bromide has also been used as a soil nematicide, but it is too phytotoxic to be used in fields with a standing crop. It should be applied in the soil as a fumigant a few weeks before the crop is sown or planted. The use of this sterilizing gas has been restricted to the treatment of nursery beds, vegetables and fruit production, and ornamentals. Methyl bromide has a low boiling point (4.5°C) and requires a plastic cover for effective confinement of the gas or for application with deep injectors. This chemical is scheduled for withdrawal from use as it affects the ozone layer in the earth atmosphere.

DD Mixture

Dichloropropene and Dichloropropene (DD mixture) and Ethylene dibromide (EDB) are two effective nematicides, which came into the market soon after the Second World War and are extensively used for nematode control.

Dazomet

Dazomet (tetrahydro 3, 5-dimethyl-2 H-1,3,5-thiazene-2-thione) is a wettable powder used at the rate of about 440 kg/ha. It is applied with a fertilizer spreader or by spraying a suspension in water, followed by thorough mixing and "sealing in" with water. In the soil, Dazomet releases methyl isothiocyanate and is effective for the control of nematodes, wireworms and some soil-borne fungi, such as *Pythium*, *Rhizoctonia*, *Fusarium*, *Verticillium*, and *Colletotrichum.*

Oils

Various oils are capable of controlling several plant diseases. The effectiveness of oils in the control of plant diseases has been reviewed by Calpouzus (1966). Sigatoka disease of bananas caused by *Mycosphaerella musicola* has been controlled by oils, which, because of their advantages have almost completely replaced conventional fungicides. Oil sprays are now widely used for the control of *M. musicola.* Nearly 50 million liters are estimated to have been used in Latin America and the Caribbean in 1964 and perhaps another 7.6 million liters in other banana producing areas.

The advantages of oils are:

- Superior disease control,
- Effectiveness at very low dosages of even less than one gallon per acre,
- Excellent spreading and sticking properties on leaf surfaces,
- Low cost, and
- Little or no toxicity to man and animals.

There are several disadvantages also, these are:

- Effective against a limited number of plant diseases, and
- Phytotoxicity may occur on sensitive plant species

The oils involved in disease control are mainly mineral oils from petroleum, and to a much lesser extent, glyceride oils from plants, and synthetic oils. It is not exactly known how mineral oils control plant diseases. Light viscosity oils atomized into very fine droplets of 50–1000 μm in diameter seem to be most effective and can apparently stop the growth of the fungi after it has become established inside the leaf. According to Calpouzus (1966), any hydrophobic fluid of low volatility, such as silicon oil, which is chemically inert, will control *M. musicola*.

The therapeutic action of oils is perhaps due to the oily physical barrier which interferes with gas exchanges in the leaf and alters its physiology so as to stop the development of the fungi. There is evidence that anti-transpirants may reduce disease by, for example, inhibiting stomatal penetration. It is also likely, but not certain, that oils exert their therapeutic action not directly on the pathogen but rather on the physiology of the host. *M. musicola* is encouraged by fairly high light intensities which no doubt favour photosynthesis and high sugar concentration. Some high sugar pathogens, such as powdery mildews and rusts, are apparently susceptible to oils.

Oils have been used successfully in the control of greasy spot of citrus caused by *Cercospora citrigrisae*. Oil alone controls angular leaf spot of tung tree (*Aleuites fordii*) caused by *Mycosphaerella aleuritides*. Oil sprays have also been successfully utilized for the control of *Septoria apii* on celery, *Puccinia graminis tritici* on wheat and several powdery mildews, such as *Sphaerotheca pannosa* on rose. Blister rust has been affected by application of fuel oil. Some synthetic oil fluids (polybutenes) are also effective against *Peronospora tabacina* responsible for blue mould of tobacco. According to Castellani and Matta (1964), mineral oil alone misted on grapes, effectively controlled downy mildew caused by *Plasmopara viticola.* Ocana and Hansen (1960) tested a non-volatile mineral oil against *Phytophthora palmivora* which attacks the pods of the cacao tree. Commercially acceptable control of the disease did not occur but it did retard the development of lesions. Thus, the use of oils in the control of plant diseases is an interesting recent development. Oils obtained from seeds of several plants such as sunflower, olive, corn, and soya bean gave excellent control of powdery mildew of apple when applied 1 day before to 1 day after inoculation.

Safeners, Spreaders and Stickers

Safeners A safener is defined as chemical that reduces the phytotoxicity of another chemical. Copper sulphate is phytotoxic, but with the addition of lime its toxicity is reduced. Lime is, therefore, a safener. Lime is used universally with chemicals to prevent the formation of, or to neutralize arsenic acid, which is phytotoxic to sensitive foliage. Glyceride oils act as good safeners for copper sprays by providing a film around each particle, but they reduce the fungicidal value of the product.

Spreaders Materials which are added to the spray mixture to establish improved contact between the fungicides, and the sprayed surface are known as spreaders. These can be classified as water soluble and water-insoluble. Mineral oils, glyceride oils, and terpene oils are water-insoluble spreaders. Soap has long been known as a spreader. Sulphated alcohols, sopamines, resinate, and petroleum sulphonic acids are some of the common spreaders available in the market. Spreaders reduce the surface tension and

improve contact between the fluid and the sprayed surface so that the fluid does not collect into large drops and run off easily.

Stickers Materials added to spray or dust, which improve its adherence to plant surfaces are known as stickers. Flour, starch, gum arabic, and dextrins are commonly used as stickers. Bentonite clays when added to dusts improve the latter's sticking quality. Oils, oil emulsions, and hydrocarbon oils have also been employed as stickers.

Carriers When the fungicide is used as a dust, the active ingredient is generally diluted with a finally divided powder called a carrier.

Disease Control by Immunizing the Host

Inoculation of plants with weak strain of pathogen leads to immunization of the plants to which the plants are generally susceptible. The resistance of plants can also be done by incorporating genes obtained from plants. Other pathogens, that code for the production of certain enzymes or toxins also interfere with infection by the pathogen. Genetic engineering technology has made possible the isolation of resistance (R) genes from resistant plants and the transfer of R genes into susceptible plant in which they induce the resistant response.

Cross Protection

This method applies to the protection given to a plant by infection with a mild strain of a pathogen from subsequent infection by a severe strain of the same pathogen that normally causes symptoms. Its application in controlling virus diseases has met with some success in cross protecting citrus with mild strain of Citrus tristeza virus, tomatoes with mild strain of tobacco mosaic virus. This method has some limitations because mild strains of viruses are often not available. In addition, there is a danger of mutation towards new, more virulent strain, and spread to other crops in which virulence might be higher.

Acquired Resistance to Fungicides

Georgopoulos and Zaracovitis (1967) reviewed the tolerance of fungi to organic fungicides. Dekker (1972, 1976) had reviewed acquired resistance to fungicides. Ashida (1965) had reviewed adaptations of fungi to metal toxicants. Development of fungal resistance to fungicides had been discussed by Georgopoulos (1977) in Vol. 2 of *Antifungal Compounds*. The term "acquired resistance" is used when, in a population which is normally sensitive to a biocide, forms arise that is less sensitive to this biocide. This resistance may have a genetical basis and then as a rule be stable, or it may be caused by a physiological adaptation and disappear again rapidly when the organism is no longer exposed to the toxicant. This temporarily reduced sensitivity is called tolerance and the former type is called resistance.

The high specificity of action of a toxicant is both a strength and weakness. Many organisms are resistant to selective organic insecticides. The dithiocarbamates and inorganic compounds are non-specific toxicants and do not penetrate the plant tissues. The development of resistance in microorganisms due to these toxicants is unusual (Dekker, 1972). However, with the introduction of selective systemic fungicides, there was a significant development of resistance. In practice, sometimes resistance is so great that the use of the compound concerned has to be severely restricted or even abandoned in the treatment of certain diseases. Resistance to many of these new systemic fungicides could be readily induced in the laboratory by treatment with ultraviolet irradiation or other mutagenic agents.

Dimethrimol was introduced in Holland in 1968 to control powdery mildew in cucumbers. During the following two years, this control measure was found to be less effective. Even after the removal of the fungicide, tolerant fungi were reported in 1971 (Bent *et al*., 1971). Georgopoulos and Dovas (1973) reported that the Benomyl was very effective in the control of heart rot in cyclamen. Within a short period, a strain of *Botrytis cinerea* developed that could tolerate 1000 ppm, while the wild type was controlled at 0.5 ppm. The tolerant strains showed cross resistance to Thiabendazole, Fuberidazole and Thiophanate methyl. Benomyl protected sugar beets in 1970 and 1971 at comparatively low levels. However, in July of 1972, resistance was noted and subsequently a large acreage of beets was lost. Dekker (1972), Wolfe (1971), and Vargas (1973) have suggested a number of ways to reduce fungicide tolerance. Among these is the avoidance of the use of one fungicide on an exclusive basis. Systemics with different modes of action would be alternated along with the protectant fungicide. An economically feasible level of fungicide dose should be used, phytotoxicity should be avoided and toxic residues should be avoided. Resistance to the fungicide Metalaxyl has also been observed. Metalaxyl fungicide is used for controlling Oomycetous pathogens.

The continuous and prolonged use of a selective, systemic fungicide may result in loss of effectiveness, which may be incorrectly diagnosed as fungicide tolerance.

Plant Defense Activators

Resistance in plants to several diseases/pathogens such as TMV, *Peronospora tabacina*, and *Pseudomonas syringae* can be induced with salicylic acid, acetyl-salicylic acid (Aspirin), Dichloroisonicotinic acid (INA) when applied into the plant by injection or spraying. Such synthetic compounds have no antimicrobial action but mimic the biological induction of systemic acquired resistance in plants against many pathogens. For example, silicon seems to protect plants from powdery mildew infection by inducing localized defense reactions including papilla formation, callose production and accumulation of phenolics along the cell wall.

Control Through Use of Transgenic Plants

DNA technology has made it possible to engineer transgenic plants that are transformed with genes coding for enzymes (chitinases and glucanases), for tolerance against specific diseases or for tolerance of adverse environmental factors against certain group of pathogens that lead to gene silencing of pathogen.

Transgenic Plants that Tolerate Abiotic/Environmental Stress

Many plants have been transformed with genes that enable those plants to tolerate abiotic stresses beyond the normal range of these plants. For example, egg plant transformed with the bacterial gene coding for mannitol phosphodehydrogenase are tolerant against osmotic stress induced by salt, drought, and low temperature. Rice gene coding for glutamine s-transferase have been transformed in maize plant that enabled maize plants to tolerate low temperature and to germinate better under submerged in water. Drought tolerance in tobacco plants was increased by introducing the yeast gene coding for trehalose phosphate synthase into the genome of tobacco chloroplast.

Transgenic Plants that Tolerate Specific Plant Genes for Resistance

There are many crops in which specific plant genes for specific pathogens have been isolated from resistant plants and transferred and expressed into susceptible plants. Such resistant plants are cloned

and multiplied that is resistant to the specific pathogen. For example, hybrid rice transformed with the rice gene Xa21 coding for resistance of rice to the bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*. Similarly, when the resistance gene DRR 206 from pea was transferred into Canola, the transgenic Canola plants exhibited resistance to *Leptosphaeria maculans, Rhizoctonia solani*, and *Sclerotinia sclerotiorum*.

Transgenic Plants that Tolerate Gene Coding for Antipathogen Compounds

Genes coding for some enzymes like chitinase and some glucanase have been identified, isolated, cloned and expressed in susceptible plants and provide resistance.

Origin of Fungicide-Resistant Strains

It is not clear how fungicide resistant strains of fungi arise, as little work has been done on their genetics. The disappearance of resistance in the absence of the fungicide could be due to sub-lethal concentrations of the latter. This results in the formation of a substance or substances which confer resistance which could otherwise not be formed. Perhaps, adaptive enzymes, the formation of which is induced by the fungicide, are involved.

Stable resistant strains perhaps arise by mutations, which occur either by chance, or in the case of certain fungicides, could be brought about by the fungicide itself. It is significant that the exposure of *Fusarium caeruleum* (potato dry rot) to tetrachloronitrobenzene (TCNB) resulted in the appearance of TCNB-resistant mutants and that variants of *Botrytis allii* (onion neck rot) were produced after the fungus was treated with the vapor of Pentachloronitrobenzene. Some aromatic hydrocarbons are said to produce gene mutation in fungi. The fungicide resistance of some fungi, including a few which attack plants, has been shown by genetical analysis to be of chromosomal origin, as in *Hypomyces solani* f.sp. *cucurbitae* (seedling blight and fruit rot of cucurbits), where resistance to TCNB is determined by three independently-inherited genes (Georgopoulos, 1962, 1963). However, there is little critical evidence that fungicides can induce mutations in fungi which bring about resistance to fungicides.

Mechanism of Resistance to Fungicides

The development of resistance to a fungicide may be due to changes in the fungal cell that inhibit the fungicide, to a greater or lesser extent, from reaching the site of action. Such changes include a decreased permeability of the protoplast membrane to the fungicide, or an increased detoxification before the site of action is reached. If the fungicide is able to reach this site, resistance may be due to a decreased affinity between the chemical and the reactive site, to circumvention of the blocked site by operation of an alternate pathway or to compensation for the effects of inhibition. Dekker (1976) had discussed examples of various types of resistance to fungicides in detail.

Breeding for Disease Resistance

The promise of resistance to pests and diseases has attracted the attention of plant breeders ever since the demonstration near the turn of the century that resistance could be selected and that it is often simply inherited. The built-in protective resistance effective throughout a crop plant's life offers a compelling alternative to fungicides and insecticides. In the early years, minimizing the use of protective chemicals

was desirable because it saved money, but today there is an added advantage of reducing their hazard against those pests and disease such as cereal rusts, soil-borne smuts, and certain nematode disease. There is considerable literature available on the breeding and inheritance of diseases resistance in plants. Hansen (1934) had given a list of papers published on the inheritance of disease resistance in plants up to 1934. The literature on the genetics of diseases resistance in vegetables has been reviewed by Walker (1965, 1969) and on field crops by Ausemus (1943) and Dickson (1956). Inheritance of resistance to viral diseases has been reviewed by Holmes (1954), resistance to rusts by Hooker (1967), and to nematodes by Hare (1965). Some more review articles are those of Hooker and Saxena (1967), Roane (1972) on trends in breeding for disease resistance in crops, Hooker (1974) on cytoplasmic susceptibility in plant disease, and Sadasivan (1975). Alien germplasm as a source of resistance to disease has been discussed by Knott and Dvorak (1976). An interesting article is by Browning *et al*. (1977) on "Managing Host Genes: Epidemiological and Genetic Concepts". The genetics of resistance to plant viruses has been reviewed by Fraser (1990). Multiple disease resistance in grain legumes has been discussed by Nene (1988). Molecular genetics of plant disease resistance has been recently discussed by Staskawicz *et al*. (1995).

The use of disease-resistant varieties for controlling plant disease has been termed the "painless method" because it does not cost the farmer anything. In an underdeveloped country like India, it is all the more important since we cannot pay for the heavy costs of spraying and dusting crops on a large scale. The use of resistant cultivars and hybrids has several other advantages. It eliminates the hazard to human health and wildlife which is caused by large scale use of dangerous fungicides and pesticides. It also reduces pollution which results from the use of poisonous chemicals and their residues. Resistant crop varieties check epidemics of pathogens and pests and thus help to maintain the biological balance in the environment.

It has earlier been seen that resistance can be varied in different hosts and parasites. It can be mechanical, such as waxiness, hairiness and toughness of the epidermis or it may be biochemical in which case it is the result of the interaction between the cytoplasm of the host and the parasite. The biochemical defence mechanism is due to the synthesis of polyphenols in the host that may be toxic to any organism present and to the plant itself. Susceptible and resistant reactions differ principally in the speed and scale of host responses which are more rapid in the latter. As a result, the development of the invading parasite is restricted.

Susceptibility and resistance to a parasite are largely inherited characteristics. The laws governing their inheritance are the same as those governing other characteristics of the host and parasite. It has been proved in several cases that the resistance or susceptibility to disease is located in the genes. The breeding of resistant varieties of crops is, therefore, based upon the laws of inheritance. The Darwinian principles of a variety's struggle for existence and survival of the fittest apply to all the wild varieties and their pathogens. Wild plants have been growing for centuries in the presence of pathogens and natural selection must have eliminated the most susceptible biotypes and left the most resistant ones. In this way, regional biological balances exist between the native plants and their pathogens and pests. The balance will tend to persist unless disturbed by new factors such as the introduction of new biotypes of pathogens or hosts. This is likely to occur if barriers such as oceans of high mountains protect the region. Often it is man who disturbs the natural balance by transporting plants and pathogens to new regions, or who, through crop improvement, produces new varieties of crop plants. Many of the serious outbreaks of plant diseases are traceable to the introduction of parasites to which non-resistant crop plants immediately succumb. The following are some of the notable examples:

1. A very superior variety of hops (*Humulus lupulus*) was cultivated in Western Europe. In 1917, downy mildew *Pseudoperonospora humuli* was observed in England in a locality, which grew on seeds and plants imported from Japan. In 1922, the fungus attacked south eastern England. The pathogen soon spread to Belgium, France, Holland, Germany, and other European countries and had to be controlled at a heavy cost, by spraying.
2. *Cryphonectria (Endothia) parasitica*, the cause of chestnut canker, does not cause serious damage in the Eastern countries. When it was introduced in the USA in about 1904, it brought heavy losses to the chestnut forests (*Castanea dentata*) in a few years time. It is estimated that within a period of about 10 years it has caused losses of about 50 million dollars.
3. Taichung Native 1 (T.N.1) is a high yielding variety but it brought with it the bacterial blight, a serious disease of this crop in 1966. T.N.1 is highly susceptible to bacterial blight.

There are so many other important diseases, which have been imported into countries which were earlier free from them. For example, *Phytophthora infestans* was brought in infected tubers from South America to England and other European countries in the 1830s and *Plasmopara viticola* from America to France in 1870.

Failures accompany successes while breeding resistant varieties (Stakman, 1954, and Caldwell, 1966). Plant breeders even now introduce genes for resistance, as they did in 1918. Borlaug (1965) has remarked that the average useful life of a rust-resistant variety is as short as five years in some parts of the world after which it succumbs to a new race of rust. Walker (1959) wrote, "By and large the development or resistant varieties must be looked upon as a continuing programme. The potential variability of most pathogens (including viruses) will not permit any currently successful variety to remain so for an indefinite period." Several other examples can be cited where resistant varieties did not stay resistant for long, such as Hessian fly on wheat, powdery mildew on barley, *Cladosporium* leaf mould of tobacco, mosaic virus of tomato, golden nematode and leaf blight of potato. The question arises as to why these varieties were so short-lived and why the long and continued series of efforts failed to give the desired results. The reason is that while the breeder tries to make plants more resistant, nature tries to make pathogens more virulent, and ultimately nature gets the upper hand.

It was earlier stated that the characteristics of an organism are governed by the laws of genetics which are common to both the host and parasite. Resistance or susceptibility (both of which are relative expressions) is the result of interaction between these characteristics. It has now been shown that the genotypes of the host as well as the parasite can be varied by segregation: recombination of genes, mutation, heterokaryosis, somatic hybridization, etc. Genetic studies have now been extended to bacteria and viruses where new strains arise due to hybridization, transformation, and transduction. The previous conception that the pathogen is a fixed entity which damages the crops year after year without change is no longer acceptable. There is a great array of genotypes of the parasite as there is of the host.

Types of Resistance

Plant pathologists commonly divide resistance into monogenic, polygenic, and oligogenic, according to whether resistance is governed by one gene, many genes or a few gene. Resistance can be described as genetic, mechanical or epidemiological.

The general behavior or the genetic behavior of disease-resistance is governed in many simple-to-complex ways.

Monogenic resistance (governed by a single gene) is governed by one gene with a 3:1 Mendelian ratio. It can be monohybrid dominant, such as the resistance of oats to *Helminthosporium victoriae* (Caldwell, 1966), of sorghums to *Periconia circinata* (Vander Plank, 1968), of cabbage to the pathogen

Fusarium oxysporum f. sp. *lycopersici* (Walker, 1965), of onion to the pathogen, *Pyrenochaeta terrestris* and to *Cercospora* leaf spot of cucumber. It can be monohybrid recessive, with resistance as a recessive character, such as powdery mildew of barley, sorghum smut, and yellow rust of wheat (Biffen, 1905).

Oligogenic resistance (governed by several genes) is determined by two-to-several genes.

Polygenic resistance (governed by many genes) involves many genes which are more difficult to analyze and which are known to be involved in a large number of diseases such as cotton wilt.

Extra Chromosomal Inheritance Extra chromosomal inheritance of disease resistance is rare but is of great importance. The widespread epiphytotic of southern leaf blight of corn in the USA was due to the sudden appearance and distribution of new race identified as race T (Smith and Hooker, 1970) of *Helminthosporium maydis*. Resistance to the new race is mostly extra chromosomal (Hooker *et al.*, 1970). Corn with *cms*-T cytoplasm is more susceptible to yellow leaf blight caused by *Phyllosticta* sp. Symptom production in potato virus X in peppers is apparently determined by cytoplasm (Nagaich *et al*., 1968).

Gene Interaction Disease resistance to pathogens is not always simply inherited. In many instances the pattern of inheritance is quite complicated. The resistant phenotype is the ultimate expression of a chain of events in which genes for resistance perhaps control only a few links. Although genes may appear to act as autonomous units, in effect gene products interact and this becomes clear in genetic analysis by the appearance of complementation, modification and epistasis.

Modifier Genes A host may carry a gene for strong resistance but this is not expressed because of the presence of an inhibitor gene. In onions, resistance to smudge disease is controlled by a single dominated gene R. In the presence of a partially dominant inhibitor gene, I, the resistance is reduced or completely suppressed, depending on whether the gene I is present in the heterozygous or homozygous state. In wheat rust, resistance is completely suppressed by a dominant inhibitor gene. Inhibitor genes affecting recessive genes for rust resistance are also known to be present in maize.

Reversal of Dominance A gene may be dominant for resistance to one race of the pathogen, but recessive for resistance to another race of the pathogen. This reversal of dominance may be due to dosage effect, i.e., one dose of the gene in a heterozygote being sufficient to provide resistance against one race of the pathogen but not against the other, or due to closely-linked genes for resistance one dominant and the other recessive.

Vertical and Horizontal Resistance When a variety is more resistant to some races than to others, resistance is called "vertical", "perpendicular", or "specific". When resistance is such that it is uniformly spread against all races of the pathogen it is called "horizontal", "lateral", "generalized", "non-specific" or "field resistance". The derivation of this term is mathematical and refers to the horizontal axis of the graph. Horizontal resistance can be demonstrated when a series of pathotypes is tested against a series of pathodemes. Horizontal resistance has been called field resistance against *Phytophthora infestans* and generalized resistance against *Puccinia graminis*. Vertical resistance (VR) confers complete but impermanent protection, whereas horizontal resistance (HR) confers incomplete but permanent protection.

The concept of VR and HR was put forward by Vander Plank in 1968. Vertical resistance is conditioned by oligogenes and is effective against only some races of pathogen, whereas HR, which is polygenic in inheritance, is effective against all races of a pathogen. The terminology is a bit confusing. All oligogenes are not necessarily major genes in the sense of being important genes. For example, the R1 gene governing resistance to potato blight merits being called major gene. But the gene R4 is weak

and of little importance to potato breeders, and hence, it would be an exaggeration to call the R4 gene a major gene. In the same way, all polygenes are not minor genes in the sense of being relatively less important.

The derivation of the term "vertical resistance" is mathematical and refers to the vertical axis of graph (see Fig.1.3). Vertical resistance is proved by analysis of variance in which there is significant differential interaction between path types and pathodemes. Vertical resistance is generally (but not necessarily) inherited by means of major genes. It often (but not invariably) produces a quantitative presence of absence effect in that it operates completely or not at all. The epidemiological characteristic of vertical resistance is an apparent reduction in the initial inoculum; as a result, the epidemic is delayed (cf. horizontal resistance which slows down the epidemic).

Methods of Selection of Resistant Genotypes

While searching for resistant genotypes, a breeder has to look for the former by one of the following methods:

Selection from Existing Crops This is done in the following ways:

Selection from Crops that Escape Damage in Infected Fields The early breeders used this as one of the prominent methods, but it should be noted that such selected material cannot be usually called a genetically pure stock. As early as 1900, Orton of the US Department of Agriculture began selecting cotton varieties against cotton wilt (*Fusarium oxysporum* f. sp. *vasinfectum*). He selected individual plants, which showed resistance to disease in a wilt-infested plot of land, i.e., even when they were drastically exposed to the disease. The seed from the selected plants was repeatedly tested for resistance in progeny tests on the infected soil. By this process a number of good yielding wilt resistant varieties were evolved.

Flax wilt caused by *F. oxysporum* f. sp. *lini* is another good example of selection being done to obtain resistant varieties. Prior to the development of resistant varieties, flax was a migratory crop in the USA, and as it spread westward to fresh lands, the crop, after a few year of cultivation, became "hard on the soil" till the soil became so "flax sick" that wilt destroyed practically every crop. In 1901, it was Bolley of North Dakota who first announced that the disease was due to *F. lini.* A special plot was established in which the disease was develop freely and flax was grown on it continuously year after year till almost no crop survived except for a few plants. From these surviving plants, seeds were collected and resistant varieties were developed In North Dakota, a similar plot of *Fusarium*-infested soil was set up at Minnesota in 1912. These two plots were constantly inoculated with every kind of *Fusarium* wilt organism from various areas and sources. Screening centres were established for testing all the resistant varieties, which were evolved from time to time.

The wilt disease caused by *F. oxysporum* f. sp. *conglutinans* has practically ruined cabbage production in parts of the USA. The disease was investigated by L. R. Jones and others. Some seeds were collected from three highly resistant plants and resistant varieties of Hollander types were developed. Further work at Wisconsin by L. R. Jones showed that temperature plays an important part in the onset of this disease. He found that some varieties became non-resistant at high temperature. He showed that when the soil was kept at 18.3ºC or lower, plants grown in infected soil did not develop disease. As the temperature rose up to about 27ºC, the intensity of disease increased after which it declined. Resistant varieties were selected on the lines given above. Later on, hybridization was also resorted to, and homozygous resistant varieties were evolved. This was the first experimental proof of the fact that in disease resistance, environment plays a very important part. Curly top of sugar beets, a virus disease, which causes devastating losses, has also been controlled by the production of resistant varieties by selection.

The curly top resistant varieties were a spectacular success and saved the sugar beet crop in western USA. It should be emphasized here that the selection methods for resistant varieties can succeed only when the population has some resistant biotypes.

Pure Line Selection The above method is followed in pure line selection also but seed from selected plants is collected and tried separately. The stock thus produced is genetically pure.

Plant Introduction Very often the genes needed to control a particular disease or race of a pathogen cannot be found in domestic varieties. It becomes necessary to introduce plant materials from other countries, especially from places where the pathogen and the host species may have evolved together and thus provide rich pools or resistance genes (Leppik, 1970).

Hybridization Among the existing varieties it is found that a variety may be resistant to a disease but may not possess suitable agricultural characteristics, i.e., it may not be high-yielding or the quality of the produce may not be satisfactory. On the other hand, there may be good varieties of high yields but they may be susceptible to disease. A breeder tries to combine the good characteristics of both the varieties to evolve a high yielding and resistant variety.

Hybridization: H- High yield
h- low yield
R- Resistant
r- Susceptible

HHrr (High-Yielding Susceptible) × hhRR (Low-yielding resistant)

Gametes Hr × hR

F_1 generation = HhRr Phenotypically high-yielding resistant

Gametes HR, Hr, hR, hr

	HR	Hr	hR	hr
HR	HHRR	HHRr	HhRR	HhRr
Hr	HHRr	HHrr	HhRr	Hhrr
hR	HhRR	Hhrr	hhRR	hhRr
hr	HhRr	Hhrr	hhRr	hhrr

(Out of these the first HHRR is high-yielding and resistant and is a pure stock).

Selection from Wild Varieties A search has often to be made for resistant genes from the wild varieties, species or related species of the cultivated plants. The best places to find wild varieties resistant to certain pathogens are the primary and secondary gene centres of cultivated plants (Vavilov, 1949-50; Zhukovsky, 1961). Vavilov (1950) used the term "gene centres" or centres of origin for primary regions of diverse cultivated species and varieties. He considered that these centres contain the greatest wealth of hereditary variants with definite naturally-inbred characteristics. Cultivated plants in these centres were frequently, but not always, accompanied by closely related wild species as possible progenitors of domesticated plants. It soon became necessary to distinguish between primary and secondary gene centres. In the primary centres the cultivated plants evolved from wild progenitors native to these regions. During cultivation, however, these plants were frequently brought to other areas, where they were subjected to different climatic and edaphic conditions. These climatic factors forced the plant population to produce new diverse genetic characteristic. This is how secondary and tertiary centres of diversity were

developed outside the primary centres. Zhukovsky and Migushova (1969) have added some new phytogeographic terms. A new term, "genofund" is used by Zhukovsky and Migushova (1969) to indicate the genetic source or gene pool for breeding.

Both the host and parasite have long been associated in their centres of origin as reciprocal selective factors in evolution. Every new and more virulent race of parasite necessarily eliminates most of the susceptible individuals in the local host population. The individuals that survive definitely carry genotypes different from these of the bulk of the population. In this way, the survivors perpetuate genotypic wild species and varieties as a result of natural selection.

It has, however, to be remembered that the gene centre of the host plant is also the centre of origin of its specialized pests and pathogens, as well as the new and more virulent idiotypes, biotypes and pathogenic races which occur in the population of the parasites.

According to Vavilov (1949–50) there are nine world megacentres of origin of the most important cultivated plants in China, India (with a secondary Indo-Malayan centre), Central Asia, Australia, the near East, the Mediterranean region, North-east Africa or Abyssinia, Southern Mexico and Central America, and South America (Peru-Equador-Boliva) with two subcentres (Chile and Brazil-Paraguay).

Although breeders have often turned to wild varieties of cultivated plants to find new sources of resistance and have isolated stocks possessing the desired resistance, it is difficult to transfer this resistance because of incompatibility problems between subject species or because of sterility in the hybrids. Breeders have not come to rely on special cytogenetic techniques and embryo culture to facilitate the more difficult crosses.

Induced Mutations New gene mutations and polyploids can be induced artificially with the help of abnormal radiations, such as X-rays, ultraviolet rays, etc., and also mutagenic chemicals like colchicine, ethyl methane sulphonate. The new mutations, which arise, may be useful or useless depending upon chance. The useful ones are selected and bred as per one of the above described methods. Muller established mutation genetics in the late 1920s and plant breeders immediately realized the possibility or utilizing it. Sigurbjomsson and Micke (1969) have listed 77 cultivars that have been developed. As a direct consequence of induced mutations, 16 of these were cited as having improved disease resistant.

Breeding Wheat for Disease Resistance

The power and potency of modern plant breeding for the improvement of crops have been amply demonstrated in wheat. Wheat improvement has played a key role in ameliorating world food shortage and also in the green revolution in India. Organized and systematic wheat breeding in India was started by the late Sir Albert Howard and Mrs. Howard in 1904 at the Agriculture Research Institute, Pusa, Bihar. Since then a number of research and breeding institutions have come up where wheat improvement work is being carried out. The major techniques used in wheat breeding are: (i) Introduction, (ii) Selection (iii) Hybridization, and (iv) Induced genetic changes.

Early work on wheat improvement in India was concentrated on developing a suitable strain from the local ones, because the varieties introduced from abroad were mostly found to be late in maturing under Indian conditions. On the other hand, introduction played a key role in the USA and Canada, because wheat was not a natural crop in these countries. A successful introduction in India was that of Ridley variety from Australia. This variety is stiff-strawed, fairly resistant to rusts and suitable for hilly areas. More recently, quite a few introductions have been made from Mexico and number of useful varieties have been selected and bred from these introductions. Some of the dwarf varieties introduced from

Mexico include Sonara 63, Sonara 64, and Lerma Rozo. From these, red and white kerneled varieties have been developed in India. Norin, the gene for dwarfness, arose in Japan, from where it was sent for testing to the USA after the Second World War. With the help of suitable crosses this gene was transferred to the American Brevor wheat varieties, which were sent to Dr. Norman E Borlaug, who introduced this gene into many Mexican varieties. Selections made by Borlaug, as well as unselected hybrid materials were later introduced in India.

Besides introduction, hybridization has also played a key role in the development of number of new varieties, especially the rust-resistant ones. Simple hybridization, back crosses, and composite crosses involving various varieties, species, and genera have been used for developing suitable varieties.

Biffen (1905) was the first to apply the Mendelian factorial analysis in the case of resistance in wheat against stripe rust. He crossed the resistant wheat variety, Rivet (American Club variety), and the susceptible variety, Michigan Bronze. He showed that resistance was inherited as a simple recessive factor independently of other factors and could be combined with other desirable characters.

Following the early accomplishments of Orton and Biffen there was a tendency to think that resistance was always governed by a simple monohybrid Mendelian factor which could be manipulated easily. Later work proved otherwise. It was found that resistance in some cases could be dominant and in others, recessive and it may be governed by a numbers of genes which segregate independently, producing great variations in progeny. Linkage and crossing over also complicate the inheritance of resistance as in the case of any other character.

In India all the three rusts of wheat are prevalent in most of the growing areas. There are also several races and biotypes of each of the three rust. Systematic work on the breeding of wheat varieties resistant to all the three rusts was started by Pal in collaboration with Mehta in 1934. The breeding work was done in several stages. Strains of wheat resistant to the races of each rust were first obtained by crossing the susceptible Indian varieties with the resistant foreign varieties. The resistant segregates obtained from each set of crosses were used for multiple crosses. The segregates resistant to all the three rusts were obtained. These resistant strains were back crossed with the original Indian varieties to concentrate their economic characters. One of the outcomes of the crosses is the variety NP 809, which has proved resistant to all the rusts in the hill stations of North India and in Uttar Pradesh, Punjab and Himachal Pradesh. Another simple cross between the susceptible but economically important NP 4 and the resistant Japanese variety, Konon, resulted in a promising variety, NP 770, which showed a high degree of resistance to the rusts at high altitudes. A large number of foreign collections of wheat varieties were also tested for resistance to the races of the three rusts and the Australian variety, Ridley, has been found to be very promising.

The introduction of Mexican dwarfs in India in 1965 brought about a green revolution and the problem of rust was considered to be almost over. However, the parents of the varieties were bred in Mexico where the main rust is black rust or stem rust of wheat. Therefore, the varieties based on the Mexican genetic stock have requisite resistance only to black stem rust. Nevertheless, some of these varieties did show some degree of resistance to more common rusts (especially leaf rust). During the last four years it has been observed that due to continuous cultivation of only selected varieties over large areas in India, resistance is breaking down.

Some of the important Mexican dwarfs varieties introduced in India and developed further are: Sonora 64, Lerma Rojo 64A, K65, Sharbati Sonora, Kalyan Sona, Sonalika, Choti Lerma (S331), Safed Lerma, Hira, Lal Bahadur, HD1918 (Pratap), HD 2009 (Arjun), HD 2122, HD 4530, HD1102, UP 262, and JNK (4-W-184).

Breeding Rice for Resistance

In India, rice breeding programmes go back to the early 1920s when varieties as such GEB24 were evolved. The greatest reservoir of breeding materials for introducing disease and insect resistance into the new varieties has come from India. This appears to be a direct result of breeding and selection programmes conducted in a country where the use of agricultural chemicals is minimum and where most of the major insect pests and diseases of rice are rampant.

In 1946, the Indian government established the Central Rice Research Institute at Cuttack, Orissa, in an effort to intensify scientific research on rice. In 1950, one of the major steps of this institute was to lead an FAO-sponsored project called the Japonica-Indica Hybridization Programme. Although this project was pursued vigorously, it appears from the reports of the Central Rice Research Institute that very little attention was given to morphological plant type and too much emphasis was placed on the performance of advanced generations of bulked seed. Irrespective of the cause, the performance of the progeny was not outstanding as compared with the local varieties used as controls.

Blast disease-resistant rice was bred in 1936 in Tamil Nadu, with the parent material Co4, which is immune or near immune to the blast disease. Co4 is a selection from the variety "Gobi Anaikomban". This was crossed with a local variety "Korangu samba" which is highly susceptible to the disease. Co4, though resistant to the disease, is a long duration variety with coarse grains and is not popular among the cultivators. "Korangu samba" though susceptible to the disease, is a high yielder, with many economic properties. Cultures obtained from these crosses were tested for resistance in the field and the green house and the highly resistant varieties, Co25 and W26, were selected from them.

In India, as far as blast disease is concerned, the resistant variety, Co25 (across between A dt 10 and Co4) was released for cultivation in 1948 and Co 29, a cross between Co 13 and a selection from "Arupathamkodai", was released subsequently. They have been grown in the fields for about 25 years. Instances of heavy infection occurring on these varieties are now being reported. Resistance may break down within a short period of time in endemic areas. The release of Co 25 brought into prominence *Helminthosporium* in the Thanjavur delta, because Co 25 was susceptible to this disease.

Several solutions, including the development of multilineal lines and horizontal or field resistance based upon polygenic resistance, have been suggested to overcome the problem created by the variability of the pathogen.

Apparently the penetration phase in blast is controlled by polygenes. Work done by Padmanabhan (Padmanabhan, *et al*. 1974) had shown that it is possible to combine a high degree of resistance to penetration (polygenic) with resistance to the spread of disease inside the tissue which is controlled by major genes. There is evidence that in some of the moderately resistant varieties, resistance may be conditioned by polygenes.

As early as 1954, a programme of breeding for resistance to blast and *Helminthosporium* disease was initiated by crossing Co 25 (resistant to blast but susceptible to Helminthosporium) and BA 410 (susceptible to blast but resistant to *Helminthosporium*). Varieties resistant to both were evolved and widely tested in India.

Some of the important varieties of rice introduced and developed in India are as follows:

IR8, IR20, Jaya, Padma, Hansa, Pankaj, and Kavery.

Table 11.3 gives a birds-eye view of the disease-resistant varieties used in the IRRI rice-breeding programme to several diseases.

Table 11.3 Disease-resistant varieties from the world collection used in the IRRI rice-breeding programme

Disease	Resistant Varieties
Blast	Zenith, Sigadis, Tadukan, B589 A4-18, Dawn, H105, T172, Nahng Mon S-4, Leuang Yai34
Tungro Virus	Peta, Sigadis, Pankhari 203, TKM6, HR21
Bacterial leaf blight	Zenith, Sigadis, TKM6, Malagkit Sungson, Tadukan, B589 A4-18
Bacterial leaf streak	Zenith, Cp 231-SLO-17, B589 A4-18, Nato Tadukan, RL, Gopher, PI 208443

The International Rice Research Institute (IRRI) has named IR-28, IR-29, and IR-30 their early maturing rice varieties. All the three varieties are insensitive to changes in photoperiod. Hence IR-28, IR-29, AND IR-30 are the only known rice varieties that are highly resistant to grassy stunt virus disease, which is a serious problem in many countries. The varieties inherit this resistance from *Oryza nivara*, a wild relative of rice. IRRI pathologists report that IR-28 and IR-29 are also resistant to tungro, bacterial blight, and blast diseases. IR-30 is resistant to tungro and bacterial blight, but is moderately susceptible to blast.

IR-32 and IR-34 have been named by the International Rice Research Institute and both varieties are resistant to blast and insect diseases. The incorporation of disease and insect resistance into improved germplasm is a major objective of the IRRI and GEU (Genetic Evaluation & Utilization) programme. Several donor parents that have been used as sources of resistance to each major disease and insect are poor plant types (tall with weak stems and droopy leaves). They are crossed with races of improved plant types, such as TN-1, IR-8, IR 262-43-8 and IR-24. Semi-dwarf lines with good grain quality and resistance to specific diseases or insects are selected and evaluated for several reasons and inter-crossed to develop lines with multiple resistances. Gurdev S. Khush and Radalpho *et al.* used several sources of resistance for each pest and disease, except grassy stunt virus, for which *Oryza nivara* is the only source of resistance. For blast, such donor parents as Gam Pai 15, H-105, Sigadis, Nahng Mon S-4, Dawn, Zenith, Kam Baunagan, and Tetep were used. For bacterial blight, Zenith, Malagkit Sungsong, TKM-6, Sigadis, hase Aikok 4^3, BJI and DZ 192 were used, and for tungro, Gam Pai 15, TKM-6, Peta, Pankhari 203, Malagkit Sungsong, HR21, Co 13 and Ptb 18 were used.

The breeding of blast-resistant varieties is now an important objective in rice-breeding programmes of Japan, the USA, India, Taiwan, Philippines and other countries. The problem of breeding for resistance is complicated by the large physiological races of the fungus. Furthermore, the races of the blast fungus appear to be different in different countries. For example, varieties resistant in India may not be resistant in Taiwan or Philippines. A comparison of races in different countries has been difficult because various differential varieties have been used in the countries for identification of the races.

Attempts have been made to develop a set of differential varieties, which may be used uniformly in all the countries. Resistance to blast has been reported to be controlled by one, two, or three genes with resistance partially dominant. Strains resistant in the seeding stage are also resistant to the same races of the blast fungus in the neck-rot stage (Ou and Nukue, 1963), for example, accession 6741 (CD 231 × HD-12), accession 9914 (MO.R-500 × Nato) and H-105 from the USA. Taichung 172 and 176, Chinan 8 from Taiwan and Kataktara from Pakistan are among the varieties resistant to blast at the International Rice Research Institute.

Bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) is one of the most destructive rice diseases in India, Indonesia, the Philippines and other countries of South and south-east Asia. The breeding of resistant varieties appears to be the principal means of control. Out of the 102 varieties tested at the International Rice Research Institute, 1 variety of rice from the USA, and 13 from Taiwan were resistant. Thirty varieties from 8 countries were rated intermediate in resistance. Resistance was noted in many of the wild species of rice. Accession 6973 and 9797, Sigadis, Kaohsiung 68, Chinan 8, and Taichung 172 and 176 are among the resistant varieties. A single dominant gene, Xo, is reported to control resistance to bacterial leaf blight.

Virus and Mycoplasmal Diseases In 1961, when the International Rice Research Institute was established in the Philippines, it was rightly believed that viral diseases caused damage to rice in south-east Asia. Since then at least four viral or mycoplasmal diseases have been reported from the Philippines. These are orange leaf, tungro, yellow dwarf, and grassy stunt (now thought to be bacterial). There is also evidence that the so-called "physiological diseases" known to occur widely in south-east Asia are caused by viruses. The sources of resistance should be located and genes for resistance incorporated into locally adopted varieties of rice.

Brown Spot In India, the varieties BAU 10, T. 141, and CH-13 are reported to be resistant. A single dominant gene was reported to control resistance in a study made in India, while polygenic control was reported in the USA.

Breeding Sugarcane for Resistance

The breeding of sugarcane has been closely related to the outbreaks of serious diseases in the crop. The sereh disease, thought to be caused by a virus, led to the abandonment of the black Cheribon variety of the noble sugarcanes in Java. Another virus disease, mosaic, combined with red rot, almost closed down the sugarcane industry in Lousiana. The disease was later controlled by the breeding of resistant varieties. Fiji disease caused severe damage to sugarcane in Fiji until that crop was brought under cultivation by breeding.

Saccharum officinarum is susceptible to mosaic, sereh, streak, red rot and gummosis, although it is generally resistant to smut *S. barberi* varieties are generally susceptible to red rot and smut, but moderately resistant to *Pythium* root rot, mosaic, sereh, and streak.

S. spontaneum forms are usually resistant to sereh, *Pythium* root rot, and largely susceptible to smut. *S. spontaneum* has been widely used in crosses as a source of disease resistance in many present day hybrids. The possibility of breeding for disease resistance was dramatically demonstrated first by a chance hybrid between noble sugarcane, Black Cheribon, and a wild cane of Java which proved to be resistant to sereh disease (Abbott, 1953).

The principal sugar cane diseases in India are red rot, smut, mosaic, ratoon-stunting and rust. Wilt and grassy shoot also occur but not much information is available on the sources of resistance to them.

Red Rot *S. spontaneum* has been the chief source of resistance. *S. officinarum* and *S. barberi* are susceptible. There are several physiological strains of the red rot organism. To meet the challenge of host-resistant breakdown posed by the emergence of new strains/races of pathogens, a number of genotypes are tested at endemic locations and varieties are available for replacement. Co 7314, Co 5767, BO91, BO99, Co 6907, and Co 7219 are the varieties currently popular for red rot resistance.

Smut Varieties of *S. barberi* and *S. spontaneum* are largely susceptible but *S. officinarum* varieties are resistant. Co 449, derived from a cross between POJ 2878 and Co 331, has in it the complements of S. officinarum, S. barberi and *S. spontaneum.* It is a fairly high yielder and is resistant to smut.

Co 527, derived from a cross between Co 349 and Co 312, is resistant to smut. Co 658 a hybrid between Co605 and Co 443 is resistant to smut. Co 7704 is a popular smut-resistant variety these days.

Ratoon Stunting Little information is available on resistance in India. A number of resistant and high-yielding varieties have been bred in Coimbatore which have become world-famous and are known as Co varieties. In these crosses, resistance, and hardiness is incorporated from the wild, grassy *S. spontaneum* which is crossed with *S. officinarum* (noble sugar canes) and other species. The P.O.J. varieties of Java are also notable in this regard. In these varieties also, *S. spontaneum* is one of the parents. P.O.J. varieties are known for their resistance against mosaic.

Breeding Potato for Resistance

A world-wide research in *Solanum* species showed that a number of them had natural resistance to *Phytophthora infestans*. One species which has proved to be an important source of resistance is *S. demissum*. Although this species is not of economic value, it is possible to cross it with *S. tuberosum*, and some of the hybrids that are obtained are resistance to the disease. Formerly, it was thought that *Solanum demissum* contained four major genes for late blight resistance in potato (Black, 1960). On this basis the possibility of the occurrence of races of *Phytophthora infestans* should be 2^6, i.e., 64 races.

In Canada, five new R genes from *S. demissum* have been identified. Another R gene has been identified in *S. stoloniferum.*

At least nine genes have now been identified (Malcolmson and Black, 1966). The unit for selection is a single tuber or the tubers from a single plant. Kufri Kundan is a good example of a hybrid variety that was developed at CPRI, Shimla, from a cross between Ekishizazu and Kalahdin, introduced from Japan and the USA, respectively. Sometimes multiple crosses are used to develop suitable varieties. In India, hybridization is done at Shimla but the F_1 seeds are sent to the regional stations in different parts of the country for performance trials. The variety, Kufri Kuver, released in 1958 was developed from the cross (*Solanum curlilobum* × *S. tuberosum*) *S. andigena*. Kufri Sindhuri is a selection from a cross between Kufri red and Kufri Kundan.

There are several resistant varieties now such as Kufri, Jeevan, Kufri Alankar, Kufri Khasi Garo, and Kufri Muthu for areas where these varieties give good yield and late blight is of regular occurrence. In 1978, the variety Kufri Badshah was released for the north-western plains of the country to replace varieties which had started losing resistance to late blight. For the hilly regions of the North and North-East, varieties K. Himabim and K.Sherpa were released in 1981. Variety Kufri Jyoti which was resistant to late blight has now become susceptible to certain races of the pathogen.

Potato Wart Varieties resistant to wart have been developed in the UK, USA and Holland. All these varieties have been utilized in Shimla as sources of resistance, which is also found in several wild species.

Several viral diseases infect the potato crop. These are virus X, virus A, virus Y, leaf roll, virus S, virus C, and a spindle tuber as well as several soil-borne viruses.

In India, breeding work has concentrated on resistance to virus X and S.

In *Solanum acaule* and *S. andigena*, a single dominant gene R controls immunity. Yet in another case of selection of *Solanum tuberosum* from Wisconsin, immunity is reported to be controlled by a gene C in the recessive homozygous condition.

The hybrid HC 294, a selection from the cross Kufri red × (Gudstone × taborky) has been reported to be resistant to *M. incognita*. The most common potato beetle is *Epilachna ocellata*. A few wild species of *Solanum*, such as *S. malinchense* and *S. polyadenium* are reported to be resistant to this beetle.

The main objectives of potato breeding in India are high yield, regional adaptability, quality, and resistance to heat, frost, drought, diseases and insects. In order to achieve their introduction, selection, hybridization, mutation, and polyploidy, breeding has been resorted to. Many varieties have been introduced in India from time to time, among them being over 1500 commercial varieties and 120 wild species of *Solanum* which are maintained at the Central Potato Research Institute, Shimla. This collection contains indigenous as well as exotic strains. Varieties, such as Magnesium, Barium and Craigs Defiance are some of the introduced varieties which are commercially grown in India. New varieties are developed by clonal selection and hybridization. The variety, Kufri Red, is selected from the Darjeeling Red Round which was introduced in India many years ago and whose history is lost. In 1975, Kufri Jyoti was introduced as a wart immune and late blight resistance cultivar. Between 1976 and 1988 many wart-immune cultivars were developed and released for cultivation. There are Kufri Muthum, Kufri Sharpa, Kufri Sheetman, Kufri Khasi Garo, Kufri bahar and Kufri Kumar. Kufri Kanchan with red skin has been found resistant to wart recently.

Breeding for Disease Resistance in Tomato

Tomato breeders have made use of several *Lycopersicon* spp. to breed a single cultivar resistant to several diseases. The following species were utilized in the breeding programme to bring in genes as follows:

L. pimpinellifolium—Fusarium, Verticillium, bacterial and spotted wilt, bacterial canker, grey leaf spot and leaf mould

L. hirsutum—Septoria leaf spot, tobacco mosaic, leaf mould

L. peruvianum—Beet curly top, root knot spotted wilt, Septoria leaf spot

L. chilense —Anthracnose

Breeding for Disease Resistance in Pulses

Such breeding has been confined mostly to blight and wilt diseases of gram and the wilt disease of pigeon pea.

Gram blight is caused by *Ascochyta* (*Phyllosticta*) *rabiei*. Studies on blight resistance have been made in India and other countries and sources of resistance are available. Adequate information is not available regarding the races of the fungus although in India there are indications of the occurrence of new races. In Punjab, the variety C. 1234, which was originally found to be blight resistant, was susceptible in later years.

Root rot of gram is caused by *Rhizoctonia bataticola. Fusarium* wilt disease has also been reported. The inheritance of *Fusarium* wilt resistance is reported to be controlled by a single pair of genes. The variety C. 24 from Punjab is reported to be resistant to wit. In Pakistan, the gram variety C. 612 is reported to be resistant to blight and tolerant to wilt.

The most important disease of pigeon pea is wilt caused by *Fusarium udum*. Resistance is reported in the types, NP-41, NP-51, and NP-80. Crosses of NP-51× NP-24 have resulted in the development of four highly wilt-resistant selections: NP(WR) 15, NP (WR) 16, NP (WR) 16, NP (WR) 38.

The wild varieties, *Atylosia lineata* and *A. sericea* genus related to *Cajanus* are reported to be sources of wilt resistance and have been utilized in crosses for this purpose. Resistance to wilt is stated to be controlled by a pair of duplicate dominant genes and also by multiple genes.

The important diseases of black gram and green gram include leaf spot, incited by *Cercospora cruenta* and chlorosis. The black gram variety, Kulu Mash 4, is reported to be resistant to leaf spot, and the green gram selections 24-2 and 24-3 are resistant to chlorosis in Punjab.

Breeding for Disease Resistance in Groundnut

Leaf spot or Tikka disease (*Cercosporidium personatum* and *Cercospora arachidicola*) and wilt or root rot (*Rhizoctonia destrens*) are important diseases of groundnut in India, the former being the most common. Resistance to leaf spot is independently inherited and a single factor inheritance for resistance to Tikka has been reported in wild species, such as *Arachis villosa*. Hybrids from the inter-specific crosses have been obtained with resistance to the disease. Varieties resistant to leaf spot and wilt include 5203 of Gwalior, G-0120, G-1032 and G-0607 of Mysore and Exotic-4 of Indore.

Jute Breeding for Disease Resistance

The stem rot fungus, *Macrophomina phaseolina*, can produce seedling blight, stem rot, or root rot in jute. Screening for existing varieties, both local and exotic, and breeding for resistance is in progress at the Jute Research Institute, Barackpore. The standard varieties, D-154 and JRC-918, a selection from the Brazilian material, are sources of resistance for stem rot.

Mishra and Mukherjee (1984) had reported *Capsularis* strains like 8694, 212, 918, and 5854 to be highly resistant to *M. phaseolina* diseases.

Breeding for Disease Resistance in Tobacco

There has been progress in disease resistance in tobacco in the USA. Some of the lessons learnt from the experiences indicate that:

1. Resistance to black shank and bacterial wilt is present in common tobacco.
2. Adequate resistance to many diseases is found only in wild species of *Nicotiana*.
3. Intra-specific resistance is frequently polygenic, although inter-specific resistance is often simply inherited.
4. Transfer of high resistance or immunity from other usually results in the production of strains undesirable in plant type, yield or quality unless back crosses are made to eliminate the undesirable genes introduced from the alien species.
5. Desirable genes in other species are sometimes linked with undesirable genes.

The black shank disease was first found in the USA in 1916. Florida 301, a resistant shade tobacco variety, was developed around 1930 by crossing and selection within local varieties of “Big Cuba” and “Little Cuba”. Florida 301 was later used as the source of resistance in the breeding of other black shank-resistant varieties. Some of the black shank-resistant varieties developed in the USA are RG, Oxford 1, 2, 3, and 4, Dixie Bright 101 and 244, Vesta and Dixie shade. Resistance to black shank appears to be controlled by multiple factors, varieties differ in the degree of resistance, and *N. longiflora* and *N. plumbagenifolia* are highly resistant or immune. Resistant genes from these are being transferred to common tobacco.

Breeding work on the resistance to bacterial wilt was started in 1934 by studying 1034 collections of tobacco from Mexico, Central America and South America.

Breeding for Disease Resistance in Sorghum

Research in breeding for resistance in sorghum has begun in India. Leaf spot disease caused by *Helminthosporium turcicum* often causes severe damage to sorghum in India. The disease is carried on the seeds or in the soil. This is the same disease that causes leaf blight in maize. Several varieties were found to be resistant in the USA, including two varieties of Sudan grass. Tift Sudan grass was developed from the cross (*Sudan grass* x *Leoti sorgo*) x Sudan grass. Only 1 of the 30,000 F_2 plants of this cross possessed the resistance of *Leoti sorgo* and the plant characters of Sudan grass.

Breeding for Disease Resistance in Cotton

Many diseases attack cotton plants in India, but breeding work has concentrated on two diseases, *Fusarium* wilt, and Bacterial blight or black arm disease.

Breeding for resistance to bacterial wilt was started before 1900. A wilt-resistant variety, Sea Island cotton, Rivers and two upland varieties, Dillon and Dixie, were developed in the USA by selection of resistant plants growing on wilt-infested soil. Seed harvested from the resistant plants was then tested on a progeny row basis. The principles of survival and progeny testing were introduced in cotton breeding. Various studies on the inheritance of disease indicated the presence of two or more dominant genes which control resistance to the wilt disease.

Resistant varieties in India include H-420 and Virhar in *Gossypium arboreum*, KFT, Digvijay and Jayawant in *G. herbaceum* and Co. 2 in *G. hirsutum*. The US varieties, such as Coker 100 wilt, Stonewilt, and Auburn 56 are nematode-resistant, besides being highly wilt-resistant.

Bacterial blight, also called black arm, angular leaf spot, and boll blight, is a bacterial disease caused by *Xanthomonas campestris* pv. *malvacearum*. The Indian arboreum and herbaceum varieties are not much affected by the disease but the American hirsutum cottons in India are highly susceptible. Extensive studies in resistance have been carried out in Sudan, the USA, India, and other countries. In India, resistant varieties have been developed from *hirsutum* x *herbaceum* crosses.

A lot of work for incorporating resistance to agronomically better and high-yielding but susceptible varieties has been done in India and abroad. Several sources resistant to bacterial blight are available such as 101-102B, BJA-592, P-14-7-128, Raba B-50, HG-9, Tamcot-CAMDE, Tx Bonham. In India, a variety BJR (Bacterial blight Jassid Resistant) has been developed.

In India, infestations in cotton due to Jassids (*Empoasca devastans*) are most severe in hirsutum varieties grown in the irrigated tracts. The arboreum and herbaceum varieties are more resistant owing to the presence of dense hairs. Much has been done on the genetics of the hairiness of cotton, and hairy genes have been identified.

Innovative Methods of Plant Disease Control

In a multi-authored book entitled *Innovative Methods of Plant Disease Control* edited by I. Chet (1987), several aspects of innovative methods have been discussed. Mehrotra (1993) has discussed the innovative methods for plant disease control. The first important innovative technique is soil solarization.

There are others such as use of siderophores, mycovirus infection and hypovirulence-induced resistance, utilization of mycorrhizal fungi, genetic engineering for plant disease resistance, pollen management and biological control. Some of these have already been discussed earlier. Here soil solarization and pollen management will be discussed in somewhat greater detail. Soil solarization: according to Katan *et al*., (1976) this technique for control of plant diseases refers to a new approach for soil disinfestations by means of solar energy. Solarization is done by covering the soil with transparent polythene sheets 100 μm thick for 6–8 weeks during summer before planting any crop. At ICRISAT, Pattancheru, Hyderabad, Chauhan *et al*., (1984) successfully controlled wilt of pigeonpea and chickpea with the help of soil solarization. Sharma *et al*. (1984) successfully demonstrated reduction in the viability of sclerotia of *Sclerotium oryzae* when soils were mulched with transparent polythene sheets 0.5mm thick. Lodha (1989) showed the reduction of the population of *Macrophomina phaseolina* in arid soils of Rajasthan as a result of solarization. Dwivedi (1993, 1997) studied the soil mycoflora of sugarcane and sugarbeet crops in relation to soil solarization and reported encouraging results. Soil solarization has also been successfully employed to control soil nematodes in India.

Pollen Management

Thakur and Williams (1978, 1980), and Thakur *et al*. (1983) have reported excellent control of ergot in pearl millet through pollen management. Based on the findings that rapid pollination reduces ergot infection, experiments were conducted at ICRISAT Centre at Pattancheru, A. P., where an ergot less susceptible early flowering line was strategically planted as a pollen donor for susceptible hybrids, ergot severity was considerably reduced and grain yields were increased in different hybrids.

Genetic manipulation (engineering) has been discussed in Chapter 29 where techniques and applications of biotechnology have been discussed in relation to biotechnology and plant disease control.

Integrated Pest Management

IPM (integrated pest management) is a paradigm that is widely used or adopted by all pest control disciplines but whose early definitions and philosophical basis belong to entomologists (Jacobsen, 1997). In phytopathology, another term IDM (integrated disease management) is being used and is a concept derived from integrated pest management. Integrated pest management aims at keeping the pest population below the level of economic injury using best combinations of various components of management. FAO has defined IPM as a pest management system that, in the context of associated environment and the population dynamics of the pest species, utilized all suitable techniques and methods, in as compatible a manner as possible and maintained the pest population at levels below those causing injury. Srivastava (1999) has briefly summarized the concept of integrated pest management for sustainable agriculture. This new concept actually describes what the farmers were doing before chemical pesticides came into vogue. In IPM the various components of management are so tailored so as to get cost-effective control without any adverse effect on the environment and ecosystems. The IPM or IDM relies on non-toxic means but does not intend to keep pesticides at bay from the management system, rather advocates minimum or requisite use of pesticides as may be appropriate to make disease management not only effective but non-hazardous, user and environment friendly. IPM or IDM thus rules out total reliance on pesticides which by and large are the strongest weapons against pathogens or pests. The various components of IDM are host resistant, cultural practices (such as physical treatment of soil by baking the soil by direct exposure to the sun or through soil solarization, removal of infected plants before they

contribute to disease severity such as removal of seedlings affected by downy mildew of pearl millet caused by *Sclerospora graminicola* within 3–4 weeks after sowing followed by gap filling by healthy seedlings). Crop rotation is immensely beneficial in reducing inoculum levels. In the case of flag smut of wheat caused by *Urocystis agropyri* (syn. *U. tritici*), manipulation in the dates of sowing is aimed at avoiding the susceptible age with congenial environmental period. For example, blast of rice in Haryana can be minimized by planting Basmati varieties by the first week of July and ergot of bajra incidence can be minimized by sowing the crop by June end or the first week of July. Integrated disease management also encourages biological control of plant pathogens. This aspect has been discussed earlier. In India efficiency of several biological control agents has been reported from TANU, Coimbatore; PAU, Ludhiana; G. B. Pant University of Agriculture and Technology, Pantnagar; University of Agricultural Sciences, Bangalore; and Kurukshetra University, Kurukshetra; and several potent biocontrol agents reported are species of *Trichoderma*, *Gliocladium*, *Chaetomium*, and certain *Pseudomonas*.

In recent years several botanical pesticides have been reported to control plant pathogens. Neem products/neem extracts have become quite popular as insecticides. The efficacy of neem has also been reported against some fungal pathogens also. Though IPM or IDM relies on nontoxic chemicals for plant disease control, it does not preclude the use of pesticides. It favours minimum and judicious use of chemicals. Of course growing resistant varieties is the best method of controlling any disease and will from an important strategy for managing plant pathogens.

Thus, IPM or IDM is system approach that combines a wide array of crop production and protection practices to minimize the economic losses caused by pests (insects, pests, nematodes, weeds, rodents, birds etc.). It emphasizes careful monitoring of pests and conservation of their natural enemies. The Indian Council of Agricultural Research (ICAR) established a National Centre of Integrated pest Management (NCIPM) in 1988 to cater to the emerging plant protection needs of agriculture in the country.

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Questions

1. What are the cultural methods of plant disease control?
2. What are plant quarantines?
3. When and where was the first quarantine act enacted?
4. What is the D.I.P. Act? When it was first enacted?
5. Name, some plant diseases, which have been introduced before and after the enforcement of plant quarantines in India?
6. Is quarantine necessary for all types of diseases?
7. Name some plant diseases, which can be controlled by crop rotation.
8. What is seed certification?
9. What is biological control of plant pathogens? Give some suitable examples where it has been successfully used to control disease.

10. What are the different chemical systemic fungicides for plant disease control?
11. How do the fungicides act in the pathogen?
12. What is chemotherapy?
13. What is the mechanism of the action of various groups of fungicides?
14. Name some important antibiotics used for plant disease control.
15. Breeding for disease resistance is the best method of controlling plant diseases. Why?
16. What is integrated Pest/Disease Management (IPM or IDM).
17. What is solarization?
18. What are the innovative methods of plant disease control? Give few examples.
19. Name some plant pathogens of plant quarantine importance to India.
20. What is resistance to fungicides by plant pathogens? What strategy will you employ to overcome this?

Plant Diseases Caused By Fungi—Characteristics and Classification of Plant Pathogenic Fungi

12

General Features Of Fungi

Although it is not easy to define fungi, biologists currently use the term "fungus" to include nucleated, spore-bearing, achlorophyllous organisms with absorptive nutrition which generally reproduce sexually and asexually, and whose filamentous, branched, somatic structures are typically surrounded by cell walls containing cellulose or chitin or both.

Although fungi are a diverse group of organisms, a feature which they all have in common is their mode of nutrition. Like animals, fungi are heterotrophic organisms, which must consume preformed organic matter. They may live as saprophytes or parasites.

Fungi are of great economic importance to man and plays an important role in the disintegration of organic matter. They affect us directly by destroying food, fabric, leather, and other commercial goods. They are responsible for a large number of diseases of plants, animals and man.

Cell Structure

Fungal cells may be minute and a single, uninucleate cell may constitute an entire organism. A single cell of *Olpidium* or yeast cell falls in this category. Alternatively, fungal cells may be elongated and strand-like and several may be joined to form a thread of cells: a hypha (pl. hyphae). A large number of hyphae collectively form the mycelium. The cell wall of fungi is generally made up of chitin or cellulose. The majority of the fungi have chitinous walls. Occasionally, both chitin and cellulose may occur simultaneously.

One cell in the hyphae may be separated from another by a cross wall or a septum. Septa in different groups of fungi have important differences in structure. For example, the septa in the lower fungi are pseudosepta, which are septa that are perforated by so many pores that they are sieve-like. In Ascomycota and some Fungi Imperfecti, the septum is perforated by a single pore. In Basidiomycota and some members of Fungi Imperfecti, the septum is more complex than that found in the Ascomycota. The septum here is called a dolipore septum.

Hyphae characteristically grow at the tips and can usually produce lateral branches in their older parts. The mycelium may remain microscopic or develop easily visible organized structures, such as strand- or cord-like rhizomorph compact resting bodies comprising fungal tissues made of prosenchyma or pseudoparenchyma.

All growing hyphae are filled up with cytoplasm, are frequently vacuolated and may be aseptate; hence, they form a single multinucleate cell (coenocyte) or are divided into segments by septa. Fungal nuclei are very small. The nuclei have well-developed nuclear membranes. The nuclear envelope consists of a double nuclear membrane and is marked by large gaps. During mitosis the nuclear envelope does not disappear ordinarily, instead it constricts in a dumbell-like fashion and eventually separates into two daughter nuclei. Meiosis conforms more closely to the norm in other organisms than does mitosis. Ribosomes are of 80S and mitochondria occur in the cytoplasm. There is also the endoplasmic reticulum. Storage products occur in the fungal cell. These, may be in the form of glycogen or lipids.

Golgi apparatus similar to those in higher plants and animals have been found in some fungi. A feature which is of widespread occurrence is the presence of lomasomes. These are made up of membrane bounded tubules or vesicles. The function of lomasomes is not known.

Reproduction

Fungi reproduce both asexually as well as sexually. Asexually reproduction takes place by a variety of methods. This may be by:

1. Fragmentation of the soma,
2. Fusion of the stomatic cells,
3. Budding of the somatic cells, or
4. Production of spores, such as conidia, sporangiospores—motile or non-motile.

Sexual reproduction is of widespread occurrence in fungi. Fertilization can be brought about in a variety of ways. The most common methods are:

1. Planogametic copulation,
2. Gametangial contact,
3. Gametangial copulation,
4. Spermatization, and
5. Somatogamy.

Life Cycle

As in other organisms, in fungi too there is generally a cycle of haploid and diploid structures, corresponding to the gametophyte and sporophyte in the green plants. The diploid phase begins with karyogamy and ends with meiosis. In the majority of fungi there is no distinct alternation of generations. Raper (1954) recognized seven basic types of life cycles. However, Burnett (1976) recognizes five basic life cycles in fungi. These are:

1. Asexual, in which sexual reproduction is lacking, namely, Deuteromycetes.
2. Haploid, in which meiosis immediately follows nuclear fusion. This is very commonly seen in fungi or some members of Ascomycota. Here the diploid phase is of minimum duration.
3. Haploid-dikaryotic, which is similar to the second type, excepting that paired, potentially conjugate nuclei persist in close physical association in the same hyphal segment (hence dikaryon). The examples of this are many members of Ascomycota. Binucleate ascogenous hyphae develop just prior to ascus development and such a dikaryon cannot exist independently of the haploid phase. On the other hand, there may be a condition where the meiospores fuse to form a dikaryon so that the fungus cannot exist independently of the haploid phase. On the other hand, there may be a condition where the meiospores fuse to form a dikaryon so that the fungus is dikaryotic throughout

its life cycle, excepting for the moment of fertilization and during the subsequent meiosis. This occurs in yeast (Endomycetales) and more commonly in smuts (Ustilaginales). In the Basidiomycota, the mycelium derived from the germination of a meiospore may persist in the haploid condition as a monokaryon, but once a dikaryon is formed, it shows unrestricted and independent growth and is the "long-lived" phase of the life cycle.

4. Haploid-diploid, in which the haploid and diploid phases alternate regularly. This is restricted to the order, Blastocladiales.
5. Diploid, in which the haploid phase is restricted to the gametes. The vegetative phase is diploid. Reduction takes place at the time of gametogenesis, such as *Pythium*, *Phytophthora, Saprolegnia* spp., in the Oomycetous fungi.

Classification of Fungi

Whittaker (1969) broke the tradition of a three kingdom system of classification of all living organisms. In doing so he recognized that the classification of all living organisms as Prokaryotes, Animals and Plants (including Fungi) did not reflect their relationships. Whittaker added two more kingdoms in the classification, viz,. Fungi and Protista (Fig. 12.1).

In an attempt to recognize monophyletic groups, the organisms once classified as Fungi are now considered in three different groups: the monophyletic kingdom Fungi, Straminipila (various names have been applied to Straminipiles also known as "Stramenopiles" and "Straminopiles") or Chromista, and four protist phyla. The kingdom Fungi includes four phyla: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. Kingdom Straminipila or Chromista includes phylum Oomycota, Hyphochytriomycota, and Labyrinthulomycota. The four phyla of the Kingdom Protista or Protictista are Plasmodiophoromycota, Dictyosteliomycota, Acrasiomycota and Myxomycota.

An outline classification of fungus and fungus-like organisms is given below:

KINGDOM FUNGI	KINGDOM STRAMINIPILA	KINGDOM PROTISTA
Phylum Chytridiomycota	Phylum Oomycota	Phylum Plasmodiophoromycota
Phylum Zygomycota	Phylum Hyphochytriomycota	Phylum Dictyosteliomycota
Phylum Ascomycota	Phylum Labyrinthulomycota	Phylum Acrasiomycota
Phylum Basidiomycota		Phylum Myxomycota

The classification scheme proposed by Blackwell and Spatafora (2004) is presented below:

Fungi	Straminipila	Slime Molds
Chytridiomycota	Oomycota	Plasmodiophorales
Zygomycota	Hyphochytriomycota	Dictyosteliomycota
Ascomycota	Labyrinthulomycota	Acrasiomycota
Basidiomycota	Thraustochytriales	Myxomycota

Schuessler and coworker in 2001 erected a fifth phylum, the Glomeromycota for arbuscular mycorrhizal (AM) fungi and their relatives that had previously been included in the Zygomycota. Thus, according to Schuessler *et al.* (2001) the five phyla included in the kingdom fungi are Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota, and Basidiomycota.

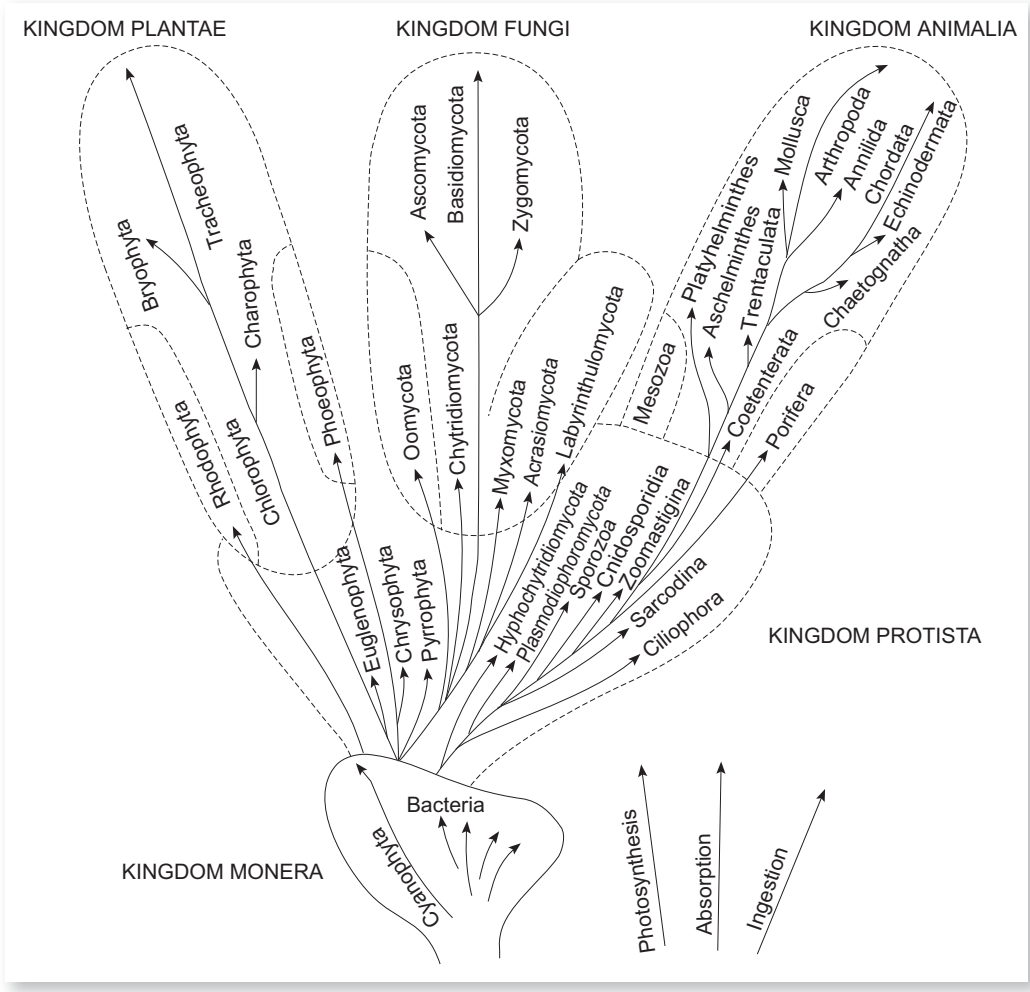


Figure 12.1 Diagrammatic representation of the 5-kingdom system of Whittaker (1969) based on three levels of organization: the Prokaryotic (Kingdom Monera) and Eukaryotic kingdoms (Protista, Plantae, fungi and Animalia)

An outline classification followed in the 10th edition of the *Dictionary of Fungi* by Kirk *et al.* (2008) is given below:

Classification of Fungi (based on 10th edition of Ainsworth and Bisby's *Dictionary of the Fungi* by P.M.Kirk, P.F.Cannon, D.W. Minter and J.A. Stalpers, 2008, CAB International, UK)

Kingdoms	Phyla	Classes/Subphyla	
FUNGI	1. Ascomycota	13 Classes	1. Arthoniomycetes 2. Dothideomycetes 3. Eurotiomycetes 4. Laboulbeniomycetes

(*Contd.*)

(*Contd.*)

Kingdoms	Phyla	Classes/Subphyla	
			5. Lecanoromycetes 6. Neolectomycetes 7. Orbiliomycetes 8. Pezizomycetes 9. Pneumocystidomycetes 10. Saccharomycetes 11. Schizosaccharomycetes 12. Sordariomycetes 13. Taphrinomycetes 14. Leotiomycetes
	2. Basidiomycota	14 Classes	1. Agaricomycetes 2. Dacrymycetes 3. Tremellomycetes 4. Entrorrhizomycetes 5. Exobasidiomycetes 6. Ustilaginomycetes 7. Agaricostilbomycetes 8. Atractiellomycetes 9. Classiculomycetes 10. Cryptomycocolacomycetes 11. Cystobasidiomycetes 12. Microbotryomycetes 13. Mixiomycetes 14. Pucciniomycetes
	3. Chytridiomycota	2 Classes	1. Chytridiomycetes 2. Monoblepharidomycetes
	4. Glomeromycota	1 Class	1. Glomeromycetes
	5. Microsporidia (Microspora)	No Classes	
	6. Zygomycota	4 Subphyla	1. Entomophthoromycotina 2. Kickxellomycotina 3. Mucoromycotina 4. Zoopagomycotina
CHROMISTA	1. Hyphochytriomycota 2. Labyrinthulomycota (=Labyrinthista) 3. Oomycota	1 Class 1 Class 1 Class	1. Hyphochytriomycetes 1. Labyrinthulea (=Labyrinthulomycetes) 1. Oomycetes
PROTOZOA	1. Percolozoa (Acrasida)	1 Class	1. Acrasiomycetes
	2. Amoebozoa (Mycetozoa)	3 Classes	1. Dictyostelia (= Dictyosteliomycetes) 2. Myxogastria (= Myxomycetes) 3. Protosetlia (= Protosteliomycetes)
	3. Cercozoa (Plasmodiophorida) 4. Choanozoa	1 Class No Classes	1. Phytomyxea

Kingdom Fungi

Phylum Chytridiomycota The phylum Chytridiomycota contains the single class Chytridiomycetes. These are the only members of the kingdom Fungi that produce motile cells at some stage of the life history. The motile cells (both zoospores and gametes) of these organisms each possess a single, posterior flagellum. Other characteristics that these species of Chytridiomycota have in common, but other fungi may also exhibit are: 1) The coenocytic structure of the thallus, whether a globose or ovoid structure, an elongated simple hypha, or a well developed mycelium, and 2) The conversion of the zygote into a resting spore or a resting sporangium, or in one order, its growth into a diploid thallus. Cell walls of these fungi contain chitin and glucan. Nuclear divisions in the group are intranuclear and centric.

Chytridiomycota are divided into following orders: Spizellomycetales, Neocallimasticales, Chytridiales, Blastocladiales, and Monoblepharidales.

Order Spizellomycetales Zoospores with several (sometimes one) lipid globules, sometimes amoeboid when in motion, rhizoids when present with blunt tips, less than 5 μm in diameter predominantly from soil. Some important genera belonging to this order are: *Olpidium*, *Rozella* and *Spizellomyces*. Two other interesting genera are: *Caulochytrium* and *Urophlyctis*. *Urophlyctis alfalfae* is a plant pathogen causing crown wart disease of alfalfa, while *Caulochytrium* is a biotrophic parasite of higher fungi.

Genus Olpidium A genus consisting of nearly 30 species of holocarpic, endobiotic plant algae, mosses, pollen and leaves and roots of higher plants. Development in the genus is monocentric. One best species, *O. brassicae*, is a vector for a number of plant viruses. This species attacks the roots of cabbage, the host for which it is named, but is capable of attacking a wide variety of monocots and dicots.

The genus *Rozella* consists of 20 species, virtually all of which are obligate endoparasites of aquatic fungi belonging to Chytridiomycota and Oomycota.

Order Neocallimasticales This order has been raised to a divisional status in the 10th edition of the *Dictionary of Fungi* by Kirk *et al*. (2008). The order once considered within Spizellomycetales consists of anaerobic chytrids that inhabit the rumen and also caecum of herbivore animals. They are placed in five genera including *Neocallimastix*. Both monocentric and polycentric thalli occur within the group. While some species have typical uniflagellate zoospores, others are exceptional within the chytrids because they are polyflagellate. Neocallimasticales is also unique among the fungi because its members are obligatory anaerobic.

Order Chytridiales In Chytridiales, the zoospore is characterized by a constellation of characters. One or more mitochondria are included in the MLC (microbody-lipid globule complex) of the zoospore and the nucleus appears to occupy the space not taken by the MLC and the ribosomes. The nucleus connected to the kinetosome. The ribosomes do not occur dispersed throughout the cell, but packaged by a double membrane in the central part of the cell. Rootlet microtubules typically extend from the side of the kinetosome into the cytoplasm.

Most members of Chytridiales are water- or soil-inhabiting fungi, many of the former parasitic on algae and water moulds, many of the latter on vascular plants. A few parasitize animal eggs and protozoa while others are saprobic on decaying remains of dead plants. Some better known genera are: *Chytridium, Chytridiomyces, Polyphagus, Rhizophydium, Endochytrium, Synchytrium, Cladochytrium* and *Nowakowskiella. Synchytrium endobioticum* causes the wart disease of potato.

In the genus *Synchytrium* belonging to the family Synchytriaceae the thallus is holocarpic, formed exogenous to zoospore cyst, at maturity developing into a sorus, prosorus or resting spore.

Order Blastocladiales They are chiefly water- and soil- inhabiting fungi. Blastocladiales genera are characterized by the production of thick-walled resistant sporangia usually with pitted walls. Another feature linking the members of this group is the prominent nuclear cap present in the zoospore and planogametes. The order is now divided into four families, Blastocladiaceae, Coelomomycetaceae, Catenariaceae, and Physodermataceae (formerly placed in Chytridiales).

Examples of the genera placed in Blastocladiaceae are *Allomyces*, *Blastocladia*, and *Blastocladiella*. *Coelomomyces* belonging to the family Coelomomycetaceae shows alternation of generations but is also heteroecious. Basically the life cycle consists of the diploid spore-producing phase which develops in the mosquito larvae while the haploid gamete-producing phase is produced in the copepod. In the family Catenariaceae, the thallus is Eucarpic polycentric producing what has been described as a catenulate thallus consisting of branched or unbranched septate hyphae with rhizoids.

The species of the *Physoderma* are obligate parasites of vascular plants producing thick-walled and thin-walled slipper shaped sporangia. *Physoderma maydis* causes brown leaf spot of maize or corn.

Order Monoblepharidales Thallus unbranched, eucarpic with a basal or sub-basal, holdfast or differentiated into a well-developed hypha-like vegetative system, oogamous and saprobic. The order is divided into two families, Monoblepharidaceae and Gonapodyaceae. These are not of plant pathological importance.

Phylum Zygomycota In the 10th edition of the *Dictionary of Fungi* by Kirk *et al*. (2008) this division has been divided into 4 subdivisions including 10 orders, 27 families, 168 generas and 1065 species. Earlier it was divided into two classes Zygomycetes and Trichomycetes. Saprobic or, if parasitic or predacious, having mycelium immersed in host tissue—Zygomycetes.

Associated with arthropods and attached to the cuticle or digestive tract by a holdfast and not immersed in host tissue—Trichomycetes.

The class Zygomycetes are divided into orders Mucorales, Entomophthorales, and Zoopogales, etc. In the order Mucorales non-motile spores are produced in terminal sporangia. No zoospores are produced. The sexual spore is a zygospore. There are several families in the order Mucorales. The genus *Rhizopus* of the family Mucoraceae and the genus *Choanephora* and *Blakeslea* of the family Choanephoraceae are plant pathogens.

The genus *Rhizopus* causes soft rots of fruits and vegetables, *Choanephora* species also cause soft rot of cucurbitaceous fruits and young flowers and of other plants including lady's finger and others. *Mucor* species also cause bread mould and storage rots of fruits and vegetables.

Phylum Ascomycota These fungi are characterized by the production of a sac-like structure containing ascospores, cleaved from within by free cell formation after karyogamy and meiosis. Eight ascospores are generally formed but this number may vary from one to over a thousand according to species. Mycelial ascomycetes are characterized further by compartmentalization of mycelium with distinctive walls, septa having simple pores. According to the 10th edition of the *Dictionary of Fungi* by Kirk *et al*. (2008) the division Ascomycota has been divided into 15 classes, 68 orders, 6355 genera and 64163 species.

Group I Archiascomycetes This is equivalent to Taphrinomycetes of Kirk *et al*. (2008) minus Schizosaccharomycetales which has been placed in a separate class Schizosaccharomycetes. Here, group of fungi are difficult to characterize with morphological and biochemical characters. Members of the group lack ascogenous hyphae and ascocarps, and the asci have been homologized with sporangia. In some

members of the group the asci are formed within thick walled ascogenous cells that have been called cysts or chlamydospores.

According to Alexopoulos *et al.* (1996) this is divided into three orders namely: Taphrinales, Schizosaccharomycetales (fission yeasts), and Protomycetales. According to some, Taphrinales contains two important genera, *Taphrina* and *Protomyces*. Some recognize the order Protomycetales to include the genus *Protomyces* separate from *Taphrina*. According to Kirk *et al.* (2008) the order Taphrinales is included in the class Taphrinomycetes. The monotypic genus *Pneumocystis* previously placed in Archimycetes has been placed in a new class Pneumocystidiomycetes. This is unique human pathogenic yeast which causes lethal pneumonia.

Order Taphrinales—Asci arise from binucleate ascogenous cells as thin-walled sacs. In the genus *Protomyces*, the ascospores are formed in a synascus. *Taphrina* species cause leaf curl of peaches and almond. The important species is *T. deformans*. *T. pruni* causes plum pockets and *T. cerasi* causes witches' broom of cherries. *T. maculans* causes leaf spot of turmeric. Species of *Taphrina* are dimorphic with a saprobic, haploid, uninucleate yeast phase that probably undergoes plasmogamy to produce their infective mycelial phase in the presence of the host.

Protomyces species cause galls. *Protomyces macrosporous* causes galls on coriander.

Group II Saccharomycetes—Order Saccharomycetales (the Ascomycetous Yeasts) Mostly unicellular that reproduce by budding. As a group this order is characterized, not by the yeast phase, but by absence of ascogenous hyphae and ascocarps. A number of ascomycetous or true yeasts also produce mycelium. Yeasts are important in fermentation, in baking, brewing, distilleries, and related industries. Some yeasts, such as *Candida albicans*, are human pathogens. In the 10th edition of the *Dictionary of Fungi*, Kirk *et al*. (2008) have kept these yeasts in the class Saccharomycetes. Schizosaccharomycetes yeasts have been placed in a new class Schizosaccharomycetes.

Group III Filamentous Ascomycetes (Plectomycetes) Kirk *et al*. (2008) recognizes a class Euratiomycetes for this group of Fungi. These include Eurotiales and related species. Plectomycetes share the following characters: (1) Asci typically thin-walled, globose to pyriform, evanescent, (2) Asci scattered at various levels in the ascocarp and not forming a hymenium, (3) Ascospores unicellular, (4) Ascocarp typically a cleistothecium when one is present, (5) Cleistothecial peridium of thin wefts to stromatic structures, (6) Various types of anamorphs and conidial forms.

Orders Ascosphaeriales Kirk *et al*. (2008) have kept this in Eurotiomycetes. Members of this order have been considered to be yeasts because they lack an ascocarp. Species of the genus *Ascosphaera* are associated with bees, most apparently are saprobic on the provisions of insects (pollen and nectar). Although a number of genera are saprobic, several members of Onygenaceae, notably species of *Ajellomyces* are important animal pathogens. Kirk *et al*. (2008) have created a separate order for these pathogens as Onygenales.

Order Eurotiales In this order, the member of the family Trichocomaceae are the most widely distributed of all fungi. The group is characterized by a diversity of coverings over the asci. The teleomorph genera usually have phialidic anamorphs, including *Aspergillus*, *Penicillium* and *Paecilomyces*. Species of *Aspergillus* and *Penicillium* are important in the processing of foods, beverages. Some are important in the production of mycotoxins, yet others produce life-saving antibiotics. Species of *Aspergillus* and *Penicillium* play an important role in the post-harvest rotting of fruits and vegetables.

Filamentous Ascomycetes (Pyrenomycetes; Ascomycetes with Perithecia) These are characterized by: (1) Perithecial or, occasionally, cleistothecial ascocarps that may be formed in a stroma, immersed in a subiculum or be unassociated with specialized stromatic structures, (2) Ovoid-to-cylindrical unitunicate asci, usually formed from ascogenous hyphae and croziers in a hymenium or becoming secondarily scattered throughout the ascocarp, (3) Persistent asci that have forcible discharge of ascospores through an ascal tip apparatus or evanescent asci with passive discharge (4) Hamathecium that may consist of one or more types of sterile hyphae and pseudoparenchymatous tissue (5) One-to-several celled ascospores of various shapes and (6) Diverse and often complex anamorphs and related conidial species which do not reproduce sexually.

Pyrenomycetes are important as parasites and symbionts of arthropods, endophytes, mycotoxin producers, mammalian parasites, plant parasites, and saprobes. The orders discussed here are Hypocreales, Melanosporales, Microascales, Phyllachorales, Ophiostomales, Diaporthales, Xylariales, Sordariales, and Meliolales. Kirk *et al.* (2008) have kept these orders in a separate class Sordariomycetes more or less equivalent to the old name Pyrenomycetes.

Order Hypocreales (1) Pale-to-brightly colored, fleshy stromata (2) Perithecial ascocarps in most species (3) Ovoid-to-cylindrical asci (4) Ascospores spherical to needle-like, one-to-several celled, breaking into parts, spores in some species (5) Ascospores forcibly discharged (6) Conidia produced enteroblastically from phialidic conidiophores. The order is now placed in the class Sordariomycetes according to Kirk *et al*. (2008). *Hypocrea, Nectria, Gibberella, Claviceps, Cordyceps, Epichloe*, and *Balansia* are some important genera from pathological point of view.

Genus Hypocrea It belongs to the family Hypocreaceae having cushion-shaped or effused stroma. Asci are narrowly cylindrical containing 8 two-celled ascospores: Several types of anamorphs including *Trichoderma* and *Gliocladium* which are biocontrol agents.

Family Nectriaceae

Genus Nectria It belongs to the family Nectriaceae. *Nectria* produces its perithecia on the surface of a cushion-shaped stroma and the ascocarps are brightly colored. *N. galligena* and related species cause serious canker diseases of a large number of hardwood trees including apple and pear.

Genus Gibberella This belongs to the family Nectriaceae. *Gibberella* is characterized by deep blue or purple perithecia and fusoid one septate ascospores. *Gibberella zeae* with a *Fusarium graminareum* anamorph causes red ear rot of maize, while *G. xylarioides* destroys coffee plantations in Africa. *G. fujikuroi* with a *F. moniliforme* anamorph causes the foolish seedling disease of rice in Asia and is a source of gibberellic acid. Gibberellic acid acts as a promoter of flowering, cell elongation, amylase production in seed germination, and inducer of seed-less fruit development.

Family Claviciptaceae and Cordycipitaceae Characterized by bright or darkly-pigmented, fleshy, stromata, long narrow cylindrical asci with thickened dome-like caps perforated by long cylindrical pores, and paraphyses formed on the lateral walls of the ascocarp but not occurring among the basally-tufted asci. Ascospores are thread like and extend the length of the ascus. Important genera are *Claviceps*, and *Cordyceps*. *Claviceps* is parasitic on grasses while *Cordyceps* is pathogenic on insects. *Torrubiella* is pathogenic on spiders, *Epichloe* is endophytic in grasses and *Balansia* is endophytic in grasses and sedges. *Claviceps purpurea* causes the ergot of rye (*Secale cereale*) while *C. fusiformis* causes the ergot of pearl millet.

Order Melanosporales The important genus is *Melanospora*. The genus is characterized by the absence of stroma, production of short-to-long necked, pale colored perithecia, hamathecia of pseudoparenchyma, absence of paraphyses but with periphyses, ovoid, evanescent asci, darkly pigmented, one-celled ascospores, fusoid lemon shaped with germ pores at each end. *Phialophora* and *Gonatobotrys* anamorphs parasitize the mycelium of many fungi, including the important plant pathogens.

Order Microascales Lack stromata, perithecia in most species but some with cleistothecia. Asci are globoid or ovoid, disintegrating. Ascospores are one-celled. *Ceratocystis* and *Ophiostoma* are important genera of the family Ceratocystidaceae. *Ceratocystis fagacearum* causes the oak wilt in North America. *C. fimbriata* causes root rot of sweet potato, butt rot of pineapple is caused by *C. paradoxa*. The genus *Ophiostoma* primarily attacks gymnosperms.

Order Phyllochorales The order is characterized by stromatic perithecial forms in most species, sometimes having a clypeus (a shield-shaped structure of differentiated stromatic tissue around the mouth of the perithecium) with oblong-to-cylindrical asci arranged basically or peripherally at the base of the perithecium, and ascus tips with pores encircled by narrow rings. Phyllachorales include the genera *Phyllachora*, *Polystigama*, and *Glomerella*.

Genus *Phyllachora* In *Phyllachora*, the perithecia are immersed in the substratum. *P. graminis* causes leaf spot of grasses.

Genus *Glomerella* *Glomerella* produces dark perithecia and there is the absence of stroma. *Glomerella* species are more encountered as anamorph in the genus *Colletotrichum. G. cingulata* causes anthracnose of many plants, bitter rot of apple is caused by *G. cingulata*; its anamorphic stage is *Colletotrichum gloeosporioides. G. tucumanensis* causes red rot of sugarcane. *Colletotrichum gloeosporioides* causes mango anthracnose and that of papaya fruit rot.

Order Ophiostomales The order is characterized by perithecial ascocarps, but with cleistothecia in one genus, globose-to-ovoid evanescent asci usually produced in a basal fascicle and released upward into the cavity or developing, from cells that line the periphery of the cavity and are released into the centre in the manner of *Ceratocystis*. *Sporothrix* and *Leptographium* anamorphs are characteristics of some species.

The genus *Ophiostoma* differs from *Ceratocystis* because it has rhamnose and cellulose in the cell walls, resistance to cycloheximide and nonphialidic anamorphs. *Ophiostoma ulmi* causes the Dutch elm disease.

Order Diaporthales Perithecial ascocarps produced in a stroma of fungal and substrate tissues or directly from somatic hyphae on the substrate. Persistent clavate or cylindrical asci with pores are present.

Genus *Diaporthe* *Diaporthe* is characterized by perithecia immersed in a stroma with long necks protruding. Egg plant fruit rot is caused by *Diaporthe vexans*. *Diaporthe citri* is the cause of citrus melanose. Species of *Diaporthe* have *Phomopsis* anamorph.

Genus *Gnomonia* Perithecia of *Gnomonia* are buried in the substrate, apparently not in stromata. *G. leptostyla* is the cause of walnut anthracnose.

Genus *Gaeumannomyces* The development pattern is similar to *Diaporthe*. It differs from other genera of the order by having multiseptate, filiform ascospores. It is a strong pathogen of plant roots, prominent appressoria referred to as hyphopodia and hyphomycetous phialophora-like anamorphs. *G. graminis* causes the very destructive take all disease of wheat, rice and oats.

Genus *Magnaporthe* *M. grisea* is best known by its anamorph *Pyricularia grisea* (formerly *P. oryzae*). It is the cause of rice blast. The ascospores are three-septate, and the two middle cells are pigmented. A non-specific toxin pyricularin is involved in the disease.

Genus *Cryphonectria* (formerly included in *Endothia*) *C. parasitica* causes the chestnut blight (*Castanea dentata*). Perithecia of *C. parasitica* are produced in a stroma, with long necks terminating at the surface in an ostiole.

Order Xylariales It includes diverse group of pyrenomycetes with dark, leathery, woody, or carbonaceous perithecial or occasionally, cleistothecial ascocarps, sometimes embedded in stroma.

Genus *Xylaria* Mostly saprobic or weak parasites on woody trees. The genus is characterized by the stromata, which are stipitate upright simple or branched; corky, leathery or woody in texture. Perithecia are produced over the entire portion of the stroma except the stipe.

X. digitata is responsible for root rot of hard woods and tree cankers.

Genus *Rosellinia* *Rosellinia* species especially *R. necatrix* causes root diseases of fruit trees and vines. The genus is characterized by a subiculum from which the anamorph, and later stromata develop.

The imperfect state of *R. necatrix* belongs to the form genus *Dematophora necatrix* characterized by the formation of synnemata.

Order Sordariales The order is characterized by dark or pallid perithecia or cleistothecia with periphyses and membranous or leathery peridia; ascocarps sometimes are embedded in a subiculum. From an economic standpoint, it is not an important order but several species have proved of great value as experimental organisms. *Neurospora* is the *Drosophila* of the fungus world.

Genus *Chaetomium* The genus has ascocarps with hyphal appendages on the ascocarp surface. In some species the hairs produced on the body of the ascocarp are different from those produced at the apex. Species of *Chaetomium* have strong cellulolytic ability.

Order Meliolales It is a small homogenous group of fungi characterized by hyphopodiate mycelium produced on the surface of leaves and stems of host plants. Economically, members of the order are the least important plant parasitic fungi and none causes enough damage.

Filamentous Ascomycetes with Apothecia (Discomycetes) Most of these fungi commonly called discomycetes are characterized by the production of ascocarps known as apothecia. Asci are cylindrical-to-ovoid, often interspersed with paraphyses. Ascospores are forcibly discharged. This is the major group of lichen-forming ascomycetes. Some of the important orders of the plant pathological importance are discussed briefly.

Order Rhytismatales Ascocarps are produced in stromata. The ascocarps are black externally and may be spherical, discoid or elongate in shape. Kirk *et al*. (2008) place this order under the class Leotiomycetes.

Genus *Rhytisma* Most species of *Rhytisma* produce numerous apothecia in each stroma. *R. acerinum* causes the disease tar spot in maple leaves.

Species of *Lophodermium* cause needle cast disease of conifers.

Order Helotiales The order is characterized by either cup- or disc-shaped apothecia, and asci that have only slightly thickened apices. The ascospores are several septate and variously shaped. *Sclerotinia*, *Monilinia*, *Stromatinia*, *Pseudopeziza*, and *Diplocarpon* are the important genera from plant pathological stand point. Kirk *et al*. (2008) place this in the class Leotiomycetes.

Genus *Sclerotinia* In *Sclerotinia*, the sclerotia develop in leaf blades or rarely in twigs; are apothecia, deep inoperculate to discoid.

S. sclerotiorum is responsible for many rot and wilt diseases in vegetable crops. The species attacks 383 plant species in 225 plant genera placed in 64 families. The maximum number is in Asteraceae (Compositae) followed by Fabaceae (Leguminosae), and Brassicaceae (Cruciferae).

Genus *Monilinia* In this genus, the conidial stages is always a *Monilia* and spermatia are formed. *Monilinia* species cause brown rot disease of stone fruits.

Order Ostropales Includes several families of discomycetes with paragymnohymenial development. Graphidiaceae is one of the families that include many lichen-forming fungi. Kirk *et al*. (2008) place this in the class Lecanoromycetes.

Filamentous Ascomycetes with Ascostroma (Loculoascomycetes) Loculoascomycetes now known as Dothidiomycetes are characterized morphologically by the production of asci within locules in a preformed stroma (ascostroma) that constitutes the ascocarp. Some are lichen-forming, and others are plant pathogens, endophytes and animal pathogens. These have been kept in the class Dothidiomycetes containing the orders Capnodiales, Dothidiales and Myriangiales (Kirk *et al*., 2008).

Order Myriangiales This is a small order of the small group Loculoascomycetes. *Elsinoe* is an important genus. In *Elsinoe* the ascostroma is simple, innate, infra- or sub-epidermal, partially erumpent at maturity. Small pulvinate-to-crustose, ascospores 3 septate with longitudinal septa in some cells. *Elsinoe mangiferae* causes scab of mango.

Elsinoe fawcetti causes scab of sour oranges, rough lemon, and other citrus fruits. *Elsinoe ampelina* causes anthracnose of grapes.

Order Dothidiales This order has 4 families with more than 55 genera and 350 species. Important genera are *Guignardia, Mycosphaerella*, and *Didymella*.

In *Guignardia* the fungus develops perithecium-like structures in which clusters of 8-spored asci arising from the base of the locule are produced. Paraphyses are absent. Conidial stage is of Phoma type.

Guignardia bidwellii causes the destructive black rot disease of grapes.

Genus *Mycosphaerella* In *Mycosphaerella* the asci are 8-spored, pseudothecial are small and immersed in the host tissue (usually on dead leaves). Ascospores are hyaline or pale green. The conidial forms are of several genera such as *Ramularia, Cercospora, Phoma, Ascochyta, Septoria* and *Phyllosticta* of Imperfect Fungi.

Mycosphaerella ribis causes leaf spot of gooseberry and currant.

M. fragariae—causes leaf spot of strawberry.

M. berkeleyii has the conidial state in *Cercosporidium personatum* which causes tikka disease of groundnut.

M. arachidicola has the conidial state in *Cercospora arachidicola* which also causes leaf spot of groundnut.

M. musicola—has conidial state in *Cercospora musae* which causes leaf spot or Sigatoka disease of banana.

M. pinodes—this species is the ascigerous stage of *Ascochyta pinodes* which causes blight and pod spot of pea.

Genus *Didymella* In the genus *Didymella* the pseudothecia are separate, innate, immersed in the substratum and ascospores are hyaline.

Order Capnodiales In this order, Ascostromata or spherical pseudothecia may be grouped or separate, and sometimes occur in a subiculum and produced in a loose mat of dark hyphae.

Genus *Capnodium* Being one of several fungi causing sooty mould on plants.

Order Pleosporales According to Kirk *et al*. (2008) this order Pleosporales belongs to the subclass Pleospomycetidae of the class Sordariomycetes. In this order the asci are surrounded by psuedoparaphyses. Ascostromata are variable. *Cochliobolus, Pyrenophora, Pleospora, Setosphaera, Leptosphaeria* and *Venturia* are important genera.

Genus *Cochliobolus* The pseudothecia are often covered with conidiophores. They lack setae. Ascospores are more or less spirally coiled within the ascus. Anamorphs are in the genus *Bipolaris* and *Curvularia* cause leaf spots and root rots on grain crops and grasses.

Genus *Pyrenophora* In this genus the pseudothecia are large, hairy (setose) and often bearing conidiophores on the surface. The ascospores are large, hyaline but commonly pale brownish, oblong with almost straight sides and broadly rounded ends, 3-,5,7-septate with a single longitudinal septum in 1 or all the cells. Anamorph is *Drechslera* species causing leaf spots on cereals and grasses.

Genus *Pleospora* Anamorph of this genus is *Stemphylium* causing black mould rot of tomato. The genus differs from *Pyrenophora* mostly in shape and size and dark color of the spores.

Genus *Setosphaera* (Anamorph is *Exserohilum*) causing leaf spots on cereals and grasses.

Genus *Leptosphaeria* (anamorph is *Phoma*). In this genus the pseudothecial wall is composed of scleroplectenchyma of isodiametric thick-walled cells. The pseudothecial beak is short or lacking. The ascospores are fusoid to rarely scolecosporous, 3-to many septate and brownish-yellow-to-uniformly hyaline.

Genus *Venturia* It has globose pseudothecia or asco-stromata with several locules. Pseudothecia are often setose at the apex. Conidial stage is found in the form genus *Spilocaea. V. inaequalis* causes apple scab while *V. pyrina* the pear scab.

Other Filamentous Ascomycetes Kirk *et al*. (2008) have placed these members in the class Leotiomycetes.

Order Erysiphales The fungi belonging to this order are obligate biotrophs that cause a major group of plant diseases commonly known as powdery mildews. Asexual reproduction is by conidia formed singly or in a chain on simple, rarely-branched conidiophores. Sexual structures the ascocarps are completely closed like cleistothecia but produce their asci in a basal layer. The asci have been described as bitunicate.

Genus *Sphaerotheca* Mycelium superficial, perithecium with one ascus only. *S. pannosa* causes powdery mildew of roses and peach, *S. fuligenea* causes powdery mildew of cucurbits in India.

Genus *Podosphaera* Mycelium superficial, Perithecium with one ascus and perithecial appendages dichotomously branched. *P. leucotricha* causes powdery mildew of apple.

Genus *Erysiphe* Mycelium is superficial, perithecium with several asci and appendages are simple (myceloid). *Erysiphe* species is causing powdery mildew of several herbaceous plants.

E. cichoracearum (on cucurbits), *E. polygoni* (on pea and *Lathyrus*, etc.) are pathogenic fungi.

Genus *Microsphaera* Mycelium superficial, perithecium with several asci, perithecial appendages dichotomously branched.

M. alphitoides causes powdery mildew of oak.

Genus *Uncinula* Mycelium superficial, perithecium appendages with several asci and perithecial appendages coiled at tip. *U. necator* causes powdery mildew of grapes while *U. tectonae* is a serious plant pathogen on *Tectona grandis*.

Genus *Phyllactinia* Mycelium partly internal, Perithecial appendages stiff with basal swelling and several asci in a perithecium. *P. acaciae* on leaves of *Acacia arabica*, *P. ferruginea* on *Acacia catechu*, *P. corylea* var. *subspiralis* on *Dalbergia sissoo* are pathogenic fungi.

Genus *Blumeria* *Blumeria* is a new genus created in the year 1973 for *Erysiphe graminis* by its larger ascocarp, two types of mycelia, and grass hosts. It is a monotypic genus having only species *B. graminis*.

Asexual Ascomycetes and other Asexual Fungi (Deuteromycetes or Imperfect Fungi) Mycelium well developed, septate, branched. Sexual reproduction and structures are rare or lacking or unknown. Asexual spores (conidia) formed on conidiophores existing singly, grouped in specialized structures such as sporodochia and synnemata, or produced in structures known as pycnidia and acervuli.

Asexual Ascomycetes and Other Asexual Fungi (Deuteromycetes or Imperfect Fungi) Classification here is mainly based on the mode of development of conidia. Some of the important genera are *Geotrichium, Aspergillus, Penicillium, Drechslera, Exserohilum, Botrytis, Monilia, Oidium, Chalara, Trichoderma, Gliocladium, Verticillium, Fusarium, Colletotrichum, Cercospora, Septoria, Phyllosticta, Alternaria, Rhizoctonia*, and *Sclerotium*. The anamorphic stage, the mode of development of conidia and the presence of teleomorphic stage plays an important role in the classification of these genera.

Phylum Basidiomycota From the 3 classes in the *Dictionary of Fungi* 9th edition in the year 2001, and in the 10th edition of the *Dictionary of Fungi* (2008), 14 classes have been recognized by Kirk *et al*. (2008). The division or Phylum Basidiomycota has been divided into 3 subphyla, viz., Agaricomycotina, Pucciniomycotina, and Ustilaginomycotina. These members are characterized by the production of basidiospores formed on the outside of the specialized microscopic spore producing structures called basidium.

The class is differently classified by different authorities in the subject. For convenience sake we have followed here the old classification used by Alexopoulos and Mims (1979) in the third edition of the book *Introductory Mycology*. Class Basidiomycetes has been further divided into a single class Basidiomycetes having 3 subclasses viz. Holobasidiomycetidae, Phragmobasidiomycetidae, and Teliomycetidae

Subclass Holobasidiomycetidae Basidia are single-celled. Basidiocarp present or, if not, hymenium is exposed.

Order Aphyllophorales, also known as Polyporales all produce single-celled, club-shaped basidia in well-developed hymenia. The hymenia are borne on various ways on gymnocarpous sporophores. The hymenium is exposed before the spores are mature.

Genus *Schizophyllum* In this genus the hymenial layer consists of thick lamellae that are split longitudinally with both edges folded back. Basidiocarps are soft textured. Not much of plant pathological importance.

Genus *Poria* Sporophore is a thick crust-like layer without pileus and resupinate.

Genus *Fomes* It produces hoofed perennial trimitic sporophore containing stratified tubes. *Fomes* species attack heart wood of various trees.

Genus *Heterobasidion* This differs from *Fomes* in the absence of clamp connections. *H. annosum* is a serious problem in freshly cut stumps and causes root rot of trees. It also causes heart rot of trees.

Genus *Lenzites* It produces radially elongated pores with gill-like structures, corky-to-leathery in texture and trimitic in construction.

Genus *Trametes* The fructifications are leathery-to-corky, effused, pileate and dimitic in construction. Pore tubes are of different lengths.

Genus *Irpex* Sporophores are effused reflexed with dimitic hyphal system. It is a lignicolous species.

Genus *Ganoderma* This genus contains lignicolous species that produce spores with an inner brown layer. It produces hard shelf-like fruiting bodies.

It causes root and basal stem rot of various trees including *Dalbergia sissoo*, *Acacia catechu* and other trees.

Genus *Polyporus* The fruit bodies in this genus are not so hard as that of *Fomes*. In addition they are annual sporophores. The fruit bodies may be stipitate, sessile or effused. Most of the species are wood rotters.

Order Agaricales These produce fleshy, sometimes tough, umbrella-like sporophores that bear their basidia on the surface of gills or plates (lamellae). In *Boletus*, the basidia are borne not on gills but instead line the inside of tubes or, more rarely shallow pits.

Genus *Boletus* Most of the species are mycorrhizal.

Genus *Amanita* Well known white spored genus. Members are characterized by free gills, and the presence of an annulus and vulva. Most of the species are poisonous.

Genus *Marasmius* The genus consists of small, rather tough mushrooms with thin stalks. It grows generally on grasses and leaves. *M. ordeades* cause fairy rings on turf grasses.

Genus *Armillaria* *A. mellea* is the honey mushroom. It produces rhizomorphs and is responsible for root rot of forest trees.

Genus *Psilocybe* It produces attached gills, and dark spores. *Psilocybe* produces conis-to- campanulate caps with attached gills. *P. mexicana* is hallucinogenic.

Genus *Agaricus* White-to-brown sporocarp, free gills and an annulus but no vulva. The genus contains edible mushrooms.

Holobasidiomycetidae The third group in this subclass contains orders Exobasidiales, Tulasnellales, Dacrymycetales, and Brachybasidiales.

Order Exobasidiales Basidiocarp is lacking basidia; are produced directly from dikaryotic between the epidermal cells of the host, push through the cuticle and form a layer on the surface host.

Exobasidium vexans is responsible for the blister blight of tea in India and Sri Lanka. The other orders are not of plant pathological importance. The fourth groups of Holobasidiomycetidae are the Gasteromycetous fungi.

Gasteromycetidae These include puff balls, earth stars, stink horns and birds nest fungi. These produce holobasidia; the development of the basidiocarps is typically angiocarpic. Most are saprobic in soil, dead wood of dung, although a few are mycorrhizal. All produce well developed septate hyphae that may or may not possess clamp connection. Dolipore septa are common in the group. The orders are Hymenogastrales, Sclerodermatales, Tulostomatales, Lycoperdales, Phallales, and Nidulariales.

Phragmobasidiomycetidae This subclass consists of fungi whose metabasidium is typically divided in to four cells by transverse or longitudinal septa. The subclass consists of the orders Tremellales, Auriculariales, and Septobasidiales. The Tremellales and Auriculariales are saprobic forms and the Septobasidiales are parasitic or parasitic-symbiotic on scale insects.

Subclass Teliomycetidae (rusts, smuts and basidiomycetous yeasts) The rusts and are characterized by the production of a thick-walled, binucleate resting spore termed the teliospore which upon germination, gives rise to a promycelium into which the diploid nucleus moves. From meiosis, haploid basidiospores are formed either directly on the surface of the promycelium or on the tips of small out growths from the mycelium.

Order Uredinales Basidia arising from thick-walled probasidium and basidia becoming septate bearing 2-4 (mostly four) basidiospores, one at each septum and one terminal. Biotrophs, several types of spores are being produced. Autoecious or heteroecious.

Genus *Puccinia* The teliospores of *Puccinia* are stalked and two-celled.

P. graminis tritici causes black rust of wheat, *P. triticina* (syn. *P. recondita, P. rubigovera*) causes leaf rust or brown rust of wheat, *P. striiformis* (syn. *P. glumarun*) causes yellow rust of wheat and barley.

P. arachidis causes rust on the leaves of groundnut.

Genus *Uromyces* The teliospores are one-celled and dark and stalked.

Uromyces fabae causes rust on *Vicia faba* and *Lathyrus* spp., *U. pisi* on pea, *U. ciceris-arietini* on gram (chickpea).

Genus *Hemileia* The teliospores in this rust are one-celled and pedicellate, round and turnip shaped. The urediniospores are reniform.

H. vastatrix causes coffee rust disease.

Genus *Gymnosporangium* The teliospores in this rust are produced in long tongue-like structure with very long pedicels.

G. juniperi-virginianae causes cedar rust.

Genus *Phragmidium* Teliospores in this rust are multicellular with pedicels covered with a gelatinous structure. *Phragmidium* causes rust on rosaceous plants.

Genus *Melampsora* The teliospores in this rust are formed in a crust or column below the surface of the host. *M. lini* causes the rust of linseed (or flax).

Genus *Coleosporium* The teliospores are laterally united,forming a crust-like structure on the host. No external promycelium is formed. The aecia form on pine needles with prominent white lip that puts them in the form genus *Peridermium*.

Order Ustilaginales The order includes the smut fungi and the basidiomycetous yeasts. The teliospores in Ustilaginales are formed from intercalary cells of the binucleate mycelium. Basidiospores are sickle-shaped, elliptical, or hyphae-like, sessile and not discharged violently. Number of basidiospores is indefinite (generally) and clamp connections are more common in the mycelium. The dikaryotic mycelium is obligatory parasitic in the host plants.

Genus *Ustilago* The sori contain 1-celled teliospores, dusty at maturity and are covered by the membrane of host origin.

U. segetum var. *tritici* (*U. tritici*) causes loose smut of wheat.

U. zeae causes smut of corn or maize.

Genus *Sphacelotheca* In *Sphacelotheca*, the sori are similar to *Ustilago* but are covered by peridium of fungal cells and have a central columella of host tissue. The genus is renamed as *Sporisorium*.

S. sorghi causes grain smut of jowar, *S. cruenta* the loose smut of jowar.

Genus *Sorosporium* In *Sorosporium* the sori consist of spore balls entirely of fertile cells, are evanescent or tending to fragment into single spores.

S. reiliana causes head smut of jowar.

Genus *Tolyposporium* In *Tolyposporium* the sori consist of permanent spore balls not tending to fragment. *T. penicillariae* causes smut of pearl millet (bajra). It has been renamed as *Moesziomyces bullatus*. *Tolyposporium ehrenberghii* is now renamed as *Sporisorium ehrenbergii* which causes loose smut of Jowar.

Genus *Tilletia* In the genus *Tilletia*, the teliospores are large in size which on germination forms nonseptate promycelium bearing 8 filiform sporidia (basidiospores). The sporidia form characteristic H-shaped pieces as a result of fusion. *T. caries* and *T. foetida* cause hill bunts or stinking smut of wheat. *T. indica* is responsible for Karnal Bunt of wheat.

Genus *Neovossia* The genus differs from *Tilletia* in the method of spore germination. A large number of sporidia are formed terminally when the spore germinates and which do not fuse in situ.

Genus *Graphiola* Sori in this genus are formed under the epidermis of the palm host. There is a cup like dark outer peridium, which surrounds the central sporogenous portion.

G. phoenicis causes spots on palm.

Kingdom Chromista or Straminipila

This kingdom is divided into 3 phyla namely:

1. Phylum Oomycota
2. Phylum Hyphochytriomycota and
3. Phylum Labyrinthulomycota.

Phylum Oomycota The characteristics that set the Oomycota organisms apart from true fungi and also define or delineate the phylum are as follows:

1. Asexual reproduction by a biflagellate zoospore with a longer tinsel flagellum directed forward and a shorter whiplash flagellum directed backward.
2. Various features of ultra-structure of zoospores.
3. The production of a diploid thallus and meiosis occurring at the time of gametogenesis.
4. Oogamous reproduction by gametangial contact results in the formation of a thick-walled sexual spore termed an oospore.
5. Cell walls made up of β-glucans but also containing the amino acid hydroxyproline as well as small amounts of cellulose.
6. Mitochondria with tubular cristae, and
7. Various biochemical and molecular characteristics.

The phylum Oomycota contains a single class Oomycetes which has six orders Leptomitales, Rhipidiales, Sclerosporales, Pythiales, Peronosporales and Saprolegniales (Dick, 1990a). However Alexopoulos *et al.* (1996) have recognized only 5 orders. However Kirk *et al.* (2008) recognize 8 orders (Albuginales, Leptomitales, Myzocystiopsidales, Olpidiopsidales, Peronosporales, Pythiales, Rhipidiales and Saprolegniales).

Key to the Five Orders of the Class Oomycetes

A. Spores always formed within the sporangium, monomorphic or dimorphic, rarely aplanetic: B,BB

B. Eucarpic, hyphae regularly constricted with "cellulin" granules at constrictions: *Leptomitales*

BB. Holocarpic or eucarpic, hyphae when present without constrictions: C, CC.

C. Thallus holocarpic or eucarpic and mycelial, periplasm minimal, usually several oospores in an oogonium: *Saprolegniales*

CC. Thallus monocentric, with rhizoids, periplasm persistent, usually a single oospore per oogonium; often in stagnant water: *Rhipidiales*

A. Spores formed within the sporangium or, if not, then usually within an evanescent vesicle arising from the sporangium; monomorphic reniform: D, DD

D. Holocarpic *Lagenidiales*

DD. Eucarpic *Peronosporales*

Aphanomyces euteiches (Saprolegniales) causes root rot of peas. Genus *Pythium* causes damping off seed decay, root rot and fruit rot, *Phytophthora* species cause blights, fruit rot, cankers, and root rot of several economic plants. Kirk *et al.* (2008) placed the genus *Phytophthora* in the family Peronosporaceae of the order Peronosporales while *Pythium* has been placed in the order Pythiales.

Genera belonging to Peronosporales such as *Sclerospora*, *Peronospora*, *Bremia, Plasmopara, Pseudoperonospora, Peronosclerospora*, and *Sclerophthora* cause downy mildew of several crop plants. *Pythium* and *Phytophthora* were earlier placed in the family Pythiaceae by Alexopoulos and Mims (1979).

Phylum Hyphochytriomycota These are fresh water or marine chytrid-like fungi whose motile cells are anteriorly uniflagellate. In species studied so far both chitin and cellulose have been shown in the cell wall of the thallus. Sexual reproduction has not been demonstrated so far. Fuller (1990) included them in the Phylum Hyphochytridiomycota, Kingdom Protictista. Barr (1992) suggested the ancestry of the hyphochytrids was within heterokont algae, which have similar flagella structures and a similar DNA sequence analysis which support this view. There is a single order Hyphochytriales containing the families Anisolpidiaccae, Rhizidiomycetaceae, and Hyphochytriaceae which are separated on the basis of mature thallus morphology.

Phylum Labyrinthulomycota The phylum has been the subject of many taxonomic debates. These have now been placed in the kingdom Straminopiles or Chromista. Porter (1990) recognized two families: Labyrintlhulaceae and Thraustochytriaceae. Several characters unite these two families; the most important character is the presence of an ectoplasmic network of branched, anastomosing wall less filaments produced by cells with a specialized organelle known as bothrosome or sagenogen. Other characters include the production of cell walls composed of Golgi-derived scales and in many species heterokont, biflagellate zoospores.

The members belonging to both these families are associated with leaves of vascular plants, algae or organic debris. Most species are saprobes or weak parasites. Some evidence has been found that species of both the families are endobiotic in algae (Raghukumar *et al.*, 1992).

Kingdom Protista

It is a kingdom of eukaryotic microorganisms exclusive of the kingdoms Animalia, Fungi, and Plantae, but including Myxomycota and other plasmodial groups (Plasmodiophoromycota, Dictyosteliomycota and, Acrasiomycota).

Phylum Plasmodiophoromycota (Endoparasitic Slime Moulds) The phylum Plasmodiophoromycota contains a single class Plasmodiophoromycetes, with a single order Plasmodiophorales and a single family Plasmodiophoraceae. There are 10 genera and a total of 29 recognized species.

Plasmodiophorids produce multinucleate unwalled protoplasts typically referred to as plasmodia. These plasmodia are significantly different from Myxomycota as they are incapable of translocational movement, lack the ability to phagocytize food materials and exist wholly within the cells or hyphae of their hosts. Their life cycle involves the production of two different plasmodial phases. Mitotic division of nuclei is called cruciform division of nuclei is called cruciform division.

Plasmodiophora and *Spongospora* are two important genera from the plant pathological stand point. In *Plasmodiophora*, the cysts lie free in the cells of the host; in *Spongospora*, the cysts are in the form of spore balls. *Plasmodiophora brassicae* causes club root disease of crucifers (also known as finger and toe disease of crucifers). *Spongospora subterranea* causes powdery scab of potatoes.

Phylum Dictyosteliomycota (Dictyostelid Cellular Slime Moulds) These are distinguished from most other organisms by the aggregation of their somatic amoebae to form a pseudoplasmodium also designated grex or slug. The component amoebae never fuse but retain their individuality while cooperating as members of a well organized community until the formation of a sorocarp. *Dictyostelium discoideum* is an important member of this group.

Phylum Acrasiomycota (Acrasid Cellular Slime Moulds) Acrasids are characterized by cylindrical amoebae exhibiting phagotrophic nutrition. The amoebae are all of the limax type. Dictyostelid and acrasid slime moulds are significantly different in a number of morphological and life cycle details.

Phylum Myxomycota (True Slime Moulds) In the assimilative phase, a plasmodium is free living and saprobic. These organisms exhibit phagotrophic nutrition and produce the following life cycle stages:

1. Three types of uninucleate cells, one of which is flagellate.
2. A multinucleate somatic phase as a plasmodium that moves and exhibits a reversible shuttle streaming of its protoplasm.
3. A resistant stage consisting of a sclerotium.
4. A reproductive phase that culminates in the production of stationary sporophores containing walled spores.

The phylum Myxomycota contains a single class Myxomycetes that is subdivided into three subclasses containing a total of six orders. These orders are Liceales, Echinosteliales, Trichiales, Physarales, Stemonitales, and Ceratiomyxales.

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Questions

1. Fungi and fungal-like organisms are now placed in 3 kingdoms. What are these three kingdoms? How are Oomycota different from other true fungi?
2. What is the criteria for classifying fungi and fungal-like organisms?
3. Give an outline classification of fungi and fungal-like organisms.
4. What is Centrum? Discuss its importance in the classification of Ascomycota.
5. What are anamorphs and teleomorphs of a fungus?
6. Give an outline classification of Chytridiomycota based on zoospore ultrastructure.
7. What are the salient differences in the classification of fungi as given in the *Dictionary of Fungi* 10th edition by Kirk *et al.* (2008) to that of Alexopoulos, Mims and Blackwell (1996)?

Rots, Damping Offs, Downy Mildews and White Rusts 13

Damping Off Fungal Pathogens, Downy Mildews and White Rusts

Damping off fungi, white rusts, and downy mildews all belong to the order Peronosporales. According to 10th edition of the *Dictionary of the Fungi* as already mentioned earlier in chapter 12, the phylum or division Oomycota of the Kingdom Chromista (Straminopila) has been divided into 8 orders, viz. (1) Albuginales (2) Leptomitales (3) Myzocystiopsidales (4) Olpidiopsidales (5) Peronosporales (6) Pythiales (7) Rhipidiales and (8) Saprolegniales. The genus *Pythium* has been separated from *Phytophthora*, the former has been placed in Pythiales and *Phytophthora* has been placed in Peronosporaceae. However, in this edition of book, the authors have followed the old classification followed in the 9th edition of the *Dictionary of The Fungi*, till some other mycologists/taxonomists accept this revised classification. Many fungi in this group are parasites of higher plants and may cause diseases of economic importance, such as late blight of potato, downy mildew of grapevine, and white rusts of crucifers. Some of them, particularly the members of the Peronosporaceae and Albuginaceae appear to be biotrophs (obligate parasites), and do not survive saprophytically in soil or decaying vegetable matter. Traditionally the family Albuginaceae is believed to contain one genus only, i.e., *Albugo*. However, Thines and Spring (2005) had recognised three genera, i.e., *Albugo*, *Pustula*, and *Wilsoniana*. Determination of white blister species was based on morphological characters of the pathogen and host. *Albugo candida* is recognised as pathogen on *Brassica* and *Raphanus* species and it is a causal agent of white rust disease (Koike *et al*., 2007). *Wilsoniana bliti* was determined on *Amaranthus* spp while *W. portulaceae* was determined on *Portulaca* sp. *Ambrosia artemisiifolia* is a host for *Pustula* spp. while *Crisium arvense* was found to be host for *Pustula spinulosa*.

Peronosporales are coenocytic mycelial fungi which are parasitic in plants or saprobic in water or soil. The cell walls are mainly made up of a glucan-cellulose complex in addition to protein containing hydroxyproline and lipid. The parasites form haustoria.

Asexual reproduction occurs by zoosporangia or conidia which vary greatly in shape. The sporangia possess one or few pores with or without a papilla and produce biflagellate zoospores or a plasma, either in a vesicle or naked, or a germ tube. Sexual reproduction is oogamous.

Key to the Families of the Order Peronosporales

Obligate parasites of plants with unbranched, club-shaped sporangiophores. Each bearing a basipetal chain of deciduous sporangia in dense subepidermal clusters on the host, white or creamish sori; oogonial periplasm persistent and conspicuous haustoria are knob-like *Albuginaceae. (Albugo)-A. candida* grows world wide on crucifers, *A. tragopogonis* is common on Compositae; *A. bliti* on Amaranthaceae and *A. ipomoeae panduratae* on Convolvulaceae—***Albuginaceae***.

Strict parasites of plants, some are biotrophs with branched tree like sporangiophores or conidiophores of determinate growth, emerging singly or in tufts usually via stomata, producing sporangia or conidia singly at the branch tips, periplasm persistent and conspicuous, haustoria varied usually branched—***Peronosporaceae***.

Non-obligate parasites or saprophytes, sporangiophores or conidiophores usually undifferentiated from the mycelium, branched, indeterminate, if determinate then the sporangiophore similar to the vegetative mycelium. Periplasm a thin layer or absent, haustoria absent or branched—***Pythiaceae***.

Key to the Important Genera of Peronosporaceae

Sporangiophore unbranched, apex swollen with short sterigmata bearing papillate sporangia, germinating by zoospores, oospores aplerotic—***Basidiophora***.

Sporangiophore 2–3 times dichotomously branched in the upper part, sporangia usually non-papillate, germinating by zoospores or germ tube, oogonial wall thick and oospore aplerotic—***Sclerospora***.

S. *graminicola* on sorghums, millets, maize, and grasses, *S. sacchari* and *S. spontanae* on sugarcane, *S. maydis*, *S. phillippinensis*, and *S. sorghi* on maize and *Sorghum*

Sporangiophores dichotomously branched at acute angles and taper to gracefully curved pointed tips on which sporangia are borne. Sporangia always germinate by germ tube—***Peronospora***. *P. parasitica* on crucifers, *P. destructor* on leeks and other alliums, *P. tabacina* on tobacco, *P. viciae* on peas, etc. *P. farinosa* on spinach.

Sporangiophores dichotomously branched at acute angles. The tips of the branches are expanded into saucer-shaped structures—***Bremia***. *B. lactucae* on lettuce.

Key to the Important Genera of Pythiaceae

Sporangiophores micronemous (undifferentiated), sporangia ovoid or obyriform or filamentous, protoplasm emerging almost or completely undifferentiated into a spherical vesicle usually at the tip of a short discharge tube. Zoospores formed in a vesicle, antheridia, always paragynous—***Pythium***.

P. ultimum and *P. irregulare* (damping-off and root rots), *P. aphanidermatum* (common in warmer areas as root rot pathogen), *P. debaryanum* is rare.

Sporangiophores macronemous, or sympodially branched, sporangia pyriform or globose. Sporangia do not mature synchronously, zoospores differentiated inside the sporangia. Antheridia are mostly amphigynous (in some paragynous), oogonia smooth or occasionally ornamented, oospore aplerotic, oospore germination direct by a germ tube—***Phytophthora***.

P. infestans (late blight of potato and tomato), *P. palmivora* (Cocao black pod and diseases of rubber and other tropical crops); *P. megasperma, P. cactorum, P. cinnamomi* and *P. nicotianae* and its variety *parasitica* (on a large number of hosts such as tobacco, castor and *Piper betle*).

Sporangiophores determinate (growth definite), not extensively branched; conidia/sporangia mature synchronously, oogonial walls thick, confluent with that of the oospore, oospore germination direct by a germ tube or a sporangiophore terminated by a sporangium—***Sclerophthora***.

Damping Off Disease of Seedlings

Damping off of seedlings is very common all over the world. It occurs in agricultural and forest soils, in tropical and temperate climates, and in almost every greenhouse. Damping off can be of two types, that is, pre-emergence damping off in which the seed and radicle rot before the seedlings emerge out of the soil and the post-emergence damping off in which the newly emerged seedlings are killed at ground level after they emerge from the soil, causing them to collapse or topple over. This is a common symptom of post-emergence damping off. This disease is most noticeable in nursery beds, greenhouse flats, and row crops because symptoms develop suddenly, killing large numbers of seedlings in local areas. Among the pathogens in this class are the species of *Pythium*, *Phytophthora*, *Corticium* and *Fusarium*; the former two will form the subject of our present discussion. Most species of *Pythium* mainly affect the juvenile or succulent tissues. This restricts their parasitism to seedlings, the feeder roots or root tips of older plants, and to watery fruits or stem tissues. They also cause fruit rots of crops, such as beans, squash, and watermelons. At a later stage, when the cells of stems and main roots have developed secondary thickenings, infection is restricted to feeder roots. This causes seedlings to become stunted and chlorotic. Peach decline, which kills thousands of trees each year, has been shown to be a feeder root problem caused by a combination of factors, including *Pythium* spp. Pectolytic and cellulolytic enzymes produced by *Pythium* and *Phytophthora* spp. play an important role in tissue disintegration and maceration. Mathews (1931) did much work to clarify the taxonomic position of the known *Pythium* spp. during his time. Middleton (1943) compiled an extensive monograph of the genus *Pythium.* Waterhouse (1967, 1968) compiled the original descriptions and illustration of all the species and published a key to the genus in 1967. Literature up to 1961 has been compiled by Rangaswamy (1961). Vander Plaats-Niterink (1981) has given a key to the species of *Pythium.* Dick (1990) gave a key to the world species of *Pythium* while Misra and Hall (1996) gave characteristics, distribution and host range of forty one species of the genus *Pythium* in India.

Tucker (1931) published a monograph of the genus *Phytophthora.* Waterhouse (1956, 1963) compiled the original descriptions and illustrations of all described species and published a key to the genus in 1963. There is an interesting article by Hickman (1958) on "*Phytophthora* as a plant destroyer". There are two recent books on *Phytophthora* diseases, i.e., *Phytophthora Diseases World Wide* by Erwin and Ribeiro (1996) and *Phytophthora Diseases in India* by Mehrotra and Aggarwal (2001). In contrast to the species of *Phytophthora, Pythium* spp. have a high degree of competitive saprophytic ability. Biodiversity of Phytophthora species in India has been recently discussed by Mehrotra (2011).

Damping Off of Vegetables Seedlings

Pythium debaryanum Hesse and several other species of *Pythium* such as *P. aphanidermatum* (Eds.) Fitz, *Pythium ultimum* Trow and *P.arrhenomanes* Drechsler are the pathogens.

Symptoms

As has already been stated, damping off of vegetable seedlings occurs in two stages, that is, the pre-emergence and the post-emergence phase. In the pre-emergence phase the young seedlings are killed before they reach the soil surface. The young radicle and plumule are killed and there is complete rotting of the seedling. The post-emergence phase is characterized by infection of the young, juvenile tissues of the collar at the ground level. The infected tissues become soft and water soaked. The seedlings topple over or collapse. Such symptoms are very common in seed beds in nurseries.

Causal Organisms

The mycelium of *P. debaryanum* is branched, hyaline, and coenocytic hyphae, the main hyphae being up to 5 µm in diameter. The ends of the blunt terminal hyphae penetrate the cell walls of the hypocotyl and ramify within and between the tissues of the cortical parenchyma; sporangia and oospores are formed in the parenchymatous tissues of the host. Sporangia are terminal or intercalary and are 15–26 µm in size. They are spherical, oval, or barrel shaped depending upon their position. Just before germination, a prominent beak is formed on the sporangium, followed by a thin-walled vesicle into which the sporangial contents are emptied. Zoospores are differentiated in the vesicle. The zoospores are reniform, with two lateral flagella. They measure upto 8 µm in diameter.

Sexual reproduction is oogamous, oogonia spherical, and terminal on the short side branches with a smooth and thin wall, 15–28 µm antheridia monoclinous or diclinous, one to six per oogonium; oospores smooth, aplerotic 12–20 µm. Among other common species of *Pythium* are *P. arrhenomanes, P. aphanidermatum*, and *P. ultimum.* These are characterized below:

P. arrhenomanes—Sporangia are inflated, filamentous, lobulate, zoospores about 12 µm in diameter. Oogonia are subspherical to spherical, usually acrogenous, smooth, thin-walled, 17–56 µm antheridia diclinous, 15–25 per oogonium, oospores plerotic, 15–54 µm. This species is responsible for seedling blight, pre-emergence rotting and root rot of maize, wheat, sugarcane and other Gramineae.

P. aphanidermatum—Sporangia inflated, lobulate branched or unbranched, zoospores 7–12 µm oogonia spherical, terminal, 22–27 µm antheridia monoclinous or diclinous, intercalary or terminal, one or two per oogonium, dome shaped, 9–11 by 10–14 µm oospores aplerotic, single, 17–19 µm. Associated with soft rot of storage organs and damping off of many host species. It is responsible for cottony leak of cucurbit fruits in storage, cottony blight of turf grasses, root and stalk rot of maize, stem or foot rot of papaya, rhizome rot of ginger, rhizome rot, and root rot of turmeric. Other hosts are sugarbeet, tobacco, sugarcane, pineapple, bean and cotton.

P. ultimum—Sporangia mostly terminal and spherical, with a diameter varying from 28 µm and more to 12 µm and less, but occasionally intercalary and barrel-shaped, with the dimensions varying from 27.8 µm × 22.9 µm–17 µm × 14 µm, antheridia monoclinous, arising just below the oogonium, usually one per oogonium; oospores aplerotic, single, spherical, 14–18 µm. It is a common causal organism of damping off, root rot and soft rot of many species.

Disease Cycle and Predisposing Factors

Pythium spp. are natural inhabitants of the soil, where they are probably low grade parasites on fibrous roots. As a plant parasite, the pathogen can infect succulent organs of plants but tissues that have matured and developed secondary thickening become resistant to its attack. In soil, *Pythium* spp. are susceptible to antagonism of a large number of fungi (*Trichoderma*) and bacteria (*Bacillus* and *Pseudomonas)*

and some amoebae (Singh and Singh, 1984). This weakness of the pathogen is compensated by its ability to form oospores. The oospores serve as the overwintering or oversummering organs. They are not vigorous competitors and their saprophytic activities are greatly restricted. Soil moisture is important in saprophytic growth of *Pythium* spp. and survival by resistant structures is more important than saprophytic persistence.

Factors influencing infection include inoculum density, soil moisture, soil temperature, pH, cation composition, light intensity and the presence of members of other microorganisms. Soil temperature and moisture are the most important factors. Generally, high soil moisture is necessary for disease development. The disease cycle of a typical *Pythium* species causing damping off and seed decay is given in Figure 13.1

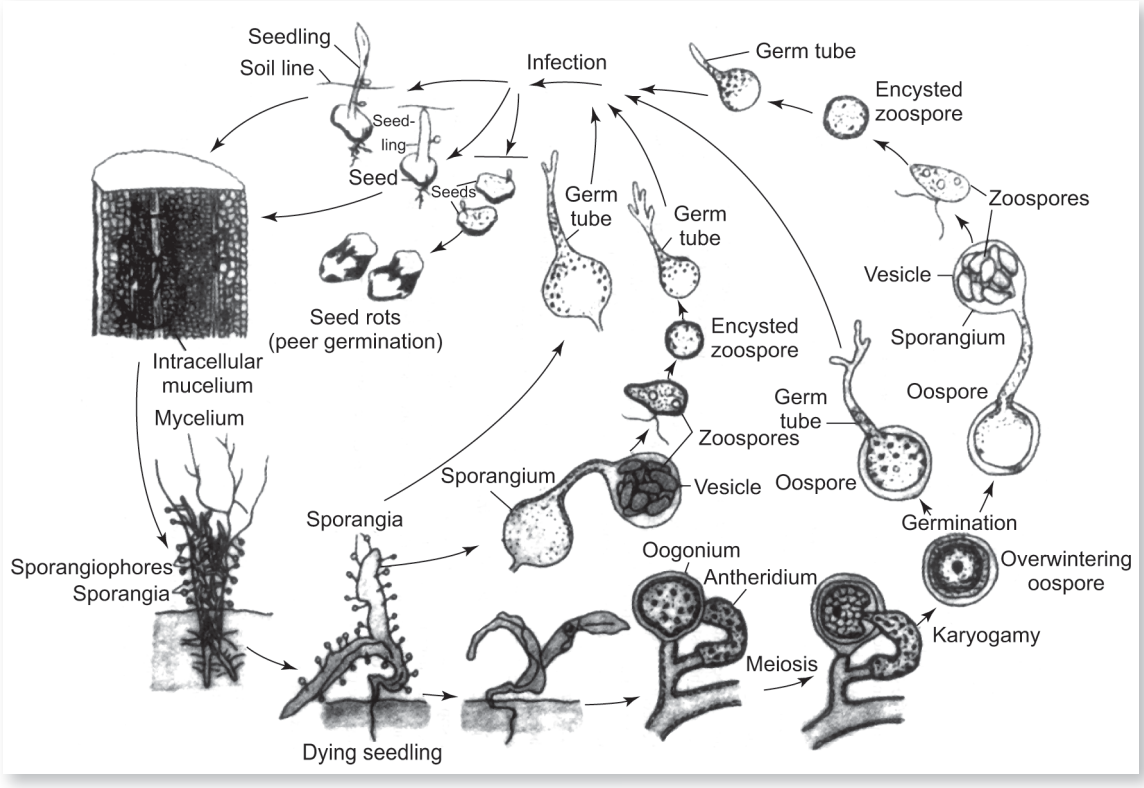


Figure 13.1 Disease cycle of damping- off and seed decay caused by P*ythium* sp. (From G.N. Agrios, P*lant Pathology, A*cademic Press, New York and London, 1969.)

Temperature has as much effect on disease development as has soil moisture. Generally, *P.irregulare, P.spinosum* and *P.ultimum* are more damaging at lower temperatures, while *P.myriotylum, P. aphanidermatum, P.arrhenomanes* and related species are damaging at higher temperatures. But the disease is most severe in poor soils like ill-aerated and ill-drained soil. *Pythium* spp. are most destructive when soil temperatures are between 24–30°C.

Disease Management

Most *Pythium* spp. produce oospores and chlamydospores and persist for many years in soil. Direct control of *Pythium* on field scale is difficult and expensive. On a small scale, *Pythium* spp. can be eliminated from the soil for greenhouse use by heating. The common method is by steam treatment or pasteurization. The latter method is preferred as it does not change the microflora drastically.

Very often the most effective measure against damping off of fungi involves the use of seed protectants to keep away the pathogens in the pre-emergence phase and to adopt sanitary precautions in the nursery to check post-emergence damping off. The most commonly seed protectant fungicides now are Thiram, Captan, Zineb and some systemic fungicides such as Metalaxyl. Drenching the soil with suitable fungicides, such as Cheshunt compound, 1% Bordeaux mixture, 0.1% Thiram, 0.3% Blitox – 50 or 0.2% Esso Fungicide 406 have been found useful in eliminating soil-borne infection.

Dexon and other soil fungicides, applied at low rates, have been successfully used to retard the rate of reinfestation of fumigated soils by *Pythium.* The use of healthy seeds in crops (ginger and turmeric) which are vegetatively propagated by rhizomes, etc., form an important control measure in such diseases.

Crop rotation is another method of reducing the populations of soil pathogens, but because the more common and destructive *Pythium* spp. have a wide host range, this practice is not of much help.

Seed treatments with spores of *Trichoderma harzianum* and *Penicillium oxalicum* and cells of *Burkholderia* (*Pseudomonas*) *cepacia, P. fluorescens* have been found very effective biological control agents. The combination of seed and soil treatment is best for management of damping off. Some cultural practices recommended are: thin sowing of seeds, light sandy soil for nurseries, use of well decomposed manure, light but frequent irrigation, and sterilization of soil by burning a 30 cm thick stack of farm trash on the nursery beds (Singh, 1998). Some new methods such as osmopriming of seeds (seed priming with inorganic salts) before planting have been found promising. Some of the other fungicides used these days are Thiram (3g per kg of seed), Captan (3g per kg of seed) and some systemic fungicides such as Metalaxyl (2g per kg of seed).

According to Manoranjitham *et al*. (2001), application of talc based formulations of *T. viride* and *P. fluorescens* reduces damping off of tomato caused by *Pythium aphanidermatum*. *Trichoderma* spp. colonize the wounds and broken roots. As a result of which it produces glucanase and cellulase enzymes which in turn inhibit germination of encysted zoospores of *Pythium*. According to Hultberg *et al*. (2000), strains of *Pseudomonas fluorescens* produce an antibiotic 2, 4-diacetylphloroglucinol (2, 4-Dapg) which inhibits the mycelial growth of *P. ultimum* and protects tomato seedlings against damping off. Other microbial biocontrol agents against damping off are binucleate *Rhizoctonia* strains, *Pseudomonas putida*, and *Bacillus amyloliquefaciens* (Harris and Adlcins, 1999; Lewis and Larkin, 1998). According to Bardin *et al.* (2003) seed treatment with the microbial strains of *Bacillus cereus*, *Pantoea agglomerans* and *Erwinia rhapontici* also control damping off diseases of safflower, pea and canola respectively.

According to Chung *et al.* (2003), amendment of the growth medium for the green house grown watermelon with *Brassica* seed pomace (cake) along with *Pseudomonas boreopolis* gives good control of damping off caused by *Pythium* sp. The mechanism of control is attributed to the hydrolysis products of glucosinolates into allyl and butenyl isothiocyanates which are toxic to *Pythium*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, and *Phytophthora capsici*. Cucumber seed treated with *Pseudomonas putida* bacteria or with the mycoparasite *Verticillium lecanii* results in the systemic production of phytoalexins and other host defense reactions that protect cucumber seedlings from attack of *Pythium*. Similarly, treatment of spruce seedlings with methyl jasmonate induced accumulation of free salicylic acid,

chitinase that protected the seedlings from infection by *Pythium*. Soil treatment with low dose of formalin (one part in 50 parts of water) at a depth of 10cm is quite effective. The low dose of formalin boosted up the population of *Trichoderma* in soil and which in turn shows antagonistic behavior against *Pythium* sp. Use of formalin dust is also effective. The dust consists of 85 parts of charcoal ash and 15 parts of formalin. Raj *et al.* (1997) reported soil solarization by polyethylene sheet mulching for control of damping off of some vegetables. As a result of solarization, the temperature of mulched soil is 49.7°C which is quite effective in killing *Pythium* and *Fusarium.*

Integrated Disease Control

Integration of cultural practices, chemical and biological control methods can be useful for management of damping off. Cultural practices include avoidance of nursery sowing in the same bed repeatedly year after year and frequent heavy irrigation. Soil solarization with transparent polyethylene mulch has been found effective for control of the disease. Application of Captan as seed dresser and for soil drenching 15 day after planting has been recommended for healthy plant stand in chili, cabbage and onion crops (Champawat and Sharma, 2003). Seed coating with conidial suspension of several biocontrol agents like *Trichoderma koningii*, *T. harzianum*, and *T. viride* have been found more effective than soil application in protecting the tomato seedlings from damping off (Hazarika *et al*., 2000). An integrated management module comprising of change of nursery site every year along with pre-sowing, soil sterilization with Formalin (5%), seed treatment with Captan or Thiram and drenching of beds after emergence with Captan (0.3%) or mixture of Mancozeb (0.25%) and Carbendazim (0.1%) at 8–10 days interval coupled with avoidance of heavy irrigation have been suggested.

Fruit Rot of Cucurbits

This is very common disease of cucurbits in India. The disease occurs on bottlegourd (*Lagenaria vulgaris*), sponge gourd (*Luffa cylindrica* and *L. acutangula*), snakegourd (*Trichosanthes anguina*), cucumber (*Cucumis sativus*), bitter gourd (*Memordica charantia*), and other cucurbitaceous fruits. Cottony leak disease is very widespread in cucurbit fruits. Sometimes 50% or more of muskmelons or watermelons undergo rotting after rains due to this disease (Singh and Chouhan, 1977).

Symptoms

A watery soft rot develops, especially in the fruits touching the soil surface. The disease is common in the field during and after the rains. It also spreads among fruits during storage and transit. As a result of infection the diseased fruits show formation of a luxuriant wooly mycelial growth. The decaying fruits also emit a bad smell.

Causal Organism

The causal organisms are mainly *Pythium aphanidermatum* and *P.butleri* but other fungal pathogens, such as *Fusarium, Rhizoctonia* and *Phytophthora* spp., may also be involved. The pathogen *P. aphanidermatum* has been described earlier. *P. butleri* and *P. aphanidermatum* are responsible for cottony leak disease (or fruit rot) of cucurbits in north Indian conditions. *P. butleri* can be distinguished from

P. aphanidermatum in more copious robust mycelium, larger and more swollen, and much branched sporangia, larger zoospores, oogonia and oospores, and larger (usually diclinous) antheridia. The susceptibility of six cucurbits, chili, tobacco and papaya for *P. butleri* had been reported. *P. butleri,* a papaya isolate causes fruit rot of many cucurbits on inoculation. Damping off of "toria" seedlings (*Brassica campestris* var. *toria*) also occurs. *P. butleri* grows best between 30°C and 35°C. This observation is in conformity with the study by Desai and Pathak (1969) regarding the effect of temperature on the growth of *P. butleri in vitro.* Desai and Pathak (1969) also reported that 30°C was the optimum for fruit rot of cucurbits due to *P. butleri.*

Disease Cycle and Predisposing Factors

The pathogen *P. aphanidermatum* and *P. butleri* are soil inhabiting ones and survive as a saprophyte on dead organic matter, causing the disease when a suitable host is available. A slight injury or wound on the surface of fruits or vegetables facilitates infection. High soil moisture and temperature favour the development of the disease.

Disease Management

Efforts should be made to keep the fruits away from the soil surface. Soil disinfection, as recommended for damping off, is not practicable due to the prohibitive costs involved.

According to Chen *et al.* (1999) some strains of *Pseudomonas*, i.e., *P. corrugata* and *P. aureofaciens* when applied to roots induce systemic resistance against the pathogen. The mode of action is perhaps through accumulation of endogenous salicylic acid and stimulation of peroxidase and polyphenol oxidase activity. Soil solarization during summer has been found quite effective against *P. aphanidermatum* causing root rot as the pathogen is soil borne.

Stem or Foot Rot of Papaya (*Carica papaya*)

It is a severe disease found in many parts of India, Sri Lanka, Cuba, Hawaii, and South Africa. The disease often appears during the rainy season. The severity of the disease depends directly on the temperature and rainfall.

Symptoms

It is characterized by the appearance of spongy, water-soaked patches, or areas on the bark, at the collar region or immediately at the soil line. The patches enlarge rapidly and girdle the stem, causing the tissues to rot. The tissues become black and the entire tree topples down under a slight wind pressure and dies. If the bark is opened, the internal tissues appear dry and give a honeycomb appearance. The rotting spreads to the roots and they are destroyed.

The typical stem rot symptoms are common in 2–3 year-old-plants but younger plants have also been seen dying due to early infection. A damping-off of papaya seedlings in the nurseries is also common. Seedlings grown in such nurseries carry the disease and when transplanted develop symptoms.

Causal Organism

The morphology of organism *P. aphanidermatum* has been described earlier.

Disease Cycle and Predisposing Factors

The pathogen is a soil inhabitant and produces abundant oospores in the soil. It is capable of growing and surviving on the residue left in the soil. According to Trujillo and Hine (1965), the optimum temperature for disease development is 36°C. Water-logging is very conductive to disease development.

Disease Management

Stem rot of papaya can be avoided if plants are grown in well-drained soil. Affected plants should be carefully pulled out and destroyed (by burning). Replanting should not be done in the same pit where disease has once appeared.

At the time of cultivation, care should be taken so that no injury is caused to the basal portion of the stem. The disease can be kept in check by drenching the soil with Bordeaux mixture with a sticker (6:6:50) or 2% Captan considerably reduces the incidence of stem rot. According to Rawal and Muniyappa (1988) soil drenching with Calixin (0.1%), Topsin-M (0.1%), and Daconil (0.2%) at bi-monthly intervals gave very good stand of the crop. The damping off phase of the disease has been reported to be effectively controlled by seed treatments with Thiram, or Difolatan (0.25%) along with soil treatments as described earlier with suitable fungicides. The affected areas on the plant should be removed and fungicidal paste should be applied.

Rhizome Rot of Ginger (*Zingiber officinale*)

Rhizome rot of soft rot of ginger (*Zingiber officinale*) occurs in India wherever this crop is grown. The disease is quite severe in south India. *Pellicularia* spp. and *Fusarium* spp. are also known to cause rhizome rot of ginger. The disease is not confined only to the crop in the field but also causes 80–90% loss of corms in storage. In some low-lying infested fields, there may be total loss but generally losses in the field vary from 8–15%.

Symptoms

The first visible symptoms are seen at the basal portion of the plant which becomes watery and soft. The leaves become slightly pale in color, their tips turn yellow and the yellowing spreads down the leaves. The rhizomes start rotting and are converted into a pulpy mass giving out a putrefying smell.

If the plants are raised from infected rhizome, the young shoots show damping-off and if infection occurs after emergence of shoots, the pseudo-stem withers and dies, this result in poor crop.

Causal Organisms

Pythium myriotylum, *P. aphanidermatum*, *Pythium monospermum*, and *Pythium gracile* have been isolated from the diseased plants. The pathogenicity of *P.myriotylum* and *P. aphanidermatum* has been proved so far.

At least 8 species of *Pythium* are known to attack ginger in different parts of world. According to Rajan and Agnihotri (1989) in India, *P.aphanidermatum, P.butleri*, *P.myriotylum*, *P.graminicola*, *P. deliense*, *P. pleroticum*, and *P.vexans* (syn *P. complectans*) have been reported on ginger. *Pythium ultimum* has been reported to be the main threat in almost all ginger growing areas of Himachal Pradesh. The losses are upto 40%.

Pythium myriotylum hyphae straight or sinous, not much branched except at the terminations, upto 8.5 µm in diameter, forming numerous clavate or knob-like appressoria, often in branching clusters. Sporangia are terminal or intercalary, consisting of long simple or branched portions of unswollen hypha or often with lateral, swollen, lobulate or digitate branches up to 17 µm diameter. Oogonia abundant, terminal or intercalary, 15–44 µm, smooth wall. Antheridia up to 10, usually 3–6 per oogonium, usually diclinous, stalks slender, often once or twice branched, investing with a single reserve globule, wall up to 2 µm thick, smooth, pale golden in color.

P.aphanidermatum—this has been described earlier.

Disease Cycle and Predisposing Factors

It is a soil- and seed- borne disease and it is transported over long distances on ginger rhizomes. Incidence of this disease is very high in virgin soil containing abundant decomposing matter.

Disease Management

The first prerequisite for checking this disease is to choose healthy seed pieces. Soft rot of ginger has proved amenable to control in India by the preplanting treatment of rhizomes and the soil with copper fungicides. Evaluation of ginger germplasm for resistance to soft rot caused by *P. myriotylum* has been done by Bhai *et al.* (2013). Wild varieties like Indonesia wild, *Zinziber* species, Kanya Kumari and Karakkal showed high degree of resistance.

Dohroo and Sharma (1983) had recommended a half hour dip of seed rhizomes in 0.25%. Antracol or 0.3 %t Fycop or 0.3% Blitox-50 is effective in controlling rhizome rot. Since the pathogen is present deep into the tissue of the host (corm or rhizome), surface disinfection of seed is not effective. Spraying of the soil with Bordeaux mixture at the rate of 7 liters per square foot once before sowing and second after germination gives partial control of the disease. Ramachandran *et al.* (1989) found best control of the disease by seed and soil treatment with Metalaxyl (Ridomil 5g or Apron 35 WS).

Regarding the biological control agent, *Trichoderma*—a known biocontrol agent provides significant control of rhizome rot of ginger. Rajan *et al.* (2002) have also reported control of rhizome rot of ginger by *T. harzianum*. Dual inoculation of *T. harzianum* along with fluorescent pseudomonads is also reported to improve plant growth and yield. According to Das *et al.* (2001), cultivars Moran and Riodegenerio are found to be resistant to rhizome rot.

Late Blight of Potato (*Solanum tuberosum*)

The greatest famine in Ireland and elsewhere in Europe during the 1840s can be attributed mainly to the failure of the potato crop. Potato is a native of North Andes (South America) and the late blight of potato was initially an endemic disease. However, due to good communication links, it spread to the USA and Europe in 1830–40. The pathogen was well established in Ireland, England, and Europe by 1842.

By 1845, the epiphytotics of late blight became widespread throughout Europe where it devastated the potato fields and brought famine.

In India, the disease was first introduced into the Nilgiri hills between 1870 and 1880. Very soon it spread to the Darjeeling district in the Himalayas after the introduction of the English potatoes. Losses due to the ravages of this disease have been recorded in different parts of our country by several workers (Bhattacharya, 1990). These have been estimated at 20–25% in Punjab, 40–50% in Haryana, 15–50% in U.P., 5–10% in Bihar and West Bengal. The overall losses range from 20–25% amounting to 4 million tonnes of potatoes worth ₹4 crores. The disease is endemic and more severe in eastern than northern plains. According to Bhattacharya (1990) with the release and large scale cultivation of Kufri Jyoti in early seventies losses came down considerably.

Symptoms

The first symptoms of late blight in the foliage of potato and tomato are hydrotic areas with indefinite margins at the tips or on the margins of the leaflets (Fig. 13.2 a, c, d). These lesions then become necrotic and turn brown to almost black. Very often a chloranemic border develops around the necrotic areas. If moist weather prevails, the entire leaf may be killed within a couple of days. If dry weather intervenes, following the appearance of lesions, the infection advances slowly and the affected areas curl and shrivel. However, under moist conditions, they remain limp and soon decay, producing a characteristic offensive odor. If the weather remains dry after the development of spots on the leaflets, the diseased areas seem hard and easily break away from the leaf blade. At this stage symptoms are very similar to those due to *Alternaria solani,* which is responsible for early blight. The wet rot in warm humid weather advances rapidly and affects all the leaves and the stem but if does not go as far down as to the tubers. Tubers are infected separately.

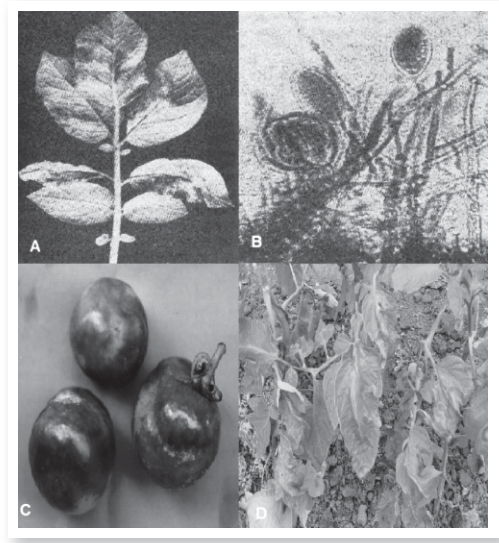


Figure 13.2 Late blight symptoms due to *Phytophthora infestans*: **(a)** on potato leaves, **(b)** Sporangiosphores and sporangia on leaf **(c)** Late blight symptoms due to *Phytophthora infestans* on tomato fruits **(d)** symptoms on tomato leaves

Slightly sunken hydrotic areas develop in the superficial tissues of the potato tubers. These areas become necrotic, and if conditions remain favourable, the infection spreads inwards so that the entire tuber turns brown and decays before harvest. In drier soils, the progress of the disease is slower and a rot may result. During wet and humid conditions the sporangiophores and sporangia can be seen as a downy growth, particularly on the abaxial surface of the leaflets. The fungus penetrates the host mostly through stomata and the morpho-anatomical characters of the host are reported to be related to resistance (Kaur *et al.*, 1990)

Causal Organism and Disease Cycle

The causal organism of late blight of potato is *Phytophthora infestans* (Mont.) deBary. The causal organism of late blight of potato is carried through tubers used as seed, volunteer plants that develop

from diseased tubers left in the field of sprouts produced from infected potatoes in cell piles or dumps. Such infected shoots form foci within the crop from which the disease spreads. Sporangiophores and sporangia formed on the diseased shoots are blown to healthy areas, where they germinate either by the formation of germ tubes or zoospores. Zoospore production is favoured by temperatures of 9 and 15°C. After swimming for a time the zoospores encyst and then form germ tubes which usually penetrate the epidermal walls of the potato leaf and occasionally enter the stomata. An appressorium is formed at the tip of the germ tube and penetration of the cell wall is probably both by mechanical as well as enzymic action. Penetration occurs within two to two and half hours.

Optimum temperature for the growth of the fungus is 16–18°C. Sporulation occurs at temperatures of 9–26°C but the optimum temperature for their production is 21°C. The optimum temperature for germination of sporangia by zoospores is 12°C and by germ tube, 21°C. The minimum temperature for sporangial germination is as low as 2–3°C, while the maximum temperature may go up to 24–30°C.

It has been noted that a relative humidity of 100% is necessary for abundant production of sporangia, none being formed at relative humidities of less than 90%.

P.infestans is a heterothallic fungus. The oospores of *P.infestans* appear to be very rare in the eastern hemisphere, the USA and Canada, and therefore, may not play a significant role in the survival of the fungus. However, in Mexico, oospores have been discovered by Gallegly and Galindo (1958). It is quite possible that in this part oospores play a significant role in the survival and recurrence of the disease from year to year. The disease cycle of late blight pathogen is given in figure 13.3.

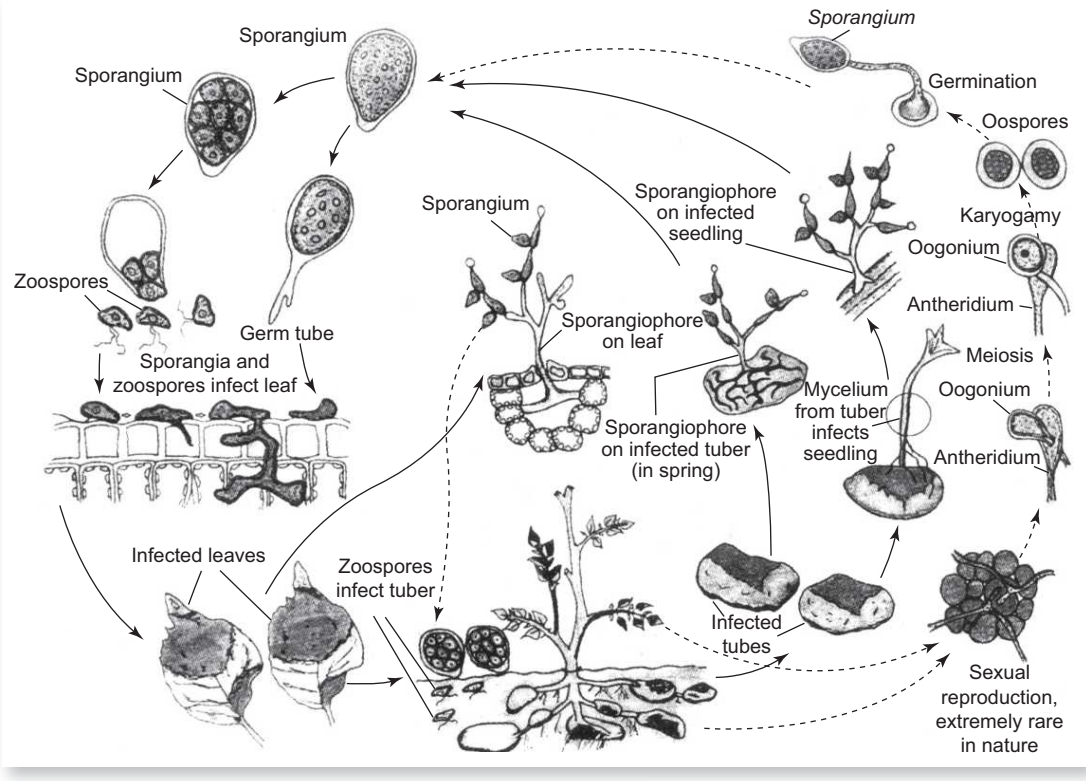


Figure 13.3 Disease cycle of late blight of potato caused by *Phytophthora infestans* (From G.N. Agrios, *Plant Pathology*, Academic Press, New York and London, 1969.)

It is not possible for the fungus to survive in the form of mycelium. The living mycelium in the tuber is killed by exposure to 40°C for four hours or to 30°C for 65 hours. The viability of the sporangia in the soil is also affected by moisture and temperature. The optimum soil moisture is between 15–20% saturation. The viability and infectivity of sporangia is present for 9–10 weeks at low temperature and optimum moisture levels. Kaung (1965) also studied the viability of zoospores in soil and found them to be viable only for two to three weeks.

In India, the main potato growing season is followed by hot summer months in the plains, and therefore, the fungus is eliminated from the soil. Potato tubers which are stored at ordinary temperatures also become free from disease. In Indian plains, the late blight is rare because the two cardinal factors: high humidity and low temperature must be simultaneously present before infection can develop. Such conditions are encountered only in the northern hills of India, and therefore, late blight epidemics are confined to the hills. However, late blight epidemics have now been regularly reported in the northern plains from places like Kurukshetra, Meerut, Patna and so far as Kanpur in Uttar Pradesh. It is believed that the primary source of infection for late blight in these areas is the seed potatoes stored in cold storages.

Oospores of this pathogen have been reported in the hilly areas of our country (Singh *et al*., 1994). It is now well established that now both A_1 and A_2 mating types occur in India and chances of their mating and consequently production of thick walled oospores are very likely especially in Indo–Gangetic plains. However, what remains to be established is the role of oospores in the biology of *P.infestans* and disease epidemiology. Series of experiments conducted at CPRI, Kufri (Shimla) have revealed that the oospores of *P. infestans* are also able to germinate and act as primary source of disease (Singh and Bhattacharya, 1999).

The environment plays a very significant part in the occurrence of late blight epidemic. Van Everdingen (1933) was one of the first to define the weather conditions necessary for an outbreak of blight in a potato crop. His work was developed by Beaumont (1947) who examined critically the meteorological data for the periods preceding blight outbreaks in south-western England. He found that if for two days the temperature remained above 10°C and relative humidity was 75% or above the blight could be expected approximately ten days afterwards. These periods are called "Beaumont periods" and are the basis of blight forecasting service in England and Wales.

Physiological Specialization and Origin of New Races

Schick (1932) and Muller (1933) discovered physiological specialization and occurrence of races of *Phytophthora infestans* on different cultivars of potato from crosses of *S.demissum* and *S.tuberosum.* In the hybridization work, resistance genes from *S.demissum* were transferred to the commercial cultivar. On the basis of this, Black *et al.* (1953) determined four major genes for resistance (R_1, R_2, R_3 and R_4). They suggested an international system for classifying races and genes for resistance. According to this system, a particular race receives the resistant genes of the plant it is able to infect. The four R genes produce 16 possible combinations, 15 of the total 16 races were identified experimentally. In India now several races are found. Later, six major genes for blight resistance in potato were identified (Black, 1960). On this basis probably 64 races of *P.infestans* can be identified.

Another type of resistance, the so-called minor gene resistance or field resistance was found in numerous cultivars of *Solanum tuberosum.* This type of resistance is relatively weaker and is not race specific. It is a recessive character and probably depends on the interaction of several genes.

Resistance of the tops is not necessarily correlated with that of the tubers. Several workers from different parts of India have reported a number of physiologic forms of this fungus. Up to 1965, races O and A_1 were predominant in north-western hills, and O, and 4 in the eastern hills. Gradually more complex races appeared since 1966 in the eastern hills and by 1971 in the Shimla hills. By the end of 1980, 8 to 9 complex races became quite prevalent particularly in the hills (Bhattacharya, 1990; Singh and Bhattacharya, 1998). A_2 type was reported from Shimla hills in 1990.

It is believed that the race flora has reached more than 100. The races from different parts of India contain 2–10 genes in different combinations. It is believed that the pathogen produces new and complex races through mutation and somatic re-combination particularly on resistant genotypes carrying major genes. The rate of development of new races is higher in areas like eastern and western hills having larger blight favourable periods as compared to in southern hills and northern hills. The identification of some high spectrum races of *P. infestans* in Khasi hills has been done by Khanna *et al.* (1982). Sokhi *et al.* (1993) reported 11 pathogenic races of *P. infestans* on potato and 3 pathotypes on tomato in plain of Punjab.

The origin of physiological races is not properly understood. Sexual reproduction is a rare phenomenon in *P.infestans.* Mutation could possibly be responsible for new races. Another possibility is that the mycelium of *P.infestans* is heterokaryotic, carrying nuclei of more that one race. It has been suggested that there is some evidence that the pathogenicity of a strain of the parasite can be increased by continued passage through resistant hosts. The discovery of both A_1 and A_2 type in our country has given a new dimension to the disease epidemiology in our country.

Epidemiology and Forecasting

Infected tubers are the main source of initial inoculum. In hills, the temperatures are favourable for survival of the pathogen in tubers in soil, and in the country stores up to the next planting season (Bhattacharya, 1990). In the plains, the pathogen does not survive in infected potato stalk buried in the soils for more than 5 days. However, it was able to survive for 150 days at Shimla and for 40 days Ootacamund in the potato stalks left in the field. According to Singh and Bhattacharya (1990), the pathogen can survive in tomato seeds. In our country, 10–20 of potato true seeds collected from fruits or berries have been found to carry the pathogen. However, the role of potato stalks and tree potato seeds as the effective primary source of infection in the hills is still to be confirmed (Singh and Bhattacharya, 1990).

In the plains, the initial source of the disease is in the tubers placed in cold stores or the seed tubers have been found to carry the late blight infection.

Moisture and temperature are the main factors limiting the development of blight and these conditions determine the time of the appearance of the diseases. The forecasting is based on weather data collected in meteorological observations. Main points under consideration are: rain fall or humidity, temperature, cloudiness, and dew operation. Relationship between *P.infestans* and weather is well understood and has been utilized for developing disease forecasting models across the globe including India. Ambient temperature, RH, light, fogginess, rainfall, dew, wind velocity etc have a strong relationship with blight pathogen and the disease.

Chaudhuri and Pal (1950) utilized rainfall data together with the dates of appearance of late blight in Darjeeling hills for 12 years using moving concept of Cook's and Hare's. Bhattacharya and co-workers in 1982 utilized daily weather data including temperature, rainfall and RH and the data of actual appearance of late blight for Shimla, Shillong and Ootacamund. They established that if a 7 day moving

precipitation of at least 30 mm for Shimla, 28.9 mm for Ooty and 38.5 mm for Shillong hills with a mean temperature of 23.9°C or less continue for 7 consecutive days, late blight would appear within 3 weeks and further if hourly temperature between 10–20°C was associated with RH ≥ 80% for continuous 18 hours for at least 2 consecutive days, late blight would appear with in a day. This model has been put to a successful use for predicting since 1983 and still working well in Shimla hills (Arora, 2016). Singh and coworkers in 2008 used moving graph concept and developed a computerized forecasting model "JHULSACAST" for western U.P. for both raining and non-raining years.

Disease Management

Certified and disease-free tubers should be used for cultivation. Seed tubers should be obtained from uninfected areas.

Harvesting of a diseased crop should be delayed until the plants are fully mature. The danger of infection of tubers by sporangia falling on to them from foliage at lifting time can be minimized by ensuring that all foliage is destroyed beforehand. This is achieved by spraying the foliage two to three weeks before harvesting with sprays, such as sulphuric acid, copper sulphate, tar acid compounds or sodium chlorate. High ridging at the time of earthing helps to reduce chances of infection.

The spread of the disease is favoured by cloudy, warm weather with occasional rains. Since the time of the appearance of the disease in the hills and plains is more or less known, spraying should be done with suitable fungicides. In advanced countries there is a regular disease forecasting service where accurate forecasts of the disease can be made on the basis of the temperature and humidity. In the plains, Paharia (1961) tried different fungicides for the control of late blight in Patna, and Dithane Z-78 gave good results. In the Nilgiri hills, Bordeaux mixture provided a good protection to the crop and helped to increase the yields. In the eastern Himalayas, experiments were conducted in Darjeeling with a few fungicides and Dithane Z-78 was found to give good protection to the crop. In the western Himalayas, at Kufri in the Shimla hills, Vasudeva and Azad (1962) tried different fungicides and found Burgundy mixture to be effective. Dutt (1962) tried 16 different fungicides in these hills during 1958–60 and found Bordeaux mixture to be the best. Dutt (1970) demonstrated that Bordeaux mixture was found to give better results than Burgundy mixture or any other proprietary product or fungicide.

According to Nagaich (1977), in the northern hills, a protective spray of Dithane M-45 at 2 kg/ha is essential in the last week of June before the disease appears. This should be followed by a spray of Bordeaux mixture 5:5:50 at intervals of 10 days. In the eastern hills, the crops should be sprayed with Bordeaux mixture. Other fungicides, such as Brestan (1 kg/ha) can also be sprayed. In the Nilgiri hills, spraying may be done with Brestan or Difolatan. For spraying in the hilly regions some sticker like Triton HE or Sandont (0.1%) should be added to the fungicides. In the plains any of the fungicides, for example, Dithane M-45(2 kg/ha), Brestan (1 kg/ha), and Difolatan 80 WP (2.5 kg/ha) can be sprayed.

The discovery of Metalaxyl, a systemic fungicide gave a new dimension to late blight control strategies. According to Bhattacharya *et al.* (1983) and Khanna and Sharma (1981) Metalaxyl (1 kg/ha) and Propineb (1.5 kg/ha) were found superior over other fungicides in inhibiting the infection completely and increasing the yield by about 3 time over unsprayed control.

Oxadixyl, a new systemic fungicide has also been found effective against the disease. At Kufri, Shillong, and Darjeeling the formulation of Oxadixyl (Oxadixyl + Mancozeb; Oxadixyl +Copper Oxychloride) were very effective. This treatment did not reduce tuber infection. According to Bhattacharya *et al.* (1987) eradicative properties of this chemical were also observed. According to Singh *et al.* (1988), the pathogen was found to be least adaptable to Mancozeb and Oxadixyl + Copper oxychloride but readily

adaptable to Metalaxyl + Ziram and Oxadixyl + Mancozeb. The pathogens in the tubers can be readily killed if these are dipped in water at a temperature of 45°C for 4–5 hours or at 40°C at 24 hours (Butler 1903; CPRI, 1987) and according to Singh and Bhattacharya (1988) dipping the tubers suspected to be carrying infection up to 5% in a solution of Oxadixyl or Metalaxyl for 30 minutes in 1500–2000 ppm of concentration of these systemics is very effective in removing infection from the tubers but this holds true only when the tubers have overcome the dormancy.

Thind *et al.* (1989) have emphasized the importance of the time of application of fungicides in the control of late blight of potato. A detached leaf technique was developed by Thind *et al.* (1989) to study the efficiency of fungicides after artificial inoculation. Their work further showed that the contact fungicides provided better control when applied before infection, therefore these should be used as prophylactic measure only. Systemic fungicides exhibited a good control potential to check established infection also. However, to avoid build up of resistant strains of the pathogen, as reported in some countries, it is advisable to use these fungicides rationally and in combination or alternation with fungicides. Metalaxyl resistant isolates were first reported by Arora (1991 a, b). Navneet *et al.* (1995) have studied the effect the effect of two effective fungicides Dithane M-45 and Ridomil on the phylloplane mycroflora of potato to know the succession of microbes on treated and untreated leaves of potato plants. Singh *et al.* (1998) have examined Metalaxyl residue in potato tubers. Accumulation of Metalaxyl within potato tubers after two sprays on foliage was within prescribed limits.

The metalaxyl-resistant population of *P. infestans* is resistant to Ofurace (acylalanine fungicide) and Captafol. These two fungicides have similar mode of action as that of Metalaxyl. According to Thind (2002), there is no resistance to Strobilurins and Cymoxanil. According to Johnson *et al.* (2000), two applications of Propiconazole and Chlorothalonil are sufficient to restrict lesion expansion and sporulation of late blight of potato. According to Baider and Cohen (2003) combination of Mancozeb with beta-aminobutyric acid (BABA) has synergistic effect against *P. infestans* in potato and tomato. Kaur *et al*. (2010) have done work on profiling of *P. infestans* populations for metalaxyl resistance and its management with novel action fungicides. Web-based decisions support systems are now available which guide the farmers in a particular region in timing fungicide applications depending on weather conditions.

However, the best control measure lies in cultivating resistant varieties. Hybridization work has been done with *Solanum tuberosum* (South American variety), *S.neoantipoviezi*, etc., showing a high and promising degree of resistance. *Solanum demissum*, a Mexican species, shows complete resistance with commercial susceptible varieties. There has been considerable progress in obtaining resistant varieties for India at the Central Potato Breeding Station in Shimla. Kufri Kundan and Kufri Kumar were the first set of varieties, possessing resistance of late blight, released for commercial cultivation. Later on hybrids were released for commercial cultivation as Kufri Jeevan, Kufri Neela, Kufri Muthu, Kufri Jyoti, Kufri Khasigaro, Kufri Naveen, Kufri Badshah, and Kufri Sherpa.

Cultural Methods of Controls

The following are some of the cultural methods, which have been found useful in controlling the disease:

1. Tubers from infected fields should be discarded for seed purposes or very carefully inspected at planting time, to remove all those tubers which might be suspected to be carrying or harbouring infection.
2. The tubers should be harvested after proper skin curing.

3. In an infected field, harvesting should be delayed until the plants are mature. The plants should be allowed to dry completely before harvesting. Alternatively, the infected haulms be cut and buried or burnt so that the tubers do not get in touch with infected portions of the potato plants.
4. Infected tubers and vines should be collected and buried deep in the soil.
5. All sanitary precautions must be enforced to keep the crop free from weeds. Under hill conditions ground keepers or volunteer potato plants harbour the infection and therefore, it is essential to remove these as soon as these make their appearance.
6. Tuber should be planted on high ridges. High ridges will cover the tubers thoroughly with soil and thus prevent growing out of the fungus from mother tuber and also infection in daughter tubers.
7. The tubers should be stored either in cold storage at low temperature or in a cool, dry and well aerated store. The best storage temperature is 38–40°F.
8. In the plains, the disease normally appears with the onset of winter rains. Therefore irrigation should be restricted during this period.
9. Planting of alternate beds of susceptible and resistant varieties reduces spread of the disease.
10. In order to reduce humidity, in the field-spacing and reduced nitrogen are recommended in endemic areas.

Biocontrol

Arora (1999) has reported the antagonism of a number of phylloplane mycoflora of potatoes such as *Trichoderma viride*, *Penicillium viridecatum*, and *Chaetomium brasilence*, while Roy *et al.* (1991) reported *Myrothecium verrucaria* and *Penicillium auranteogriseum.* Jindal *et al.* (1988) have reported *Epicoccum purpurascens*, *Stachybotrys atra*, and *Trichoderma koningii.* Evaluation of the fungal antagonists of potato late blight disease has been done in greenhouse (Roy *et al.*, 1994) while mass culture of antagonists and their application on a large scale in field conditions have been done by Arora (1994) with some success. Khanna (1992) and Anju Puri (1995) have done field experiments in small plots against some fungal antagonists of *P.infestans.* Daayf *et al*. (2003) reported the biological control of *P. infestans* by using *Bacillus*, *Pseudomonas*, *Rahnella* and *Serratia.* The mode of action included antibiosis and induction of resistance in the host. Finally cultivation of resistant varieties where disease is common not only ensures best check of the disease but it also reduces the cost of fungicide sprays.

Blight of Colocasia (*Colocasia esculenta*)

Taro (*C. esculenta*) is an important tropical tuber crop for millions of people in developing countries. Leaf blight and corm rot are the important diseases of Taro causing yield losses of 25–50 percent. Besides, the pathogens also causing a serious post harvest decay of corms. Comprehensive reviews have been published by Ghosh *et al.* (1983), Thankappan (1985), and Aggarwal *et al.* (1990). The disease was first reported in India by Sydow and Butler (1907). Butler and Kulkarni (1907) were the first to make detailed investigation on the disease and causal organism, via. *P. colocasiae* Raciborski.

In India, two *Colocasia* types, viz., *C. esculenta* var. *esculenta* and *C. esculenta* var. *antiquorum,* are generally cultivated and both are equally susceptible to *Phytophthora* blight. The pathogen affects at crucial stages of crop growth. In *C. esculenta* var. *esculenta,* the crop is in the stage of tuber (corm) development whereas in *C. esculenta* var. *antiquorum,* it is initial stages of crop growth at the time of infection. Infection at these stages in both taro leads to heavy yield losses. Misra (1993) reported 33–64%

yield losses in the farmers field in Orissa whereas in the experimental farm 50.39% and 26.26% yield loss was recorded in susceptible and tolerant cultivars, respectively due to leaf blight. The local variety (Jankhari) in Orissa seems to have some tolerance to the disease.

Symptoms

The disease first shows itself ordinarily on the leaf, the earliest attack occurring in August or September, in the form of small, dark, roundish specks, which increase in area rather quickly by centrifugal growth (Fig. 13.4). The spots may remain circular or assume an oval or irregular appearance. Several such spots may appear on the leaf and the whole leaf lamina may get infected. Clear, yellow liquid drops ooze from the surface of the spots in the initial stages; later the central portions become dry and drop off, and thus holes in the leaf. The periphery of the spots has a clear zonation pattern of different shades of brown, green, and yellow.

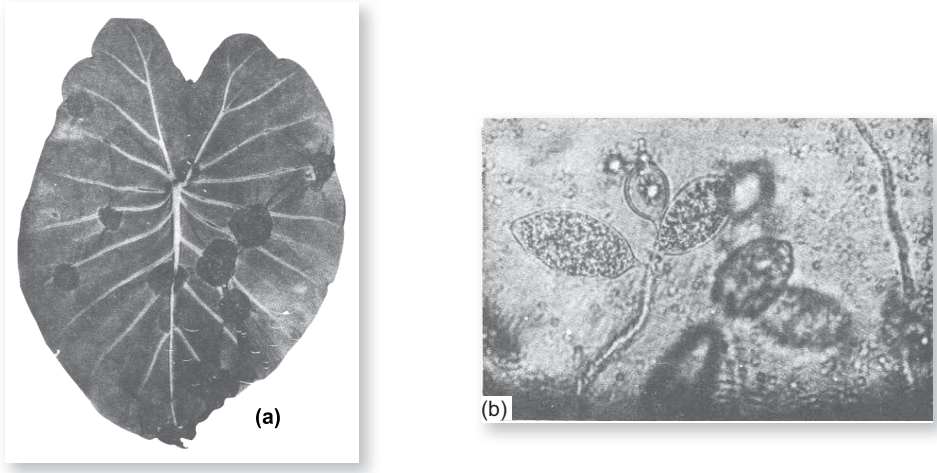


Figure 13.4 **(a)** Symptoms of *Colocasia* due to *Phytophthora colocasiae* **(b)** Sporangiophore and sporangia

Towards the periphery these zones reveal on careful observation, a delicate white haze which is caused by the sporangial stage of the pathogen. As the spots increase in size or diameter, sporangia continue to be produced, in a zonate fashion at progressively increasing distance from the center. In severe cases of infection when humidity is high and temperatures are congenial for the development of blight symptoms, the petioles of the leaves get infected. The tissues at the infection region often become so softened as to be unable to bear the weight of the leaves, which fall over the even break off completely from the plants. Extension may continue down to the sheathing base of the petiole and probably into the short stem. Inflorescence is also attacked. In severe attacks, all the leaves in fluorescence are completely destroyed and the underground corms are infected and rot completely.

Causal Organism

P. colocasiae Raciborski is the pathogen responsible for the leaf blight and corm rot of *Colocasia esculenta* (Linn.) Schott, which was first described by Raciborski in the year 1900 as the causal agent of the leaf spot of taro in Java (Indonesia). *P.colocasiae* is primarily a foliar pathogen and there is little information on the origin of *P.colocasiae,* but there are indications of Asiatic origin. According to the latest

revised key to the species of *Phytophthora. P.colocasiae* belongs to group IV of Stamps *et al.* (1990). The parasitic mycelium grows within the tissues, the hyphae being coenocytic, unseptate and branched profusely. In the leaf they grow between the cells, except in the epidermis. Butler (1918) has given a detailed account of the histopathological account of the pathogen inside the tissues. Longish, unbranched, slender haustoria are sent into the cells. Hyphae have not been seen to enter the fibro-vascular bundles in the leaf or petiole.

In the corms on the other hand, not only hyphae are found in storage cells, the mycelium being rather within the cells than between the cells, but the bundles are commonly penetrated. The effect of infection leads to the gradual disappearance of the green coloring matter and the collection of the cell contents in to a shapeless and granulous mass. Gradually, the whole starch is destroyed and the cells turn brown in color.

From the internal hyphae, branches of sporangiophores grow out to the surfaces which ultimately produce sporangia. The sporangia are found on the both surfaces of the leaves, petioles, or inflorescence. These are produced in a successive manner as the spots continue to grow. According to Butler (1918), similar sporangia can be induced to form on the corms in water or in moist air, for some months after growth elsewhere has ceased. These incipiently infected corms can further rot in storage and spread the pathogen.

Sporangiophores are slender (2–4 μm), narrowing at the tip and unbranched. On the leaf they are short, sometimes scarcely longer than the sporangium. In culture, branching of the sporangiophores is irregular to sympodial with swellings at the point of origin of the branches as well as the sporangiophore. These branches bear single, elongated, ellipsoidal, ovoid, or pear-shaped sporangia which measure 38-60 × 18-26 μm; sporangia are semi-papillate (<3.5 μm), deciduous with pedicel predominantly between 5-20 μm in length. L/B ratio of the sporangia is 1.2-2.4, av. 1.6, discharge pore 7 μm or less in diameter. Chlamydospores are usually produced in culture, sometimes abundantly, usually 26–30 μm in diameter, wall up to 3 μm thick, yellow.

Regarded as prevalently homothallic in the past is now considered heterothallic. According to Misra and Thankappan (1994) both A1 and A2 mating types have been reported from our country. However, Narula and Mehrotra (1980) found that the isolates from 3 states of North India namely Punjab, Haryana, and U.P. belong to A1 mating type only. Butler and Kulkarni (1913) reported the oospores in host tissues. Existence of races of *P. colocasiae* is not known.

It is interesting to note that Umbala and Rama Roa (1984) detected *P. palmivora* attacking *Colocasia* leaves at Hyderabad and also reported sexual structures in host tissues. Narula and Mehrotra (1984) have investigated the influence of different environmental and nutritional factors on *P.colocasiae* on agar media and a correlation was made of the meteorological records at three years when there were epidemics of *P. calocasiae.* The epidemics of *Colocasia* are mainly due to relatively high temperatures (20–30°C), high relative humidity (90–100%) and free water available on the surface of leaves of *Colocasia* plants.

Disease Cycle

It is presumed that the disease mainly perpetuates through infected corms and also through oospores persisting in the soil wherever these are produced. Attempt to isolate the pathogen by using selective media was not very encouraging. Narula and Mehrotra (1984) could isolate the fungus from the field soil with or without *Colocasia* crop only during the rainy season. In artificially infested soil, the pathogen survived for 51 days but infected leaves buried in soil did not yield the pathogen after 5 days. The

pathogen appears to possess poor competitive saprophytic ability and not soil borne in free state for long. Thus, the infected corms used as seed are the major source of primary inoculum.

According to Misra and Thankappan (1994) self sown *Colocasia* seems to play the most important role in the recurrence of the disease. Secondary spread of the disease is through zoosporangia, zoospores, and cysts, which are shed in water and carried by rain splashes between plants and plantings. Secondary inoculum is produced in abundance and is capable of causing infection and producing the symptoms within two or three days. Cysts are more damaging from disease point of view as they are produced in large numbers, small in weight compared to zoosporangia.

Disease Management

Since the disease is mainly carried over from season to season through corms, selection of disease free seed should be given due priority. Sanitation of the field, including destruction or badly affected plants should be done. Copper fungicides and Metalaxyl have proved to be quite effective (Misra and Thankappan, 1994), Metalaxyl (Apron 35F), Captafol (Difolatan), and Chloroneb (Demosan 65w) used as spray at 15 days interval gave good control of the disease (Aggarwal and Mehrotra 1987, 1988 a, b). Deshmukh and Chhiber (1968) have reported field resistance to this disease in certain varieties of *C. esculenta* var. *antiquorum* (*C. antiquorum*). Variety Ahina is fairly resistant. Pharia and Mathur (1964) have reported resistance to the disease in various regions of India. Bio-control potential of control of *P. colocasiae* was investigated by Narula and Mehrotra (1987).

"Koleroga" or "Mahali" of Areca Palms (*Areca catechu*)

Koleroga (Kole = rotting, Roga = disease) as is called in Karnataka is otherwise called as Mahali (heavy devastation) in Kerala or fruit rot in a number of places. The disease was first recorded in India in 1906. It occurs in severe form in heavy rainfall areas of the country wherever arecanut crop is being grown. Shastry and Hegde (1987 a–c) present evidence that support reclassifying *P. arecae* as *P. meadii* and according to them, it is one of the most serious disease in the arecanut plantation in Karnataka.

Symptoms

"Koleroga" usually makes its appearance 2–3 weeks after the beginning of the rains. Water soaked areas develop on the nuts, starting from the base. This is the chlorosis stage (Neergole); in advanced stages, the fungus develops on the outside of the fruits (Bhusargole). Attacked nuts soon begin to shed and this is a sure sign of the disease in the plantation. Fallen nuts show felty whitish mycelial mass on their surface and very soon the entire nut is enveloped by it. While the disease is predominantly on the nuts, the tops of trees are occasionally attacked. The infection travels up the stalks and sheaths and reaches the growing tops. As it advances on the crown of the plant the trees may dry up, resulting in a withering of the leaves and bunches. The main loss is due to the nuts and there is hardly 1% annual loss due to the death of the plants.

Causal Organism

The fungus that is responsible for Koleroga is *Phytophthora arecae* (Coleman) Pethybridge. The mycelium is coenocytic and hyphae vary in diameter from 8 μm to 9 μm. The sporangiophores are about 2.5

μm wide, irregularly sympodial, sporangia broadly ellipsoid or obturbinate to nearly spherical, mostly in the range of 40.50 × 35-40 μm, ratio 1.1-1.4:1, deciduous, pedicel 1–6 μm papilla and apical thickening hemispherical or slightly less; chlamydospores (in culture) variable in numbers, sometimes absent or rare, never abundant, 35–40 μm in diameter. For the development of sporangia, strong light appears to be necessary. Four hours after formation, the protoplasm within the sporangia begins to divide into uninucleate pieces which ultimately are transformed into zoospores. Zoospores emerge out of the sporangia in the presence of abundant light and humidity in the air. Sexual reproduction is oogamous and antheridia are diclinous. The oogonia develop sometime after the antheridia are produced. The oospores are smooth, spherical, amphigynous and 23–26 μm in diameter. Their germination has not been seen so far.

In North Kanara (Bombay), Uppal and Desai (1939) did pioneering work on Koleroga disease of areca nuts caused by *P.arecae.* They demonstrated the heterothallic strains in the species and obtained abundant oospores by mating different strains isolated from areca nuts.

Disease Cycle and Predisposing Factors

The fungus is endophytic. The areca palms harbour latent infection in their crowns during the non-monsoon period. Sexual spores are not produced in nature. Oversummering is carried out by mycelium persisting on tree tops and chlamydospores in fallen nuts. Heavy rainfall and constant moist conditions are undoubtedly the chief factors for "Koleroga". Intermittent rainfall and sunshine are highly favourable for infection. Narasimhan (1927) recorded some wild hosts of *P.arecae* from Mysore.

Disease Management

The earlier practice of controlling Koleroga was to cover the arecanut branches, made of arecanut leaf sheath (called Kotte in Malnad region or kind of grass in other parts named "Karada") but did not help in controlling the disease. However, covering the arecanut branches with polythene bags as a prophylactic measure give cent percent control of fruit rot though it is costlier than fungicide spray (Saraswathy and Nambiar, 1994).

Various workers tested the efficacy of Bordeaux mixture with several adhesives and spreaders such as potash alumn with casein, vegetable oil such as ground nut gingelly, coconut or sunflower oil. However, it was shown that plain Bordeaux mixture (1%) without any adhesive was equally effective in controlling the disease. It has been recommended to have prophylactic sprays of Bordeaux mixture 2–3 times in a year which is quite effective in keeping the disease under control (Nambiar, 1971). Copper oxychloride checked koleroga. However there were reports that copper oxychloride or nickel chloride were not effective. According to Saraswathy and Nambiar (1994) systemic fungicides such as Aliette and Ridomil at 0.2% are also good in checking fruit rot of arecanut. Adopting phyto-sanitary conditions in the plantations go a long way in reducing the incidence of the disease. The measures include removal and destruction of fallen nuts, diseased bunches tree tops and other plant parts in the field.

Bud Rot of Palms

In southern India, toddy palms (*Borassus flabellifer*), and coconut palms (*Cocos nucifera* L.) suffer seriously from bud rot caused by *Phytophthora palmivora* Butler. The disease though sporadic in nature causes severe losses. Veena *et al.* (1997) reported that during 1992–93, a severe bud rot incidence

was noticed in Kannur, Kasaragod and Kozhikode districts of Kerala, which killed about five thousand plants. Nambiar and Ramanujam (1994) have reported that the disease incidence was up to 35–40%. A survey of disease in non-traditional coconut growing areas of eastern M.P. by Dantre *et al.* (1997) has shown that the incidence of bud rot varied among horticultural units from 4.72% to 12%. Radha and Joseph (1982) have reviewed the work done on this disease up to that period.

Symptoms

The young leaves and shoots are affected by the disease. Discolored spots are seen on the leaves and at the bases of leaves. The central expanding leaf turns yellow and dries up. In the beginning, the lesions appear as tiny and water soaked to begin with which later become dark brown and sunken in advanced stages of disease. The tender part of the crown including the bud completely rots into a mass and omits fowl smell while older leaves often remain green and fall after maturity.

Causal Organism

Phytophthora palmivora (Butler) Butler is the established species. This fungus has a very wide host range which includes various palms, cacao, areca nuts, rubber, *Citrus* spp., cinchona, castor and safflower.

The mycelium of the pathogen is intercellular, producing haustoria in the cells. Hyphae are fairly uniform, undulating, and branched with inverted pear shaped, rarely sound and always terminal sporangia. Sporangia are mostly 50–60 µm, tapering slightly to the stalk, deciduous, pedicel 2–10 µm, chlamydospores are up to 55 µm, mostly 30–55 µm, forming promptly in a few days in a culture and are terminal or intercalary. Sporangia germinate in water to produce large zoospores. Zoospores measure 8–10 µm in diameter. Oospores are spherical, measuring 35–45 µm in diameter, the wall being up to 4 µm. Oospores germinate readily, giving rise to a short branch on which secondary sporangia are produced.

Disease Cycle and Predisposing Factors

The disease perpetuates through mycelium in diseased tissues and through oospores (if formed) and lies in the dead parts. Once the disease has started in the beginning of the rainy season, the production of sporangia and zoospores on tree tops and their dispersal to neighboring trees takes place. Toddy tappers may also help in the spread of the inoculum. The rhinoceros beetle (*Orycles rhinoceros*) and rains are also responsible for the spread of the pathogen. The possibility of survival of *P. palmivora* in the leaf during the dry months exists was suggested by Menon and Pandalali (1958). Radha and Joseph (1982) have reported survival of the pathogen in infected coconut crown for over 5 months.

Disease Management

Cutting down and burning diseased trees was the method of control adopted earlier. Later, spraying with Bordeaux was found to be very effective. Affected tree should be sprayed all around with Bordeaux (5:5:50). As soon as the disease appears, copper fungicides should be periodically sprayed. Ramachandran *et al.* (1982) found Ridomil to be effective against *Phytophthora* from coconut. According to Nambiar and Ramanujam (1994), Mancozeb application in the spindle stage has been found effective. Palm injection with Fosetyl aluminium has been found effective in Ivory Coast.

Root Rot, Crown Rot, Foot Rot, Gummosis and Brown Rots of Citrus Fruits

Several species of *Phytophthora* are associated with this disease. *Phytophthora palmivora*, *P. parasitica*, and *P. citrophthora* are responsible for gummosis in *Citrus* spp. in almost all the citrus growing parts of the world. Sweet varieties are more susceptible than the sour ones. The diseases occur sporadically in India but are common in Maharashtra and Punjab.

Symptoms

Infection starts from the ground level. The earliest symptom is the appearance of a water soaked area in the basal part of the plant from, where at a later stage a brownish gummy substance exudes (Fig. 13.5). In course of time, cracks appear in the bark, which slowly peels off leaving the wood exposed. Fruits are also infected and they rot and drop down. The intensity of symptoms varies with the citrus species. For example, there may be considerable gum formation in sweet oranges but relatively little on grapefruit. On the other hand, the bark in the case of sweet oranges turns black and deep cracks are formed in the bark and sometimes in the stem cortex. When gumming starts close to the soil, the disease spreads on the main root and then around the base of the trunk. Exudation of the gum is not conspicuous always as it is partly absorbed by the soil. As result of severe gumming, the bark becomes completely rotten and the tree dies owing to girding effect.



Figure 13.5 Symptoms of *Citrus* gummosis/foot rot (Courtesy: Dr. T.S. Thind, P.A.U. Ludhiana)

Causal Organism

Several species of *Phytophthora* are responsible for this disease: *P. palmivora, P. nicotianae* var. *parasitica* and *P.citrophthora.* The first two species have been described earlier. A historical account of different *Phytophthora spp.* on *Citrus* spp. has been given by Fawett (1936). Hyphae up to 6 μm or 7 μm, sporangiophores in water delicate (1–2 μm), scarcely widening at the base of sporangium, irregularly branched with a swelling at the point of branching, sporangia in water very variable in size and shape, often with two widely divergent apices, average 40-45 × 27 μm, papilla scarcely hemispherical, deciduous with a pedicel 10–12 μm long, chlamydospores absent or few, sex organs unknown.

Disease Cycle and Predisposing Factors

Phytophthora citrophthora occurs in the soil to a depth of a meter or more, and is disseminated to the fruit and foliage by rain. Infection can take place when the bark is wet only through the tissue where the scion has been budded. Other species of *Phytophthora* can also survive in the pool for a while. The testa of seed from infected citrus fruits can also carry the pathogen to new seed beds, and on transplanting, to the nursery.

Disease Management

Management of *Phytophthora* diseases of citrus involves and integrated approach which includes the:

1. Use of resistant roots stocks
2. Appropriate cultural practices to mitigate disease development.
3. Use of Chemicals as prophylactic measure for cure of the diseases and
4. Biological control.

In general Mosambi and Pomelo are more susceptible than mandarin. Lime and Jambheri (*Citrus jambheri*) are resistant. Troyer citrange and trifoliate oranges seem to offer protection against brown rot, and gummosis incited by *Phytophthora* species. Adoption of tolerant root stocks like sour orange, Cleopatra mandarin, trifoliate orange, Rangpur lime (*Citrus limonia*), etc., had been advocated. A variety resistant in one region may be susceptible in another area. The use of nursery stock grown under *Phytophthora* free conditions and prevention of water standing in contact with susceptible portions of the bark above the bud union by way of good drainage is extremely helpful in checking the disease. Prasad and Rao (1985) emphasized the need to screen citrus for *Phytophthora* tolerance only after the plants cross the juvenile phase.

For prevention of gummosis and collar rot, Bordeaux paste on stem above ground level up to (60.9 cm) at least once a year is recommended. Replanting citrus in fields/lands where this crop has been growing previously grown is hazardous and should not be done. Use of resistant root stocks will give permanent solution to the whole problem. For control of foot rot and gummosis application of Bordeaux paste is quite effective. Spraying with Difolatan 0.3% or Bordeaux mixture (4:4:50) was found to be quite effective.

The disease can be further checked to some extent by dusting the walls of the pots as well as bottom of the pits with Zinc-Copper-lime 5:1:4. The exposed wood can also be covered with Zinc Copper with lime ($3:1:3^{1}/_{2}$) wash or Bordeaux paste which should be followed with the application of white lead or any wound dressing agent. Complete control of the disease has been obtained by spraying Kagzi lime trees infected with *P.citrophthora* with 3 gm. Aureofungin 30 ml of liquid soap/30 gallons of water 2–3 times. Somani and Patel (1970) got good control of the disease (gummosis) incited by *P. nicotianae* var. *parasitica* by Aureofungin as soil drench and spraying of foliage twice at an interval of one month. Drenching of Ridomil MZ (0.2%) and Aliette (0.2%) has also being found effective against gummosis. Leaf fall and fruit rot can be controlled by spraying Bordeaux mixture (1%) or Bordeaux mixture + tin Sulphate or Difolatan (0.3%). In addition, spraying Fosetyl Al (Aliette 80 WPO), Metalaxyl + Mancozeb 1:8, (Ridomil MZ-72), copper oxychloride, Bordeaux mixture and Bordeaux paste were tried by Naqui (1994) and were quite effective.

Matheron and Porchas (2000) studied the effect of systemic fungicides on growth and sporulation of *P. parasitica* and *P. citrophthora*. Mycelial growth was inhibited by Dimethomorph, Fosetyl-Al and Metalaxyl fungicide while sporangial production was minimum in the presence of Dimethomorph (1mg/ml). Germination of encysted zoospores was most sensitive to Dimethomorph, less to Fluazinam and Metalaxyl and least to Azoxystrobin and Fosetyl-Al. Matheron and Porchas (2002) studied the effect of six fungicides *in vivo* conditions and reported that Dimethomorph, Fosetyl-Al and Metalaxyl inhibited cankers caused by *P. citrophthora* on the trunk better than Azoxystrobin or Fluazinam. Arora *et al.* (2002) compared the effect of systemic and non-systemic fungicides and reported Ridomil MZ as soil drench in combination with Aliette as spray best in controlling the disease. These chemical treatments should be done before the onset of rains because the pathogen multiplies rapidly during rainy season i.e. during high humidity.

Guar *et al*. (2002) have purposed integrated disease management of Phytophthora disease in Mandarin cv Kinnow which are as such:

1. Ridomil MZ (1g/l water) sprays twice in February and August along with trunk painting with Ridomil MZ (20 g/l of linseed oil).
2. Topsin-M (1g/lwater) soil treatment along with trunk painting with Ridomil MZ.
3. Soil application of potash (500g/tree) or zinc sulphate (150g/tree) in February and August along with trunk painting with Ridomil MZ.

Thind *et al.* (2000) also reported Ridomil MZ (2g/100ml linseed oil) more effective than Bordeaux paint. According to Barkai Golan and Phillips (1991) hot water treatment of grapefruit (48°C for 3 min.), Lemon (52°C for 5–10 min.) and orange (53°C for 5 min.) has been quite effective. According to Sonavane *et al.* (2015) treatment of trees with Bordeaux paste and drenching with Al-Fosetyl (0.2 %) was found most efficacious for the management of *Phytophthora* root rot of mandarin in Coorg in Karnataka.

Leaf Rot and Foot Rot of "Pan" (*Piper betle*)

Betel vine is an important commercial crop of India, Bangladesh, Sri Lanka and to some extent, of Malaysia, Singapore, Thailand, Philippines, and Papua New Guinea. The betel vine is a perennial creeper, cultivated for its leaf, which is used as masticatory. In India, it grows over an area of 40,000 hac., providing livelihood to hundreds of thousands of families engaged in its cultivation and trade (Balasubrahmanyam *et al.*, 1994). Root rot collar rot and leaf rot of *P. betle* (Pan) is a serious disease throughout the betel vine growing areas in the country and is a great threat to the cultivation of this crop in our country. Some reviews have appeared on this disease, which are of importance. Saksena (1977) gave a presidential address to the members of Indian Phytopathological Society and discussed in details the havoc brought out by this disease and summarized the work dine on this disease till then. After this several other important reviews and articles have appeared. These are Mehrotra (1982, 1984), Johri *et al.* (1984), Bhale *et al.* (1987), Khare *et al.* (1988) Balasubrahmanyam *et al.* (1994), and Rao and Singh (1996). Foot rot and leaf rot disease was first describe by Dastur (1935) named the pathogen as *P. parasitica* var. *piperina.* Although the name is still used (Mehrotra 1982). Waterhouse (1963) rejected the variety name since it was invalidly published without a latin diagnosis and reduced it to a synonym of *P. nicotianae* var. *nicotianae.*

Symptoms

The pathogen infects almost all portions of the plant. The most common symptom in an orchard is the appearance of wilted vines. This is not the typical vascular wilt caused by *Fusarium* species. It is due to the fact that the root and basal foot rot or collar rot occurs which results in wilting symptoms. The first symptom of a diseased plant is the loss of luster, a little later the upper leaves and tender shoots of the plant begin to show signs of drooping. The whole plant shows loss of green color, which is replaced by yellow. The plant later rapidly dries up. The underground parts are more or less completely rotted (Fig. 13.6 b). If a newly diseased plant is uprooted it is found that the smaller roots are infected first and gradually the rotting spreads to the bigger roots and then collar region of the plant. As the rotting develops the soft tissues are readily eaten up by the fungus and the fibrous remains give the parts a shredded appearance.

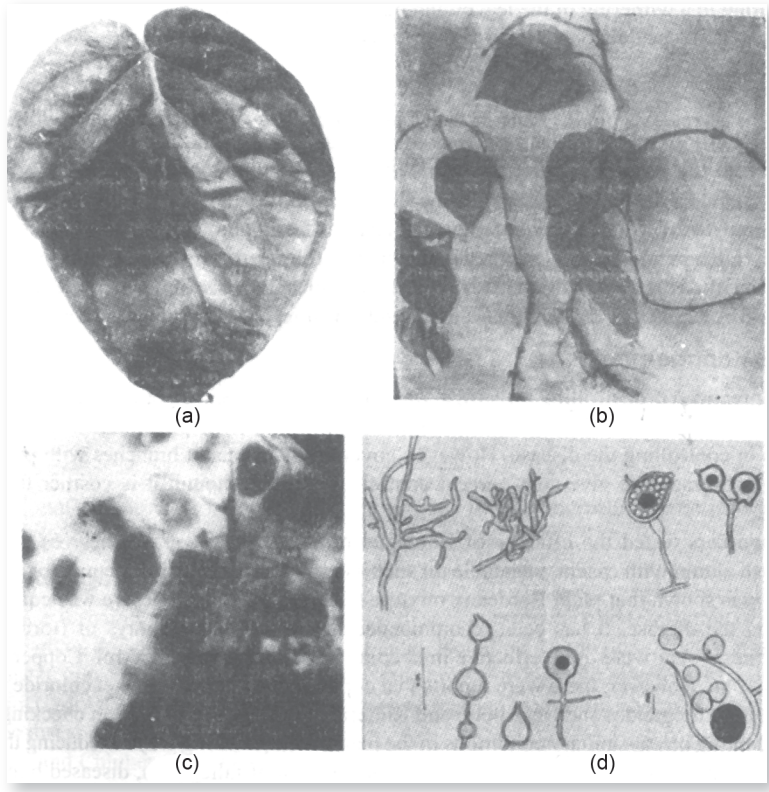


Figure 13.6 Leaf rot and foot rot of *Piper betle* caused by *Phytophthora parasitica* var. *piperina* **(a)** leaf rot **(b)** root rot and foot rot **(c)** sporangia on leaves **(d)** mycelial knots, sporangia and chlamydospores

In the leaf rot symptoms (Fig. 13.6 a), the first symptom of the disease is the presence of a circular black or brownish spot which are more or less wet in appearance. Under continuous wet and humid conditions the black spot rapidly increases in diameter and develops a wet rot of a major portion to the leaf. The rot extends to the petiole and from the petiole to the stem. It has been observed that when the infection reaches a midrib or one of the main veins, it spreads very rapidly along their length than between the veins. If the atmospheric conditions do not remain humid and wet continuously and if the rain lasts only for a day or so with warm dry periods in between, the diseased areas show distinct concentric puckering and wrinkles on account of the unequal growth of the tissues. If the condition becomes dry, the progress of the disease is checked and the black spots usually surrounded by a brownish halo or ring remain dry and localized. More than one spot are often found on a leaf and the spots may coalesce with each other. The leaf at any position in the plant is attacked. It may be noted that in an orchard the leaves within a couple of feet from the ground surface are particularly liable to infection in the beginning of the season and start of the disease. The top most leaves are generally seldom infected.

Causal Organism

There is no unanimity with regard to the pathogen/pathogens responsible for the diseases (Fig. 13.6 b and c). Dastur (1935) named the pathogen as *P. parasitica* var. *piperina* which has not accepted by Waterhouse (1963) as it was invalidly published without Latin diagnosis and therefore it became

P. nicotianae var. *parasitica.* Maiti and Sen (1977) considered it as *P. palmivora,* while Marimuthu (1991) called the pathogen *P. palmivora* MF_4. In recent literature *P. palmivora* MF_4 has been called as *P. capsici.* It appears that more than one species of *Phytophthora* are infecting this crop in different parts of the country.

Sporangia develop only at 20–31°C when relative humidity is 100%. Free water is essential for the liberation and movement of zoospores which is maximum at 21–23°C.

Disease Cycle

The pathogenicity of *Phytophthora parasitica* to the pan crop was well established by Dastur (1935), Mahmud (1952), Chowdhury (1994 a, b, c), and Asthana (1947). Mahmud claimed the isolation of the pathogen from the soil and concluded that the fungus lives saprophytically in the soil and can survive for years. Mehrotra and Tiwari (1967) repeatedly tried to isolate *Phytophthora* from the soil but the results were always negative. They however, succeeded in isolating *Phytophthora* from the soil by the method adopted by Ocana and Tsao (1966). Mehrotra and Tiwari decided to measure the competitive saprophytic ability of *Phytophthora parasitica* var. *piperina* by Wastie's method (Wastie, 1961). The results of these experiments revealed that "*pan" Phytophthora* is a poor saprophytic competitor and is easily suppressed by other soil organisms.

The survival of *Phytophthora* as mycelium in the host tissue was studied by Mehrotra and Tiwari (1967). The pathogen could be isolated up to 17 weeks from the host tissues. Chowdhuri (1944 a, b, c) and Asthana and Mahmud (1945) found that the disease is primarily carried through the plantation of infected cuttings. The work of Mehrotra (1961), Tiwari (1968) and Saksena and Mehrotra (1970) clearly show that the infected part rots rapidly and that the mycelium cannot be detected in the healthy parts to any appreciable distance on either side. Mehrotra and Tiwari (1967) demonstrated that after five weeks, chlamydospores or resting sporangia were formed and the pathogen could be isolated 17 weeks after burial. Besides chlamydospores, oospores of the pathogen have been detected in nature for the first time in India by Chaurasia (1976).

Disease Management

Effect of Manure on the Disease Nayak *et al.* (1984) recommended combined application of phosphorus and nitrogen. The mortality of vines was more when high dozes of nitrogen was applied but was considerably lowered with phosphate alone or in combination with low dosage of nitrogen.

Host Resistance Betel vine is propagated vegetatively. In most places, the vines do not flower. A living female vine has been located in Bongaon (West Bengal) and therefore there is possibility of evolving resistant lines using various breeding methods. Generally, the vines produce only the female flowers and therefore there is the difficulty in the breeding programme. Some varieties are tolerant to the disease in different parts of the country.

Chemical Control Earlier workers recommended the use of Bordeaux mixture (2:2:50) strength for controlling the disease by treating the ridges before planting and later subsequent application at monthly intervals or bi-monthly intervals. Hector (1930), McRae (1934), and Chowdhury (1944 b) however recommended 5:5:50 Bordeaux mixture in West Bengal and Assam. These results have been subsequently substantiated by Subramaniam and Venkata Roa (1970), and Nayak and Jain (1984). Narasismhan et

al. (1976) recommended 0.5% Dexon (sodium-p-dimethyl-amino-benzene) to be effective. Chaurasia (1976) obtained best results by dipping the cuttings in streptomycin sulphate solution followed by Bordeaux mixture application twice a month. Johri and Chaurasia (1955) have recommended Akomine which is an inorganic source of plant nutrient with phosphorus as an active ingredient. Being inorganic in nature no residual or side effects are there. The product has been tested for its efficiency in management of betel vine Phytophthora along with Aliette and Captaf. About 80% mortality of the vines was checked. Ayyavoo and Samiyappan (1981) reported the efficacy of Deconil-2787, 0.1% during winters (October to February) 30 liters fungicidal solution for every 65 sqms at 15 days interval.

Some homeo-drugs against betel vine *Phytophthora* disease and found some encouraging results. Botanicals for the management of the betel vine diseases were tried by Johri *et al*. (1994) with varying successes. Tiwari and Mehrotra (1973) obtained promising results by using carbon disulphide (CS_2) and formalin as soil fumigants in controlling the disease in a limited scale in pots. The fumigant formalin was found to be phytotoxic at the dosages used to control the disease. Fumigation of soils brings about the dominance of the antagonists *T.viride* in soil, which indirectly acts as a controlling agent.

Cultural Practices The importance of phyto-sanitary approach to reduce the disease incidence in the orchards is emphasized. Collection, removal, and destruction of dead and dying plants or burning or burying with lime, deep in the soil is recommended. Since it is believed that there are chances of the disease being carried through contaminated cuttings carrying the infection both internally as well as externally. Planting of healthy cuttings had been recommended. According to Asthana (1947) only the tops of the vines up to 1.5 meter should be utilized for plantation purposes as cuttings. Saksena and Mehrotra (1970) recommended the removal of collateral hosts such as Colocasia species growing around the orchards. Nayak (1974) and Singh and Chand (1973) suggested judicious use of water to prevent the dissemination of the pathogen.

Since the pathogen overwinters or over summers in the soil, judicious rotation of the crop in betel vine orchards may check the spread of the disease. Paddy and banana rotation in Salem and Tanjore districts in Tamil Nadu state had been found to considerably safeguard the crop against the incidence of disease. According to an anonymous report in Maharastra, the cultivars grow onion, gram, chilies, tobacco, wheat, or jowar as rotational crops before planting betel vine. Combined effects of flooding, following hot weather, and deep ploughing have been found to reduce the incidence of the disease (Tiwari and Mehrotra 1974).

Biological Control Tiwari and Mehrotra (1968) recommended dipping of cuttings in a suspension of Trichoderma viride spore suspension prior to plantation. Mehrotra and Tiwari (1976) experimented with organic amendments and antagonists in soil to control foot rot of P. betle. Incorporation of corn straw into the soil gave best control. The effect of different antagonists growing on corn straw and til (Sesamum indicum) oil cake amendments showed that T. viride gave best control. Corn straw supplemented with nitrogen gave better control. Chaurasia (1976) demonstrated the effectiveness of Aspergillus flavus, A.oryzae, and Penicillium sp. in the control of the disease. Nayak et al. (1984) however found that T.viride was not effective in field experiments. Ayachi (1983) tried to control the disease (leaf rot) by using phylloplane microorganism, 17 of these organisms caused inhibition of Phytophthora to varying degrees in vitro condition. Tiwari and Mehrotra (1973) obtained promising results by using CS2 (Carbon disulphide) and formalin as soil fumigants in controlling the disease in pots. Formalin however is phytotoxic under the dosages used for controlling the disease.

Collar Rot of Apple

The disease is also known as *Phytophthora* trunk or crown rot and is universally present in all the apple-growing regions of the world. Butler and Bisby (1931) were the first to report the occurrence of *Phytophthora cactorum* on pear and plum in India. Bose and Mehta (1951) and Agarwal (1960) have found it attacking apple fruits and trees in Kumaon hill, Himachal Pradesh during 1960, wherever soils are capable of sustaining its growth in susceptible varieties, the losses are extensive, sometimes resulting in death of apple trees within a few years.

The major fungus involved in collar rot symptoms is *P. cactorum* though other fungi namely *P. citricola*, *Pythium ultimum*, *P. polytylum*, *P. debaryanum* are also associated with the collar rot of apple in Himachal Pradesh.

Symptoms

There are two phases of the disease, i.e., crown rot (fungal attack on root-stock below soil level) and collar rot (fungal attack on the scion above the soil level). The disease is evident on the collar portion near the soil level or the graft union. Initially, small necrotic areas are produced which later turn brown and have a characteristic alcoholic odor. In moist conditions, such lesions exude a brown watery substance at the bark surface. Wood below such lesions turns dark brown. The dead bark dries out and splits away from the wood. Collar rot phase does not normally extend into crown region but, can extend high on stem which may sometimes bleed. After some time, these lesions girdle the tree, which may produce a variety of symptoms on the aerial parts. In crown rot, the bark below soil level turns brown in early stages of infection, which may start at the point of root emergence. The disease can be initiated from injuries in the crown region, which cover most of the crown. The tissues start rotting. The tissue becomes spongy resulting in girdling of the whole tree, which may be killed in due course of time. Apple tresses initially infected by the pathogen are difficult to distinguish from healthy trees unless one looks very carefully for the symptoms on the crown (the root stock), collar (the scion), or roots. Fruit rot due to *P. cactorum* is of minor importance. Fruit infection usually follows contact with soil particles that splash upward during heavy rain. The infected tissues become spongy, mealy, or grannular. The infected fruits become mummified and may become a source of infection for fresh infections. Fruit rot due to *P. cactorum* also does damage in storage of fruits.

Causal Organism

The major fungus involved in collar rot symptoms is *P. cactorum* though other fungi namely *P. citicola*, *Pythium ultimum*, *P. polytylum*, *P. debaryanum* are also associated with the collar rot of apple in Himachal Pradesh.

Epidemiology and Disease Cycle

The main pathogen *P. cactorum* is soil borne and is known to survive in the soil either as mycelium or through oospore production. Soil temperature of 12°C associated with pH 5–6 is found to be best for its survival. The zoospores of the pathogen were found to survive up to 20 days while sporangia survived upto 32 days at a temperature of 7°C and 27°C. The crown rot pathogen enters the host directly through cracks, injuries created by insect pests or indirectly via un-thickened roots.

Disease Management

Amongst the cultural practices, general recommendation exists for having graft union of plant at 30 cm or above soil level so that infection does not take place. Proper drainage should be provided to avoid prolonged and periodic water-logging. According to Utkhede (1987) use of plants like canola (Brassica napus), mustard, and turnip as inter-cropping reduce disease. To check the spread of the disease in nursery, the site must be changed to avoid further infection (Gupta, 1999). Approach graft (inarch) in case of trees whose bark gets damaged is helpful in saving them.

Cleaning of wounds at soil level followed by application of copper or Bordeaux paint on the cleared area help in containing the wounds and reduces the spread of the disease as well. Harris (1991) has showed several fungicides like Difolatan, Dexon, Maneb, Captan, Dicholone, Thiram, Ferbam, Acylalanines, Ethyl phosphonates, Metalaxyl, and Fosetyl-Al to be effective. Rana and Gupta (1981) showed the efficacy of Dithane D-14, Dithane M45 and Agallol to be effective in reducing the lesion size on apple twigs. Gupta and Mir (1983) further demonstrated that Ridomil, Aliette, and Euparen M inactivated fungal mycelium in the soil besides inhibiting the production of reproductive structures.

Regarding biological control, *Enterobacter aerogenes* and *Bacillus subtilis* have been shown to inhibit *P. cactorum* and their use in soil was helpful in recovery of collar rot affected plants (Utkhede, 1987). Gupta and Garg (1990) showed that *B. subtilis* and *E. aerogenes* in combination with Metalaxyl reduced the growth of the pathogen in *in vitro* and also reducing the seedling blight in pots. Apple root stocks like M2, M4, MM102, MM113 and MM114 are resistant under Indian conditions.

Downy Milew of Peas (*Pisum sativum*)

Downy mildew is a common disease in the Indo-Gangetic plain and may also occur in other parts of India. Although it is widespread in Europe and North America, it is considered to be unimportant.

Symptoms

The disease appears on young plants in the form of downy growth on the under surface of the leaves, in patches of varying size, sometimes covering most of the leaf surface, of a greyish-violet color. The growth is composed of conidiophores, arising from the mycelium of fungus which is buried within the leaf tissues. On the pods the disease is first noticed during the flat-pod stage as pale green, more or less elliptical blotches on the sides, or more irregular, elongated lesions along the dorsal surface of the pods. Gradually the blotches darken to a bright brown color, mottled with light green islands. Seeds inside the infected pods adjacent to the infected tissues, especially those under the lesions, are generally aborted and considerably reduced in size. Systemically infected plants are very much stunted.

Causal Organism

The fungus which causes the downy mildew of peas is *Peronospora pisi* Sydow (Fig. 13.7).

The fungus produces coenocytic intercellular mycelium which branches profusely. Finger-shaped or branched haustoria penetrate into the host tissue. Conidiophores arise from the intercellular mycelium through the stomata and bear the conidia. Conidiophores arise in clusters. They are unbranched for two-thirds or more of their length and bifurcate two to seven times at the apex, the ultimate branches diverging from each other at right angles. Conidiophores are hyaline, nonseptate, long and thin, measuring

400-700 × 9-11 µm. They bear conidia that are hyaline, thin-walled and elliptical, measuring 22-27 × 15-19 µm. The fungus also produces oospores. Oospores are embedded in the tissues of old and withered leaves or in the tissues of the pods. They are almost spherical, greenish yellow, 26–43 mm in diameter and have a thick epispore with large reticulations.

Disease Cycle and Predisposing Factors

Infection in pea, downy mildew can be of two types: one is due to systemic infection from the oospores, and the other due to local infection. The possibility that the mycelium and oospores (found in the seed coat) also cause primary infection has not yet been definitely proved. Moist and cool weather is conducive to the development of the disease, while warm, dry weather retards development. The pathogen also attacks *Pisum arvense* L., *Vicia hirsute* Koch, *Lathyrus sativus* L., *Trigonella polycerata* L., and *Lens esculentus* Moench. Secondary spread is by conidia (sporangia).

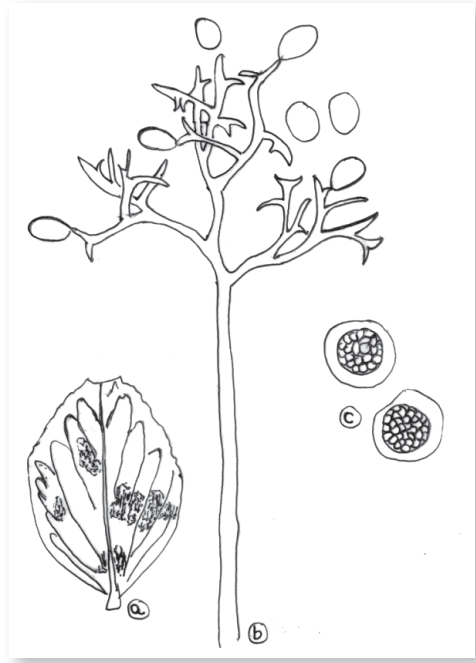


Figure 13.7 **(a)** Symptoms on pea due to *Peronospora pisi* **(b)** Conidiophores and conidia **(c)** Oospores

Disease Management

It is difficult to control this soil-borne disease. It is a good practice to destroy the debris which may contain the oospores and rotation of two to three years is also recommended. Spraying and dusting peas with fungicides is not of much help in controlling this disease. Since the fungus also attacks other hosts, it may multiply on those hosts which then are a source for peas the next season.

Downy Mildew of Crucifers

The disease attacks those parts of turnip, radish, cabbage, cauliflower and the oil seed *Brassica* species that are above the ground. It is common among young plants, but also appears on mature plants.

Symptoms

The disease is characterized by the appearance of purplish-brown spots on the underside of the leaves. These may remain small or become enlarged (Fig. 13.8). The upper surface of the leaf on the lesion is yellow. Sometimes *Albugo candida* is also found side by side on the same leaf and the symptoms of the two look very much similar from a distance. The stems also get infected and swell up. The young ovary may be very much elongated into a twisted body as a result of infection and the floral buds are atrophied.

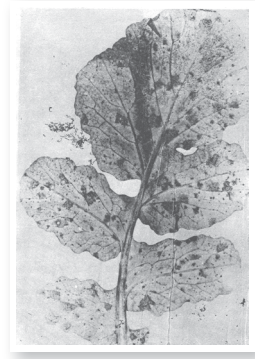


Figure 13.8 Downy mildew symptoms on a crucifer due to *Peronospora parasitica*

Causal Organism

The fungus causing the disease has generally been described as *Peronospora parasitica.* However, according to Gaumann, it is a composite species consisting of many smaller morphological and biological species. He has named the fungus occurring on *Brassica campestris* as *Peronospora brassicae.* The pathogen shows host specialization.

The mycelium of *P. parasitica,* an obligate parasite, is strictly intercellular with large, finger-shaped or clavate, branched haustoria which nearly fill the cell cavity (Fig. 13.8). Numerous branched conidiophores emerge through the stomata on the undersurface of leaves. Conidiophores being absent from floral organs, oospores are produced in them. Conidiophores are 100–300 μm long and unbranched for a major portion of their length. Dichotomous branching occurs 6–8 times at the tip region. Sterigmata are long, slender and pointed. They are at an acute angle with each other. A single conidium is borne at the tip of each branch. The conidia are broadly oval, ellipsoidal, hyaline and measure 24-27 × 15-20 μm. They are shed very readily and germinate by a germ tube.

Oospores are produced in the interior of the host tissues at a later period than the conidia. The oogonium is spherical and hyaline. It is fertilized by a single antheridium. The oospores are globose, 26–45 μm in diameter, pale yellow in color and enclosed in crest-like folds.

Disease Cycle

The pathogen is capable of surviving in the form of oospores in the soil. Seeds contaminated with trash may also carry the disease. The pathogen has several physiologic races. Some wild plant hosts also serve as a primary source of inoculum. The disease is spread by means of conidia. Vishunawat and Kotle (1993) have reported that oospores of the pathogen occur on the surface and in the hypodermis of seed coat of rapeseed mustard. Such infected seeds produce seedling with downy mildew symptoms. Some wild hosts may also serve as source of primary inoculum. Conidia are responsible for the secondary spread of the disease.

Disease Management

Among cultural practices, eradication of weed hosts, crop rotation, and deep ploughing during summer are important. The fungicides recommended against the disease are: Dithane Z-78(0.3%), Dithane M-45(0.1-0.3%), Daconil (0.1%), Difolatan (0.3%), Blitox – 50(0.3%) and Ridomil (0.1%). Treatment of seed and soil with Metalaxyl provide protection against the disease.

Ziadi *et al.* (2001) have reported the production of PRP (pathogen related protein) in cauliflower in response to acibenzolar-S-methyl. Silue *et al.* (2002) have also reported induction of resistance to downy mildew in cauliflower by treating it with DL-β-amino–n–butyric acid (BABA). Induction of resistance to downy mildew in cauliflower by treatment with spray of water solution of the elicitor, Benzothiadiazole (BTH) is reported by Godard *et al.* (1999). Application of Phytogard (58% dipotassium phosphate + 42% water) also inhibits the sporulation of the downy mildew pathogen. According to Monot *et al.* (2002), treatment of broccoli (*Brassica oleracea* var. *botrytis*) with avirulent strains of *P. parasitica* also induces systemic resistance. Dang *et al.* (2000) have reported seven varieties/genotype, i.e., *Brassica alba*, *B. carinata* (HC-1), *B. juncea* (DIR-1507, DIR-1522), and *B. napus* (GS-7027, Midas and Tower) showing multiple disease resistance including resistance to downy mildew.

Downy Mildew of Cucurbits

This is an important disease which affects many cucurbits. According to Butler (1918), "This parasite is an interesting example of a fungus, described on what seems to have been a wild plant, in an isolated part of the world and considered of not economic importance, which by its gradual spread to other countries and the damage that it has caused on new hosts has won for itself a position amongst the major enemies of cultivated plants." The disease was first discovered in Cuba in 1868. It has now been reported from various parts of the world. It is found on almost every cultivated cucurbit, but most frequently on cucumber. In addition, the pathogen can infect watermelon (*Citrullus laratus*) round melon (*Citrullus vulgaris*), parwal (*Trichosanthes dioca*), ridge gourd (*Luffa acutangula*), muskmelon (*Cucumis melo*), bottle gourd (*Lagenaria siceraria*) and vegetable marrow (*Cucurbita pepo*).

Symptoms

The pathogen attacks the leaves, causing at first pale yellow, angular patches, which deepen to a brownish yellow color as they grow older. On the lower side of these spots, a purplish downy growth appears during periods of high humidity. The growth is scanty and hardly discernible. Fruits are not directly attacked, but once the leaves are well infected, few fruits develop and those that appear often small.

Causal Organism

The causal organism is *Pseudoperonospora cubensis* (B. & C.) Rostow. This is an obligate parasite (Biotroph).

The mycelium is coenocytic and intercellular between the mesophyll cells of the leaf, which are penetrated only by short ovate haustoria. Sporangiophores arise in groups of one to five through the stomata. They are 200–300 µm long and 5–9 µm broad. The upper third is 3–5 times branched, either dichotomously or intermediate between dichotomous and monochotomous branching habits. The spore-bearing tips are sub acute; sporangia are greyish to olivaceous purple, ovoid to ellipsoidal, thin walled and with a papilla at the distal end. They measure 21-39 X 14-23 µm. The germination occurs through biflagellate zoospores. Oospores are not commonly formed.

Disease Cycle

The exact disease cycle is not known. According to Butler (1918), the pathogen is able to survive from season to season and cause infection because of the great diversity of climate and season in different parts of India. The abundance of suitable wild hosts would probably ensure the continuous growth of the parasite. Bains and Jhooty (1976) studied this aspect in Punjab and reported that during winter season, pathogen perpetuates in the form of active mycelium on self sown or cultivated sponge gourd. In areas where oospores are found, these may be an important source of survival and primary inoculum for initiation of the disease. The secondary spread of the disease is through dispersal of sporangia.

Disease Management

The control of the disease becomes difficult with protectant fungicides like Dithane –M-45, Copper oxychloride, Zineb, Difolatan and Chlorothalonil effectively check the disease if sprayed before the appearance of the disease. In order to control the disease in established infections, spray systemic fungicides, Ridomil MZ (0.25%) or Aliette (0.3%) (Jhind *et al.*, 1991).

Downy Mildew of Grapes (*Vitis vinifera*)

The disease is prevalent in all the grapevine areas of the world. It also affects other species of *Vitis.* In India, the disease is quite common in Maharashtra state and South India (Karnataka and Andhra Pradesh).

Symptoms

All the succulent tender parts of the vines are attacked, leading to a partial or total destruction of the foliage, dwarfing and killing of the shoots and rotting and checking of the berries. The fungus spreads rapidly, with profuse downy growth on the undersurface and corresponding greenish patches on the upper surface, which turn chlorotic with age. The mildew growth may cover the entire leaf blade which turns brown and withers. The disease may also involve floral parts and the fruit. Infected flowers die and drop off. The fruit becomes greyish and the skin hardens and shrivels. Very often they are mummified while still attached to the bunch. (Fig. 13.9).

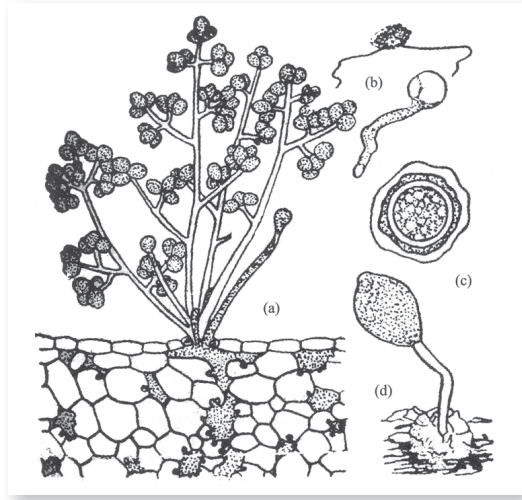


Figure 13.9 Downy of grapes: **(a)** Sporangiophores and sporangia emerging out of the grape leaves, intercellular mycelium with haustoria **(b)** Zoospores **(c)** Oospore **(d)** Germinating oospore.

Causal Organism

The pathogen responsible for grape downy mildew is *Plasmopara viticola* (Berkeley and Curtis) Berlese and de Toni. The mycelium of the pathogen is intercellular with vesicular haustoria. The pathogen produces large and branched sporangiophores with sporangia on small, rectangularly placed branches. The branches of the sporangiophores and their subdivision occur at right angles and are irregularly spaced. The sporangia germinate to produce biciliate, egg shaped zoospores, which swim in the film of water and germinate to enter the host tissue, causing fresh infections. Oospores are 25–35 mm in diameter with a rough epispore. They germinate after a period of rest by producing germ tubes on which sporangia are borne and which germinate in the usual manner and initiate the disease.

Disease Cycle

In the areas where grapevine is evergreen, the fungus produces sporangia (conidia like) throughout the year, otherwise the disease is carried from year to year in the form of oospores formed on leaf and leaf shoots. Climatic conditions primarily influence the occurrence and spread of this downy mildew. The most favourable temperature for the germination of sporangia is between 10°C and 23°C. Cool temperatures are necessary for infection. Humid and cloudy conditions are also essential for the germination of sporangia and zoospores.

Disease Management

Sanitary measures, such as cutting the primary source of infection by deep cultivation and ploughing, thus preventing the germination of oospores, are desirable practices. Removing and burning the diseased leaves, shoots and berries that may contain hibernating oospores helps in preventing epidemics.

The disease can be effectively controlled by giving three to five prophylactic sprays with 1% Bordeaux mixture.

The applications of copper and dithiocarbamate fungicides have been successfully used against the disease. In addition to Bordeaux mixture, the disease has been controlled with well-timed application of Blitox 50 (0.3%), Maneb, Zineb and Mancozeb (0.2%). According to Singh (1998), fruit sprays have been recommended at the following stages:

1. Immediately after pruning the grapevine leaves
2. 3-4 weeks after pruning the leaves
3. Before the vine buds open
4. When berries have formed and
5. Finally during growth of roots.

Combination of systemic and non-systemic fungicides is one such strategy for disease control. Hence application of Metalaxyl (Ridomil) with either copper or Mancozeb fungicides has been found to give good control of the disease.

Aureofungin (50 ppm) has been reported to be quite effective. Spraying of Ridomil (0.1%) and Aliette (0.2%) among the systemic group and Daconil (0.2%) and Delan (0.2%) among the non-systemic group have been found to be effective by Rawal and Muniyappa (1982).

The use of Metalaxyl fungicide along with either copper fungicides or dithiocarbamates has been found quite effective in controlling the disease. This practice is followed to avoid resistance development in the pathogen against Metalaxyl fungicide. Metalaxyl fungicides (Ridomil) inhibit the mycelial growth and sporangial formation through action of its volatile. According to Wicks and Hall (1990) Dimethomorph has been better than Metalaxyl–Dithiocarbamates combination because of its protective and curative activity. Another systemic fungicide Phosphorus (phosphonic acid) or its derivatives Phosethyl-Al is considered better than Metalaxyl group of fungicides because this fungicide is translocated from leaves via xylem or phloem to shoots and fruits where this fungicide breaks down to phosphorus acid and carbon dioxide. The phosphorus (phosphonic) acid acts directly on the fungus or indirectly affects the fungus, thereby inhibiting the fungal growth.

According to Wong and Wilcox (2001), Azoxystrobin—a strobilurin wide spectrum systemic fungicide when applied 0.5 mg/ml gives 61% control of the disease. Even the mixture of Azoxystrobin with Cymoxanil was more effective. Trifloxystrobin another Strobilurin fungicide is also effective against downy mildew of grapes. According to Reuveni (2003) Benthiavalicarb is a more recent fungicide effective against Oomycetes and has been used in the control of downy mildew of grapevine. This fungicide inhibits zoospores encystment, mycelial growth, and a sporangial production. It has a strong prophylactic and local activity.

Induction of resistance to downy mildew of grapes by treatment of DL-β-amino–n-butyric acid (BABA) has been reported by Reuveni *et al.* (2001). Elicitation of defence mechanism in grapevine against downy mildew by the application of Laminarin (beta-1-3 glucan laminarin) a phytochemical derived from *Laminaria digitata* (brown algae) is reported by Aziz *et al.* (2003).

According to Weltzien and Kettere (1986), the application of water extract of compost horse manure–straw soil mixture develops resistance in plant against *P. viticola.* The mode of action is the result of biological control through enhanced microbial activity. Even *Fusarium proliferatum* has been identified as

biocontrol against *P. viticola*. The hyphae of the mycoparasite (*Fusarium* sp.) coil around and penetrate the sporangiophores of *P. viticola*. Cultivars Amber Queen, Champion and Digraset have been found highly resistant while cultivars Athens, Buckland, Sweet Water, Goethe, James, Malaga are moderately resistant to downy mildew of grapes.

Downy Mildew of Bajra (*Pennisetum typhoides*)

Downy mildew and green ear disease of bajra is a very serious disease in all bajra growing areas of India. In also occurs in many parts of Africa. In India, it was first reported by Butler in 1907 and Kulkarni in 1913. The disease was not considered serious and did not attract much attention since damage was only severe when pearl millet was grown in low-lying, poorly drained areas. With the release of new high-yielding varieties and hybrids, the disease attracted much more attention. Downy mildew is now considered a major problem in the pearl millet crop, not only in India, but in the African countries too. Losses may be as high as 30% in high yielding varieties in India. During the Kharif season of 1975, there was an outbreak of the disease on an unprecedented scale on hybrid bajra covering several thousand hectares in Karnataka and Maharashtra, recording almost 100% loss. Bhat (1973) has given information on the geographical distribution of this disease. Nene and Singh (1976) and Thakur (1992; 1996) have reviewed the work on this disease.

Symptoms

The infected plants are dwarfed because of shortening of the internodes, and tillering is excessive. The foliage becomes pale and chlorotic and on the underside the leaves are covered by the whitish cover of the sporangia (Fig. 13.10 a) which are formed in abundance during dewy nights. The leaves may become distorted and wrinkled and tend to split into threads. Shedding of the leaves is more common in the jowar downy mildew caused by *Sclerospora sorghi.* The tearing and shedding of sorghum leaves has been shown to be due to the activity of pectolytic enzymes of *S.sorghi* (Vidhyasekaran *et al.*, 1973). The oosporic stage of the fungus is more predominant and the ears are transformed into leafy bearded structures. Three types of symptoms are seen (Fig. 13.10 b): (1) normal cob length and the whole cob converted into green leafy bearded structure, 2) normal cob length but the lower half converted into leafy bearded structures and the upper half bearing normal grains and 3) the whole inflorescence reduced and converted into leafy bearded structures without bearing any grains. The infection converts the various floral parts, including glumes, palea, stamens and pistils into green, linear, leafy structures of variable lengths.

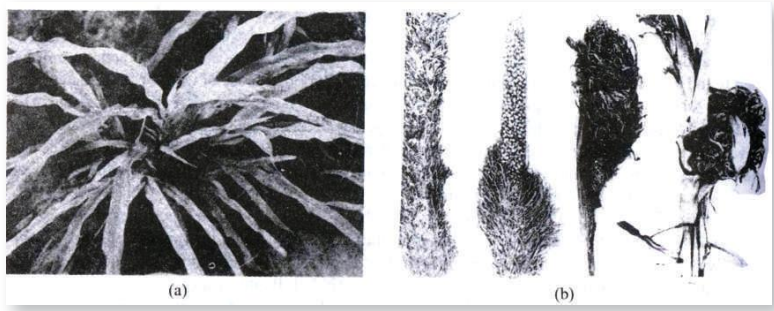


Figure 13.10 **(a)** Downy mildew symptoms on bajra due to *Sclerospora graminicola* (a) on young seedlings and **(b)** Ears showing symptoms

Causal Organism

Sclerospora graminicola (Sacc.) Schroet. is the causal organism of the downy mildew and green ear disease of bajra. The fungus is an obligate parasite. Arya and Tiwari (1969), and Tiwari and Arya (1969) reported saprophytic growth of *S.graminicola* on White's basal medium supplemented with casein hydrolysate, 2,4D and kinetin and on callus tissue. The mycelium is found in all parts of the systemically infected parts. The hyphae are intercellular with bulbous haustoria. They are large aseptate and up to 10 μm in diameter. These hyphae are found in the ground tissue of the stem and the mesophyll of the leaves. Branches may penetrate into the bundles of the leaves. The sporangiophores emerge in clusters through stomata. Each sporangiophore measures about 100 mm in length and 12–15 μm in breadth. It is unbranched in the lower part but usually gives out a few thick, short branches di-or tri-chotomous at the tip. Sporangia are usually produced during the night. They are hyaline, broadly elliptical, slightly pointed or papillate at the free end and with a free smooth wall. They measure 13-34 × 12-23 μm in size. The mature sporangia fall off soon and germinate in the surrounding film of water by producing zoospores. There seems to be an agreement that the temperature below 15–25°C favours the formation of the sporangial stage (Safeeulla and Thirumalachar, 1956). Suryanarayana (1965) considered low temperature (even10°C) to be favourable. But Safeeulla and Thirumalachar (1956) claimed that temperature below 15°C is unfavourable. Suryanarayana (1965) observed the sporangial stage at a temperature above 28°C.

Relative humidity above 75% is considered necessary for the stimulation of sporangial production. However, in general, a saturated atmosphere with a film of water on the leaf is considered best. An optimum combination of high humidity and favourable temperature usually occurs in July, August, and September in North India and the disease occurs more frequently during these months.

Weston (1924, 1929) and Weston and Weber (1926, 1928) studied *Sclerospora graminicola* in detail, Weston (1924) claimed that asexual sporulation takes place only nocturnally and this was accepted for nearly 30 years. Safeeulla and Thirumalachar (1956), however, demonstrated that nocturnal production of sporangia is result of the coincidence of natural factors, and the important factors regulating the sporangial stage are: 1) An interval of 15–20 hours between two successive crops of sporangia (this period is essential for the organization of primordia of the sporangiophores beneath the stroma), 2) Moisture film on leaves and 3) Temperature around 25°C. Under these conditions it should be possible to manipulate the artificial production of sporangia at any time. Bhat (1973) confirmed these observations. According to Suryanarayana (1965), rainfall appears to be of no consequence in the prevalence of the disease. Sporangia, on germination produce 3–13 zoospores.

Sexual reproductive organs are mostly produced in the leaves and malformed spikelets. The oospores are produced in large numbers in the host tissues. The oospores are round and have three walls. The oogonial wall is persistent and is visible in the form of regular folds on the mature oospores. Although the germination of oospores by 1–4 germ tubes has been observed by Chaudhury (1932), Hiura (1935), McDonough (1938), and Suryanarayana (1956), but the subject of oospore germination is still unclear. Arya and Tiwari (1969) established the fungus *S.graminicola* on healthy callus of *Pennisetum typhoides* on Casein hydrolysate medium (CHDK). Singh, S. D. (1995) has given the size of oospores as 22–35 (av. 32 μm).

Disease Cycle

The disease is primarily soil-borne. The oospores which are abundantly present in the diseased leaves fall on the ground and perennate during summer. It is reported that oospores can survive from eight

months to ten years. Oospores give increased infection (therefore germinate better after weathering) and one-year-old oospores give more infection (98%) than fresh or more than one-year-old oospores (Bhat, 1973). Earlier it was thought that oospores have a dormant period. Bhat reported that newly formed oospores gave 55 % infection but he did not mention clearly how "new" these oospores were. Safeeulla and his group (Safeeulla, 1970; Bhat, 1973) have detected oospores in the roots of pearl millet. These are formed late in the season and can thus survive in the same plots in the left-over roots. Sundaram (1970) stated that the oospores passed through the intestine of cattle and further claimed that these oospores could produce diseased plants even after the composting of cattle dung. This was confirmed by Bhat (1973).

Although sporangia are produced in millions on plants, yet the role these play in the spread of the disease is not clear. Most reports indicate that their role is not significant. Bhat (1973) also stated that the role of asexual spores in epidemiology is rather limited under certain conditions, such as those prevalent in Mysore (South India) and that sporangia are unsuited for long distance dissemination. However, according to Thakur (1992, 1996), positive role of sporangia/zoospores in the secondary spread of the disease has been established in the north west Indian states where it has been observed that atmospheric temperature between 25–30°C and RH above 85% during the months of July–August favoured high level of secondary infection.

The disease is thought to be seed-borne, and Butler (1918) was the first to suggest that the oospores might be carried on the seeds during harvesting and threshing. He failed to collect any mycelium or oospore in sound seeds collected from partly infected pearl millets. Arya and Sharma (1962) detected traces of the mycelium in the embryo in the pearl millet seeds, and Tiwari and Arya (1966) obtained diseased plants from seeds collected from heavily infected ear heads. Suryanarayana (1962) also observed the mycelium inside the seeds of pearl millet and could not obtain diseased plants form such seeds. Singh and Pushpavathy (1965) failed to observe mycelium in embryos but did find it in the ovary wall. Sundaram *et al.* (1973) reported that the percentage of embryos of seeds of HB-4 and others showing the presence of mycelium varied between 9.5 and 13.6. To confirm whether the mycelium observed was of *Sclerospora graminicola,* these workers took seeds of one type of hybrids and male sterile in pot culture and recorded the incidence of the disease on the seventeenth day after germination until flowering. Bhat (1973) failed to observe oospores of mycelium in microtome sections of pearl millet seed collected from infected ears. Studies made by Govindu and Kulkarni using ultrasonic vibration technique indicated that even if bajra downy mildew is internally seed borne as reported by some workers, it is not of much consequence in starting primary infection (Govindu and Kulkarni, 1977). According to Siddiqui and Gaur (1978) and Muthuswamy and Narayanasamy (1981) oospores have been seen on the seeds and such seeds planted in sterilized soil produced diseased plants. According to Siddiqui and Gaur (1970) oospores carried with the seed and present in the soil play the main role in the disease. According to Shetty *et al.* (1970), the pathogen may be present in bajra seeds either as oospores externally or in the form of dormant mycelium internally. Arya and Tiwari (1970) studied the amino acid composition of bajra tissues in healthy and diseased states *in vivo* and *in vitro.*

Disease Management

Alterations in the date of planting (early planting), modification in planting methods (transplanting instead of direct sowing), avoidance of ratooning, roguing and gap filling, deep ploughing and sun baking of soil, removal of infected crop residues, avoidance of monoculture. Crop rotation, avoidance of low-lying fields and water-logging will reduce the disease incidence. Application of balanced fertilizers particularly nitrogen and cultivation of trap crops help considerably in disease management. Since the

disease may be carried partly with the seeds, seed treatment may be of help in destroying the spores sticking to the surface of the grains. Seed treatment with Ceresan and Captafol is recommended. The all India coordinated Millet Improvement Programme had recommended seed treatment with Agrosan GN (0.1% + Thiram 0.4%) which gave about 50% control of the disease. However, organomercurials are not recommended. Recently, metalaxyl compounds have been found very effective. Muthuswamy and Narayanasamy (1981) had reported seed treatment with Ridomil (8 g/kg of seed) followed by one spray of 0.1% Ridomil 20 days after planting. Appaji *et al.* (1989) recommended that seed dressing with Apron SD-35 (8 g/kg of seed) followed by a foliar spray. Twenty-five days after sowing, with Ridomil-MZ-72 WP (Metalaxyl + Mancozeb) at 500 ppm concentration or with Ridomil-MZ-280 FW (Metalaxyl + Zineb) at 1000 ppm concentration was the best combination. Gupta and Verma (1991) also recommended that seed treatment with Apron SD-35 (2.5g/kg of seed) controlled the diseased up to 30 days after sowing. There are some reports that seed treatment with crude extracts of *Vinca rosea*, *Ocimum sanctum*, *Allium sativum*, *Datura stramonium*, *Azadirachta indica*, and *Thuja sinensis* reduces incidence of disease.

Breeding of resistant varieties is an important control measure for this disease. Singh, S.D. (1995) reported ICMH 451, and Pusa 23 resistant to downy mildew. Hybrid ICMH88088 produced by ICRISAT has also remained free from downy mildew. Some of other resistant varieties are HB-5, NHB-10 and NHB14. Cultivars WC-C75 and ICTP-8203 have shown durable resistance.

Downy Mildew or Leaf Shredding Disease of Sorghum

The downy mildew of Sorghum is prevalent in many countries in Asia and Africa and in Italy. In India it is common in the states of Tamil Nadu, Andhra Pradesh, Madhya Pradesh, Maharashtra, and Uttar Pradesh. The disease is more severe in fodder Sorghum varieties than in grain crops. The pathogen also attacks maize and teosinte. Severe infection occurs every year in parts of Mysore and Maharashtra with infection ranging up to 25%. Infected plants rarely bear ears and therefore, the loss in grain yield is considerable. Anahosur and Laxman (1991) have reported 74 percent loss in yield in a certain genotype with 100% infection.

Symptoms

Disease symptoms (Fig. 13.11) vary with the stage at which the plants are affected. When the infection is systemic and initiated by oospores lying in the soil or lodged in the whorls of the young plants, the conidia are produced on the growing leaves. This is seen as a whitish soft downy growth mostly on the lower surface of the leaves. There is a corresponding yellowish discoloration on the upper surface. Such plants continue to grow but finally dry up and die before the ears are formed. The infected leaves become brown and the tissues tear into strips. The midrib remains intact. Oospores are formed in the vascular bundle (sheath), the leaf blades tear into strips along the brown streaks, producing characteristic symptoms. However, the tearing and shredding of sorghum leaves has been ascribed to the actions of the pectolytic enzymes of pathogen (Vidhyasekaran *et al.,* 1973).



Figure 13.11 Downy mildew symptoms due to *Sclerospora sorghi* on *Sorghum vulgare*

Causal Organism

Downy mildew of sorghum is caused by *Peronosclerospora sorghi* (Ito) Shaw, earlier known as *Sclerospora sorghi.* The mycelium is systemic in primarily infected plants. Intercellular, non-septate hyphae are present in the parenchymatous tissues of the roots and stem and the mesophyll of the leaves. The haustoria are finger-shaped.

The conidiophores arise singly or in clusters through the stomata. Each conidiophore has a stout basal portion with one or two transverse septa. The conidiophores measure 200 μm in length and branch profusely into a series of pointed sterigmata. The ultimate branches of the conidiophore bearing the conidia spread out in a hemispherical plane. Conidia are globose, hyaline, 15–29 μm in diameter and lack the apical papilla. These never form zoospores. The conidia are smaller than those of *Sclerospora graminicola.* The oosporic characters resemble *Sclerospora graminicola.*

According to Uppal and Desai (1932), the optimum conidial production occurs at night between 3 a.m. and 4 a.m. in Pune (India) under field conditions. Safeeulla and Thirumalachar (1956) observed that the natural development of the conidia under field conditions depends on the coincidence of a saturated atmosphere with a film of water on the leaves and a temperature of 21°C. Conidial formation is suppressed at a temperature of less than 15°C.

Oospores of the pathogen germinate when mature by means of one or more germ tubes. The oospores are believed to retain viability for 3–4 years. Oospores exposed to field conditions, as in the case of *S.graminicola* (Suryanarayana, 1954), germinate better than if kept in the laboratory. Maize and teosinte are infected but not pearl millet and *Setaria* spp. Varieties of sorghum are differentially susceptible to the pathogen.

Disease Cycle

The pathogen is soil borne (oospores in the soil) as well as seed-borne. Oospores can remain viable in plant debris for 15 months. According to Rajasab and Ramalingam (1985), the hay stacks and shady places near field serve as source of inoculum. The pathogen can multiply by some weed hosts in off season. Secondary spread of the disease is carries out by conidia and the disease is more severe in the welter parts of the field.

Disease Management

The pathogen is soil-borne in the form of oospores lying in the soil. It is, therefore, very difficult to control the disease. Seed treatment is helpful in eliminating the externally seed-borne infection (in the form of oospores sticking to the surface). Cultural control by deep ploughing, rotation of crop, and eradication of the diseased plant before the formation of oospores reduces the inoculum potential.

Anahosur and Patil (1983) had recommended spraying of metalaxyl 25WP (Ridomil) at the rate of 2 g/litre of the water for complete control of the disease. Potassium azide (soil fungicide) was found useful in controlling the disease. Seed treatment with Ridomil at 1 or 2 g/kg is not very effective but higher doses are phytotoxic. The disease can be effectively controlled by breeding resistant varieties.

The disease can be effectively controlled by breeding resistant varieties/germplasm, i.e., IS 1547, IS 4696, IS 5665, IS 5743. These germplasms have been found free from downy mildew disease in India and abroad.

White Rust of Crucifers

White rust of crucifers is common on several cruciferous crops, such as cabbage, turnip, rape seed, mustard and radish and occurs throughout India. According to Saharan *et al.* (1984) the yield loss due to floral infection may be up to 54.5%.

Symptoms

All aerial parts of the plant are attacked. White or cream-yellow pustules of various sizes and shapes appear on the surface of the leaves (Fig. 13.12 a). In some crucifers, the leaves may become thick, fleshy and inrolled and when infection is severe, the size of the leaves may be reduced and even the entire plant may be stunted. The spots are generally produced on the lower surface of the leaves. When young stems and inflorescence are infected, the fungus becomes systemic in the tissues and stimulates various types of deformities of which hypertrophy is most pronounced. Due to the hypertrophy and hyperplasia of the tissues the floral parts show swelling and distortions. The peduncle, pedicel, sepals, petals and carpels are hypertrophied. The petals may become like sepals and the stamens may become leaf like or occasionally like carpels (Fig. 13.12 b). The latter may be open, while the ovules are usually atrophied. The parasite also stimulates cell activity, leading to and increase in cell size, cell division and formation of chlorophyll and starch at places where none is usually formed.



Figure 13.12 White rust of crucifer: **(a)** Symptoms on the leaf **(b)** Hypertrophied inflorescence

Causal Organism

Albugo candida (Lev.) Kunze, also known as *Cystopus candidus* Lev., is an obligate parasite. The mycelium is intercellular, forming knob-shaped haustoria in the host cells. The fine structure of haustoria of *A. candida* has been studied by Berlin and Bowen (1964). Hyphae form the endophytic mycelium collect beneath the epidermis. These dense masses of hyphae form the sporangial beds (sori). The sporangiophores are formed by the vertical growth of broad, short, clavate stalks under the epidermis which is raised up due to the pressure of these structures. The raised epidermis is separated from the underlying

cells. The sporangiophores are free from each other laterally and are very thick, especially towards the base. Sporangia are formed in basipetal succession in chains (Fig. 13.13). Sporangia are hyaline, nearly spherical and 14-16 × 16-20 µm in size. They germinate by means of a germ tube or by the formation of zoospores. The germination of the sporangium is clearly dependent on the prevailing temperature, the maximum being 25°C. The optimum temperature for germination is 10°C. The oogonia and antheridia are formed from the intercellular mycelium in the intercellular spaces (Fig. 13.13). Oospores are formed only in some hosts and their formation is conditioned by certain environmental factors. They are globular, provided with a tuberculate brown of rest and by the formation of zoospores (Fig. 13.13). The rest period can be shortened if they are chilled at 4°C for a week.

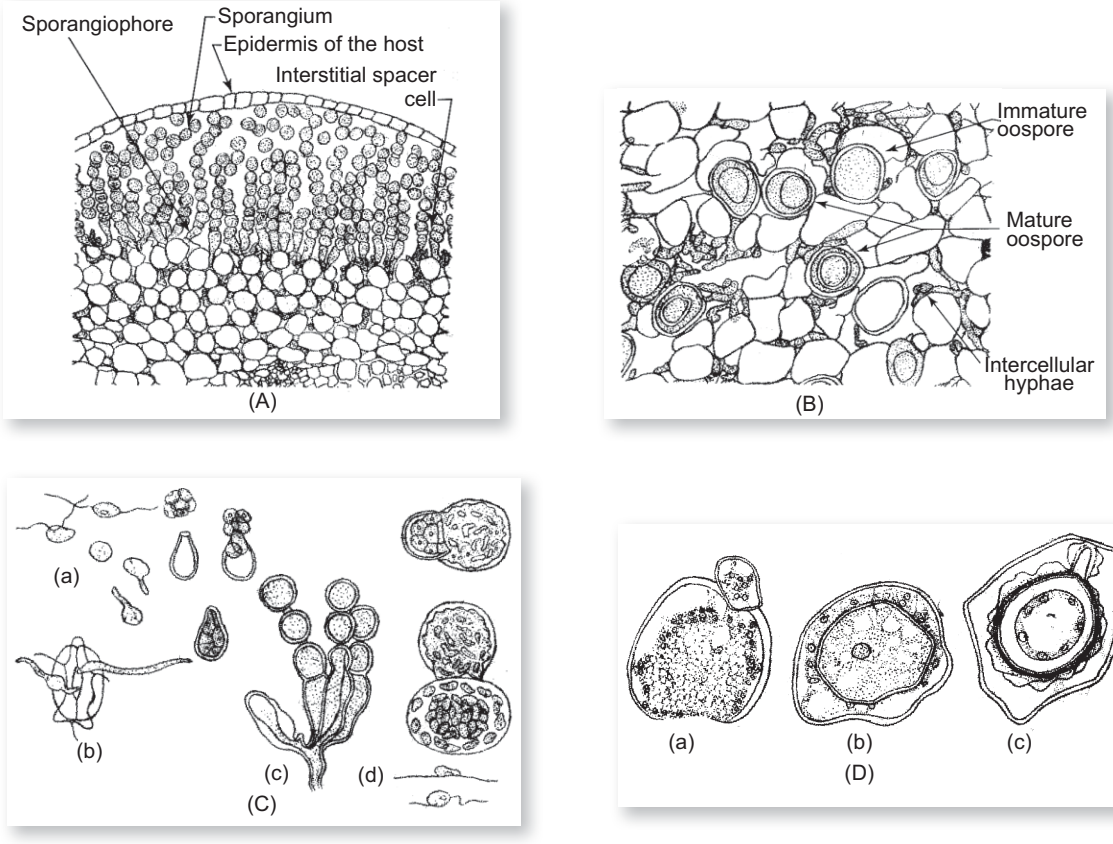


Figure 13.13 **(A)** *Albugo candida* section through a conidial (sporangial) pustules showing conidiophores, conidia and intercellular mycelial with haustoria **(B)** Intercellular mycelial and various stages of oospore development **(C)** Stages in the life cycle of *A. candida* **(a)** Zoospore formation **(b)** Stomatal penetration by germ tube **(c)** Conidiophores **(d)** Oospore germination **(D)** Formation of oospores **(a)** Oogonium and antheridium **(b)** Formation of oospore wall (c) Mature oospore in section

Disease Cycle

The pathogen perpetuates through the oospores lying in the soil in diseased plant debris. Perennial weed hosts serve as a source of primary inoculum. Secondary spread of the pathogen is carried out by

sporangia and zoospores. Cool, moist weather favours the development of the disease. Lakara and Saharan (1990) while working at CCS H.A.U., Hisar, reported that the leaf blisters progressed at a faster rate on susceptible cultivars from December 15 to February 15 when the RH was more than 65% and temperature less than 15°C. Saharan *et al.* (1988) and Kumar *et al.* (1995) further confirmed and reported that crops sown late, after 19th October showed increased incidence of disease. Mehta and Saharan (1998) have developed the prediction model for white rust of crucifers.

Disease Management

A. Cultural Methods

1. Clean cultivation should be encouraged.
2. Destruction of weeds in and around the field should be given top priority.
3. The disease can be controlled by crop rotation.

B. Chemical Control Kapoor and Sugha (1995) have recommended Ridomil and Aliette for white rust of crucifers. Other fungicides which are also effective include Bordeaux mixture (0.8%), Difolatan (0.3%), Daconil (0.1%), and Dithane M-45 (0.2%).

Sources of resistance to the disease in *Brassica napus* and *B.juncea* are reported by Saharan and his associates (1988).

Good sources of resistance have been listed in some species and genotypes in rapeseed-mustard having multiple disease resistance and these include *Brassica alba*, *B. carinata* (Hc-1, Pcc-2), *B. juncea* (DIR-1507) and *B. napus* (Midas, Tower). According to Singh U.S. *et al.* (2002) pre- or co-inoculation with *Peronospora parasitica* or avirulent strain of *Albugo candida* provide protection against the development of *Albugo candida*.

Some Other Diseases Caused By Oomycota

Name of the Disease	Causal Organism	Symptoms	Disease Cycle	Management
Downy mildew of maize	*Peronosclerospora sorghi*	Development of long, rather broad, chlorotic stripes on the leaf which are initially yellow in color and finally turn brown	Pathogen can perennate through oospores in soil and on alternate host	Long crop rotation, burning of crop debris, Dithane M-45, Ridomil 25WP, Apron-35 (as seed treatment) are effective. Phil-DMR-1 is a resistant variety
Brown stripe Downy mildew of maize	*Sclerophthora rayssiae var. zeae*	Narrow chlorotic spots with well defined margins which extent in parallel fashion between the veins appear on the leaf plates	Soil borne	Fungicides like Dithane M-45, Demosan in combination with rouging of infected leaves, neem oil and systemic fungicides like Ridomil 25WP are effective

(*Contd.*)

(*Contd.*)

Name of the Disease	Causal Organism	Symptoms	Disease Cycle	Management
Sugarcane Downy mildew of maize	*Sclerospora sacchari* (Now called *Peronosclerospora sacchari*)	Main symptom is the development of long rather broad chlorotic stripe along the entire length of leaf, young leaves show downy growth more clearly than older leaves	Pathogen can perennate through oospores in soil and on alternate host	Diseased crop residue should be destroyed. Dithane M-45 is effective in checking the disease.
Philippine Downy mildew of maize	*Sclerospora philippinensis* or *Peronosclerospora philippinensis*	Leaves become pale yellow with wooly white growth on under surface, plants possess shorter internodes, infected plants may not produce any cob.	Pathogen survives on alternate host	Long crop rotation is recommended

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Questions

1. What are downy mildews and how do they differ from powdery mildews?
2. What do you understand by physiological specialization? Discuss the phenomenon, giving suitable examples from downy mildews.
3. Suggest the various methods of controlling downy mildews.
4. Name some important plant diseases caused by *Pythium* and *Phytophthora* spp.
5. Describe the predisposing factors and the disease cycle of late blight of potato.
6. Enumerate the major differences between the symptoms of downy mildew and rots.
7. Describe the mechanism of disease development or the host–parasite relationship in white rust of Crucifers.
8. What is obligate parasitism? Describe the life cycle of *Albugo candida* along with its methods of disease control.
9. Write notes on the following with special reference to controlling the diseases:
   (i) Late blight of potato
   (ii) White rust of crucifers
   (iii) Downy mildew of grapes
   (iv) Downy mildew of cucurbits
   (v) Leaf blight of Colocosia
   (vi) Collar rot of apple
   (vii) Damping of diseases

Powdery Mildews

14

Powdery Mildews

The members belonging to the order Erysiphales are responsible for diseases which are popularly called powdery mildews because of the powdery appearance of the conidia on infected plants. They are all obligate parasites of angiosperms, often showing great physiological specialization that is having strains or races confined to a narrow range of host plants. The members of Erysiphaceae extend from the tropics to the Arctic and from below sea level to 4000 m (Hirata, 1966). The major modern taxonomic treatments are by Salmon (1900), Homma (1937) and Blumer (1967). Other treatments, exclusive of routine treatments in text books, are by Brundza (1933), Sawada (1914), Hirata (1986) Yarwood (1957) and Heffer *et al*. (2006). The genetics of Powdery mildew has been discussed by Moseman (1966). Formerly, fruiting structures of powdery mildew containing asci were called as cleistothecia but now a better term is used for these: Chasmothecium. Chasmothecium is a closed fruiting body having no predefined opening but with the asci arranged in a single basal fascicle.

The taxonomy of powdery mildew fungi (order *Erysiphales*) recently underwent extensive revision based on DNA sequence data. According to Heffer *et al.* (2006), powdery mildew genera are now grouped into five tribes: *Phyllactineae* (for *Phyllactinia* and *Leveillula*); *Erysipheae* (for *Erysiphe, Microsphaera*, and *Uncinula*); *Blumeriae* (for *Blumeria*); *Golovinomyceteae* (for *Golovinomyces*) and *Cystotheceae* (for *Podosphaera* section *Podosphaera*, *Podosphaera* section *Sphaerotheca*). Currently these genera are identified using the following five features: (i) location of mycelium in the host tissue (epiphytic or endophytic); (ii) Conidial production (singly or in chains, pseudochains or true chains; (iii) number of asci in the chasmothecium (cleistothecium) one or several; (iv) type of appendages (mycelial, bulbous base, circinately coiled, or dichotomously branched; and (v) host specificity.

Key to the Genera of Powdery Mildew Fungi (Based on Five Features) (Aneja and Mehrotra, 2011)

Conidia formed Singly

Mycelium Partially Endophytic

Chasmothecium Contains Several Asci

Appendages straight, bristle-like with bulbous base……*Phyllactinia*

Appendages simple, hyphae like……*Leveillula*

Conidia Formed Singly (or in Pseudochains)

Mycelium Epiphytic

Chasmothecium Contains Several Asci

Appendages simple, hypha-like……..*Erysiphe*
Appendages coiled or hooked at tip……*Uncinula*
Appendages branched dichotomously at tip……*Microsphaera*

Conidia Formed in True Chains

Mycelium Epiphytic

Chasmothecium Contains Several Asci

Appendages short, simple, hypha-like; infect grasses……..*Blumeria*
Appendages hypha-like, flexuous, simple or irregularly branched; infect dicots.....*Golovinomyces*

Chasmothecium Contains a Single Ascus

Appendages simple, hypha-like…….*Sphaerotheca*
Appendages bristle-like, branched dichotomously at tip….*Podosphaera*

The old genera *Uncinula* and *Microsphaera* have been considered as subsection of *Erysiphe* (e.g. *Erysiphe* section *Uncinula*, *Erysiphe* section *Microsphaera*). *Sphaerotheca* is considered as section of *Podosphaera* (e.g., *Podosphaera* section *Sphaerotheca*). However, at present the older concept of the genera has been followed in this text in order to avoid confusion in the naming of the pathogens.

Erysiphaceae are parasitic on some 40,000 plant species representing well over 40 orders of angiosperms (Hirata, 1986). Examples of economically important hosts include cucurbits, apples, grapes, cereals and various ornamental plants such as roses etc., while some powdery mildew diseases are extremely destructive. Some of the important and destructive powdery mildew diseases are as follows:

1. *Uncinula necator* → Powdery mildew of grape vine
2. *Sphaerotheca humuli* → Powdery mildew of hops
3. *Sphaerotheca pannosa* var. *rosae* → Powdery mildew of roses
4. *S. fuliginea* → Powdery mildew of cucurbits
5. *S. mors- uvae* → Powdery mildew of gooseberries
6. *Podosphaera leucotricha* → Powdery mildew of apples
7. *Erysiphe cichoracearum* → Powdery mildew of cucurbits and many other plants
8. *Blumeria graminis* → Powdery mildew of cereals and grasses
9. *E. polygoni* → Powdery mildew of peas
10. *E. cichoracearum* → Powdery mildew of cucurbits
11. *Microsphaera alni* → Powdery mildew of lilac
12. *Leveillula taurica* → Powdery mildew of *Medicago sativa*
13. *Phyllactinia dalbargiae* → Powdery mildew of *Dalbergia sissoo*

The Erysiphaceae have dark, spherical perithecia as well as colorless septate, hyphae and conidiophores and conidia, which appear white on plant surfaces. They occur primarily on leaves, buds, flowers, and fruits. Some of their other characteristics are the occurrence of haustoria in the epidermal cells of their hosts; rapid development in rain free seasons; high water content of their large, turgid, passively liberated, air-borne conidia; diurnal periodicity with respect to several characters; compatible association with their natural hosts, macroscopically visible hypersensitive reaction shown by some incompatible host reaction; slow injury to their hosts; and vulnerability to control by sulphur fungicides. The powdery mildews are favoured by cool, shaded environments and by succulent host tissues. The

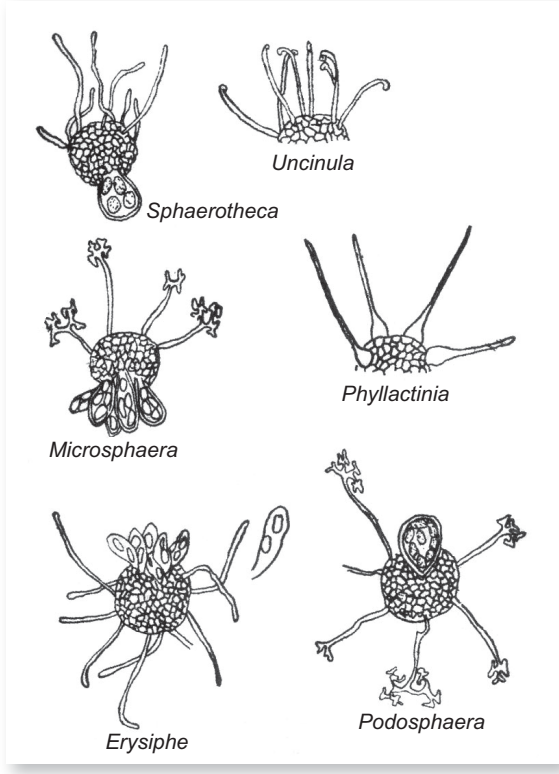


Figure 14.1 Some important genera of Erysiphaceae

appearance of powdery mildew is due to the conidiophores and conidia. Three types of conidiophores can be recognized within the family.

1. The Oidium type, which consists of a short strip of one of more cells, a cell which gives rise to the conidia, that is a generative or spore mother cell, and chain of maturing conidia. This is found in species of *Erysiphe.*
2. The Oidiopsis type, in which the conidiophores are branched and typically arise from a stroma, as in *Leveillula.*
3. The Ovulariopsis type with clavate conidia, and conidia are not in chains as in *Phyllactinia* species.

There are two principal types of haustoria: the globular, formed by most Erysiphaceae; and the digitate, formed by *Erysiphe graminis* now *Blumeria graminis.* Usually the fungus penetrates only the epidermal cells, but in those species where hyphae enter the stomata, haustoria develop from hyphae in the intercellular spaces. One species, *Uncinula salicis,* is unique in that several haustoria may arise from one appressorium and may extend to the subepidermal layer of cells.

Once penetration has taken place, further germ tubes are produced from the conidium, and haustoria are developed from appressoria borne laterally on them. The fungus grows out laterally from the point of inoculation. About four days after inoculation, sporulation starts near the site of inoculation and then extends outwards. An interesting feature is that many species show a marked diurnal cycle of conidial production.

After the conidial stage has been apparent for some time, usually late in the season, cleistothecia develop in the same mycelial felt. The factors influencing cleistocarp production have been much studied but there is little indication of any general features.

Diseases Management

The powdery mildews are effectively controlled by sulphur dust. On certain plants, such as cucurbits and some apple varieties, sulphur powder cannot be used as it causes injury. Such plants are called sulphur shy and for them there are various new alternatives, the most important of which is Dinocap (2-[1-methyl-n-heptyl]-4, 6-dinitrophenyl crotonate) known commercially as "Karathane". This is used as a wettable powder or dust. Dinocap is both protective as well as therapeutic. In 1960s, 2-aminopyrimidine derivatives- dimethrimol, and ethirimol were introduced as effective seed dressing fungicides. Calixin or Tridemorph, a morpholine derivative is quite effective against barley mildew and rose powdery mildew. Triazole fungicides such as Triadimefon (Bayleton), Triadimenol (Bayton), Etaconazole (Vangard), Flusilazole, Myclobutanil, and Penconazole are currently being used for controlling powdery mildews.

Powdery Mildew of Wheat (*Triticum* spp.) and Barley

The diseases occur throughout the world. In India it causes considerable damage in some areas when the weather is cool and cloudy. The diseases are common in the sub-montane areas of northern India and in certain parts of Rajasthan, Haryana, and Punjab. Powdery mildew occurs on wheat, barley, rice, and oats and also on grasses, such as species of *Agropyron*, *Bromus, Dactylis*, and *Elymus*. According to Kapoor and Singh (1993), the disease adversely affects ear length and grain weight and is quite common in low lying fields. Losses on well drained lands are less but if the crop is cultivated on the same land every year, the damage may be very high. Grewal and Aujla (1982) reported the occurrence of powdery mildew along with downy mildew and leaf rust on wheat in Punjab.

Symptoms

A greyish-white powdery growth occurs on the leaf. Such symptoms are also found on leaf sheaths and floral parts. The pathogen develops numerous superficial colonies. The mycelium forms a flocculent growth, which is white when the conidia are being formed and then changes to grey or black when the cleistothecia are formed. The leaf size and the number of leaves get reduced in infected plants. They are not shed become weak, twisted and deformed. In the diseased plant, transpiration and respiration are increased but photosynthesis is reduced considerably (Fig. 14.2).

Physiology of the Diseased Plants

Infection results in temporary stimulation of respiratory activity of the tissue and in chlorosis underneath and around the fungus. There is also an increase in transpiration of the host and further water loss due to the transpiration of the fungus. The host plant then becomes thin, which is reflected in reduced yield and in some reduction in the normal plumpness of the kernel. After chlorosis of the tissue beneath the fungus mycelium has occurred, the reproductive activity of the mycelium declines, the sporulation is more profuse around the advancing edge of the lesion.

Chlorophyll may then reappear, giving rise to conspicuous green spots. Apparently this chlorophyll does not become active in photosynthesis.

Causal Organism

The pathogen responsible for the powdery mildew of cereals is *Blumeria* (*Erysiphe*) *graminis*. *Blumeria* as a new genus was created in 1973 for *Erysiphe graminis* by its larger ascocarps, two types of mycelia digitata haustoria, bulbous swelling of the conidiophores and grass hosts. The variety of the species that attacks wheat is known as *E.graminis* f. sp. *tritici* while on barely it is known as *B. graminis f.* sp. *hordei.* The primary mycelium forms superficial, scattered, elongate to ellipsoid effuse patches, which are at first white but turn to pale brown or grey; the haustoria are elliptic and long finger-shaped appendages radiating from both ends. Conidiophores arising from the primary mycelium are rather short with a terminal generative cell and a swollen basal cell. Conidia are in long chains, ellipsoid and are 25-37 × 12-17 mm. Secondary mycelium is persistent and is interlaced. It is at first white, but turns grey-white to brown, is sparingly branched, shiny, 4–6 mm wide, bearing numerous unbranched, thick walled rather rigid bristles, 200–400 mm long and 4–7 mm broad, forming a thick felt. Cleistothecia (a better term is now used is chasmothecium, a closed fruit body having no predefined opening but with asci arranged in a single basal fascicle) immersed in the felt, at first globose, become strongly depressed often cuptulate, 135–250 mm in diameter; wall cells small, indistinct. Appendages poorly developed, rudimentary myceloid, subhyaline to pale brown. Asci 8-25, ovate to more or less distinctly pedicellate, 70-108 × 25-40 µm. Ascospores 8, rarely 4, elliptic subhyaline to pale brown, 20-25 × 10-14 µm, formed only after the host plant has completely dried up.

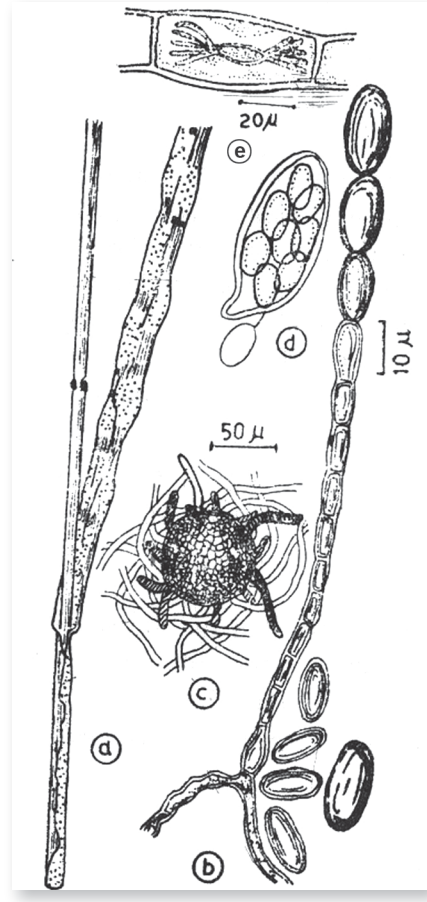


Figure 14.2 Powdery mildew of wheat caused by *Erysiphe graminis*: **(a)** symptoms **(b)** conidiophores and conidia **(c)** cleistothecium with rudimentary appendages and enveloping mycelial bristles **(d)** ascus and ascospores **(e)** haustorium with finger-shaped appendages

Development of powdery mildew on different wheat cultivars has been critically studied by Sharma and Singh (1990c) and a mathematical correlation was worked out between incidence and severity of the disease.

Diseases Cycle

The disease is soil borne through cleistothecia where conditions are favourable. Conidia do not retain their viability at high temperatures. Conidia are produced most abundantly in a relatively cool and moist

environment. They are dispersed readily by air currents and are the chief secondary inoculum during the usual cereal growing season. Upon germination, the germ tube forms an appressorium in juxtaposition with the host cuticle. A hyphal peg penetrates the cuticle and the subcuticular wall forms a haustorium in the epidermal cell. There is no further advancement of the pathogen within the host tissues. The remainder of the mycelial thallus as well as the conidiophores, conidia and cleistothecia are entirely extramatrical (i.e., on exterior of the host substrate).

Ehrlich and Ehrlich (1963) studied the ultrastructure of the haustorium with the aid of an electron microscope. They suggested that a sheath is formed around the haustorium from the host or pathogen or both, which may become a source of host metabolites used by the fungus and of substances secreted by the later, which in turn may enter the host cell matrix.

Mycelium of the pathogen grows best at 20° or 21°C. Low temperatures of 5-9°C are more favourable for the germination of conidia. The cardinal temperatures for the discharge of ascospores of *B. graminis* f.sp *hordei* are 8°, 16–29° and 24°C respectively. Ascospores, germinate best at 16-20°C. According to Moseman and Powers (1957), cleistothecia can survive up to 13 years at low temperatures. Arya (1962) holds that a temperature of 30°C or above has deleterious effect on the disease. Ascospores are formed most rapidly at 22–27°C if the cleistothecial material is exposed to alternate drying and wetting in the soil (Arya, 1964). Although most of the powdery mildews are favoured by dry weather, the powdery mildew of cereals is an exception in this respect, and thrives at low as well as high humidity. According to Prabhu *et al.* (1962), a 100% relative humidity and a temperature of 15–20°C is optimum for conidial germination. As noted by Yarwood (1939), for several species of *Erysiphe*, Cherewick (1944) also found that diseases development could be reduced by sprinkling plants with water. Yarwood (1939) attributed this response to a reduction in sporulation as a result of injury to the conidiophores. The severity of the disease is increased by the application of nitrogen fertilizer (Last, 1954).

The complete disease cycle in India is still not known. According to Mehta (1930), and Arya and Ghemawat (1953), asci and ascospores, even if formed, have practically no chance to geminate and cause infection. However, in his later work, Arya (1964) showed that asci remain immature in the cleistothecia of fallen leaves, but ascospores form in them after 10 months if the leaves are subjected to alternating dry and wet soil conditions. Such conditions are available in low lying fields. According to Mehta (1930), the disease is introduced in the plains in the form of conidial infection blown down from the hills where the fungus can survive in a cooler environment. Sharma *et al.* (1992) have reported that cleistothecia are a source of primary inoculum in temperate regions like Lahaul and Spiti in Himachal Pradesh whereas in other parts of North India conidia introduce the disease.

Physiological Specialization

The existence of distinct physiologic races of *E. graminis* now *B. graminis* was first proved by Marchal (1902–03). He distinguished seven forms on the basis of the principal host genera which were affected, i.e., *B. graminis tritici, hordei, avenae, secalis, poae and agropyri.* Pathogenic races with *E. graminis hordei* were described by Mains and Dietz (1930), and within *E. graminis tritici* (now *B. graminis tritici*), by Mains (1933).

Eighteen races have been reported on barley in Germany, 12 in Sweden, 22 in the USA and Canada (Moseman, 1964), and 11 in Japan; while on wheat, 13 races have been recorded in Britain, 19 in Germany, and 5 in India. Arya (1962) reported races 3 and 4 and a new race named X from Jodhpur (India). Sharma and Singh (1990b) have reported 14 races from 37 conidial isolates and 5 races from 12 ascosporic isolates in Himachal Pradesh.

Disease Management

The disease can be controlled by the use of sulphur dusts or sprays containing potassium or sodium sulphide (1% solution in water) or copper sulphate (1.05% solution in water), to which is added a suitable spreader. These methods are not economical except on an experimental basis. Some systemic fungicides, that is, Milicurb, Milstem and Benomyl (Bent, 1969; Kushwaha and Chand, 1971; Pathak and Joshi, 1972; Reddy and Apparao, 1971; Suryanaryana *et al.*, 1972) and other non-systemics have been used by other workers against powdery mildews. Bahadur *et al.* (1974) reported that Calixin at 0.0001-0.001% concentration controlled the powdery mildew very effectively and proved superior to Benomyl (0.01%).

Foliar spray of α-amino-isobutyric acid has been found to reduce the disease severity. According to Kettlewell *et al*. (2000) foliar application of potassium chloride (KCl) is reported to reduce disease severity by reducing spore germination on leaf. The triazole fungicides, Bayton, Bayleton, and Vigil gave 100% control when used as seed treatment or foliar spray. Other fungicides used are Prochloraz and Epoxicinazole. Triadimefon have shown promise as systemic fungicide for control of powdery mildew by seed or foliar application. A major gene for resistance from wild Einkorn wheat (*Triticum monococcum* subsp. *aegilopoides*) has been successfully transferred to hexaploid common wheat (*T.aestivum*) and resistant lines were obtained (Shi *et al*., 1998).

Resistant varieties will provide the best control measure of the disease. No work on this aspect has been done in India. According to Arya (1962), varieties NP710, NP718, K53 E750, and C591 are moderately resistant. Evaluation of powdery mildew resistance in some Indian wheat was done by Sharma and Singh (1999a). According to Sharma and Singh (1990b), the variety HD 2204 is resistant in Himachal Pradesh. They also observed that CPAN 1922 was resistant to all races of *B. graminis tritici* known in this country.

Powdery Mildew of Peas (*Pisum sativum*)

This is distributed throughout the world. The disease appears in epidemic form almost every year when the plants are in the pod stage towards the end of January and in February. Unlike the downy mildew which is favoured by moist weather, this mildew disease is at its worst when conditions are dry. Several other leguminous crops, such as *Medicago, Vicia, Lupinus, Lens esculenta, Trifolium dubium,* etc. are attacked. Uppal *et al.* (1953) observed heavy reduction in pod formation in pea due to severe infection of powdery mildew in Bombay and reported that even one picking was not possible, whereas 6–7 pickings were obtained from a normal crop. According to Munjal *et al.* (1963), it attacks cabbage, sugar beet, turnip, clover and lentil. Losses depend on the stage of maturity of the pea crop when infection occurs. The losses in yield in a 100% infected crop were estimated by Munjal *et al.* (1963) to be 21–31% in pod number and 26–47% in pod weight. There is a recent interesting article on "Pea—Powdery Mildew—An Ideal Pathosystem" by Singh (2000).

Symptoms

In the early stages, small irregular powdery spots appear on the upper surface of leaves. When plants are in the flowering and pod stage (in January and February), the disease assumes epiphytotic proportions. The powdery whitish spots completely cover the leaves, petioles, stem and even the pods, the plant

assumes a grayish white appearance, and the leaves turn yellow and are finally shed. The yield from the crop may be greatly reduced.

Histological changes in the host include collapse of the necrotic epidermal cells in case of sub infection and collapse of tissues below the penetrated epidermal cells.

Causal Organism

The organism responsible for the powdery mildew of peas is *Erysiphe polygoni* DC. The mycelium consists of delicate and persistent hyphae which are attached to the leaves by means of appressoria. Hyphae penetrate the epidermis and swell into a lobed and round sac in the epidermal cells. Conidiophores arise vertically from the leaf surface. Conidia usually form singly (rarely in chains), are ellipsoid, and 31-28 × 17-21 µm. Later in the season, cleistothecia appear as sharp, black specks scattered on the surface of the white mycelium; globose, 85–126 µm in diameter. Appendages very variable in number and length (10–30), myceloid, sometimes knotty and frequently geniculate, rarely irregularly branched, brown, usually as long as the diameter of the ascocarp, at the most 2–3 times as long. Asci are usually 4–8, ovate to broadly ovate or subglobose, 50-60 × 30-40 µm. Ascospores are 3–5 rarely 6, 22-27 × 13 × 16 µm. According to Paul and Kapoor (1983), the various species described under the name *Erysiphe polygoni* on different hosts in India comprise of eight species, namely *E. polygoni, E. pisi, E. berberidis,, E. betae, E. heraclei, E. martii, E. ranunculi and E. salviae.* Chitale *et al.* (1981) had reported the perfect stage of *E. polygoni* on lentil in India.

Physiologic Specialization

Hammarlund (1925) distinguished four biological forms of *E. polygoni* (Fig. 14.3)

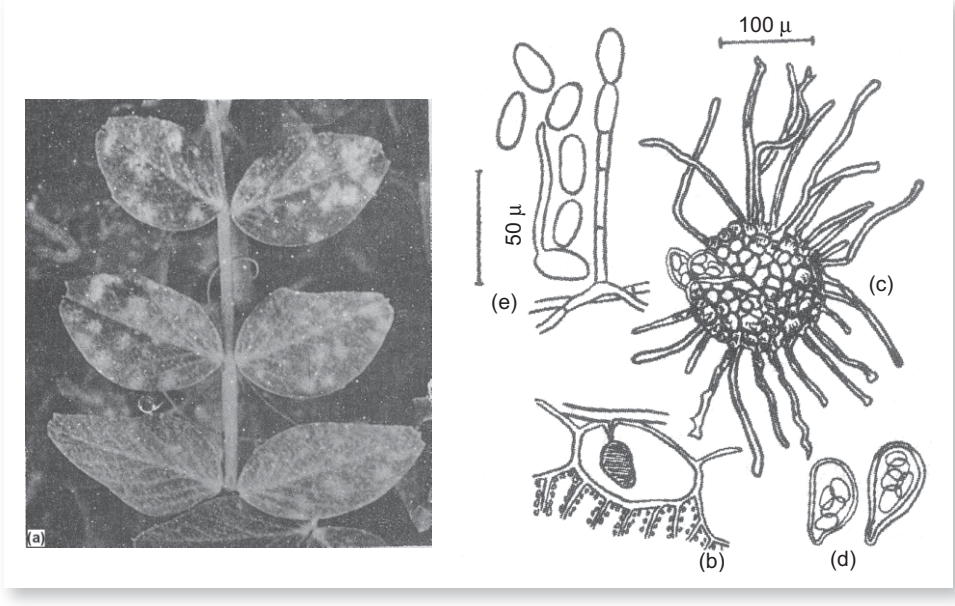


Figure 14.3 Erysiphe *polygoni* on pea: **(a)** symptoms **(b)** haustorium **(c)** cleistothecium with appendages **(d)** asci with ascospores (e) conidiophores and conidia

Disease Cycle

The fungus is an obligate parasite and the disease perennates through cleistothecia in the soil. Ascospores from the perennating cleistothecia first infect the lowermost leaves near the soil. The conidia formed abundantly during primary infection, are responsible for the secondary spread of the disease. This is also suspected to be seed borne (Mundkur, 1949).

Jhooty and Munshi (1990) have reported that the disease in India has a cycle similar to that of wheat rusts and reported that the pathogen is not seed-borne as reported earlier. These were no reports of occurrence of cleistothecia in the plains of Punjab and rarely in the hills, suggesting that perpetuation through sexual stage does not occur. They excluded the possibility of survival of the pathogen through alternate host because fungus from lentil could infect pea but not pea to lentil. They have further reported that since the pea is grown throughout the year in Punjab and Himachal Pradesh, the pathogen is present in conidial stage throughout the year. The pathogen survives in mycelial and conidial stage on the main host in the plains of Punjab during the winter season and in cooler regions of Himachal Pradesh during the summer seasons. The primary inoculum in the form of conidia comes from these places by wind currents to infect the pea crop in the plains. The conidia can germinate at temperature from 20°–24°C, with relative humidity up to 70%.

Disease Management

Field sanitation is important. Diseased plant refuse should be collected and burnt in the field. For vegetable crops, early varieties are preferred. Dusting with sulphur (200 mesh) at the rate of 25–30 lbs per acre is recommended when heavy losses are feared. Uppal *et al.* (1935), Peralta (1949), and Starker (1954) found that 1% Karathane dust was superior to sulphur dust in controlling this disease. Singh and Mishra (1975) recommended sulphur dust, Cosan, Elasol; Karathane WD and Thiovit sprays controlled the disease in Rajasthan.

Khatua *et al.* (1977) made a field assessment of systemic and contact fungicides for the control of powdery mildew of pea and concluded that the most effective fungicides were Calixin and Karathane followed by Milstem and Sultaf. Aureofungin, Morocide and Mucuprax are also effective. The use of seed and disease free areas is recommended (Chupp and Sherf, 1963). Seed treatment with organo synthetic fungicides, especially Captan, Cerenox and Thiram also gives good control. Varietal resistance of pea to powdery mildew has been investigated in India by Singh and Singh (1977).

Suhag and Rana (1984) approved 2–3 sprays of 0.1% Bavistin, 0.1% Karathane or 0.2 to 0.25% Sulfex. On the basis of power or efficacy one spray of Calixin (0.1% to 0.05%) was better than Bavistin or Sulfex. Mishra and Krishna (1990) have reported 24.22 to 51.81% control of the disease with Calixin and Karathane. Hooda and Grover (1984) had reported that one spray of Bitertanol (Baycor) checked the disease intensity up to 20%. Triarimenol (Bayton) and Dichlobutazole reduced the disease up to 50%.

Bitertanol, Triarimenol and Dichlobutazole are some of recent fungicides used. They are not only protective but curative also. Several fungal biocontrol agents such as *Ampelomyces, Cladosporium*, *Tilletiopsis*, and *Verticillium* are natural control agents of powdery mildew of pea. Singh *et al*. (2003) have reported that *Oidium*, *Phyllactinia* which are non pathogenic to pea induce resistance to pea against powdery mildew. The pea cultivars which are resistant to powdery mildew in Himachal Pradesh are DPP- 54, DPP-26, Pant-8, Sugar Giant, JP-71.

Control of powdery mildew of pea by some novel chemicals, plant products and biological agents has been tried with varying success (Singh, 2000). The inhibitory effect of bergenin a naturally occurring

isocoumarin isolated from *Flueggea macrocarpa* against *E. pisi in vitro* and also in glass house has been seen (Kumar *et al.*, 1985).Control of powdery mildew of pea by seed bacterization of *Pseudomonas fluorescens* and *P. aeruginosa* alone and the two bacterial species or Neemazal (a natural product of "neem") has been found to be quite efficacious (Singh, 2000).

Out of the 670 genotypes tested, P185, P6583, P6587, P6588, T10, T56, P388 proved resistant. It was interesting to note that all the strains which exhibited resistance to powdery mildew belonged to the table pea group (*Pisum sativum*). The germplasm lines could be utilized for breeding pea varieties resistant to powdery mildew. In Rajasthan, Mathur *et al*. (1991) have reported resistance in P6583, P6587, P6588, P383, P388, P185, DPS3, DRS6, HUP2, DMR9 and Rachna.

Powdery Mildew Of Grapevine

This is worldwide in distribution. The damage to vines is extensive in Europe and western USA. There are frequent reports from Europe about the complete destruction of the grape crop. In Afghanistan, the loss in 1958 was estimated about 50–80%. In India, this disease is common and destructive in Maharashtra, Gujarat, Madras and Andhra Pradesh.

Symptoms

The disease first appears on leaves as white patches on both the surfaces. The affected young leaves may be distorted. Similar symptoms are also seen on young blossoms and young berries. When flowers and berries are affected, there is considerable reduction in yield. Young berries, when infected, turn dark, become irregular in form and develop cracks. Diseased vines have a wilted and dwarfed appearance.

Causal Organism

The pathogen responsible for this disease is *Uncinula necator* (Schw.) Bur. Mycelium is usually semi-persistent, very thin and effused or forming patches, sometimes evanescent. Conidia are usually in long chains, ellipsoid to almost cylindric and 32-39 × 17-21 µm. Cleistothecia are rarely formed, usually epiphyllous, sometimes hypophyllous, globose, depressed, more or less scattered, 84–105 mm in diameter, wall cells 10–22 µm wide. Appendages 8–30 equatorially inserted, 1–6 times as long as the diameter of the ascocarp, septate, thin walled, brown towards the base, flexuous and flaccid, apex more or less helicoid when mature often strongly so. Asci are 4–6, rarely 6–9, broadly ovate 50-60 × 25-40 µm. Ascospores are 4–7, ovate to ellipsoid, 15-35 × 10 × 14 µm (Fig. 14.4).

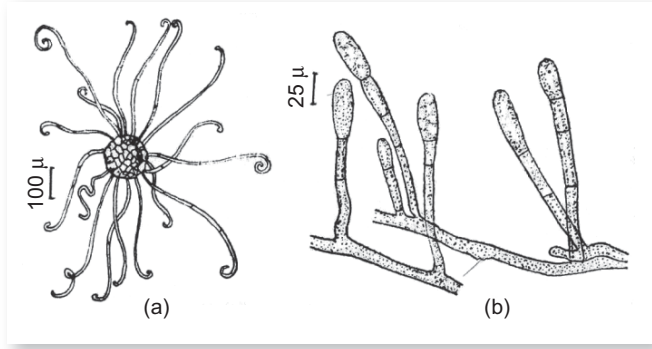


Figure 14.4 *Uncinula necator* **(a)** cleistothecium **(b)** conidiophores and conidia

Disease Cycle

Conidia and mycelium overwinters in diseased buds, fallen berries, or on over

wintered stems. Cleistothecia, when formed, are also capable of over wintering. Rains disperse the cleistothecia to bark of the vines where they are retained between leaf fall and bud break in the next season (Cortesi *et al.*, 1995). Influence of weather parameters on conidial formation in powdery mildew of grapes has been worked out by Chavan *et al.* (1995). According to Munshi and Singh (1994) extremely low humidity adversely effected the growth of the pathogen on the host. Mycelial growth and production of conidia is very rapid at 25°C followed by 20°C and 30°C. No powdery development occurs at 35°C and 40°C.

Disease Management

Cultural practices including the trimming of developing shoots to reduce shading and allow free ventilation of vines are important for control. Pruning, after the shedding of leaves, thinning out and cutting back of laterals, and removal and destruction of all diseased parts are important aspects of a clean cultivation.

Dusting with sulphur is a universal practice for control in regions where temperatures are not high. Bordeaux mixture and fixed coppers are also recommended (Anderson, 1956). Chandrasekhara Rao (1989) tested Sulfex (0.25%), Karathane (0.056%), Calixin (0.05%), Topsin, M. (0.1%), Thiovit (0.25%) and Sulphur dust (15 kg/h) against this disease and reported all these fungicides were effective but Bayleton was most effective. Person *et al*. (1994) have recommended a single application of 0.2 to 4g a.i. of these fungicides such as Triadimefon (Bayleton), Triadimefon (Bayton), Etaconazole (Vangard), Flusiliazole, Myclobutanil, and Penconazole between bloom and two weeks after shatter gives effective control.

In vertical shoot positioned leaves where pruning was not done, the disease incidence was higher (30%) than in free positioned topped vines where pruning, thinning out and cutting back of laterals was done. Excessive dose of nitrogen fertilizers tends to promote the vegetative growth of plant which is also responsible for increased incidence of disease.

Dusting with sulphur has been an effective control method. The following recommendations should be followed for disease control:

- The first dusting should be done when new shoots are about 7–15 cm long.
- Second dusting should be done during or just before blossoming.
- Third application can be made 40–50 days later.

In dry climates, sulphur dust whereas in area with high humidity or high rainfall, wettable formulations are preferred for their better retention qualities.

The different strobilurin fungicides, i.e., Azoxystrobin, Kresoxim-methyl, Pyraclostrobin, and Trifloxystrobin is reported to give better control to grapevine powdery mildew. Polar, a polyoxin B compound at a concentration of 1, 10 and 100 mg/l is highly effective and gives as good control as sulphur fungicides (Reuveni *et al.* 2000). Some of the non conventional chemicals and plant products like mineral oil and plant oils have been used against grape powder mildew with positive results. Petroleum oil when applied as emulsion (1%v/v in water) has provided moderate protection as it is anti sporulant. Rape oil derivatives and oils of *Allium sativum*, *Cyperus scariosus*, *Cuminum cyminum*, *Cymbopogon citrates*, *and Eucalyptus camaldulensis* prevented powdery mildew on grapevine plants.

Spray of 0.1 M solution of phosphate is reported to induce systemic protection against *Uncinula necator* (Singh, U. P. 2000). The control was as good as that given by systemic fungicide. The efficiency of these inducers of systemic acquired resistance (SAR) is increased by the application of 0.1% sulphur. According to Yildirim *et al.* (2002) sodium silicate, potassium silicate, potassium phosphate or sodium

carbonate could be included in spray schedules with sulphur or some systemic fungicides for better disease control.

In biological control populations of *Bacillus*, *Pseudomonas*, *Serratia, Penicillium* and *Trichoderma* are enhanced on the host surface when the aqueous extracts of compost have been used. Even the fermented cow dung, various neem formulations and rape oil product gave reasonable control of powdery mildew. Suppression of *Uncinula necator* pathogen by the mycoparasite *Ampelomyces quisqualis* is reported by Falk *et al*. (1995). English *et al.* (1999) reported reduction in powdery mildew disease by applying the mite *Orthotydeus lambi* because the formation and development of Cleistothecia is inhibited by mites.

The varieties/cultivars which are resistant to disease are Black Prince, Sultana Red, Isabella, Champion, Perlett and Cardinal. According to Bhardwaj *et al*. (1997), cultivars Chholtu White and Chholtu Red are resistant while Skibba white, Skibba Red and Bhatla Ribba are resistant with 0.1 to 5% disease severity (Bhardwaj *et al.*, 1997; Singh R.S. 2007).

Partial control of grape downy mildew has been reported by Falk *et al.* (1995) utilizing the mycoparasite *Ampelomyces quisqualis.* Sohi and Sridhar (1976) reported Flame Muscat to be resistant under Bangalore conditions. Datar (1979) had shown resistance in certain cultivars.

Powdery Mildew of Cucurbits

This is worldwide in distribution. The disease occurs throughout India almost every year. The same fungus has been reported to attack potato and tobacco seedlings, lettuce, sunflower, mango, castor (*Ricinus communis*), *Antirrhinum orontium, Hyssopus officinalis, Sedum spurium* and *Sonchus asper* (Stone, 1965).

Symptoms

Humid conditions are favourable to the disease. The first symptoms are tiny, white superficial spots on leaves and stems which become powdery as they enlarge. The superficial white powdery mass may ultimately cover the entire host surface. Black, pin-point-like bodies representing the ascigerous stage of the fungus, appear late in the season on the host surface but are not commonly formed in the Indian climatic conditions. Severe infection may lead to premature defoliation of the plant. The fruits remain undersized (Fig. 14.5).



Figure 14.5 Powdery mildew of Cucurbits: symptoms

Causal Organism

Powdery mildew is a serious widespread disease of cucurbits in India (Khan *et al.*, 1971, 1972, 1974). *Erysiphe cichoracearum* DC and *Sphaerotheca fuliginea* (Sc.) Poll. infect cucurbits in various parts of the world. In India, in as early as 1918, Butler reported the occurrence of both the organisms in cucurbits. On the basis of conidial characters, Jhooty (1967) claimed that

S. fuliginea was responsible for the disease in Punjab. In CMI description, Kapoor (1967) described that *S. fuliginea* exclusively attacks cucurbits, whereas *E. cichoracearum* is confined to the members of Compositae (Asteraceae) and other non-cucurbitaceous hosts. While Khan and Khan (1970) reported the production of perithecia of *S. fuliginea* on several varieties of *Luffa leucantha* and *Cucumis sativus* under glass house conditions at Aligarh, Uttar Pradesh. In the opinion of Khan and Khan (1970), *E. cichoracearum* may be responsible for cucurbit powdery mildew in other countries but not in India. However, Siradhana and Chaudhari (1972) reported the occurrence of both the species on cucurbits at Udaipur (Rajasthan). *Erysiphe cichoracearum* is the main causal organism of this disease. Taxonomic evaluation of anamorph characters in identification of powdery mildew of fungi on cucurbits in India has been done by Khan and Sharma (1995). Mycelium is usually well developed and evanescent, but sometimes persistent and effused. Conidia in long chains, are ellipsoid to barrel-shaped and vary is size, 25-45 × 14-26 µm. Cleistothecia are formed in autumn, gregarious or scattered, globose, becoming depressed or irregular, 90–135 µm diameter, wall cells usually indistinct, 10–20 mm wide. Appendages are numerous, basally inserted myceloid, interwoven with mycelium, hyaline to dark brown, 1–4 times as long as the diameter of the ascocarp, rarely branched (Fig. 14.6). Asci 10–25, ovate to broadly ovate,

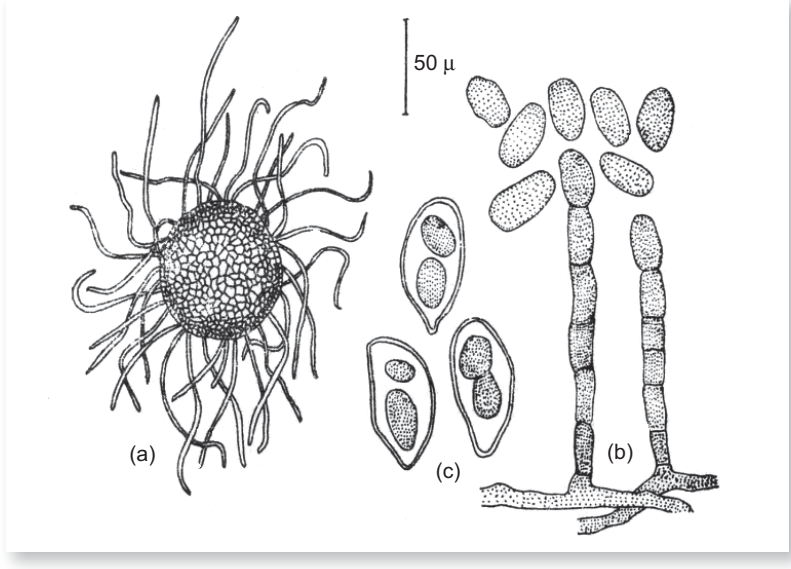


Figure 14.6 *Erysiphe cichoracearum*: **(a)** cleistothecium **(b)** conidiophore and conidia **(c)** asci and ascospores

rarely subglobose, more or less stalked, 60-90 × 25-50 µm. Ascospores 2 very rarely 3, 20-30 × 12-18 µm. *Sphaerotheca humuli* var. *fuliginea* (Schl.) Poll.—this fungus usually occurs in the conidial state, particularly on cucurbitaceae, and has often been confused and *Erysiphe* cleistothecia are different in structure from those of *Sphaerotheca*. Powdery mildew of cucurbits caused by *S. fuliginea* is a serious disease in northern India (Jhooty, 1967). Sohi and Nayar (1969) obtained perithecial stage of *S. fuliginea* on *Cucurbita moschata* from Himachal Pradesh. Khan *et al.* (1974) reported the widespread occurrence of powdery mildew of cucurbits due to *S. fuliginea* in Kashmir (Fig. 14.7).

It generally has only one ascus in each cleistothecium. The ascus is broadly elliptic to subglobose and the ascospores are hyaline, continuous, measuring 12-15 × 20-25 µm.

Kaur and Jhooty (1985) have reported three different pathological forms of *S. fuliginea* on the basis of differential reactions of cucurbit hosts. Khan and Sharma (1993) have confirmed and reported that three races of *S. fuliginea* are present. Out of the three races, race 3 is most widespread and infects most of the cucurbits in different states of India. Kabitarani (1993) reported the existence of heterothallism in the species and prevalence of 2 pathological races in Assam.

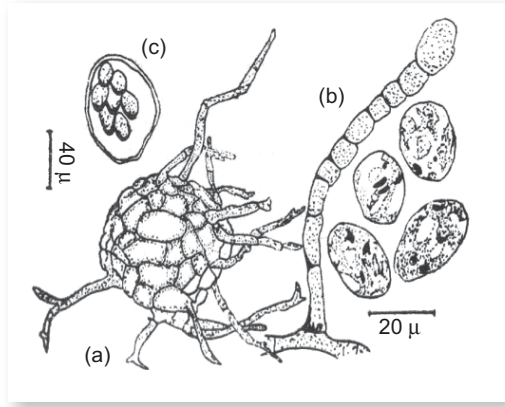


Figure 14.7 *Sphaerotheca fuliginea:* **(a)** cleistothecium **(b)** conidiophore and conidia **(c)** ascus and ascospores

Disease Cycle

The means of subsistence of the fungus between successive crops in not very clear. In the areas where cleistothecia are formed, these might be the main mode of perennation from one season to the next. It is possible that the fungus survives in its conidial stage on wild hosts in a suitable locality and from there the conidia are wind borne to cultivated areas. Heavy dew favours penetration by the germ tube. Penetration is confined to the epidermal cells where spherical haustoria are present.

Physiological Specialization

Blumer (1967) distinguished 13 formae species of *E. cichoracearum* based on a single species or a single section of a genus. Jaczewski (1927) distinguished 84 "formae" of *S. fuliginea.*

Disease Management

A safe protective measure for controlling *E.cichoraccarum* in the greenhouse is 1% Ba-polysulphide with temperature at 24–25°C and air humidity at 80%. Sprays with colloidal sulphur and Thiram effectively control *Sphaerotheca fuliginea*. Karathane has been reported to be effective against powdery mildew of cucurbits (Mandloi and Khare, 1969). Systemic fungicides, i.e., Benomyl, Bavistin, or Triozoles have been found to be equally effective in controlling this disease. Since the introduction of the systemic fungicides on field scale it was realized that *S. fuliginea* quickly developed resistance to systemic fungicide like Benomyl. To overcome this difficulty, it is now recommended that fungicides like Triadimefon should be applied once or twice with a protective fungicide and then subsequent spray should be done with protectant fungicides only. Roy (1973) claimed that Benomyl as a soil drench and Jhooty and Behar (1972) held seed treatment to be quite effective in controlling this disease. Waraitch *et al.* (1975) reported that Benomyl as foliar spray is also quite effective in controlling powdery mildew of bottle gourd.

Thind *et al.* (2002) have reported that the three strobilurin fungicides, i.e., Azoxystrobin, Kresoxim methyl and Trifloxystrobin are highly effective against *Sphaerotheca fuliginea* on the basis of inhibition of conidial germination. Reuveni and Reuveni (1998) have reported that mono or dipotassium phosphate and potassium nitrate were effective against *S.fuliginea*. According to them, a single spray of 0.1 M phosphate solution can induce systemic protection against powdery mildew of cucumber (*S. fuliginea*). Some of the micronutrients like boron, copper, manganese also provide protection from

S. fuliginea. Some mineral oil, milk powder, potassium and sodium bicarbonate, white wash and clay also reduce mildew on leaves.

In biological control, suppression of *Erysiphe* and *Sphaerotheca* by the mycoparasite *Ampelomyces quisqualis* is quite effective because the mycoparasite ramifies throughout the host hyphae resulting in reduced or death of the powdery mildew. *Verticillium lecanii*—another biological agent, its hyphae parasitizes on *S. fuliginea* and by 72 hours hyphal cell of pathogen collapse. Dik *et al.* (1998) have reported that another biocontrol agent *Sporothrix flocculosa* gave the best results in comparison to *Ampelomyces* and *Verticillium*.

Prior inoculation of cucumber leaves with a non-pathogenic *Alternaria cucumerina* or *Cladosporium fulvum* or tobacco necrosis virus induces systemic resistance to powdery mildew. Daayf *et al*. (1995) found that plant extract of *Reynoutria sachalinensis* had no effect on conidial germination of *S. fuliginea* but induced rapid accumulation of phenols in leaves which protected the plant. In muskmelon, cultivars Digurai and Haragola are immune to the disease. A phytoalexin has been isolated from the endocarp of detached open pea pods inoculated with *E. cichoracearum.* In muskmelon, varieties Diguria and Huragola are reported to be immune to powdery mildew (Amin *et al*. 1982).

Powdery Mildew of Apple

This disease is not confined to any specific region. It is common in the apple orchards of northern India. Nursery plants are damaged more than the adult plants. Peaches (*Prunus persica)*, quince (*Cydonia vulgaris*) and *Photinia* spp. are also attacked (Hirata, 1986).

Symptoms

The disease appears soon after the buds develop into new leaves and shoots. Sometimes buds are so heavily infected in the previous season that they are killed before they develop into leaves. The affected leaves are longer and narrower than normal leaves and are covered with a whitish growth (Fig. 14.8). In nursery plants, the disease prevents the formation of wood in the stem. Fruit buds suffer more damage than vegetative buds.

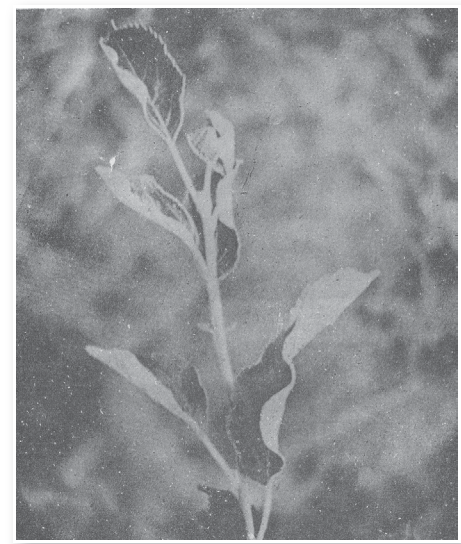


Figure 14.8 Powdery mildew of Apple (*Podosphaera leucotricha*): symptoms

Causal Organism

The disease is caused by *Podosphaera leucotricha* (Ellis and Ever) Salm. The ectophytic mycelium sends saccate haustoria in the epidermal cells. Aerial conidiophores arise form this mycelium on leaves and shoots. Each conidiophore bears a chain of oval hyaline conidia which measure 25-30 × 10-12 µm. Perithecial stage is rare in India. Cleistothecia are densely gregarious, rarely scattered, globose or sometimes lightly pyriform. Appendages on the cleistothecia are of two kinds: one set springing from the apex of the cleistothecium, the other inserted basally. The apical appendages are

3–11 µm, more or less widely spreading, 3–7 times as long as the diameter of the ascocarp, dark brown towards the base and taper towards tips, the apex usually being undivided or very rarely dichotomously branched 1–2 times, basal appendages rudimentary, short, more or less tortuous, pale brown, simple or irregularly branched. Ascus oblong to subglobose, 55-70 × 44-55 µm. There are eight ascospores in the ascus which are ovate to elliptical and measure 22-26 × 12-15 µm.

Disease Cycle

Cleistothecia play a very little part, if any, in the perennation of the fungus. In most places, the fungus survives in the form of resting mycelium or encapsulated haustoria in the buds. Secondary spread is by wind-borne conidia (Anderson, 1956).

Disease Management

Effective fungicides include microfine sulphur, lime sulphur, wettable sulphur and Karathane. Lime sulphur sprays should be undertaken according to the following schedule:

- 1: 15 dilution when the buds are green.
- 1: 35 dilution at the open cluster stage.
- 1 : 60 dilution at the blossoming or full pink stage.
- 1 : 100 dilution when about half the petals have fallen.

Breeding resistant varieties appears to be the most promising control measure, some good sources of resistance for breeding varieties are known. Susceptibility to mildew in apple varieties is governed by one completely dominant gene and is related to weather conditions.

Sharma and Gupta (1980) found that four sprays of Bavistin (0.05%) or Morocide (0.01%) starting at bud swell stage and repeated at 15 days internal give control of the disease. Among newer fungicides, Gupta and Gupta (1991) reported that 0.05% Triadimefon (Bayleton) gives best control by persisting on the host surface for up to 15 days. Sharma and Gupta (1994) reported maximum reduction of germination of spores and germ tube growth by Triforine followed by Tridemorph, Bitertanol and Carbendazim. According to Sharma *et al.*, (1992), in a comparative study Baycor and Karathane were found superior to Bavistin.

The Strobilurin or Trifloxystrobin, a formulation of Strobilurin are highly effective against powdery mildew (Singh, 2008). The fungicide inhibits mitochondrial respiration and inhibits conidia and conidiophore formation. Bupirimate (Nimrod) and Triadimefon (Bayleton) are reported to give better control. Podem –TM, a disease forecasting model developed in UK is effective in optimizing timing of sprays.

Plant oils of sunflower, olive, maize, rapeseed, and soybean have provided effective control against powdery mildew. According to Ko *et al.* (2003), these oils especially sunflower oil inhibits conidial germination and mycelial growth of the pathogen.

In biological control, suppression of apple powdery mildew by the mycoparasites *Ampelomyces quisqualis* is reported. The cultivars Red delicious and Golden delicious are slightly resistance but apple cultivar with Vf resistance to apple scab are resistant to powdery mildew of apple.

Powdery Mildew of Mango

The disease has been reported from all parts of the country i.e. Uttar Pradesh, Punjab, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu. The losses can vary from 5– 20% depending upon the

weather conditions. According to Rawal (1998) this is a serious problem in the hills, valleys and Tarai regions of Uttar Pradesh. Serious outbreaks of the disease have been reported during the last decade.

Symptoms

The pathogen attacks the leaves, flower scales, buds of tender flower heads, axils, stalks and fruits. The rachis of the panicles is infected. Wefts of white mycelium appear on the affected parts. The whole surface is later covered with white powdery by winds. The pathogen is ectophytic, and draws its nutrition from the plant cells through the haustoria which penetrate the epidermal layer and reach the cells. The affected fruits do not size and drop before attaining pea size. Sometimes the growing tips of the shoots also get infected while other parts of the plant remain free from the disease.

Disease Cycle

In Bangalore region the disease has been found to be prevalent throughout the year on mango varieties such as Prairie, and Alphonso. In the Indo Gangetic region the infection can occasionally be found on malformed inflorescences and there is a possibility of the survival of the pathogen in submountaneous region of Uttar Pradesh. Warm weather with heavy morning dew and cloudy conditions predispose the trees to infections.

Causal Organism

The pathogen responsible for the powdery mildew of mango is *Oidium mangiferae.* The genus *Oidium* is the anamorph of Erysiphaceae where conidia are formed by breaking up of hypha into cells.

Disease Management

Earlier the most effective method of controlling the disease was dusting the plants with fine sulphur (250–300 mesh) at the rate of 1–3 lb of sulphur per tree. The fungicide should be sprayed at pre-bloom, full bloom, and post bloom stage at 15 days intervals. Karathane 25 WD, 0.06 per cent to 0.09 per cent spray concentration, is slightly more effective. Spraying of Guesarol (an insecticide) has been found successful in Tamil Nadu, Andhra Pradesh, and Maharashtra. According to Sohi and Sridhar (1973) best control was obtained with Cosan (0.2%) and Benlate (0.2%) when sprayed at fortnightly intervals during January to May. Out of the several fungicides tried at IIHR, Bangalore, best control was achieved by wettable sulphur (0.2%), Calixin (0.1%), Anvil (0.05%), Systhane (0.05%) and Saprol (0.2%) when sprayed at fortnightly intervals.

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Questions

1. Can you distinguish the genera of powdery mildews on the basis of the oidial stages? How do these oidial stages differ from each other?
2. What do you understand by parasitic specialization or physiological specialization? Discuss the phenomenon, giving suitable examples from powdery mildews.
3. What are the various types of haustoria found in powdery mildews?
4. What group or groups of fungicides are used for controlling powdery mildews?
5. Powdery mildew conidia can germinate under extremely xeric conditions. How is this possible?
6. How will you distinguish the following pairs of plant pathogens?
   (i) *Sphaerotheca* and *Erysiphe*
   (ii) *Uncinula* and *Phyllactinia*
   (iii) *Podosphaera* and *Microsphaera*
   (iv) *Blumeria* and *Golovinomyces*
   (v) *Blumeria* and *Erysiphe*

Smuts and Bunts

15

In the 10th edition of the *Dictionary of the Fungi* by Kirk *et al.* (2008), the phylum or division Basidiomycota has been divided into 3 sub-phyla, i.e., (1) Agaricomycotina, (2) Pucciniomycotina, and (3) Ustilaginomycotina. The sub-division Ustilaginomycotina contains two orders, i.e., (i) Urocystidiales and (ii) Ustilaginales. The members belonging to these orders are smuts and bunts.

The Ustilaginales are the "smuts" and "bunts" numbering approximately 1200 species in more than 50 genera. These fungi generally infect the ovaries and sometimes other meristematic growing tissues also of the flowering plants, especially Gramineae and Cyperaceae and develop in them the characteristic thick-walled brown or black spores—the "brand spores"—which consequently impart a sooty appearance. The origin of the term "bunt" is unclear. According to Duran and Fischer (1961) it may have been derived from a local pronunciation of "bunt" as "buhnt". They spend a part of their life cycle (sporidia) saprophytically and a part (mycelium and smut spores) parasitically within the plant tissues. They are, thus, facultative parasites. Many smuts have been grown in culture and a few have been induced to complete their entire life cycle—from teliospore (teleutospore) to teliospore—on artificial media. The smuts resemble the rusts in several ways and the two orders appear to be closely related. The biology of these fungi has been dealt with by Fischer and Holton (1957) and there are excellent monographs (e.g., Ainsworth and Sampson, 1950; Mundkur and Thirumalachar, 1952; Fischer, 1953). Vanky's (1987) *Illustrated Genera of Smut Fungi,* and *Smut Fungi of World* (2012) are excellent monographs. A general account about smuts and bunts and their control is by Goel and Singh (1975). The main features of the two orders may be compared and contrasted as follows:

	Smuts	**Rusts**
Perfect spore	intercalary	terminal
Sporidial number	indefinite	definite (4)
Mechanism of discharge	passive	active
Basidiocarps	rare	absent
Parasitic mycelium	intercellular with haustoria	intercellular with haustoria
Alternation of generations	indistinct	distinct
Parasitism	facultative	obligate-(Biotroph)
Clamp connection	common	rare

(*Contd.*)

Sex organs	absent	specialized
Heteroecism	absent	common
Polymorphism	absent	distinct

The smuts most commonly attack the reproductive structures of the plant but may also be found on the stems, leaves and occasionally the roots. The smut may be confined to a very small part of the plant, such as the ovaries or anthers. Economically, the smuts are very important causing enormous monetary loss by damaging crops. Smut spores may also accumulate in threshing machines that may explode violently, thus destroying the machine and the grain. Allergies may also be caused by the inhalation of smut spores by human beings.

The smut life cycle is considerably less complicated than that of the rusts. The smut mycelium which initiates the infection is usually dikaryotic, although in *Ustilago maydis*, the infecting mycelium is haploid in the beginning but the dikaryotic condition is soon established by mycelial fusion. Usually there are no specialized haustoria, but short branches of the mycelium may enter host cells. The slender, septate mycelium develops intercellularly. Clamp connections and haustoria may be present. The mycelium in the sorus breaks apart into dikaryotic cells, which differentiate into teliospores. The teliospores have dark, thick walls, which may be smooth, spiny or reticulated. In some cases the spores are borne in spore balls. The two nuclei in the chlamydospore fuse, and at maturity, the spore contains a single diploid nucleus. Unlike its regularly triseptate counterpart in the rusts, the smut chlamydospore or brand spore or teliospore, on germination, produces a metabasidium which may be non-septate or, if septate, any number of septa may be formed. The majority of septate species form a three or four celled metabasidium. Meiosis occurs, and the metabasidium usually gives rise to uninucleate haploid basidiospores. Depending upon whether the apex of the metabasidium is septate or not the basidiospores are from each cell or from the apex of the metabasidium. The basidiospores may give rise to additional spores and in culture will form a yeast-like colony of spores. In this yeast-like phase many smuts are capable of prolonged saprophytic growth, but parasitic growth by the haploid phase does not usually occur.

Sori may develop in various parts of the inflorescence, including the ovaries, ovules, anthers, petals, etc., or they may form in leaves, fruits, stems, axillary buds, rhizomes and even roots. In *Entyloma*, *Burrillia*, *Doassansia*, *Tracya*, and *Tuburcinia*, sori are almost invariably formed in the leaves where they tend to remain embedded, even after maturity. However, the sori of other leaf smuts, when mature, may erupt to various degrees, as in some species of *Urocystis*, *Ustilago*, *Schizonella*, *Tolyposporella*, and *Tilletia.* Some smuts however, sporulate on most host organs, as does *Mundkurella heptapleuri* which forms sori in fruits, stems, petioles and leaves. *Melanotaenium* spp. similarly sporulates in most parts of the host which are above the ground and in the roots as well.

In the 10th edition of the *Dictionary of Fungi* by Kirk *et al.* (2008) smuts and bunts have been placed in the class Ustilaginomycetes but for the convenience sake and till the new classification becomes accepted by various authors, the present authors have followed the old classifications to avoid any confusion.

Some important genera of smuts of two families are as follows:

Family *Ustilagianaceae* – Promycelium septate, basidiospores produced laterally from each cell of the promycelium. Important genera are *Ustilago, Sphacelotheca, Sorosporium, Tolyposporium,* etc.

Ustilago – Sori almost never enveloped in a fungus peridium nor formed around columella of host tissue.

Sphacelotheca – Sori enveloped in a peridium of fungus cells, forming around a central columella of host tissue, at times with the vestiges of vascular elements remaining.
Sorosporium – Spore balls evanescent or tending to fragment into single spores.
Tolyposporium – Spores in the balls held together by interconnected thickenings of the exospores walls; germination as in *Ustilago.*
Melanopsichium – Sori forming in hard, gall-like masses, mostly in the inflorescence, spore mass embedded in a gelatinous mass.
Family *Tilletiaceae* – Promycelium non–septate, basidiospores in a terminal cluster. Important genera – *Tilletia, Neovossia, Entyloma* and *Urocystis.*
Tilletia – Spores large, mostly 16–54µm in diameter, exospores reticulate, smooth, and verrucose usually in ovaries or occasionally in the leaves, probably limited to Gramineae.
Neovossia – Sori less dusty at maturity, spores with a large hyaline appendage; on Gramineae.
Entyloma – Sori mostly forming in most parts other than roots. Sori tending to remain embedded in the host tissue after maturity, forming more or less distinct spots in leaves or other parts above the ground.
Urocystis – Spore balls formed in saccate cavities in galls, the balls consisting of one to several fertile spores surrounded by bladder-like sterile cells; spore germination Tilletiaceous.

Modes of Infection

There are three ways in which smut fungi infect their hosts. These are: (1) embryo infection, (2) seedling infection, and (3) shoot infection.

Embryo Infection

The best example is loose smut of wheat caused by *U. segetum* var. *tritici* (*U.tritici).* Here infection takes place at the time of blossoming. Penetration usually takes place through the ovary wall and not via the stigma and style, as was previously supposed (Batts, 1955). The hyphae cross the cells of the pericarp (fruit coat), enter testa (seed coat) and then move towards the ovule. After reaching the ovary, the hyphae turn sharply from the testa, enter the scutellum and pass into the growing point of the embryo. Within the pericarp and testa, the mycelium is intercellular but becomes intracellular within the tissues of the embryo. The mycelium of the pathogen remains dormant until the seed is sown, and begins to germinate after which it becomes active. It passes into the crown node of seedlings and permeates the tissues. When the tillers lengthen due to the growth of the lowest internodes, the pathogen is carried upon the inflorescence primordia.

Seedling Infection

In many smuts, the infection takes place at the seedling stage of the plants. However, the exact mode of infection varies with the species involved. In the case of loose smut of oats, caused by *U. avenae*, the spores are dispersed at flowering, similar to *U. nuda.* On germination, the mycelium becomes

established in the glumes and outer parts of the seed, that is, in the pericarp; it does not invade the embryo. When such seeds are sown, this dormant mycelium becomes active and invades the young seedling. In the case of *T.caries* responsible for bunt of wheat (also called stinking smut), the spores *en masse* have an odour of bad fish due to the presence of trimethylamine. The bunt balls often remain intact until threshing, when they are broken and the spores released in this manner contaminate the healthy seeds. When such contaminated seeds are sown, the spores on the seed coat germinate and the binucleate hyphae which, results from the fusion of sporidial cells infect the young coleoptile. Further development of the pathogen is similar to that of *U. segetum* in wheat or barley. The inflorescence primordia are colonized at an early date.

Seedling infection from spores and/or mycelium also occurs with stripe smut of grasses caused by *U. striiformis* and flag smut of wheat and grasses caused by *Urocystis agropyri.*

In certain other smuts where seedling infection takes place, seed-borne inoculum is relatively unimportant. In the case of dwarf bunt of wheat (*Tilletia contraversa*) and onion smut (*Urocystis cepulae*) infections arise mainly from the soil-borne spores which may persist for many years. Infection takes place during the first 15–20 days after germination after which the cotyledons become resistant and provide a barrier to infection.

Shoot Infection

In a few smut fungi, the pathogen gains entry through the young shoots, particularly the young buds. Fisher and Holton (1957) described this for the first time in *U. violaceae* on *Melandrium album.* In the case of *Ustilago scitaminea* on sugarcane, the entry is again through buds on the young off-shoots or setts. Shoot infection also occurs in long smut of sorghum caused by *Tolyposporium ehrenbergii.*

In the smuts mentioned so far, the infection of the hosts is "systemic" but with some smuts infection is localized and sporulation is restricted to the part of the plant it has penetrated. One good example is common smut of corn caused by *U. maydis.* Another example is bunt of rice caused by *Neovossia horrida.* In the case of leaf smut of rice caused by *Entyloma oryzae,* there is localized infection on rice leaves.

Effect on Host

In the smut of cereals, particularly loose smut of wheat and barley, there is a direct loss in yield through the replacement of the flowering parts. There are very few indications of any adverse effect on the host growth. Excessive tillering and dwarfing may also take place. Of the two species, *T. caries* causes more dwarfing than *T. foetida. T. contraversa* causes a high degree of dwarfing.

U. maydis induces large, blister-like galls on corn, and *Sphacelotheca reiliana* on the same host results in leaf-like structures in the staminate and pistillate flowers which is a phenomenon known as phyllody. When ovulate plants of *Melandrium* are infected by *U. violacea,* the normally vestigial stamens develop and at maturity the anthers contain smut spores instead of pollen; this may be accompanied by some atrophy of the gynoecium. A similar induced hermaphroditism in pistillate flowers of *knautia arvensis* infected with *U. scabiosae* has been reported. Thus, the pathogen changes the sex of the female plants. The nature of this morphogenetic stimulus is not known. According to the effect on the host the smuts may be of two types: (1) Ovariculous, when the inflorescence develops normally and ovary also functions in the normal manner but the contents of the ovary are ultimately destroyed and replaced by smut spores, (2) Culmuculous, where the full development of the inflorescence is suppressed.

Loose Smut of Wheat

Loose smut of wheat has worldwide distribution but it is a serious problem in the humid and semi-humid wheat growing areas. According to an old estimate by Luthra (1953), it caused an annual loss of fifty million rupees in Punjab alone. Misra and Singh (1969) estimated 3–6% losses in some newer high yielding varieties. According to Gothwal *et al.* (1972), smutted plants produce 22.9% less tillers, 27.5% shorter peduncles and 18.7% reduction in height of plants. Chatrath (1996) has reviewed the work on loose smut of wheat and its management in India.

Symptoms

The disease manifests itself only when the plants are in ears. Diseased ears emerge out of the boot leaf a little earlier than the healthy ones and a black powdery mass of spores replaces the flowers. The pathogen is internally seed borne. The growth of the plant and its general appearance is not affected. Smutted heads consist of deformed spike-lets filled with a black, dry, powdery mass of spores which entirely replace all the floral parts and glumes; only the ends of the glumes escape transformation (Fig. 15.1). These spores easily separate from the diseased host and are blown off by wing leaving a bare rachis behind. In many varieties, partially smutted ears or only few tillers show smutted ears while others are free from the disease.

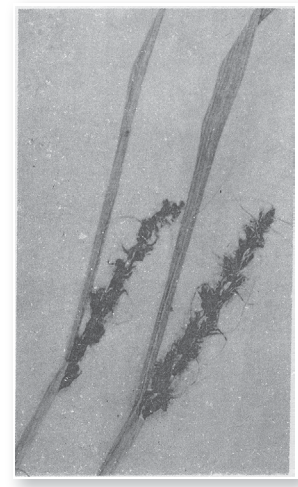


Figure 15.1 Loose smut of wheat (*Ustilago segetum* var. *tritici*): smutted ear heads

Causal Organism

The pathogen responsible for loose smut of wheat is *Ustilago segetum* (Pers.) Roussel var. *tritici* Jensen which was earlier known as *Ustilago tritici* (Pers.) Rostr. Spores which, are produced in great abundance are pale olive, spherical to oval, 5–9 µm in diameter and have minute echinulate walls. On germination, they do not produce sporidia but the promycelium gives rise to threads. The incitant is similar in morphology to that of loose smut of barley caused by *U. segetum hordei (Ustilago nuda*). Loose smut from wheat does not infect barley and *vice versa.* During the early stages of growth of the pathogen, the rate of photosynthesis and respiration is greater in diseased than in healthy plants. Later, the photosynthetic rate declines which results in considerable reduction in the dry weight of the affected plants. In comparison to healthy plants, the diseased plants contain more soluble sugars but the amount of starch is proportionately less.

Disease Cycle

Spores fall on the feathery styles of the stigmas, they germinate and produce promycelium from which infection threads enter the style and grow forward intercellularly until the ovary is reached. By the tenth day, successful entry is made into the ovule, where the germ tubes branch and the mycelium becomes well ramified in it and in the embryo. However, work by Malik and Batts (1960) in the case of *U. nuda* (now renamed as *U. segetum* var. *hordei*) on barley shows no indication of hyphae in the style. According to them, direct penetration of the ovary takes place. Thus, the fungus resides in the kernel as a

dormant mycelium and from superficial examination at harvest time; infected grains cannot be distinguished from the healthy ones. The fungus survives when the infected grains are sown and the hyphae just behind the growing point keep pace with the apical meristem. At the time of ear formation, the fungus enters the reproductive phase (Fig. 15.2).

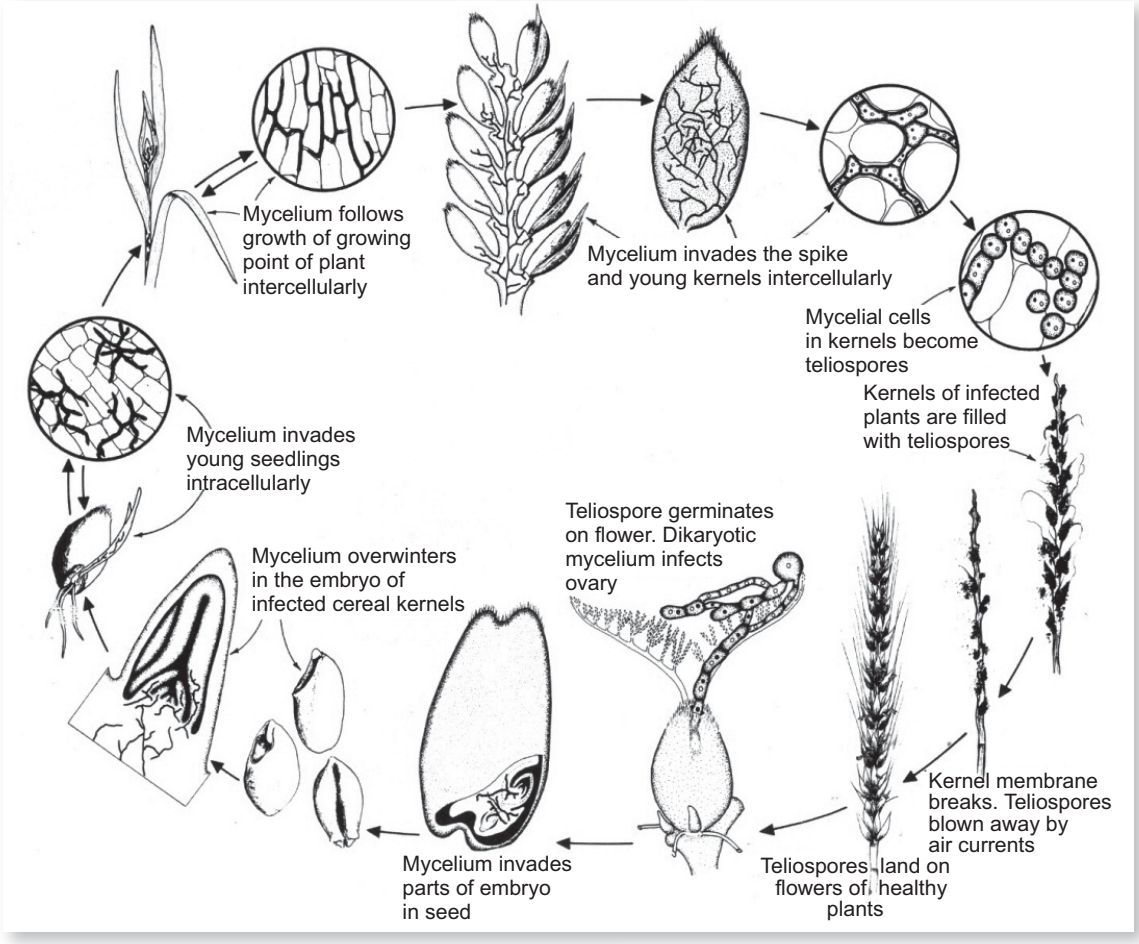


Figure 15.2 Life cycle of loose smut of wheat (*Ustilago segetum* var. *tritici*). (From G. N. Agrios, *Plant Pathology*, Academic Press, New York and London, 1969)

Although the pathogen is internally seed-borne but its active phase is visible only in the floral parts. Environmental conditions are important in determining expression of the disease. A high humidity of 65–85% and 23°C is essential for maximum disease development (Aggarwal, *et al.,* 1984). Singh and Aggarwal (1991) have reported that the flowers of cultivars with loose spikelets are more susceptible than those having compact spikelets.

No comprehensive studies have been made to determine the effect of environmental factors on floral infection. Maximum disease development occurs at 23°C. It is usually observed that the disease is less frequent in areas where humidity is low at flowering time. Physiologic specialization occurs in the

fungus and the race flora of one country differs quite appreciably from those of another. Pal and Mundkur (1945) reported two races, namely, L_1 and L_2, but later, three races were found when six Indian smut collections were examined in Canada. Gothwal and Pathak (1977), using the Cherewick's set of international differentials, identified eight pathogenic races of *U. segetum* var. *tritici* (*Ustilago tritici*) in smut collections from the north-west plain zone of which 3 were similar to 3 races in the world list. Rewal and Jhooty (1986) reported the occurrence of 3 races namely T_1, T_{10}, and T_{11} from Punjab.

Disease Management

In 1908, Jensen, a Danish investigator, found that if the dormant mycelium is induced to germinate, it is vulnerable to heat, by the application of which it can be killed. The seeds are soaked in water at a temperature of 26–30°C for 4–5 hours which induces the dormant mycelium to germinate. The seeds are then quickly transferred to hot water at 54°C for ten minutes which helps to kill the intraseminal mycelium. The thermal death points of the fungus and the embryo are very near to each other, and therefore, extensive care has to be taken in this treatment. There are several other modifications of this procedure. The seeds are then finally dried. It is claimed that the efficacy of the hot water treatment is largely due to the exclusion of oxygen, inducing anaerobic respiration. Alcohol and other catabolic products are set free which are lethal to the fungus.

In the plains of northern India, the summer temperatures are very high (42–44°C). Luthra and Sattar (1934), and Luthra (1953) suggested the following simple and economical procedure: the seeds are soaked in water for four hours in the forenoon on a bright summer day followed by drying for four hours in the sun. This is called solar energy treatment. It has been found that even drying for one hour is sufficient to kill the intraseminal mycelium under conditions prevalent in Punjab.

Weibel (1958) reported a good method of controlling loose smut which entails the immersion of seeds in water soak to which Ceresan or sodium hypochlorite has been added, or by treatment in a water soak, followed by storage in airtight containers. Gera *et al.* (1963) showed that the fungus could be eliminated by soaking the infected seeds in water for 23–29 hours at 33°C or for 41–48 hours at 25°C.

Tyner (1951, 1953), working with loose smut of barley, a disease similar to loose smut of wheat, found that the smut was eliminated to the extent of 99.8% by pre-soaking seeds for six hours in water and then immersing them in a 0.2% suspension of Spergon SL (having 98% tetrachloro para benzoquinone) for 40–50 hours at 50°–72°F. Later, Tyner (1953) discovered that loose smut of barley was effectively controlled by soaking seeds in simple water at room temperature for 56–64 hours. The same treatment controlled loose smut of spring wheat. It is believed that the soaking of the seeds results in the formation of certain quinones inside the seeds due to anaerobic respiration. It is because of this that the fungus is killed inside the seed (Tyner, 1957).

A more recent successful method in the USA could also be adopted in India. This consists of soaking the seeds for 2–4 hours in water between 60–70^{0}F and keeping the moist seeds in the airtight containers for 65–70 hours and thereafter drying them. This method is called anaerobic seed treatment. Soaking of seeds in 3 and 4% extract of onion or garlic at 30°C for 29 hours brought down infection from 12.5–1.4%.

Excellent control of loose smut of wheat is now obtained by seed dressing with systemic fungicides, such as Carboxin (Vitavax) and Benomyl at 0.2-0.25% (Chatrath and Mohan, 1971, Chatrath *et al.*, 1969; Nene *et al.*, 1971; Tyagi, 1972 a, b). According to Verma *et al.* (1984) Bavistin SD (25% a.i.) has been found as effective as Bavistin WP (50% a.i.) and is also cheaper. The combination of Vitavax and Thiram or copper quinolate has been found equally effective and reduces the cost.

Varietal differences in the reaction to loose smut of wheat have been found in different countries. Mundkur and Pal (1941) and Pal and Mundkur (1941, 1945) found several immune or resistant varieties. Since then, several resistant varieties have been bred at IARI, New Delhi. The high yielding varieties, such as Kalyan 227, PV 18, WG 307, and C 302 have been found to be resistant to this disease and with the widespread cultivation of Kalyansona; there has been a considerable reduction in the loose smut of wheat.

Trichoderma viride is a promising biocontrol agent of several plant diseases. A strain of this antagonist, viz., TV–5 showed potentiality against loose smut of wheat (Aggarwal and Nagarajan, 1992). However, strain TV–5 was incompatible to commonly used fungicide, Carboxin. Studies on development of mutants which would be compatible with commonly used fungicides are in progress (Selvakumar *et al*., 2000).

The advent of some new systemic fungicides like Azoxystrobin and Picoxystrobin (Strobilurin fungicides) when applied as spray reduce disease incidence and increase grain yield (Ruske *et al*., 2003). Johnson *et al*., (1998) had reported that when seeds of cereals (barley, oat, wheat, and rye) were treated with bacterial cultural of *Pseudomonas chlororaphis* (*Pseudomonas aureofaciens*), seed borne diseases caused by *Drechslera* spp. and *Ustilago* spp. were found to be controlled effectively. Singh and Maheshwari (2001) have reported that biocontrol agents *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*, and *Gliocladium virens* in combination with half dose of Vitavax were active loose smut of wheat. However Beniwal *et al.* (2002) have reported that biocontrol agents like *Trichoderma* spp. and *Bacillus subtilis* control the disease but not better than Vitavax. Sinha *et al*. (2000) claimed that varieties PBW65, HDR 70, VL421, and WL 410 were resistant to loose smut of wheat.

Flag Smut of Wheat

It is prevalent in almost all the important wheat growing areas of the world. It was first found in Australia in 1868 and since then it has been reported in China, Japan, India, Pakistan, Egypt, S. Africa, Italy, Spain and the USA. In India, Butler was the first person to report this disease from Layallpur in West Punjab (Butler, 1918). It is believed that the disease was introduced into India from Australia. The disease is not widespread in the former but its importance cannot be underestimated. The smut can persist in the soil for long durations and under favourable environmental conditions, it assumes serious proportions. It is estimated that the incidence of disease in some parts of Punjab, Himachal Pradesh and Haryana is very high (upto 75%). The disease is not widespread due to unfavourable environmental conditions in other parts of the country. Flag smut of wheat was considered earlier as a minor disease and was endemic in some areas only. The incidence of the diseases is gradually increasing in states of Haryana, Punjab, Rajasthan, and Kumaon Hills of Uttrakhand (Goel and Singh, 1996).

Symptoms

The disease attacks the stem, culm, and leaf. The leaf and leaf sheaths are most commonly affected. The disease manifests itself from the late seedling stage to the time the crop is mature. Grey or greyish black linear sori appear on the leaf blades and leaf sheaths.

Leaves get twisted and begin to droop and then wither. Sori develop under the epidermis in the mesophyll tissue. Later, the epidermis ruptures, exposing a black powdery mass of spores and finally the leaf tissue disintegrates along the linear sori. Later, the leaves are shed and the whole plant dies; very often

the culm remains sterile. If the grains are formed, they are shrivelled and do not germinate or germinate very poorly. In the susceptible varieties, the plants are dwarfed (Fig. 15.3a).

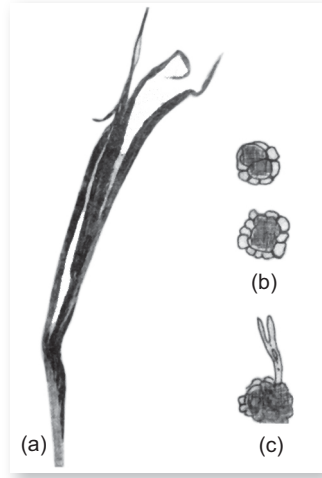


Figure 15.3 Flag smut of wheat caused by *Urocystis agropyri* (earlier called as *Urocystis tritici*: **(a)** part of the affected shoot **(b)** spore balls **(c)** spore ball germinating

Causal Organism

The pathogen responsible for flag smut is *U. agropyri* (Prueuss) Schroet (earlier known as *Urocystis tritici*).

The spores of the flag smut fungus are formed in tiny balls consisting of one to six (mostly three) fertile cells. The bright brown spores are covered by a layer of sterile peripheral cells. The fertile cells are globose and 8–18 µm in diameter. Spore balls measure 18–52µm in diameter. The sterile peripheral cells are smaller. The spores germinate in situ to produce a short promycelium with or without septation. Three to four cylindrical sporidia, hyaline in color, are produced near the apex. The sporidia germinate while still attached to the promycelium and this forms the infection thread (Fig. 15.3 b, c).

Disease Cycle and Predisposing Factors

The spores of the pathogen are soil and seed borne. The spores on germination infect the seedlings (primary shoot or the young buds) and become systemic. Sori develop later on the leaves (Fig. 15.4). Seed infestation is more important than soil inoculum. After infection, the fungus grows both inter- and intra-cellularly. Seedling infection is influenced by weather conditions such as soil temperature, soil pH, soil moisture as well as by such cultural practices as sowing depth, host variety, planting date and growth stage of the host. Sowing in dry soils favour infection. Deep sowing increases chances of infection due to the fact that the germinating seedlings remain beneath the soil surface for longer period and are thus exposed to infection for longer time than in shallow sowing.

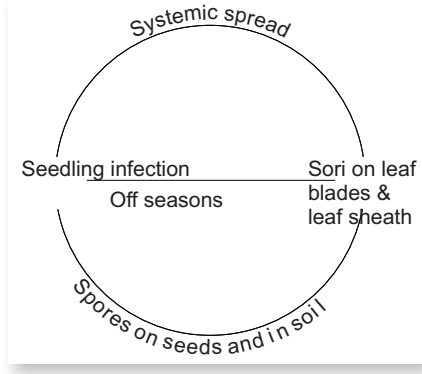


Figure 15.4 Diseases cycle of *Urocystis agropyri* (*U. tritici*) causing flag smut of wheat

Physiologic Specialization

Twelve races have been reported from China and two races from the USA. Pal and Mundkur (1941) believed that the Indian strain is different from the Australian strain. According to Goel (1992) race 4 is reported to be common in India.

Disease Management

Seed treatment with copper carbonate dust is a very effective method of controlling the flag smut of wheat. TCNA has been found to be quite effective in the USA (Purdy, 1965) and in India (Tyagi, 1972 a, b). Purdy reported that Tafasan (methyl ethyl Hg chloride), PCNB and Vitavax are effective

chemicals for controlling this smut. In the past, steeping the seeds in formaldehyde solution (half kg in 200 L water) had also been recommended for disease control. Goel and Jhooty (1985) reported that seed treatment with Benlate, Bavistin, Vitavax, Fenfuran, Triadimefon and Triadimefon gives good control of seed-borne as well as soil-borne inoculum.

Cultural practices, such as early sowing, burning of stubble, crop rotation, and hot weather cultivation, have been reported to check the incidence of the flag smut considerably. Kalyansona, PV 18, Lerma Rojo and Sonora 64 which are some of the high yielding varieties are susceptible to the flag smut. Screening for disease resistance has shown that *T. durum* wheat in general, has a few degree of resistance to flag smut.

Urocystis agropyri incited flag smut of wheat could be managed by growing resistant cvs like WH 293 or HD 2329. Seed treatment with Tubeconazole at 1.0, 1.5 and 2.0 g/Kg or Carboxin and Carbendazim at 2.0 and 2.5 g/Kg seed, reduced cent percent disease incidence. Application of poultry manure at 4–6 t per hectare managed flag smut.

Hill Bunts or European Bunts or Stinking Smuts of Wheat

Hill bunts of wheat are of importance not only because they cause serious loss to the crop in the hills and temperate climates, but also because a study of this disease helps to develop the science of plant pathology. In India the disease is normally restricted to the northern hilly region. It has also been reported sporadically from the plains. This disease is worldwide in distribution. Bunt possesses poisonous properties, and therefore, the flour of contaminated seeds is harmful for human consumption. The straw is harmful and dangerous to cattle. The disease also infects rye and some grasses such as *Agropyron* spp. Extensive cultivation of Kalyansona or material from Kalyansona has considerably reduced hill bunt problem.

Symptoms

The stinking smut is of two types, one or usually both are present in the same grain. One type is caused by *Tilletia caries* (low smut) and the other is caused by *T. foetida* (high smut) in which the culms are of the same height as those of the healthy plants. A dwarf bunt caused by a variety of *T. caries* has also been reported. This has now been named as *T. contraversa.*

The symptoms (Fig. 15.5) of the disease are not usually evident until the heading stage. A microscopic examination a few days prior to the emergence of the head from the boot shows differences between normal and smutted florets. The pistil from the smutted heads is larger, with an ovary twice the length of the normal, and becomes green instead of white as in the normal. The length and breadth of the stamens is also reduced and the anthers become pale yellow, containing sterile pollen cells.

After the emergence of the ears, the disease can be detected easily. In the *compactum* types, the normally compact or



Figure 15.5 Hill Bunt of wheat: symptoms

square heads are generally changed to a more slender type. In the *vulgare* type the change is not evident until the production of smutted grains. The tillering capacity of bunt-affected plants is increased considerably. The foul smell caused by trimethylamine, a volatile compound, is a good indication of infection in the field. As a rule, all the ears in a diseased plant and all the grains in an ear are turned into smut balls.

Causal Organism

Two species of *Tilletia*, i.e., *T. caries* (DC) Tul. and *T. foetida* (Wallr.) Liro are responsible for hill bunt or stinking smut of wheat. These species have a wide host range. These can infect rye, *Aegilops, Lolium, Agropyron* and *Hordeum.* The life history of the two species is similar. One of the chief morphological distinctions is the markings on the teliospore. In *T. caries* the spores have walls with reticulate surface patterns, while in *T. foetida* the wall is smooth. The spores are intermixed with hyaline sterile cells.

The spores germinate by producing a promycelium which comes out after rupturing the spore wall and bears a cluster of filiform, hyaline primary sporidia at its tip. These uninucleate primary sporidia fuses in pairs while still attached to the promycelium and form H-shaped structures. After fusion, these primary sporidia become binucleate. They germinate by giving out hyphae which produces secondary sporidia, which are sickle shaped.

Physiological Specialization

Twenty races of *T. caries* and ten races of *T. foetida* are known (Halisky, 1965). In India, several races have been identified.

Disease Cycle and Predisposing Factors

Primary inoculum consists of the spores carried on the seed or present in the soil. However, under Indian conditions the disease is mainly seed borne. The bunt spores do not survive even one season under field conditions (Prasada, 1965). The life cycle and disease cycle of the pathogens are given in figures 15.6 and 15.7 respectively.

Temperature ranging from 5–15°C is highly favourable for infection. Temperatures lower or higher than this are unfavourable temperature conditions. High soil moisture, sandy and humus rich soils favour infection; on the other hand, soils which are clayey and acidic are detrimental to infection. Irrigated wheat has less bunt than non-irrigated wheat. Good tillage appears to reduce the severity of attack. Potassium and Phosphatic fertilizers increase the severity of infection, while nitrogenous, superphosphate and calcium cyanamide reduce it. Spore load on the seed has a direct relationship with the severity of the infection.

Disease Management

Prevost (1807) observed that copper sulphate is very effective in controlling the disease. Being an externally seed-borne disease, seed treatment with copper fungicides, formaldehyde, mercurial fungicides and hexachlorobenzene and antibiotics, such as Actidione and Helixin B were reported to be quite effective in the past. Sharma *et al.* (1971 a, b) obtained good results with 0.3% Benlate and 0.2% Vitavax. Bavistin (2-Methoxy carbamoyl benzimidazole) was reported by Singh *et al.* (1977) as a seed dressing fungicide at 0.1% concentration giving complete control of hill bunt of wheat.

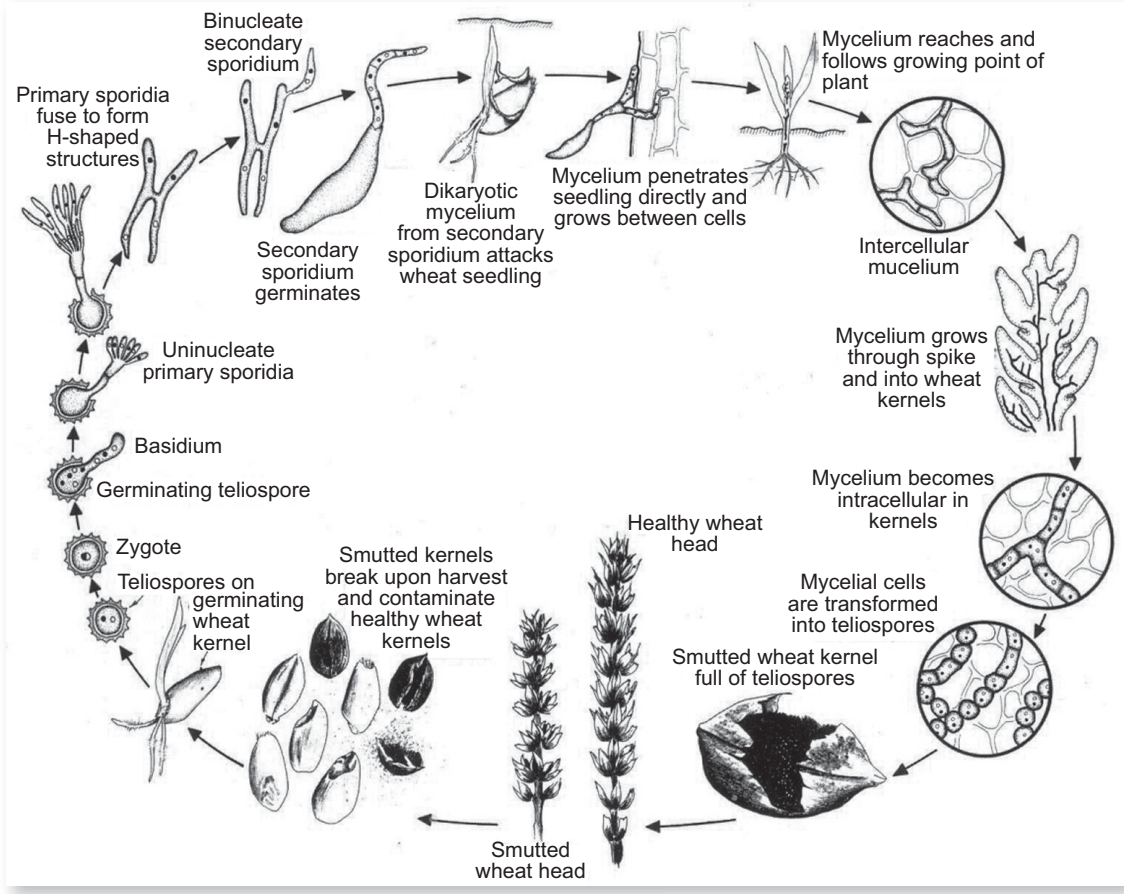


Figure 15.6 Life cycle of hill bunt of wheat (*Tilletia caries*). (From G.N. Agrios, *Plant Pathology,* Academic Press, New York and London, 1969)

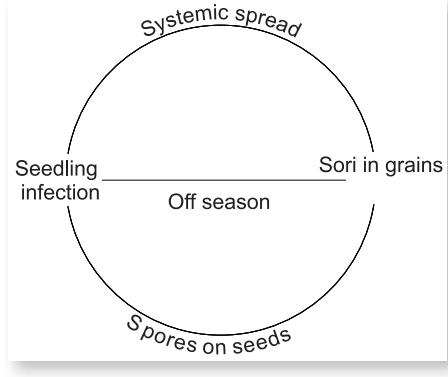


Figure 15.7 Diseases cycle of hill bunt of wheat caused by *Tilletia caries* and *T. foetida*

According to Gaudet and Puchalski (1992), the efficacy of Vitavax depends on its proper concentration for the level of seed infestation which was determined by the level of disease intensity in the parent crop. McManus *et al.* (1993) reported the inhibition of teliospore germination of *T. laevis* by a strain of *Pseudomonas fluorescens.*

Some resistant varieties are available. Kalyansona is resistant. The disease is now of minor importance due to the widespread cultivation of Kalyansona and other resistant varieties. Early sowing when the soil moisture is high and shallow sowing are effective in reducing the disease. Crop rotation is also helpful.

Organic seed treatment with wheat flour, Skimmed milk powder and skimmed milk at 160g per kg of seed reduced common bunt infection: However skimmed milk gave as good control as chemical seed treatment. Even seed treatment with yellow mustard meal (60g/kg seed) is also effective against pathogen. *Bacillus subtilis, B. pumilus* and *B. licheniformis* are some potential biocontrol agents against *Tilletia* sp. Some strains of *Pseudomonas* suppressed common bunt (*T. caries*). Bahadur *et al.* (1984) claimed that varieties UP2002, HB383, WL 410, WL885, WL1581, IWP 129, 127, 87, and 72 were immune to the disease.

Karnal Bunt of Wheat

Karnal bunt of wheat was first reported by Mitra (1931) from Karnal (Haryana), India. This bunt was formerly restricted but of late it has become more widespread and common in Punjab, Haryana, Delhi, Himachal Pradesh and the western districts of U.P. It is sporadic in its occurrence, but in certain years it assumes serious proportions. The disease has now been reported from several other countries in Asia, in the early 1980s from Mexico and since 1996, from a few places in the south-western United States of America. Karnal bunt of Wheat as a disease of international importance was recognized by Warham (1992) and Bonde *et al.* (1997). The smutted seeds are likely to have lower survival rate in storage compared to healthy seeds. The fungus causing Karnal bunt of wheat is reported on several wild species of *Triticum* (Aujla *et al.* 1985). During 1969–70, most of the dwarf wheats were affected by Karnal bunt. Again in 1974–75 crop season, Karnal bunt attained epiphytotic proportion in many places in the northern part of the country. Gogoi *et al.* (2001) showed the role of phenols as a basis of biochemical resistance in wheat against Karnal bunt.

Symptoms

The symptoms of Karnal bunt are entirely different form the other two hill bunts. Here, all the ears are not infected in a stool and in the ears all the grains are not infected. The disease is evident when the grains are formed only then can it be known whether the grains have been partially or wholly converted into black powdery masses enclosed by the pericarp. The smutted grains are very irregularly distributed, suggesting air-borne, localized infection. When mature kernels are broken, they are found to be full of black powdery mass of fungus spores that give off a foul smell due to trimethylamine resembling that of decaying fish. The transformation of the kernel into small sori is not even complete in many cases, only a part being destroyed and the rest remaining healthy (Fig. 15.8 a and d).

Causal Organism

Karnal bunt or partial bunt of wheat caused by *Tilletia indica* Mitra [*Neovossia indica* (Mitra) Mundkar] was first reported by Mitra in 1931 on *Triticum aestivum* from Karnal town of India hence the Karnal bunt.

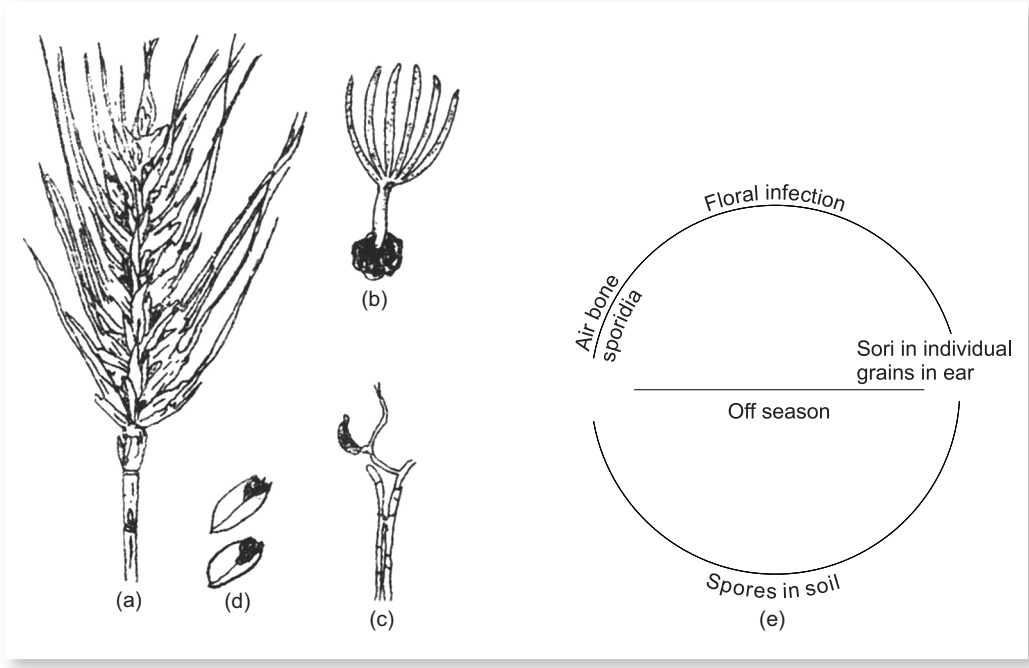


Figure 15.8 Karnal bunt of wheat *Tilletia indica (*earlier called as *Neovossia indica)*: **(a)** infected ear, **(b)** germinating spore with sporidia, **(c)** paired sporidia germinating to form secondary sporidium, **(d)** partially infected grains **(e)** diseases cycle of Karnal bunt of wheat.

The spores are dark brown and measure 20–49 µm in diameter, with reticulations on the epispore which appear as curved spines. A thin hyaline membrane covers the spines on the epispore. Sterile cells are intermixed with the fertile spore cells.

A short promycelium is produced at the apex, out of which a cluster of 60–185 sporidia are produced (Fig. 15.8 b and c). These sporidia are long, sickle-shaped and do not fuse into pairs. They germinate by producing germ tubes which act as infection threads.

Germination of the spores occurs after a long rest period.

Disease Cycle and Predisposing Factors

Mitra (1937) reported that Karnal bunt is a soil-borne disease, but later it was shown to be air borne. The spores of the fungus get into the soil during threshing or as external seed contaminants. These germinate in February or March when suitable soil moisture and temperatures are present. Sporidia produced as a result of the germination of the spores are wind borne and are carried to the wheat florets. These germinate and enter the developing grain through the ovary wall. The mycelium of the pathogen penetrates the embryonic tissue but does not inflict damage. Disease cycle of the pathogen is depicted in figure 15.8 (e).

Excessive irrigation or rains at the time of anther formation increases chances of infection. During January and February, if the weather becomes wet and cloudy, the incidence of Karnal bunt increases. An increase in nitrogenous manures predisposes the plants to infection. Seven physiologic races of the

pathogen have been identified so far (Munjal, 1975). As already indicated, the environmental factors play a major role in the occurrence of the disease. The climatic and field data on Karnal bunt from India and Mexico over ten years were analyzed following multiple regression analyses of variance and t-test of significance by Nagarajan (1991). The analyses showed that it is the number of rainy days at boot leaf stage that is important in development of the disease. Jhorar *et al.* (1992) have also attempted to develop a forecasting system. Higher dozes of nitrogen fertilizer, heavy manuring and late sowing favour increase in the incidence of the disease.

Disease Management

The pathogen is seed and soil borne and at the time of flowering becomes air borne as well. Therefore, an integrated approach including strict quarantine measures, cultural practices, chemical application, use of disease free seed and resistant cultivars can check the disease to a satisfactory level (Chahal, 2011).

Cultural Practices Crop rotation, use of disease free seed, judicious use of nitrogen fertilizer and adjustment of date of sowing, irrigation management and intercropping with gram or lentil have been worked out to minimize the disease incidence (Goel *et al*., 1977; Aujla *et al*., 1981; Gill *et al.,* 1993).

Deep ploughing, fallow after harvest, reduced plant density and planting of wheat on light soils have generally been advocated which are merely based on observation instead of long term experiments and therefore have limited practical utility.

Chemical Control Different chemicals have been tested against seed-borne inoculum. Chemicals like Butrizol, Copper carbonate, Formalin, Thiram, Trifenilentin acetate, Triphenile hydroxide have been found to reduce the teliospore germination. The fungicides as seed dressers have limited practical utility because no chemical eradicates the seed borne inoculum completely. Thus, seed treatment with systemic fungicides is moderately effective.

Spray Application According to Singh *et al.* (1985) and Smilanick *et al.* (1987) fungicides like Biteratanol, Bentizol, Carbendazim, Carboxin, Copper oxychloride, Fentihydroxide, Mancozeb, Pentachloronitrobenzene, and Propiconazole have been found effective in controlling the air-borne sporidial inoculum when applied at the time of heading stage of the wheat crop. According to Singh (1997), Tilt (Propiconazole), Baycor (Biteratanol), Bavistin (Carbendazim) and Dithane M-45 (Mancozeb) have also been found effective against the disease. Folicur 250 EW has also been found effective.

Host Plant Resistance Breeding for Karnal bunt resistance is the best and most effective method to control the disease. According to Chahal *et al.* (2001) efficient screening techniques have been developed by various workers. Cultivation of Karnal bunt tolerant varieties like WL 1562, PBW 54, HD 2281, several durums and efforts to diversify the cultivars grown by farmers have contributed in reducing the disease incidence over years. Several wild species of *Triticum* have been found to possess resistance to the pathogen. Durum and aestivum wheats show promising resistance while *T. vulgare* is susceptible. In screening done in Mexico, no bread wheat were found resistant but 41 of the 448 durum wheat and 151 of 710 triticale lines were bunt free. In varietal trials at IARI, New Delhi varieties HD 1907, HI 358, HP-743, L176, L191, M-137A, MW 59-4 X6A had shown resistance to *N. indica*. At Pantnagar, UP270, UP368, HD 2222, 2227 and 2235 remained free from bunt. Singh D.V. *et al.* (1985) had suggested that the increased cultivation of susceptible varieties like Arjun, WG 357, WL 711, etc., have increased the disease incidence.

False Smut of Paddy

False smut does not belong to the order, Ustilaginales. It is not a smut in the true sense. It was first reported in India by Cooke in 1818 from Tinnevally in South India and is now rampant in almost all the rice growing areas of India. It is now known to occur in almost all the rice growing areas of the world, including America, Burma, Ceylon and Fiji, excepting Australia. Epiphytotics of the disease have been reported from India and the Philippines. In Punjab, Losses varied from 1.6 to 6.8% in different varieties (Dhindsa *et al.* 1991).

Symptoms

Symptoms of false smut are confined to the ears. The effect of the pathogen is visible only on the grains. Due to the development of the fructification of the pathogen, scattered individual grains are converted into large, velvety green masses or balls (Fig. 15.9 a). The transformed kernels are several times the size of the normal kernels. Their color is orange-yellow on the periphery and white in the centre. When mature, the surface of the ball cracks. Glumes remain unaltered and are found closely sticking to the centre of the mass which burst out above. The ball consists of lightly woven mycelium together with the glumes and other host tissue. There are three outer layers at different stages of development. The innermost layer is yellowish with radiating mycelium, and spores in the process of formation. The next layer is orange in color and is composed of the mycelium and spores. The outermost layer is green and consists of mature spores.

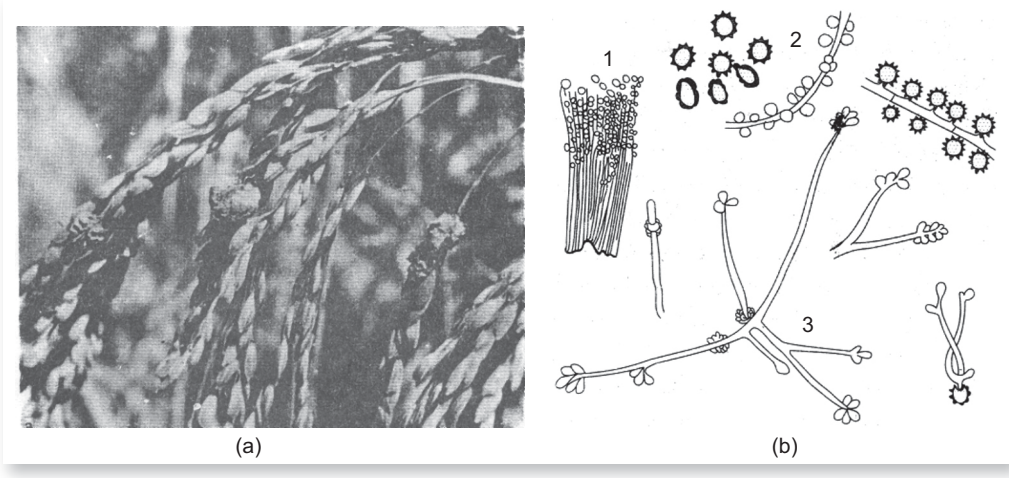


Figure 15.9 False smut of rice (*Ustilaginoidea virens*): **(a)** symptoms **(b) (1)**: section of outer part of Sclerotium, **(2)**; formation of spores, **(3)**: spores germinating

Causal Organism

The disease is caused by the fungus *Claviceps oryzae sativae* Hashioka. Its conidial stage is *Ustilaginoidea virens* (Cooke) Tak—the fungus which was first described under the name *Ustilago virens* (Fig. 15.9 b). Chlamydospores formed on the balls are borne laterally on minute sterigmata on radial hyphae and are spherical to elliptical, waxy, oliovaceous and 3–5 x 4–6 µm. The younger spores are smaller,

paler, and almost smooth. Some of the green spore balls develop one to four sclerotia in the centre. These sclerotia overwinter or oversummer in the field. The fructifications replacing the grains represent the conidial and the sclerotial stages of the pathogen. They are at first yellow to orange and later turn olive green to black. When young, they are fleshy inside but become hard after some time. The perfect stage was described by Sakurai in 1934 as *Claviceps virens* (Cooke) Sakurai. Hashioka (1971) further confirmed the observation of Sakurai and renamed the fungus as *Claviceps oryzae–sativae.* Singh and Dube (1984) studied the process of germination of sclerotia under controlled conditions. They have shown the production of stroma (perithecial head) terminating into a cap with perithecia embedded in it.

Disease Cycle and Predisposing Factors

The fungus perennates through the hard sporiferous masses which become sclerotia. The germination of the sclerotia is undescribed so far. The infection of the ovaries takes place through the conidia at an early stage.

Survival of *Claviceps oryzae–sativae*, the incitant of false smut of rice had been worked out by Singh *et al.* (1985). According to them, conidia remain viable for 3 months in a refrigerator while these survive for 2 months at room temperature and under field conditions. Pseudosclerotia which are dried and hardened conidial masses remain viable for 9 months at 10°C under field conditions. These true sclerotia are the major source of primary inoculum. In nature, infection occurs through ascospores produced by overwintering true sclerotia and by conidia. Survival and perpetuations of the pathogen has been recently worked out by Hegde and Anahosur (2000). Presence of true sclerotia and their germination through stromatic head bearing ascospores were observed in Karnataka. There is a possibility that the life cycle may be similar to *Claviceps.* An ascomycetous state of *Claviceps* type has been described from Japan where it is regarded important in the overwintering of the pathogen. The disease cycle of the pathogen is believed to be as given in Fig. 15.10.

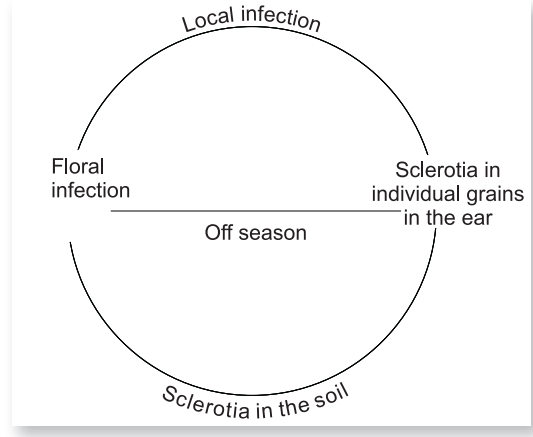


Figure 15.10 Disease cycle of false smut of rice caused by *Ustilaginoidea virens.*

Factors favouring infection by *U. virens* include high humidity, high rainfall and cloudy days during flowering.

Disease Management

Clean cultivation, crop rotation and steeping the seeds in brine solution are recommended as control measures. Apart from this, dipping the seeds in 1:1000 mercuric chloride solution for 30 minutes for 6 hours and followed by rinsing in water had been recommended for disease control in the Philippines.

Singh and Singh (1985) have reported that Bordeaux mixture (0.4%) or Blitox (0.25%) gives about 90% disease control when sprayed thrice at 10 days interval. In varietal trials, varieties IR22, IR26, IR28, Surya and Vijaya had shown complete resistance to pathogen. Eighteen strains of Bacillus bacteria showed biocontrol effects on *Ustilaginoidea virens in vitro* conditions.

Bunt of Rice (Syn. Black Smut, Kernel Smut, Rice Smut)

This is widespread in India; the far east, North (Mexico), central (Trinidad) and South (British Guiana, Venezuela, British Honduras) America; and the Pacific.

Symptoms

Only a few grains in the panicle but not more than 3–4 (as in Karnal bunt of wheat) are infected (Fig. 15.11). Not all the ears in a stool show the presence of bunt balls. Very often the entire grain is not transformed into a sorus; a portion may be free from the spores. Most of the sori are hidden by the glumes, but sometimes they burst through at maturity. Early maturing varieties are more susceptible than late maturing varieties.

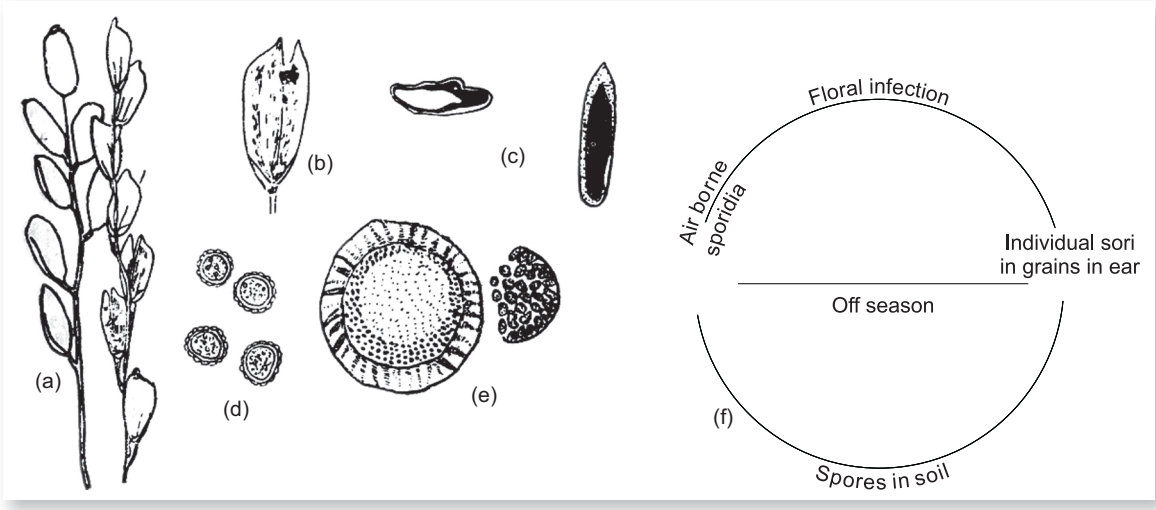


Figure 15.11 Bunt of rice caused by *Tilletia barclyana* **(a)** parts of bunted ears **(b)** bunted grain **(c)** bunted grains in cross and longitudinal sections **(d)** spores **(e)** section and surface view of spores **(f)** disease cycle

Causal Organism

This is caused by *Tilletia barclayana* (Bref.) Sacc. and Syd. (*Neovossia barclayana* Bref. *N. horrida* Tak). The pathogen produces spore mass which are granular or agglutinated black. Sterile cells are intermixed with the spores, few to many, mostly more or less globose but rather variable in shape, hyaline to yellowish tinted, smooth, wall 2.4–4.0 µm thick and 10–30 µm in diameter. Spores globose to subglobose, in the beginning light brown, becoming dark brown at maturity, enclosed in a tinted or hyaline sheath with or without a shoot apiculus, exospore, when young, ornamented with curved spines which become truncated, scales at maturity, mostly 11–25 µm in diameter (Fig. 15.11). Sporidia 10–12 in number, needle-shaped or filliform, curved in various ways, measure 31–53µm in length. These sporidia do not fuse in pair or form H-shaped structures.

Disease Cycle and Predisposing Factors

The disease is soil borne and externally seed borne. Infection occurs in the opening flower. The sporidia are wind borne to the flowers (Fig. 15.11 f). Little is known with regard to the physiologic specialization of the fungus. The host range includes species of *Brachiaria*, *Digitaria*, *Echinochloa*, *Panicum*, and *Pennisetum.* It has been found that a high rate of nitrogenous fertilizers, especially at a late stage of growth, increases the incidence of smut. Weather conditions also affect the incidence of the disease, frequent light rainfall and humid weather when the plants are flowering, being conducive to attack.

Disease Management

Cultural Practices The thick walled teliospores have been reported to survive for more than three years under normal conditions (Ou, 1985). There is no effective chemical which has killing effect on these structures. According to Chahal (2001) air-borne nature of the pathogen and multiplication of sporidia on non-host plants also delimit effectiveness of chemicals against pathogen. However, the disease can be effectively checked by integrating certain cultural practices which are as follows:

The teliospores remain adhered to the surface of healthy seeds. Use of disease-free seed helps reducing primary inoculum as well as introduction of the disease to newer areas. The detection procedures are given by Chahal and Pannu (1997).

Excessive use of nitrogen has been shown to favour the disease development. Therefore, use of nitrogen fertilizer should be judicious; well within the recommended limits to avoid increased vulnerability of the crop to the disease. The Kernel smut is more on early sown/transplanted crop in Punjab. Therefore, the crop in this region should not be transplanted earlier than 20th June to minimize losses. Irrigation should be minimized during flowering period.

Use of Chemicals According to Grewal *et al*. (1996) Triazole fungicides, i.e., Tilt, Folicur, and Baycor are quite effective in controlling the disease in spite of the fact that the disease is air-borne.

Resistant Varieties Shorter anthesis period and narrow opening of florets contribute towards resistance. Some newly developed hybrids have been found with less than 1% mean coefficient infection (Kumar *et al*., 1997). However, Sharma *et al.* (1992) and Chahal *et.al.* (1993) have observed higher susceptibility in hybrids and CMS lines as compared to varieties. In UP, some varieties were found resistant to bunt of rice which are T6, T8, T10, T27, T72, HR22, BJI, N12, N10B, NCH 7

Leaf Smut of Rice

Leaf smut is not a disease of great economic importance but it is found in India, Myanmar, Japan, China, Taiwan, Philippines, and Afghanistan.

Symptoms

The smut is characterized by the appearance of distinct, not confluent, leaden black colored spots on the leaves. The spots are linear, rectangular or angular, and elliptical. They remain covered by the epidermis, which is ruptured when the leaves are soaked in water for some time. The sori under the epidermis appear as a black mass resembling sclerotia. This sorus consists of a mass of closely packed spores (Fig. 15.12).

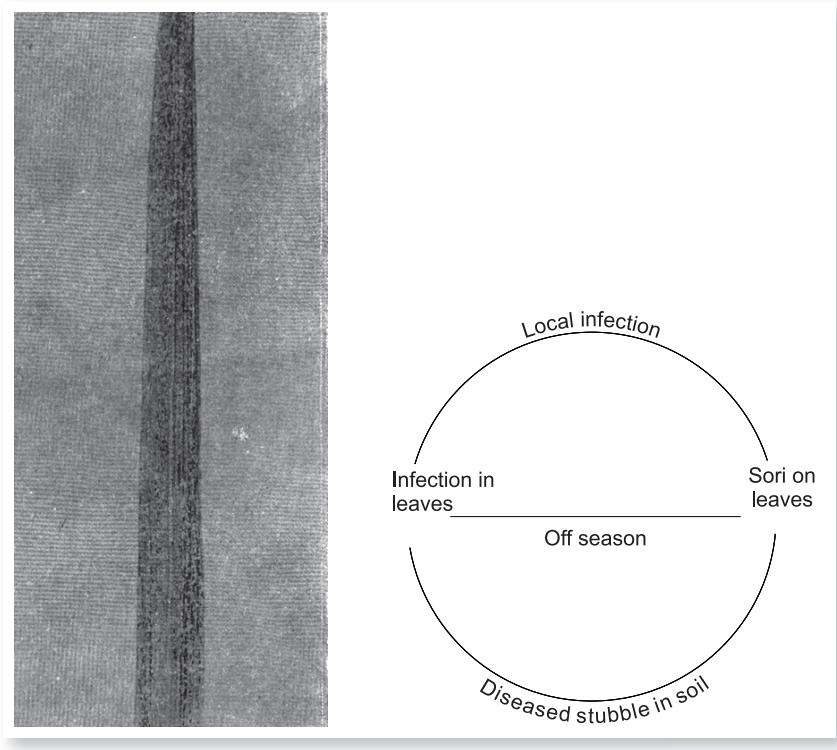


Figure 15.12 Leaf smut of rice caused by *Entyloma oryzae*: symptoms and disease cycle

Causal Organism

The pathogen responsible for leaf smut of rice is *Entyloma oryzae.* H. & P. Sydow. Spores are angular to globose, smooth walled, light brown in color and measure 6–9 x 7.5–11.5µm. The epispore is 1–1.5µm thick. The spores germinate *in situ* to form a promycelium with sporidia.

Disease Cycle and Predisposing Factors

In all probability, the disease perennates through hypophyllous sori lying in the field in the diseased leaf tissues. Infection is caused by the sporidia reaching the leaves near the soil level and there is local infection (Fig. 15.12). It also infects wild rice (*Zizania aquatica*). In the USA, it has been seen that infection is more severe when heavy nitrogenous manures are present.

Disease Management

Clean cultivation and cultivation of resistant varieties are recommended. In the USA, the variety, C 19187 has been found to be resistant. According to Reddy and Reddy (1992), cultivars T6, T8, T10, T10, T19, T36, NCH2, NCH20, NCH43, HR22, BJ1, N12, AD15426, Pankaj, Jagriti, Garima, Patel 5, and Safari are resistant.

Covered Smut, Kernel Smut, Short Smut or Grain Smut of Jowar (*Sorghum vulgare*)

It is present in Africa; Asia; Australia; and central, North, and South America and is coextensive with the cultivation of sorghum. In India, it is one of the most serious diseases of the crop in the states of Tamil Nadu, Mysore, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, and Bombay. Up to 25% of the plants have been found to be affected in certain areas, and the value of the grain destroyed was computed at several million sterling (Butler, 1918).

Symptoms

As a rule, the majority of the grains in an ear are converted into sori. In place of the normal grain, an oval, cylindrical, grey sac, measuring 5–15 mm in length and 3–5 mm in breadth, is found. Subtending the sac and the glumes of the spikelets, the sorus is covered by a tough membrane and remains intact without rupturing early (Fig. 15.13). The interior of the sorus is completely filled with the spore powder, except a slender sometimes curved, central column of hard tissues, the columella which is hollowed in to depressions at the surface. The columella is composed of the host tissues and consists of parenchyma traversed by fibro vascular bundles.

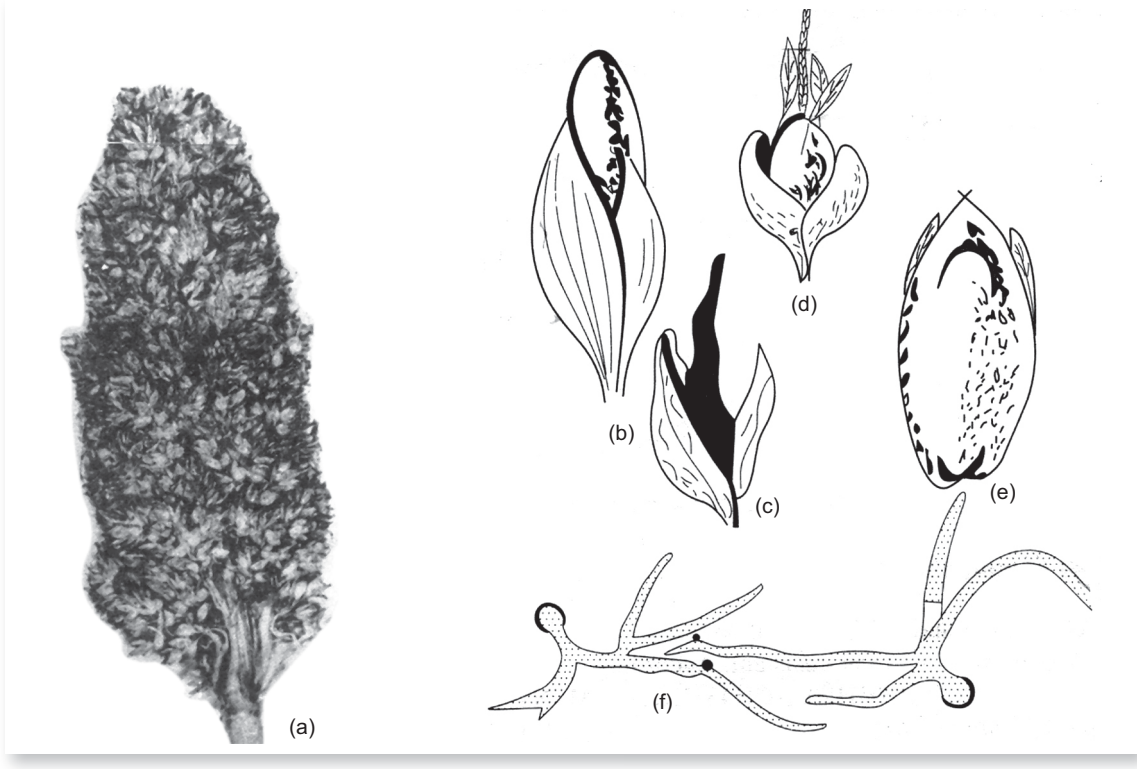


Figure 15.13 Grain smut of jowar (*Sphacelotheca sorghi*): **(a)** symptoms **(b)** a sorus magnified **(c)** columella **(d)** sorus not involving stamens **(e)** sorus with stamens involved **(f)** germinating spores

Causal Organism

The pathogen responsible for grain smut of "Jowar" is *Sphacelotheca sorghi* (Link) Clint. The pathogen is renamed as *Sporisorium sorghi* (Vanky, 1994). The spore mass is dark brown, surrounding a central columella of the host tissue. The spores are globose to subglobose, olivaceous brown, finely echinulate, 5–9 (mostly 6–7) μm in diameter. The spores germinate immediately. The spores retain their viability for a considerable period of time. Sporidial cultures may be obtained on potato dextrose agar. Studies on grain smut caused by *S. sorghi* have indicated the presence of variations in the types of sori, varietal reaction and cultural characteristics of monosporic isolates obtained from different localities (Ranganathaiah and Govindu, 1970).

Physiological Specialization

Tarr (1962) has tabulated the reactions of eight physiologic races on ten differential sorghum varieties. Hybridization has been recorded between *S. sorghi, S. reiliana* and *S. cruenta.*

Disease Cycle and Predisposing Factors

It is an externally seed-borne disease. Seeds get contaminated at the time of the threshing of the grains. Infection takes place between the commencement of the germination of the seed and the appearance of the seedling above the soil in the field. Penetration is chiefly through the radicle or mesocotyl region. The disease cycle is given in Fig. 15.14.

Environmental conditions influence the intensity of infection. Based on germination studies, Kukarni (1922) concluded that temperature plays and important part in the infection and distribution of the disease. A temperature of 25°C or so is very congenial for the development of this smut. Medium to low soil moisture is conducive to maximum infection.

The smut infects all the cultivated species of *Sorghum* and also *S. halepense* and *S. sudanense.*

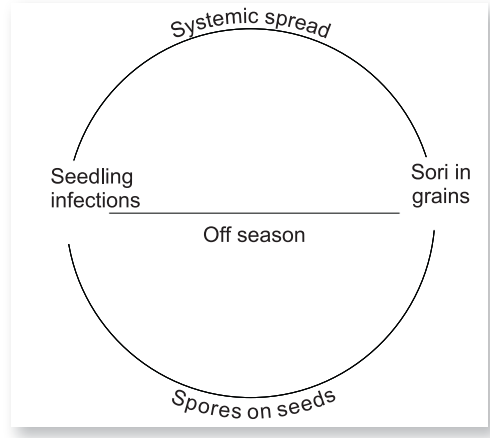


Figure 15.14 Disease cycle of grain smut of jowar caused by *Sphacelotheca sorghi*

Disease Management

Infection is mainly seed borne and occurs only at the seedling stage. Butler (1918) stated that there is field evidence to show that the spores from a previous crop do not persist in the soil. Therefore, with the help of seed disinfection, the disease can be practically controlled.

Different methods have been advocated. The methods recommended in earlier years, namely, steeping the grains in 3% solution of copper sulphate for 15 minutes or 0.5% formaldehyde for 1–2 hours, have given place to more efficient ones. Of the several chemicals recommended form time, copper carbonate has been found to be very effective. The dosage varies from 56.7–113.4 g per bushel.

Sulphur in the form of fine powder (200 mesh) from 56.7–113.4 g/1.6kg of seed is very effective. Thiram at 2–4 g/kg of seed is also effective. Other non-systemic fungicides such as Agrosan, Cerasan, Tillex, Captan and systemic fungicides such as Carboxin and Bavistin are better than other fungicides.

Asthana (1947) recommended solar energy treatment of seeds for Uttar Pradesh conditions and according to him, the seeds should be soaked in water at ordinary temperature during summer for 4 hours in the morning and then spread out in the sun to dry for better disease control.

A few varieties of Milo, Hegari, Feterita and Shantung have been found to carry the genes for resistance. Kadam and Shinde (1982) had reported the varieties CSH-9, SPV-104, SPV-102, SPV-115, SPV-297, SPV-138, RSV–1R, SDM–9, CSH–7–R as resistant in Maharashtra.

Loose Smut of Sorghum

It is distributed in Africa; Asia; Europe; and North, central and South America. It is less common than the grain smut. In India, it occurs in the states of Andhra Pradesh, Bombay, Karnataka, Madhya Pradesh, and Tamil Nadu.

Symptoms

The affected plants can be detected before the ears come out. They are shorter than the healthy plants by about a foot, with thinner stalks and marked tillering. The ears come out much earlier and are looser than the healthy ones. The glumes are hypertrophied (2.5 mm in length). All the spikelets are infected and sori may be borne on glumes and pedicels, besides the essential organs of the spikelets. The sorus may include the transformed pistil and the stamens. The size of the sorus varies with the varieties of the host (Fig. 15.15).

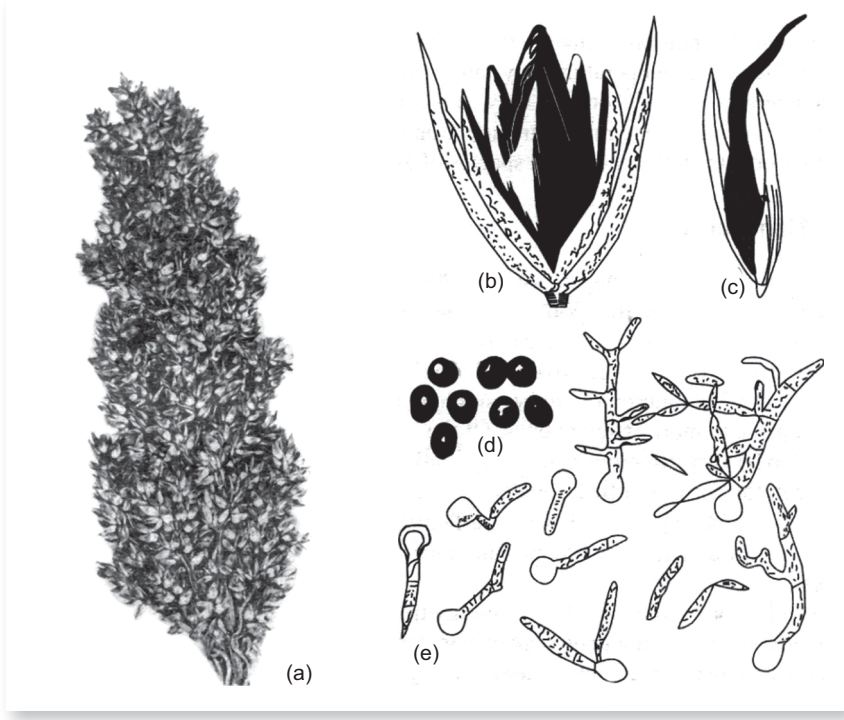


Figure 15.15 Loose smut of jowar (*Sphacelotheca cruenta*): **(a)** smutted ear **(b)** sorus involving stamens **(c)** exposed columella after the spores have fallen **(d & e)** spores and their germination

Causal Organism

The pathogen responsible for loose smut is *Sphacelotheca cruenta* (Kuhn) Potter. It has been renamed as *Sporisorium cruenta* (Vanky, 1994). The sorus is covered by a thin membrane which ruptures very early, exposing the spores even as the head emerges from sheath. In the centre of each sorus is a long conical columella as in grain smut, but longer and more curved. The spore mass is dark brown, surrounding well developed, unbranched columella of host tissue. The spores are globose to subglobose, light yellowish brown, wall 1 µm thick, finely echinulate under oil immersion, 6–10 µm in diameter. Sporidial cultures may be obtained on Czapek's agar. This species has two sex groups and can hybridize with *S. sorghi* and *S. reiliana.*

Disease Cycle and Predisposing Factors

As in grain smut, *S. cruenta* is mainly externally seed borne. It is stated to be soil borne to some extent in dry soils. Spores remain viable for four years in the laboratory, but as they germinate readily in water, soil transmission is unimportant. Spread within the crop by floral infection by air-borne spores has been observed (Fig. 15.16).

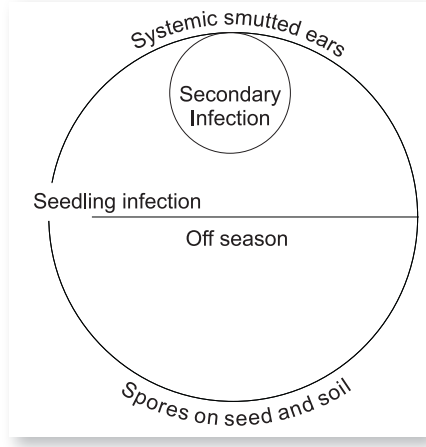


Figure 15.16 Disease cycle of grain smut of *Sorghum* caused by *Sphacelotheca cruenta*

Physiological Specialization

Three physiologic races have been described and their characterization by the reaction of five different varieties is tabulated by Tarr (1962). Hybridization has been recorded between *S. cruenta* as well as both *S. reiliana* and *S. sorghi.*

Disease Management

Control measures are the same as those for grain smut of sorghum. The disease can be controlled by sanitation and crop rotation where the spores survive in the soil.

Head Smut of Sorghum and Maize

It is found in Africa; Asia; Australia; Europe; and North, central and South America. In India, it is prevalent in Andhra Pradesh, Karnataka, Bombay, Madhya Pradesh, Uttar Pradesh, Punjab, and Tamil Nadu. In Kashmir, it causes significant damage to the maize crop.

Symptoms

The disease is not noticeable by a difference in either the size or the growth of the plant. The entire inflorescence is converted into a big sorus, about 10–13 cm in length and 4.6 cm in breadth. The large smut sori replace the tassel and ear in corn. The sorus consists of the conductive elements of the suscept surrounded by the spore mass and the fragile exterior fungal membrane (Fig. 15.17).

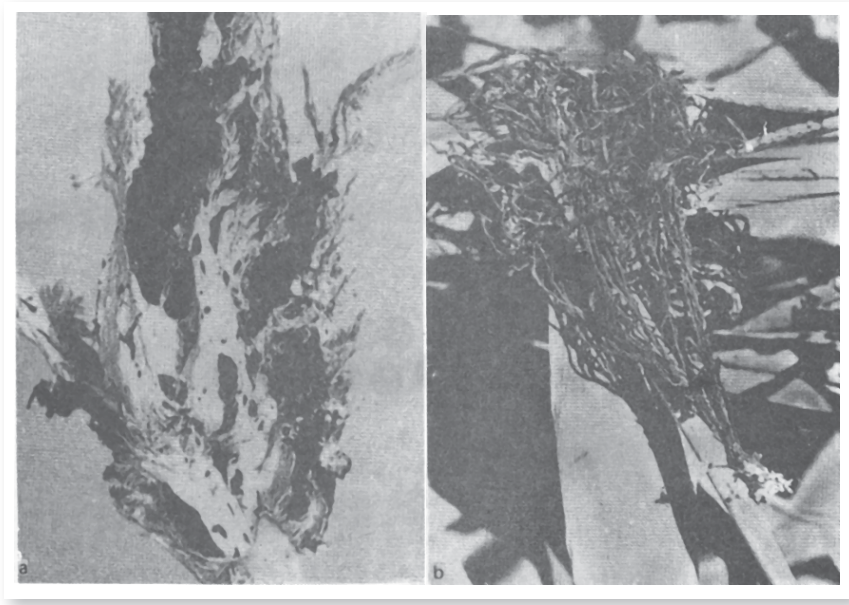


Figure 15.17 Head smut of Sorghum (*Sphacelotheca reiliana*): **(a)** smutted ear head covered by a membrane **(b)** the same with membrane ruptured showing long, thin, dark colored filaments (vascular bundles)

Causal Organism

The pathogen responsible for head smut of maize or sorghum is *Sporisorium relianum* [Syn. *S. reiliana* (Kuhn) Clinton and *Sorosporium relianum*]. The sorus is composed of loosely united spores and the conductive tissues of the suscept which is initially enclosed by a fragile fungal membrane. The reddish-brown to black chlamydospores are finely echinulate, irregular to spherical and 9–14 µm in diameter. The spore mass is powdery and dark brown, and is quickly dispersed to expose a tangled mass of vascular strands of the host or sometimes a singly central columella.

Disease Cycle and Predisposing Factors

Spores present in the soil cause infection. Only young plants are susceptible. The fungus becomes systemic (Fig. 15.18). Infection is favoured by soil temperatures of 21–28°C. In dry soil the spores remain viable for a considerable period, at least until the following crop season. Application of low C:N organic amendments reduces inoculum in soil and there is more disease at relatively highly soil temperature and low soil moisture than in wetter and cooler soils. Frequent irrigation after sowing has been found to reduce disease incidence.

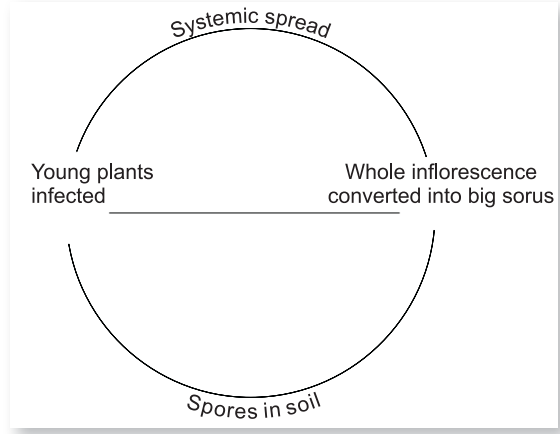


Figure 15.18 Disease cycle of Head smut of Sorghum caused by *Sphacelotheca reiliana*

Disease Management

Crop sanitation to reduce the inoculum potential and crop rotation are of primary importance. Resistant varieties are available and most of these are also resistant to covered smut. Application of urea, ammonium sulphate and triple super-phosphate reduces disease considerably.

Disinfection of seeds has been found to be partially effective in disease control in USA. Growing barley for 2 years has reduced infection of maize grown subsequently. Some insects like *Phalacrus*, *Brachytansus*, and *Lystronynchus* feed on the spores of *S. reiliana*. *Bacillus megaterium* is effective biocontrol agent against smuts as it produces antibiotics.

Long Smut of Sorghum

This has been reported in Africa (widespread) and Asia (China, India, Iraq, Israel, Pakistan, Turkey, and USSR). In India, it occurs in Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Madhya Pradesh, and Uttar Pradesh. Losses due to this disease are usually slight.

Symptoms

The presence of long smut can be discovered only by a close examination of the ears in the field. Only a few smut sori develop, scattered sporadically throughout the ear. Sori are more or less cylindrical, with tapered ends, up to 4.0 cm long, 0.5–1.0 cm wide, surrounded by a false membrane of fungal tissue which ruptures from the apex (Fig. 15.19 a).

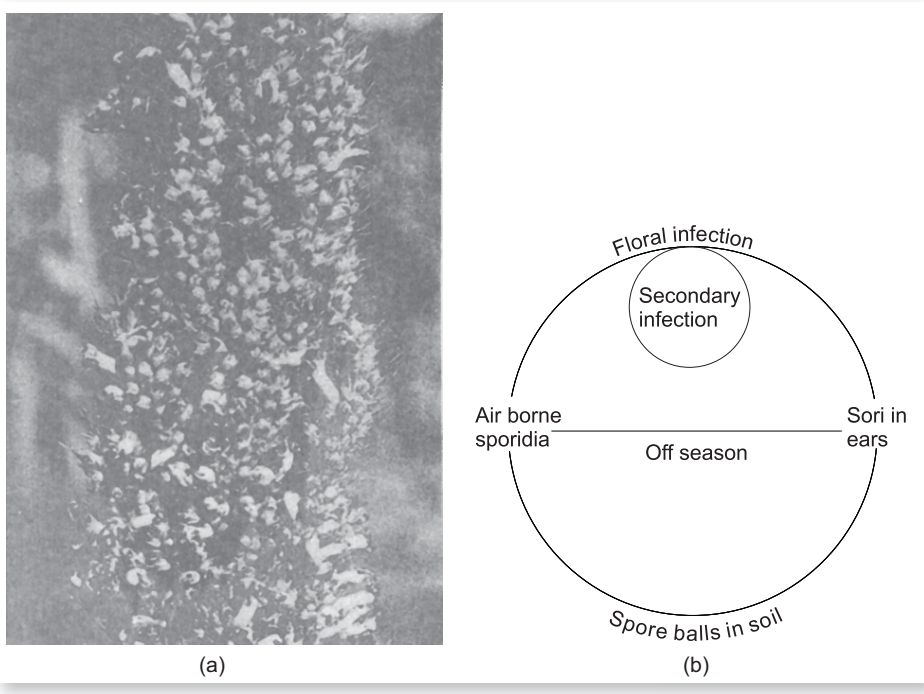


Figure 15.19 Long smut of jowar (*Tolyposporium ehrenbergii*), **(a)** portion of the head showing scattered, long cylindrical sori **(b)** Disease cycle

Causal Organism

The pathogen responsible for the long smut of Sorghum is *Tolyposporium ehrenbergii* (Kuhm) Pat. The spore mass is granular, black, intermixed with shreds of hosting tissue. Spore balls are many spored, permanent, rather irregular in size and shape, globose to elongated, dark brown in patches, opaque, 45 µm or less, with a maximum diameter of more than 200 µm. The spores at the surface of the ball are dark brown with the free surface having papillae. The inner spores are pale in color, smooth, globose to subglobose or angular and 10–15 µm in diameter.

Sporidial cultures may be obtained in 1% potato dextrose agar or carrot agar.

Disease Cycle

It is believed that floral infection occurs by means of sporidia which enter the floral parts, producing sori about 12–15 days later.

The spores in the soil may germinate to produce clusters of sporidia which become air borne and cause primary infection. Secondary infection may also take place through the spores released from the smutted ears in the field (Fig. 15.19b).

Disease Management

The use of healthy seed and sanitary practices to reduce the infection potential are advisable. Ramakrishnan (1963) reported varietal resistance. In the south, variety Irungu is usually free from this smut.

Comparison of the Four Smuts of Sorghum

Characters Pathogen	Grain smut *S. sorghi*	Loose smut *S. cruenta*	Long smut *T. ehrenbergii*	Head smut *S. reiliana*
Host	Stunted, heading premature	Not stunted, heading normal	Not stunted, heading normal	Not stunted, heading premature
Ear infection	All or most grains smutted	All or most grains smutted	Very few grains are infected	The entire inflorescence is converted into a big sorus
Site	Ovary	Ovary	Ovary	Inflorescence
Sori	Small	Small	Long	Very large
Membrane	Rather tough and persists	Ruptures easily	Relatively thick membrane	Ruptures easily
Columella	Short columella present	Long columella present	Columella absent, but 8–10 vascular strands present	Columella absent but a network of vascular tissues present
Spores	6–7 µm, spore surface apparently smooth	7–8 µm minutely echinulate	10–15 µm spore balls, free surface papillate	9–14 µm conspicuously echinulate
Viability of spores	More than 10 years	About 4 years	About 2 years,	Up to 2 years
Spread	Externally seed borne	Externally seed borne	Air borne	Soil borne and seed borne.

Smut Of Bajra (*Pennisetum typhoides*) or Pearl Millet

Bajra smut occurs in Africa, India, Pakistan, and Mesopotamia. In India, it is more prevalent in Tamil Nadu, Andhra Pradesh and Maharashtra. According to Ramakrishnan (1963), it causes loss from less than 1% to nearly 30%.

Symptoms

Only individual grains, irregularly distributed in an ear, are infected. Sori are oval or top shaped, generally two to three times the size of the grains, 3–5 mm long and limited by a dark brown membrane of the host tissue which disintegrates at maturity. They are bright green to dirty black in color (Fig. 15.20 a–d).

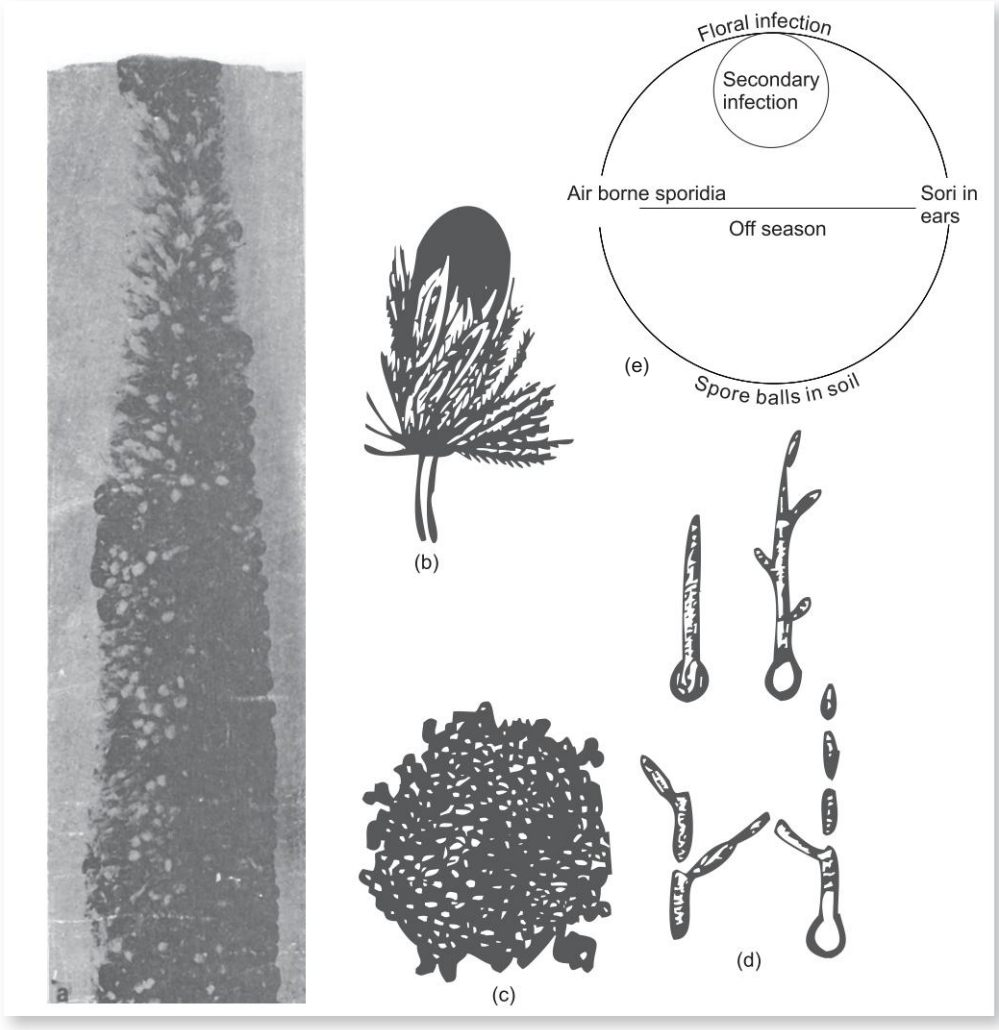


Figure 15.20 Smut of bajra *Moesziomyces bullatus* (*Tolyposporium penicillariae*) **(a)** portion of the affected ear showing scattered sori **(b)** single sorus **(c)** spore ball **(d)** germinating spores **(e)** disease cycle

Causal Organism

The pathogen responsible for this disease is *Moesziomyces bullatus* Syn. *Moesziomyces penicillariae* (Bref.) Vanky (earlier known as *Tolyposporium penicillariae* Bref). The pathogen is mostly confined to the sorus, the wall of which consists of a tough combination of host tissue and fungal hyphae which surround the powdery spore mass. The spore mass is granular and black. The spore balls are many spored, permanent and about 100–150 µm in diameter. The spores are globose to subglobose or ovoid, light brown, smooth and 6–12 µm in diameter.

Disease Cycle and Predisposing Factors

Floral infection occurs initially by sporidia from spores in the soil and later by spores from early infected plants. The fungus invades the ovule through the nucellus and mesophyll, and neither mycelium nor spore is carried on or in the healthy seed. Spikelets are most susceptible before the anthers and stigmas emerge. Once the flowers are pollinated, even subsequent entry of viable smut spore in after successful pollination does not produce much infection. It had been opined that further infection in already fertilized ovary does not take place because of the production of organic acids which may be toxic for the fungal invasion. The smut balls remain in the soil to germinate during the next season, producing masses of sporidia which become air borne and infect the ear (Fig. 15.20 e).

Disease Management

According to Ramakrishnan (1963), seed treatment has given conflicting results. There is no effective method for the control of bajra smut, and all the agronomically important varieties are susceptible. Attempts had been made to control this disease by the cultivation of resistant host varieties (Singh *et al.*, 1977).

Other control measures suggested are the removal of diseased ears, clean seed, hot weather deep ploughing, field sanitation and crop rotation. Use of resistant varieties is recommended. Intercropping of Mung bean with pearl millet reduces smut incidence. Spray of fungicides such as Carboxin Captafol and Carbendazin at boot stage has been recommended.

Common Smut of Maize

It is prevalent wherever maize is grown, with the exception of Australia and New Zealand. Common smut is one of the worst diseases of corn in the USA. In India, it is mostly confined to the Himalayas, the Kashmir Valley, and West Bengal. A complete monograph of this disease was published by Christensen (1963).

Symptoms

U. maydis produces galls on almost all the parts of the maize plant which are above the ground. Galls are formed on the ears, axillary buds, tassels, stalks and sometimes on the leaves (Fig. 15.21). The infection first becomes apparent when galls of different sizes are seen. The galls may reach the size of 10 cm or more in diameter. As they begin to enlarge, they are light colored, almost white. As the inner tissue darkens, owing to spore formation, the white outer membrane remains intact until a late stage when it breaks and exposes the powdery black mass of spores. Galls on young seedlings may result in extreme dwarfing or death of the plants. Embryonic tissues of the plant are affected.



Figure 15.21 Smut of maize (*Ustilago maydis*): galls on seedlings

Causal Organism

The disease is caused by *Ustilago maydis* (DC) Corda. The spores are spherical to ellipsoidal, 8–11 µm in diameter, black and wall shows spiny ornamentation. These spores germinate to produce septate promycelium which bears ovate, unicellular sporidia.

Physiologic Specialization

U. maydis comprises a very large number of biotypes which differ in pathogenicity and other characteristics.

Disease Cycle

The smut spores may be carried on the seed coat, but this type of perennation is not usually responsible for infection. Manure heaps and crop refuges are the chief sources of primary inoculum. Infection of the host via any actively growing tissue is localized. Few other smuts are known to grow as vigorously as a saprophyte. The saprophytic life can be prolonged almost indefinitely by transferring the fungus at intervals to a fresh nutrient medium.

Infection of the host takes place through wounds or natural openings and the initial infection of stems occur from the air-borne spores and sporidia. In *U. maydis*, the sporidia germinate and produce a uninucleate mycelium which infects the host and then dikaryotization takes place within the host by means of hyphal fusions (somatogamy). It is this dikaryotic mycelium which further invades the tissues. The fungus is heterothallic and when compatible haploid hyphae fuse in the host tissue they produce the pathogenic hyphae. The life cycle and disease cycle of the pathogen given are given in Figs. 15.22 and 15.23 respectively.

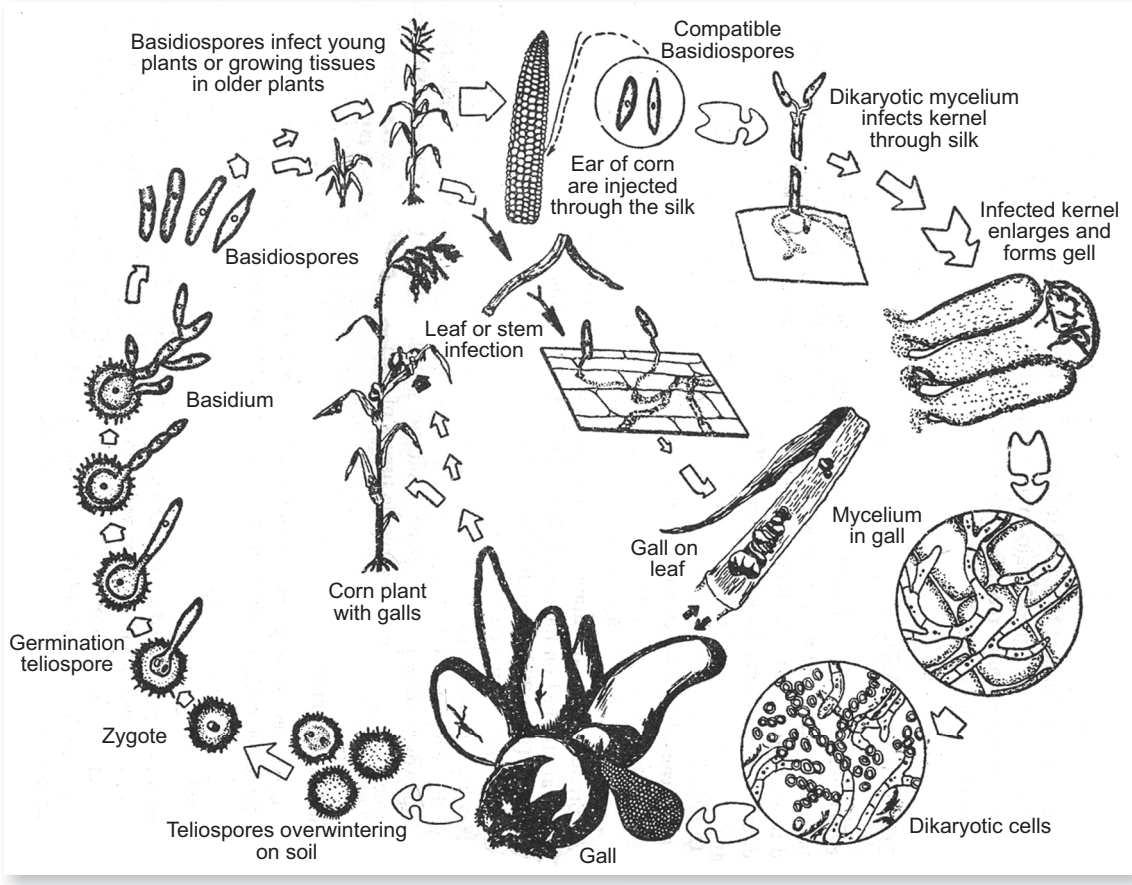


Figure 15.22 Life cycle of common smut of maize (*Ustilago maydis*). (From G.N. Agrios, *Plant Pathology,* Academic Press, New York and London, 1969)

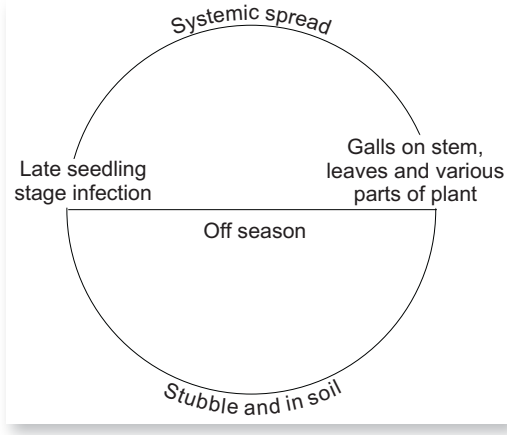


Figure 15.23 Disease cycle of common smut of maize caused by *Ustilago maydis*

Disease Management

Breeding of resistant varieties is the most important measure. The disease can, however, be kept in check by crop rotation and field sanitation.

Fungicide sprays of Carboxin + Thiram and Benomyl have given good control. As biocontrol agent, a mycobacterium and an amoeba have controlled the disease in inoculated soil (Singh, 2008).

Whip Smut of Sugarcane

It is distributed in Africa (Egypt, east and South Africa, Madagascar), Asia (widespread), and South America. It is primarily a disease of wild canes and those which approximate the wild varieties, are more susceptible than the improved thick canes. However, thick canes of the tropics are also not immune. According to Padmanabhan *et al.* (1988) the losses due to smut disease depends upon the stage or age of the plant. During early stages (40–60 days) of crop growth, there is complete loss but if it appears in a 80–120 days crop. There is loss in yield and quality when plants are attacked after 210–270 days old. Agnihotri and Sinha (1996) have recently reviewed the work on this disease.

Symptoms

Affected plants, at the growing axis, are characterized by the production of a long whip-like, dusty, black shoot, often several feet in length and much curved on itself. This is probably the floral shoot that gets transformed into a long whip-like structure. The whip is covered by a silvery, thin membrane in its earlier stages which soon flakes away (Fig. 15.24a).

Affected plants usually have slender, thin, taller canes than the rest of the crop and can be identified before the production of smutted whip. According to Bhansali and Singh (1986) the affected canes may show stem galls and multiple buds.

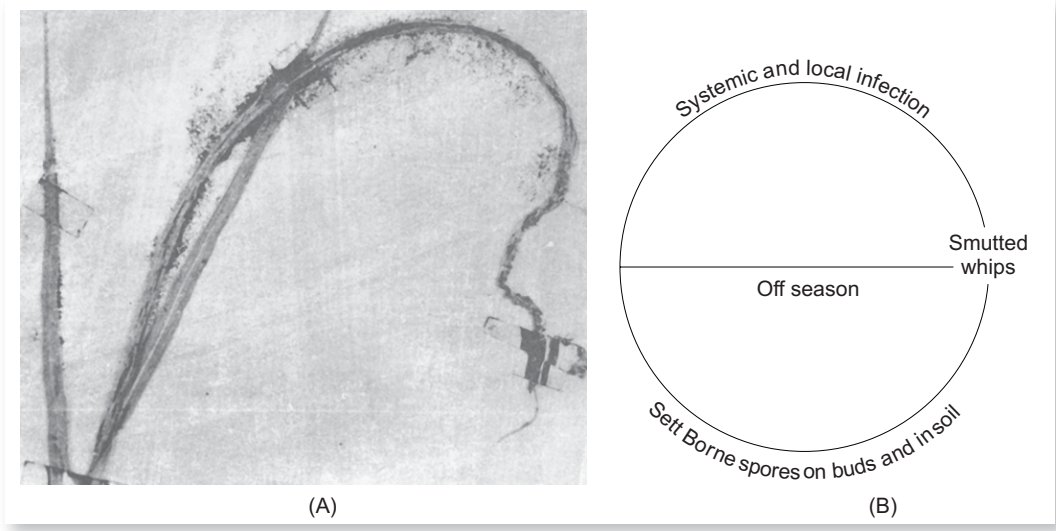


Figure 15.24 Whip smut of sugarcane (*Ustilago scitaminea*): **(A)** Smutted whip **(B)** Disease cycle

Causal Organism

Ustilago scitaminea Sydow is the pathogen. The mycelium of the fungus infects the tissues of the cane below the whip smut and is intercellular. The hyphae collect in dense masses towards the surface of the spore-bearing shoot where the spores are formed. As these are produced in abundance, they rupture the silvery membrane, turning it into flakes and shreds. The spores are globose to subglobose, reddish brown, typically smooth or punctate and 5–10 μm in diameter. Sporidial cultures on malt or potato dextrose agar frequently develop typical smut spores.

Disease Cycle

Sugarcane smut can be transmitted by the mycelium that is present in the cells from diseased clumps and also through the spores that are in the soil. Field experiments have shown that the smut is perpetuated by any one of the following ways:

1. Planting sets of smutted canes.
2. Spores borne on buds.
3. Infection of buds on standing canes.
4. Ratooning the smutted canes.

The sugarcane crop has no dead season and so the disease easily gets a ready host. The disease cycle of the pathogen is given in figure 15.24 (b).

The optimum temperature for spore germination is about 25–30°C. A 100% relative humidity is essential for spore germination. According to Saxena and Khan (1964), no germination occurs at 90% relative humidity. Saxena and Khan (1962) have also reported the stimulatory effect of diffusates from buds upon the germination of spores. Infection can also occur through the young germinating shoot.

Disease Management

Removal of Infected or Smutted Whips This method is effective when the attack is sporadic. The infected whips should be removed and burnt. This also reduces the infection.

Discouraging Ratooning Here the susceptible varieties or crop which have shown high degree of the disease should not be ratooned.

Avoiding Planting of Setts from Diseased Canes The seed setts should be selected from healthy fields.

Chemical Treatment of Setts before Planting If proper care is not taken at the time of harvest, whip smut spores may also contaminate healthy buds. To prevent this type of infection, many fungicidal/chemical treatments have been recommended from time to time.

Among chemical treatments, 5-minute dip of setts in 0.25% water suspension of Agallol or Aretan or Mercuric chloride (0.1%), and Formalin (1.0%) for a 5-minute dip or a minute dip in 4:4:50 Bordeaux mixture were recommended. These treatments ensure only inactivation of spores present on the surface of setts or buds. To eradicate internally present dormant mycelium, some of the systemic fungicides like Vitavax, Benlate, and Bavistin have been used for sett treatment. Out of these systemic fungicides tested, Benlate is highly effective against many pathogens of sugarcane. Waraitch (1986) had reported that dipping of setts in Vitavax or Agallol, Bavistin (0.5%) or Dithane M-45 (0.3%) controls both internal and external inoculum. It may be mentioned that organomercurials like Agallol are on the banned list of fungicides now.

Amongst the other control measures include hot water treatment (HWT) which includes a 10-minute dip of setts in water at 55–60°C before planting. Apart from hot water, dry or moist hot air treatment (MHAT) of setts has been found effective in the control of smut of sugarcane. Recently Vala *et al.* (1992) reported that MSMC (Methoxy ethyl mercury chloride) hot dip (250 ppm, 40°C for 10 minutes) is highly effective.

Use of Resistant Varieties Sugarcane varieties have been grouped according to their degree of resistance. As far as possible only resistant varieties should be grown. Apart from this, the susceptible varieties should not be grown in these areas where pathogen is known to be well established in the field. Wala (2003) obtained control of smut by giving dip treatment to seed setts for 10 min in solution of Pyroquilon (4g/l) and Carbendazim + Maneb (4.5g/l). Varieties Co6806, CoLK7807, 8001, 8004, 8102, CoH29, are highly resistant.

Covered Smut of Barley (*Hordeum vulgare*)

Covered smut is worldwide in distribution. Covered smut of barley is common in the northern parts of India where it causes considerable damage to susceptible varieties. It is more common than the loose smut of wheat and barley.

Symptoms

A characteristic symptom of the covered smut on barley is a rather persistent membrane enclosing the sorus until the plants are fully mature. The smutted heads emerge at about the same time as those of healthy plants but remain shorter and are usually retained within the sheath for longer time or may sometimes fail to emerge at all. Every ear in a diseased stool and every grain in a diseased ear is affected. The black spore masses of this smut are held together more or less firmly by the persistent membranes of the grains and the basal part of the glumes. The awns remain intact (Fig. 15.25 a, c). The spore masses are held together by a deposition of a fatty substance. This renders the treatment of seeds difficult, unless the fat is removed.

Causal Organism

Ustilago hordei (Pers.) Langerheim is the pathogen. The spores are light colored on one side, subspherical to angular, with a smooth outer membrane and range from 5–9 μm in diameter. The epispore is smooth. On germination, the spores give out a four-celled promycelium which gives rise to sporidia near the septa and the apex. Sporidia germinate to form the infection thread.

Diseased Cycle and Predisposing Factors

The disease perennates through the externally seed-borne smut spores which contaminate the healthy seeds during threshing. Infection of the young seedlings occurs from the seed borne chlamydospores. Penetration is through the young coleoptile into the embryonic growing point, and further development of the pathogen occurs in association with differentiating tissues of the crown buds and floral structures (Fig. 15.25 b).

Soil moisture and soil temperature influences seedling infection. Optimum temperature for spore germination is 20°C. Deep sowing results in greater infection. Several physiologic races of the fungus are known.

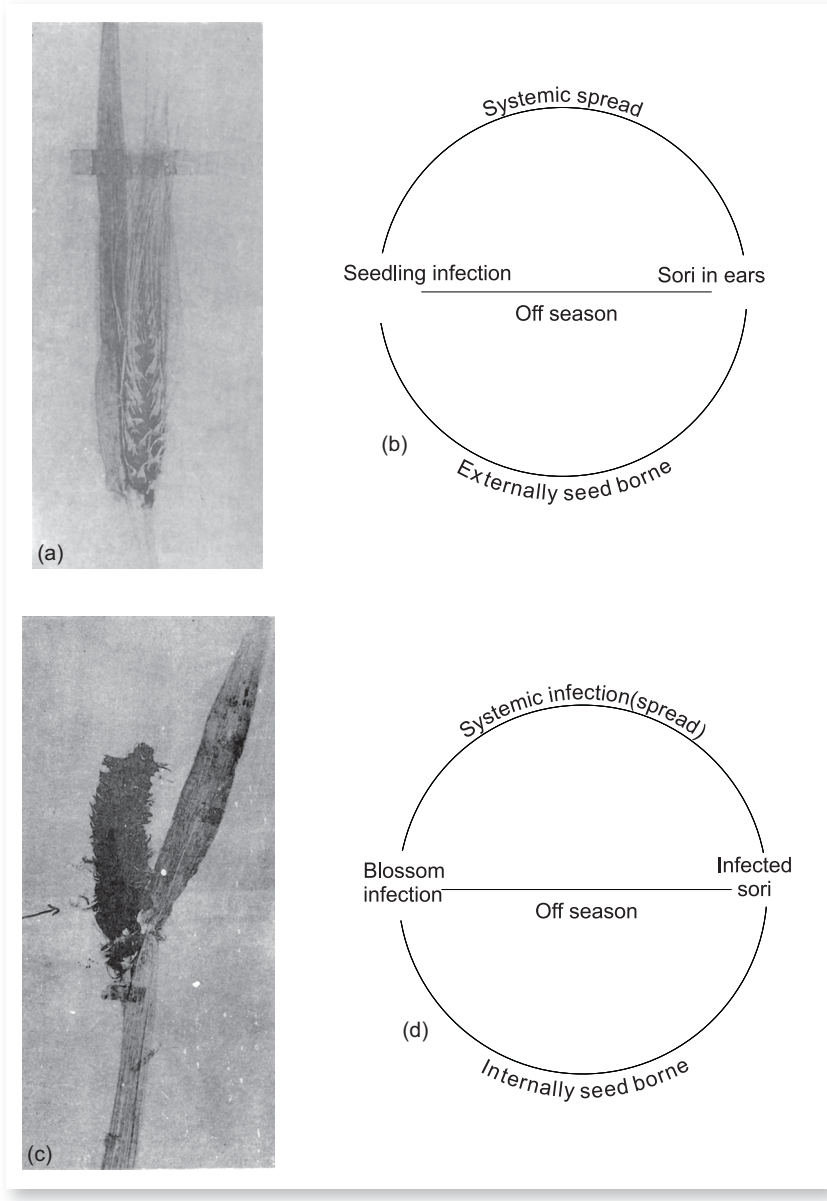


Figure 15.25 Symptoms of spot of barley: **(a)** Covered Smut caused by *Ustilago hordei,* **(b)** Diseases cycle of covered smut. **(c)** Loose smut of barley caused by *Ustilago segatum var. tritici* (U*stilago nuda*) **(d)** Diseases cycle of loose smut of barley.

Disease Management

The externally seed borne spores were controlled by fungicidal seed treatment, such as Agrosan GN applied at the rate of 113.4 g per 37 kg of seed. According to Grewal and Dharam Vir (1964), Cerasan and Agrosan 5W gave good control of the disease. But according to Bedi and Singh (1974) systemic

fungicides like Vitavax give complete control of the disease. Treatments with Mancozeb, or Thiram or Zineb are also effective for the control of covered smut of barley. Apart from chemical treatment, Atheya (1974) had reported the following varieties on the basis of field trial.

Leaf extract of *Azadirachta indica, Melia azadirachta* and *Cannabis sativa* reduce or suppress spore germination of *U. hordei* (Malik and Singh, 2002).

Highly resistant (no infection): BHS4, BM 23, LB873, EB4003, EB4032, EB4032.
Moderately resistant: HBL52, HBL53, K135, K401-5.

Loose Smut of Barley

This smut resembles the loose smut of wheat in every aspect but it is not very common as loose smut of wheat and covered smut of barley.

Symptoms

In the early stages of growth, the sori are formed in the spikelets which are covered by a thin silvery membrane. In the later stages, the spikelet ruptures and spore mass is shed leaving behind the rachis (Fig. 15.25 c).

Causal Organism

It is caused by *Ustilago segetum* var.*hordei* [earlier known as *Ustilago nuda* (Jens.) Rostr]. The spores are nearly round, olive brown, finely echinulate and measure 5–9 μm in diameter.

Disease Cycle

The disease is internally seed borne and the dormant mycelium can survive for considerably long periods in the embryo (Fig. 15.25 d).

Disease Management

Control measures are the same, as those applicable to the loose smut of wheat: like use of resistant varieties, hot water or solar energy treatment or fungicide treatment of the seed. Varieties C4, C50, CN292, CN294 and NP13 are tolerant to loose smut of barley (Mathur *et al.*, 1960).

Loose and Covered Smuts of Oats (*Avena sativa*)

Both the smuts are important diseases of oats. In some areas the incidence of covered smut was noted as high as 30% (Nene, 1964).

Causal Organism

Loose smut of oats is caused by *Ustilago avenae* (Pers.) Rostr., which has been renamed as *Ustilago segetum* var. *avenae*. Covered smut of oats is caused by *Ustilago kolleri* which has been renamed as *Ustilago segetum*. The characteristic symptoms are the transformation of grains into black powdery

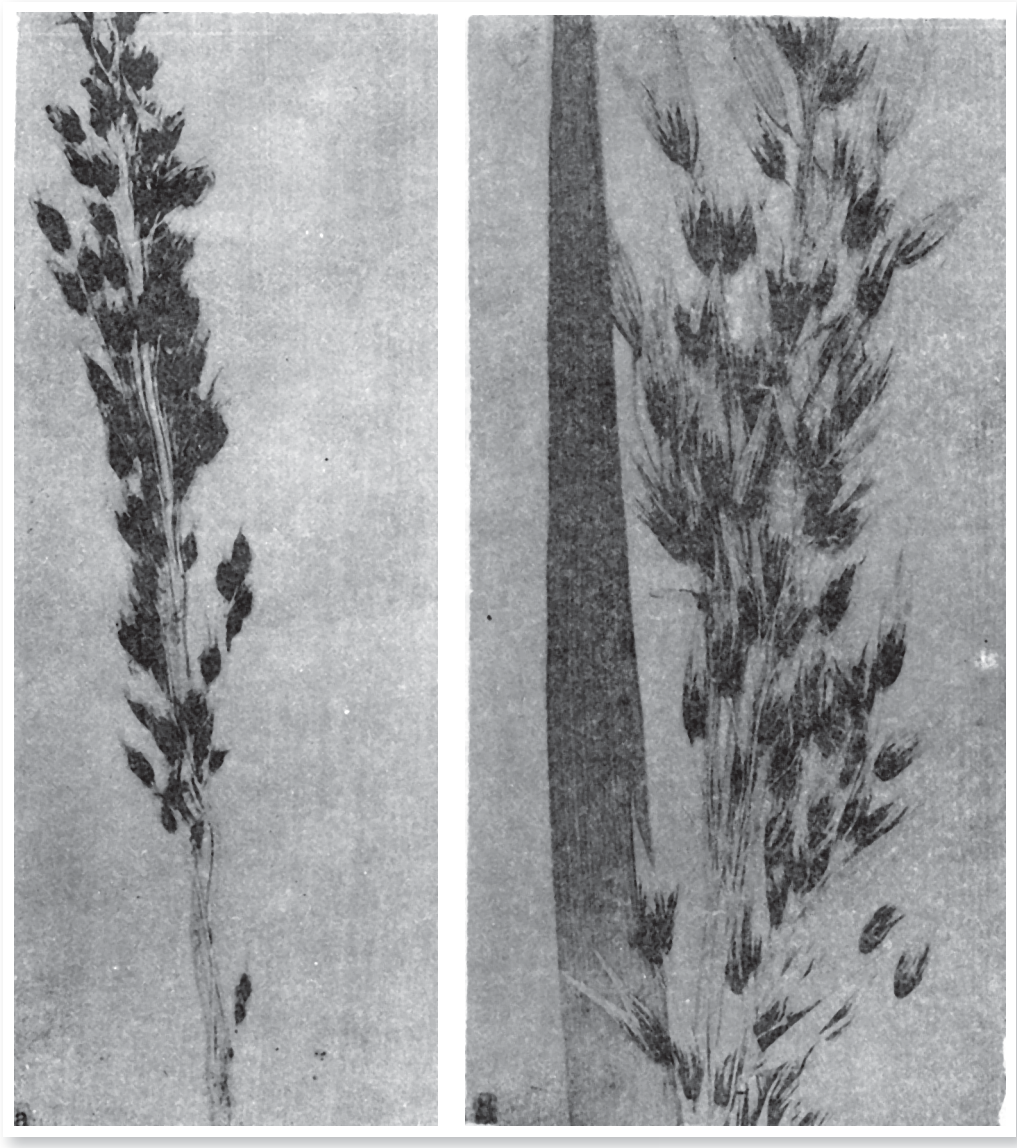


Figure 15.26 Smuts of oats: **(a)** loose smut (*Ustilago segetum* var. *avenae*) **(b)** covered smut caused by Ustilago kolleri

mass of fungus spores. In case of covered smut, the black spore mass remains enclosed in a membrane, whereas in case of loose smut the membrane ruptures and the spore mass is released at the time of threshing of the grains.

Both the fungi survive externally on the seed in the form of teliospores (Rusch, 1957). The infection takes place at the time of seed germination and these fungi become systemic (seedling infection) and later transform the grains into black powdery mass.

Management of the Two Smuts of Oats

Both the diseases can be controlled by treating the seed with organomercurials fungicide before planting. The systemic fungicide carboxin (Vitavax) at 0.2 percent has also been found effective.

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Questions

1. What do you understand by biological specialization? Explain it with reference to smuts.
2. How will you differentiate between the following:
   (i) *Ustilago* and *Tolyposporium*
   (ii) *Ustilago* and *Sphacelotheca*
   (iii) *Urocystis* and *Tolyposporium*
   (iv) *Neovossia* and *Ustilago*
   (v) *Entyloma* and *Tilletia.*
3. What is meant by systemic infection? Describe the life cycle of *Ustilago segetum tritici* along with the various control measures of the disease it causes.
4. What is the major difference between smuts and bunts?
5. Name the causal organisms responsible for the following diseases along with the major methods of disease control in each case:
   (i) Loose smut of wheat
   (ii) Karnal bunt of wheat
   (iii) Flag smut of wheat
   (iv) Whip smut of sugarcane
   (v) Smut of bajra.
   (vi) Common smut of maize.
6. Name at least three types of smuts found in *Sorghum,* indicating their causal organisms and the symptoms produced. What is the major method of control in each case?
7. Who discovered solar heat treatment? Mention its special features?
8. Would you advise seed treatment with chemicals in all seed borne diseases?
9. How will you differentiate between grain smut, loose smut, long smut and head smut of sorghum?
10. How systemic fungicides have now helped in controlling internally seed borne smuts?

Rusts

16

Plant Rusts

There are about 7798 species of rust fungi distributed among the 166 genera in use today. In nature, all are obligate parasites (biotrophs), although there has been a breakthrough in artificial culture (Scott and Maclean, 1969). They parasitize green plants—from the ferns and conifers to most of the more highly evolved families of both dicotyledons and monocotyledons. Earlier attempts to grow them in pure culture or in tissue culture (Staples and Wynn, 1965; Brian, 1967) met with limited success. It was, therefore, surprising when it was shown that *Puccinia graminis tritici* could be grown on Czapek-Dox, yeast extract agar (Williams *et al*., 1966). Several rusts such as *Gymnosporangium juniperi virginianae*, *Melampsora lini*, *Uromyces dianthi*, and several others have been cultured axenically (Narisawa *et al.,* 1992). They grow internally with intercellular mycelium, which rarely bears clamp connections. Sporulation is by means of sporogenous hyphae emerging through stomata by breaking through the surface of the host (erumpent), or in some cases, by internal production of spores, released only when the host decays. They are highly destructive and are responsible for widespread epiphytotics all over the world. The cereal rust, the white pine blister rust, the cedar rust of apple, and the rust of coffee and linseed are known for their destructive nature. Parasitism and specialization are highly developed among these fungi. According to the 10th edition of the "*Dictionary of the Fungi*" by Kirk *et al.* (2008), plant rusts have been kept in the order Pucciniales in the class Pucciniomycetes, of the phylum Basidiomycota.

Some Important Milestones in the Progress of Rust Studies are as Follows

1665—Teliospores of *Phragmidium disciflorum* were first observed under a microscope by Robert Hook in Micrographia.
1865—Heteroecious nature of stem rust of wheat was demonstrated by de Bary.
1894—Physiological specialization in stem rust of wheat was shown by Eriksson.
1896—Conjugate nuclei and their fusion in the teliospores were shown by Sappin Trouffy
1905—Inheritance of rust resistance in wheat in a Mendelian fashion was demonstrated by Biffen in England.
1914—Biological forms in cereal rust were distinguished by Stakman.
1927-31—Function of pycnia was discovered by Craigie.

1930—Origin of new physiological forms of *P. graminis tritici* by hybridization was shown by Stakman *et al*.
1931—Life cycles and evolutionary tendencies were discussed by Jackson.
1929-34—Taxonomy of plant rusts was dealt by J.C. Arthur.
1940—Cereal rust studies in India were made by K.C. Mehta.
1950— The sexual process in Uredinales was discussed by Buller.
1951—The first artificial culture of cedar rust of apple fungus was done by Hotson and Cutter.
1955—Origin of new physiological forms of *P. graminis* through heterokaryosis was demonstrated by Nelson, Wilcoxson and Christensen.
1966-67—The first successful axenic culture of black stem rust from urediniospores (uredospores, Urediospores) was done by Williams *et al*.

Some relevant and somewhat recent literature on rusts include that by Bushnell and Roelfs (1984): "*The Cereal Rusts, Vol. I*" and Roelfs and Bushnell (1985): "*The Cereal Rusts, Vol. 2*". Laundon (1973) had reviewed the work on the Uredinales till them. Zhang and Dickinson (1994) have reviewed the occurrence of double-stranded RNAs in the rust fungi.

Life Cycles

The rusts have a pleomorphic life cycle. Although morphologically simple, some rusts have the most complex life cycle found in the fungi, in addition to a high degree of specificity for hosts of a single species and sometimes of particular genetic strain. Many rusts are long-cycled, passing through regular alternation of generations and a succession of five spore stages to complete a life cycle. Life cycle of rust fungi has been discussed in Littlefield's (1981) short book title: "*Biology of the Plant Rusts"* and "*The Rust fungi*", edited by Scott and Chakraborty (1982). The spore stages may be indicated as follows.

Stage 0 – Pycnium or Spermatium

A pycnium (or spermatium), bearing pycniospores (or spermatiophores), is a haploid structure that is often flask-shaped. The pycniospores are haploid, uninucleate structures, which function as spermatia when transferred to another pycnium.

Stage 1- Aecium or Aecidium

An aecium (or aecidium), bearing aeciospores (or aecidiospores), is frequently open, cupulates sori, which bear dikaryotic aeciospores in chains. The aeciospores are the first dikaryotic spores. Aecia can be of the *Caeoma*, *Aecidium*, *Roestelia* and *Peridermium* types. The form genus *Caeoma* includes all aecial stages in which a peridium is lacking. In the Aecidium type, the aeciospores are produced in a structure shaped like a cup with a short straight lip. In *Roestelia,* the lip protrudes much beyond the surface of the host and soon becomes shredded, often bending back and forming an ornamental fringe around the mass of spore chains. In the *Peridermium* type, the aecial lip is large and conspicuous. It is generally white and appears as a thin structure.

Stage II—Uredinium or Uredium

A uredinium (or uredium), bearing urediniospores (or urediospores), is usually a sorus and the urediospores are dikaryotic. This stage is repetitive, as a urediospore may germinate, forming a mycelium, which again gives rise to uredia.

Stage III—Telium

A telium, containing teliospores or teleutospores, is usually a sorus. The teliospores function as probasidia and are the site of karyogamy and meiosis.

Stage IV—Basidiospore (sporidia)

The haploid basidiospores produced from the teliospores are capable of reinfecting the host and initiating the life cycle again.

Normally, a rust fungus produces five different spore forms in a definite sequence, which is fixed and is not reversible. However, one or more forms may be omitted from the life cycle, thus indicating the extreme plasticity and flexibility of these fungi.

Heteroecism and Autoecism

Various authors have discussed the origin of the phenomenon of heteroecism. Gaumann and Wynd (1952) have given an excellent presentation of this problem. The present trend is to regard autoecism as having been derived from the original heteroecious mode of life and the various modified life cycles as having given rise to short-cycled and microforms, and the endo-condition as originating from the autoecious forms. Heteroecism has been demonstrated in a large number of rust fungi, notably cereal rusts, white pine, blister rust, cedar rust of the apples, and bamboo rust in India (*Dasturella divina* and *Uromyces rumicis*). Autoecious rusts include *Uromyces appendiculatus*, *Melampsora lini*, *Puccinia helianthi*, *Uromyces hobsoni*, and *Puccinia asparagi*.

Physiological Specialization

Schroeter (1879) first noted the phenomenon of physiological specialization, also designated specialization of parasitism, in the rust fungus, *Puccinia graminis*. Eriksson (1894), in Sweden, followed with an experimental evidence of the presence of this phenomenon in *Puccinia graminis* based on cross-inoculation experiments. He recognized five varieties in this rust: *tritici* on wheat, *avenae* on oats, *secalis* on rye, *agrostidis* on *Agrostis* sp., and *poae* on *Poa* sp., and called them "*formae specialis*". Since then, extensive knowledge has been accumulated on this phase in a large number of other rust fungi. Specialization of parasitism of an extreme type, now recognized as physiological forms or biotypes, has been demonstrated by Stakman (1914) at the University of Minnesota, USA, within the varieties *P. graminis tritici* and *P. avenae*, and by other scholars in different countries. Stakman and Harrar (1957) and many other scholars have defined physiological specialization as the "presence of entities within morphologic species, not readily distinguished by structure, but differing from each other physiologically including pathogenicity, host morphology, biochemical properties, cultural variability, spore germination and ecological relationship." These entities have been named formae specialis, biologic species, parasitic strains, racial strains and physiologic forms.

The rust races, in turn, can be further segregated into the ultimate sub-divisions—the biotypes. A biotype is a group of organisms that are genetically identical. The descendants of a single non-sexually produced stem-rust spore constitute a biotype, unless mutation occurs and results in genetic diversity.

Most species of plant pathogenic fungi comprise many biotypes and attempts have been made to group the most closely related ones into races, to group the most closely related races into varieties, which in turn, are grouped into species. The procedure is usually undertaken in the reverse direction. The larger groups are recognized first and the successive smaller groups are discovered by more refined methods.

Occurrence and Identification of Varieties, Races and Biotypes

Puccinia graminis can be taken as an example of the way in which varieties, races and biotypes can be identified. There are at least six different varieties of *Puccinia graminis* in North America, which differ in spore size and in the kind of cereal grains and grasses that they can attack. The spores of all varieties have some characteristics in common. However, the differences between some of the varieties are so pronounced that an experienced observer can easily identify them by microscopic inspection. The principal differences between the varieties are given in Table 16.1.

Table 16.1 Size of Urediniospores and Host Range of Six Varieties of *Puccinia graminis*

Variety	Size of Urediniospores *(microns)*	Host Plants
tritici	32 × 10	Wheat, barley, and many wild grasses
secalis	27 × 17	Rye, barley, and many wild grasses, mostly like those above
avenae	28 × 20	Oat and wild grasses, different from above
phleiprantensis	24 × 17	Timothy and certain wild grasses
agrostidis	22 × 16	Red top and other species of *Agrostis*
poae	19 × 16	Kentucky blue grass, *Poa pretense* and related species

Within the varieties *tritici*, *avenae* and *secalis* races are known. There may be slight morphological differences among races of each variety, the most important and distinct differences being in pathogenicity on certain varieties of wheat, oat and rye, respectively. For the determination of races, a standard differential set of cultivars are used, which is as follows:

Triticum compactum (Club wheats)
Little clubs (C.I. 4066)

Triticum vulgare (Bread wheats)
Marquis (C.I. 3641)
Reliance (C.I. 7370)
Kota (C.I. 5878)

Triticum monococcum (Einkorn)
Einkorn (C.I. 2433)

Triticum durum (Durum or Macaroni wheat)
Arnautka (C.I. 1492)
Mindum (C.I. 5296)
Spelmar (C.I. 6236)
Kubanka (C.I. 2094)
Acme (C.I. 5284)
Triticum dicoccum (Emmer)
Vernal (C.I. 3686)
Khapli (C.I. 4013)

When inoculated with urediniospores or aeciospores of different races, these varieties are designated as susceptible (S), resistant (R) or mesothetic (M), depending on the infection type produced. The infection types produced by rust races on the different varieties of wheat indicate the degree of resistance or susceptibility of the wheat varieties (Fig. 16.1).

Infection Types

0 – Immune —No uredinia or flecks of dead tissue, hypersensitive flecks represented by a semicolon (;).
1- Very resistant —Uredinia minute, isolated and scattered, surrounded by sharp, continuous, hypersensitive necrotic area.
2- Moderately resistant —Uredinia small to medium in size, isolated, hypersensitive areas present in the form of necrotic haloes or circles, pustules usually in green, slightly chlorotic islands.
3- Moderately susceptible —Uredinia medium, no necrotic areas –chlorotic may develop under unfavourable conditions.
4- Very susceptible —Uredinia large, generally coalescing, numerous; no necrosis but chlorosis may be present.
X- Mesothetic —Heterogenous-Uredinia variable, sometimes including all infection types and inter-gradation between them on the same leaf.

In identifying races, only three varietal reaction classes are used, as shown below:

Varietal Reaction	Infection Types
Resistant (R)	0, 1 and 2
Susceptible (S)	3 and 4
Mesothetic (M)	X

For identifying rust races of *Puccinia graminis* var. *tritici*, seedling plants of twelve standard differential varieties are inoculated in the greenhouse with rust spores, after which the plants are kept in a moist chamber for 12-24 hours to provide moisture for spore germination and entrance of germ tube. It takes from one to two weeks after inoculation for the rust to produce a new crop of spores. The races are designated by numbers and are identified by means of a trichotomous key.

Many races have been subdivided. Race 59 is an example. The collections of rust from USA and Mexico that were identified as race 59 between the years 1929 and 1944 produced the types 0 and 0;

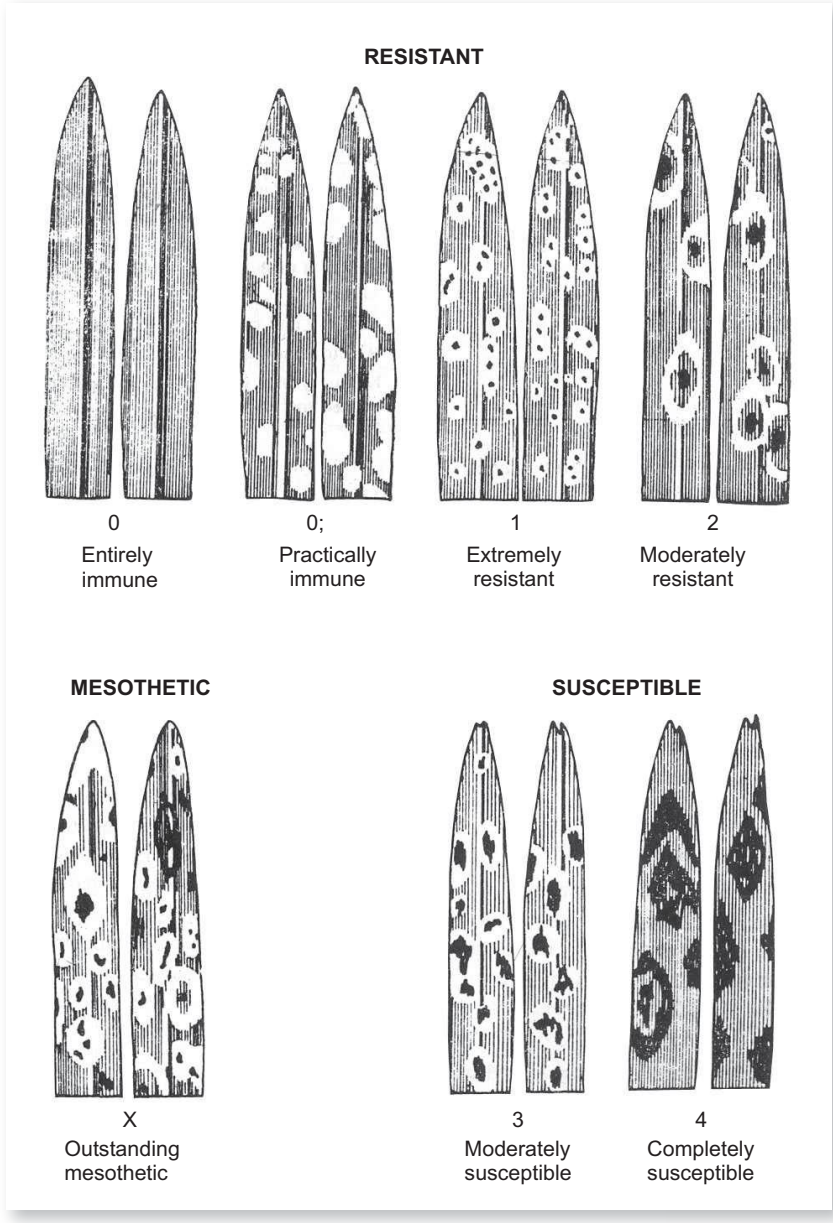


Figure 16.1 Infection types of *Puccinia graminis* f. sp. *tritici*

on Reliance and Kota. But in 1944, isolates obtained from the barberry bushes in Massachusetts and Washington consistently produced infection type 2 on Reliance wheat instead of the type 0, which was characteristic of the race. The new isolate was designated 59A. Later, two other isolates obtained from barberry differed slightly but consistently from both 59 and 59A, and were designated 59B and 59C. The infection types produced by these kinds of isolates on Reliance and Kota are given below:

Isolate	Reliance	Kota
59	0	0;
59A	2	0;
59B	2	2
59C	0	2

These minor differences in infection types on Reliance and Kota seemed significantly interesting rather than practically important, until it was found that 59A is much more virulent than 59 on certain hitherto resistant varieties of barley. 59, 59B, and 59C are the biotypes.

Use of Benzimidazole and Kinetin for the Detached Leaf Culture of Leaf and Stripe Rust

Using compounds such as Kinetin or Benzimidazole, which exert a cytokinin effect, it is possible to culture detached leaves on cytokinin solution for even a month (Person *et al*., 1957). Rusts grow well in leaf cultures of this type. On the basis of these findings, several investigators tried to determine the races of rust and mildews, as well as maintain obligate parasites on detached leaves floated on cytokinins.

Physiology and Host-parasite Relation of the Rusts

Allen (1953, 1959), who in 1959 also discussed the biochemistry of resistance has reviewed the host parasite relation of the rusts. The respiration of diseased plants had been dealt with by Millerd and Scott (1962), and Uritani and Akazawa (1959); several other aspects of physiology of disease by Farkas and Kiraly (1962), and Rubin and Artsikhovaskaya (1964); the problem of obligate parasitism by Yarwood (1953); the genetics of rust by Johanson (1953); the complementary genetic system in flax and flax rust by Flor (1956); the physiology and host-parasite relations of rusts by Shaw (1963); the genetics and expression of resistance in plants to rusts of the genus *Puccinia* by Hooker (1967); culturing of rusts fungi by Scott and Maclean (1969); and various aspects of host-parasite relationship in rust by Prasada (1964). Research on the rust fungi during the twentieth century had been reviewed by Staples (2000). Long distance dispersion of rust pathogen has been discussed by Nagarajan and Singh (1990).

Studies on rust physiology have been carried out with special reference to the following aspects:

1. Metabolism of spores.
2. Alteration translocation of infected hosts.
3. Respiratory pattern and photosynthetic activity of infected tissues.
4. Nucleic acid metabolism of the host-parasite.
5. Metabolism of growth substances in infected host.

Culturing Rust Fungi

Hotson and Cutter made *Gymnosporangium juniperi virginianae* in axenic culture, the first successful culture of rust, in 1951. Later in 1960, Cutter reported successful axenic culture of *Puccinia malvacearum* (a microcyclic rust infecting hollyhock) on the callus of *Althaea rosea* and observed teliospore formation on three-month-old callus tissues.

Williams *et al.* (1966, 1967) reported the first successful axenic culture of rust from urediniospores. In 1966, they successfully obtained the first saprophytic culture of an Australian isolate of *Puccinia*

graminis tritici. They incubated urediniospores on an agar medium containing Czapek's minerals, 1% yeast extract and 3% sucrose. The urediniospores germinated germ tubes elongated after 48 hours and branching commenced after 96 hours. The addition of Evan's peptone enhanced the vegetative growth of the fungus. Uredio and teleutospores were produced after 2-4 weeks. If in place of sucrose, glucose and citrate are added to the yeast extract peptone medium, it results in better growth.

Successful reinfection by spores formed in axenic culture was obtained only when urediniospores were placed under the epidermis, in contact with mesophyll cells (Williams *et al*., 1967). When placed directly on the intact leaf, spores gave a high percentage germination but germ tube elongation was limited. It appears that the inability of urediniospores to infect hosts by stomatal invasion is due to a low potential for independent growth rather than a lack of pathogenicity.

Several other rust fungi have been cultured and there is a considerable amount of literature on the nutritional studies of these rust fungi with regard to axenic cultures. Scott (1976), Williams (1984), and Fasters *et al.* (1993) have reviewed the growth of biotrophic parasites in axenic culture.

Rusts of Wheat

Wheat crop occupies nearly 200 million hectares across the world, which is estimated to produce nearly 250 million tons. In India wheat is grown on over 24 million hectares, which nearly yields 57.0 million tons (Goel and Sinha, 1996). Wheat rusts are regarded as the most destructive pathogens on the crops in many countries. Wheat rust epidemics in India have been recorded as early as 1827 in the Central Provinces (Sleeman, 1839) and in 1907 by Milligan. In 1947, the black rust epidemic in Madhya Pradesh (then known as Central Provinces) almost caused a famine. There also were reports of a black rust epidemic from Bihar in 1957. In 1971-72, a brown rust epidemic was reported from the northwestern part of the country and according to Joshi (1976), it caused an estimated loss of 1.5 million tons of wheat. Wheat rusts created an economic disaster in 1916 in the USA and Canada. However, an even worse disaster struck in 1935, when about 100 million bushels of wheat were lost. Another wheat rust epiphytotic swept through the wheat belt of the USA in 1937.

In our country, the major area is under bread wheat (*Triticum aestivum* L.), followed by *T. durum*, the macaroni wheat. The increase in area as well as in productivity in the past three decades has helped the country in not only achieving self sufficiency but also to build up buffer stocks of wheat.

Black Stem Rust of Wheat

In India, black rust occurs in almost all the wheat growing areas. In northern India, this rust does not appear before March and by this time the crop reaches the 'dough' stage. Therefore, the damage to wheat crop by the rust is usually not severe. On the other hand, in the peninsular and southern parts of the country, it may appear as early as the fourth week of November and hence losses to the crop are generally higher. Black rust has been extensively studied in the USA, Europe and Australia, where it often causes heavy losses in yield. Severe infection results in premature ripening and shrinking of the grain and sometimes may result in as much as 90% reduction in grain yield.

Symptoms

In black rust of wheat, the stems are most severely attacked, followed by the leaf sheaths, leaves, and ears. Uredinia are the first to appear and are very prominent on culms, leaf sheaths, and also leaves. The urediosori are large, elongated, coalescing and dehiscing early, and breaking up large fragments of the epidermis.

Telia arise later in the season. Teleutosori are like urediniosori but black in color. These burst rather early and are found on all green parts of the plant, but least on the leaf blades [Fig. 16.2].

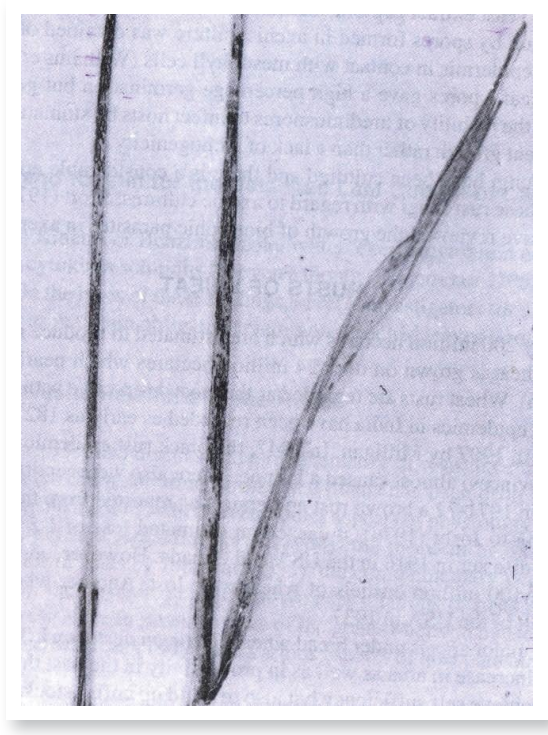


Figure 16.2 Black stem rust of wheat (*Puccinia graminis tritici):* symptoms

Apart from these physical changes on the plant, in case of severe attack, plants fail to produce normal ears. The grains are shrivelled and lighter in weight.

An increase in respiration rate accompanies the infection of plant tissue by rust pathogens. The rate of photosynthesis shows an initial increase in the early stages of infection, then is transitory and goes down finally.

Causal Organism

Puccinia graminis tritici (Pers.) Erikss. and Henn. (Fig. 16.3), is a heteroecious rust and its pycnial and aecial stages are found on species of *Berberis* and *Mahonia*, and these form the alternate hosts for this rust. *Berberis vulgaris* is the most common alternate host. Wind-borne sporidia infect the young tissues of these plants, where the binucleate stage is initiated by sexual fusion. This phase is followed by the formation of the aeciospores.

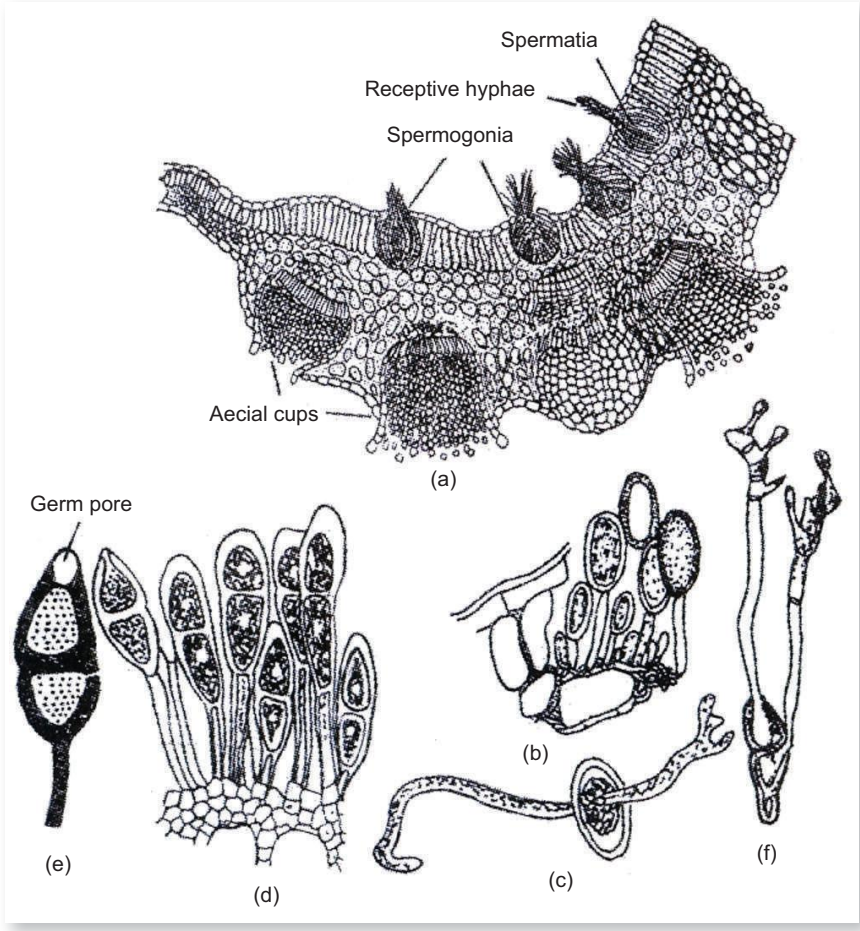


Figure 16.3 *Puccinia graminis tritici*: (a) Pycnidial and aecidial stage in section of barberry leaf (b) uredostage on wheat (c) a uredospore germinating (d) part of a teleutosorus (e) an enlarged teleutospore showing the germ pores (f) a germinating teleutospore

Pycnia—Pycnia are flask-shaped and consist of pycniosporophores and receptive hyphae with a pycnial chamber in the centre.

Aecia—Aecia are cup-shaped and are enclosed by a peridium. The aeciospores are echinulate, have six germ pores and arise in chains from the base of the aecial cup. Aeciospores are capable of infecting the grass host, that is, wheat.

Uredinia and telia are produced on the wheat plant. The urediniospores are brown in colour, oval in shape, 25-30 × 17 µm in size and have a single thick wall with tiny spines, and four equatorially-placed germ pores.

The teleutospores are two-celled, chestnut brown, 40-50 µm long and 15-20 µm broad, and have a thick smooth wall. The apex is rounded or sometimes pointed, with a germ pore through which the promycelium extrudes. The germ pore of the lower cell is at the side, just below the septum. The teleutospores have a long rest period and before they can germinate they have to be exposed to freezing temperatures. On germination, they form long four-celled promycelium and from each promycelial cell a strigma develops, which bears sporidia.

Epidemiology of Black Stem Rust of Wheat in India

In India, *Berberis* spp. do not occur in the plains. In the hills, moderately susceptible species of *Berberis* are found; highly susceptible species of *Berberis*, that is, *B. vulgaris* does not occur. However, the condition prevalent at the time of formation of teliospores (April, May) and their subsequent exposure to warm weather, as well as the time of appearance of the new leaves on *Berberis*, when temperature and humidity are unfavourable for the germination of teliospores and infection of leaves, exclude the possibility of natural infection of *Berberis*. So far, in spite of extensive search, not a singe case of *Berberis* infection observed in nature has been connected with wheat. Such aecia have been found to belong to *P. graminis* var. *agropyri* (Prasada, 1947).

We owe whatever information we now possess on the physiology and epidemiology of wheat and barley rusts in India largely to the pioneering work carried out by Mehta (1929, 1940, 1952).

In the plains of India, cereals are sown in October-November and harvested in March-April, or May. Experiments carried out here and other countries have shown that weather conditions as well as the age of the host are quite favourable for infection of crops. However, initial outbreaks of rusts are delayed by 2-3 months from the time of sowing of the wheat crop in the plains.

There is hardly any chance of survival of urediniospores of any of the three rusts of wheat from the previous crop in the plains of India due to the intense summer heat. As already pointed out, the role of *Berberis* has been fully discussed by Mehta (1940). He (1933, 1940) supplied detailed information regarding the survival of the uredo stage all the three rusts of wheat on "out of season" wheat during summer in the hills. He has also dissertated on the outbreak of rust on new crops sown earlier in the year in the foothills. A detailed comparison shows that there is heavier infection in the foothills than in the neighboring plains and also that the periods of rust appearance in the foothills and the plains in different parts of the country are not the same.

Mehta (1933, 1940, 1952) safely concluded that the rust inoculum is reintroduced into the plains of India, year after year, from some other source, and this source, in all probability, lies in the hills where all the three rusts are able to over summer in the uredo stage (altitudes 4000-8000 ft.). On account of milder summer and requisite humidity, the rust over summer in the uredo stage on volunteer wheat, stubbles, and so on and on the regular crops (summer and winter) in the Nilgiris, Pulney hills, and the submountainuous regions of the Himalayas in the north. These were regarded as the chief foci of infection, but regular surveys conducted in 1953-54 and 1954-55 have shown that the Panchgani-Mahabaleshwar range in the Western Ghats is also a focus of infection.

Earlier, it was believed that the primary inoculum of black rust comes from the north as well as the south (Mehta, 1929, 1940, 1952). However, Joshi *et al*. (1974) suggested, on the basis of data collected by survey teams and a large number of cooperators, that south India gets black rust infection much earlier than the north and that certain regions of central India may serve as reservoirs of inoculum for the main wheat belt in the north. Although the black rust can survive in the northern hills throughout the summer months, a number of factors seem to indicate that this inoculum does not play any significant part in the spread of the rust to the plains in the north. The main reason for this is that the average minimum temperature for the months of December, January and February in North India ranges from 7–11 and 9-13^{o} C, respectively. The incubation period of the rust in these temperatures will be as long as 40-50 days in the months of January and February. With the coming of favourable environmental conditions for black rust, very little time is left for its development and spread as the crop reaches maturity. The inoculum builds up in the south and its dissemination towards the north appears to be the principal

source of black rust to the Northern wheat crop. Karnataka, Madhya Pradesh and Maharashtra are the states that serve as the chief areas of multiplication of black rust. Joshi (1976, 1978 and 1986), Nagarajan and Joshi (1977, 1978), and Nagarajan *et al.* (1975) have discussed the epidemiology of wheat rust in India. Nayer *et al*. (1998) have recently critically discussed the epidemiology of wheat rust in India. Long distance dispersal of rust pathogens has been reviewed by Nagarajan and Singh (1999).

Role of Collateral Hosts

Although several grass hosts such as *Bromus patulus, Brachypodium sylveticum, Hordeum* spp*., Aegilops* spp., and *Lolium* spp. are infected with rust, their growing period coincides with that of wheat and hence, they are not of importance in the annual recurrence of the rust in the plains.

Another aspect that has been intensified during the last few decades is the study of physiological races and biotypes. In the case of black stem rust of wheat, there are five possibilities of the origin or appearance of new races. These are: (1) hybridization, (2) mutation, (3) heterokaryosis, (4) somatic hybridization, and (5) introduction from neighbouring countries.

As discussed earlier, the *Berberis* species seem to play no functional part in the annual recurrence of rust in India and not a single case of infection on *Berberis* spp. in the hills can be traced to *P. graminis tritici* infection. Hence, the question of appearance of new races by hybridization is almost completely ruled out.

Another major cause in pathogenic variation in the rusts is mutation. Mutation in rusts can no longer be regarded as a rare phenomenon because it has been reported in several cases. Johnson and Newton (1946) reviewed the subject of mutation in cereal rusts and believed that its significance lies in creating variations in wheat rusts. Work done by Sharma and Prasada (1970) indicated that changes in pathogenicity may occur through mutations in wheat rusts. Many specialists have also observed similar pathogenic changes. As yet, there is no direct evidence that new races have arisen in nature by this method.

Nuclear reassortment and somatic changes (somatic hybridization) may also play a crucial role in producing genetic diversity, particularly in association with hyphal fusion and nuclear exchange. Nelson *et al.* (1955) demonstrated for the first time that new races and biotypes of black rust arose when seedlings of wheat were inoculated with a mixture of two known races. Since then these studies have been repeated by many experts in the field (Watson, 1957a, b; Bridgmon, 1959; Ellingboe, 1961) and many new and previously unknown races have been isolated. Vakili and Caldwell (1957) isolated new races from known mixtures of brown rust of wheat.

According to Prasada (1970), there is also a possibility that races are introduced in India from neighbouring countries.

In spite of the fact that these possibilities have been put forward for the production and appearance of newer races, it is still not known exactly how newer races appear in India. Over 250 physiologic races of *P. graminis tritici* are known from the world. Around 31 races and biotypes have been reported from India.

These are 11, 11A, 14, 15, 15-C, 17, 21, 21-A, 21-A-1, 21A-2, 24, 24A, 34, 34A, 40, 40-1, 40A, 42, $42B_1$, $42B_2$, $42B_3$, 72, 75, 117, 117A-1, 122, 184, 184-1(55G-1), 194, 222 and 295.

Disease Management

In a country like India, where rusts are perpetuated annually through the agency of urediniospores in the hills, Mehta (1929) has suggested that rust epidemic can be controlled to a considerable extent by reducing the initial inoculum at the source. To achieve this, he emphasized the importance and urgency

of breeding rust-resistant wheat varieties and gave priority to the hills, which act as foci of infection. Until these suitable varieties are available and the hilly areas are saturated with them, he advocated the cleaning up of the volunteer wheat and barley plants, the suspension of summer crop in the Nilgiri and Pulney hills, and the replacement of wheat and barley cultivation with that of oats in the hills.

As pointed out by Chester (1946), adjustment of the time of sowing, fertilization, destruction of volunteer wheat plants and application of fungicides are but adjuncts to the fundamental problem of breeding cereal varieties that are resistant to rust, which is the most certain, effective, and economical means of controlling the rusts.

Breeding rust-resistant wheat is said to have originated with the pioneering work of Farrer, at the close of the 18th century. In the early years of the last century, Biffen at Cambridge, applied the newly rediscovered Mendelian principle of heredity to breeding wheat that is resistant to yellow rust. This was followed by extensive work in other countries, especially in the USA and Canada.

In India, Pal started a systematic work on plant breeding for rust resistance, in collaboration with Mehta, and several rust-tolerant varieties were released for general cultivation (1935).

Pal attempted a large number of interspecific and intergenic crosses (1948). These studies have yielded useful information regarding the feasibility of crossing different species and genera, fertility of the hybrids and relative utility of the progenies from practical and fundamental viewpoints. In this work, Pal showed that some of the resistant species of *Triticum* referred to earlier, *T. timopheevi* (resistant to yellow rust) have been successfully crossed with *T. vulgare* (common bread wheat) or other species—crosses which may indirectly prove useful in breeding rust-resistant varieties. From some of these fertile crosses, selections have been made, which show a high degree of resistance to certain rusts combined with good ear and grain characteristics. Thus, the New Pusa series were developed. Some of the varieties of the series, like N.P. 700 and N.P. 800, are resistant to one or the other of the three rusts.

Breeding for rust resistance is complicated by the emergence of newer races. Breeding for resistance is, therefore, a continuous process. The newer Mexican dwarf varieties introduced into India seem to be resistant to all or some of the rusts. Lerma Rojo is reported to be highly resistant to all the three rusts in the field in North India, though it is susceptible to some races occurring in South India, particularly in the seedling stage; Safed Lerma and Sonalika are also highly resistant to all the three rusts; Sonora 64 is susceptible to yellow rust but is moderately resistant to black and brown rusts; and Choti Lerma is highly resistant to all the three rusts in the field.

It may be stated that research for breeding rust resistant varieties has resulted in the development of resistant/tolerant forms from time to time and it has played a significant role in containing the rust problem to a great extent. During the last twenty-five years, with the release of resistant dwarf high-yielding wheat varieties, the stem rust problem has significantly reduced throughout the country, especially in the central and peninsular part of the country, where rust has been an important problem in the past. Goel and Sinha (1996) gave a list of rust-resistant or tolerant wheat varieties that are recommended for cultivation in agro-climatic zones.

According to Kumar *et al*.(2011), the wheat and its pathogens causing black, brown and yellow rust diseases (*Puccinia* spp.) survive the whole year in Nilgiri hills of south India. The rust pathogens particularly causing black and brown rusts over- summering on self sown and off-season sown wheat plants in these hills disseminate with cyclonic winds in the month of November and infect winter sown wheat crop in central and peninsular India. Therefore, cultivars released for central and peninsular India must possess resistance to pathotypes of black and brown rust pathogens prevailing in Nilgiri hills to grow rust free wheat crops in these areas, which will come a long way in controlling these rusts in north India.

Mixed cropping of wheat and barley with suitable crops gives good crop insurance even if the main crop fails. The susceptible surface area is reduced in mixed cropping and this reduces the inoculum for secondary infection and spread of the disease.

Nitrogenous fertilizers tend to increase the susceptibility of wheat crop to rusts. While potassium has the opposite effect, reduction of N in the NPK ratio helps in reducing the incidence of rust in a susceptible variety of wheat.

Dickson (1969) has reviewed the work on chemical control. According to Forysth and Perurson (1958), effective control is obtained with 4-5 application of Nabam and zinc sulphate. Dithane has been successfully employed in Japan.

In Uttar Pradesh, experiments have shown that sulphur dusting at the rate of 13.6 kg per acre, at intervals of four days during the period when there are chances of the appearance of rust, is effective in controlling cereal rusts. But, this requires an efficient disease-forecasting service. Also, sulphur has to be imported, which is a drain on India's foreign exchange reserves. In India, Grewal and Dharm Vir (1959) have reported that four sprayings of Parzate liquid + zinc sulphate at fortnightly intervals is a good control measure. Wallen (1955, 1958) has controlled rust through the antibiotic cycloheximide derivatives of Actidione at 50 ppm (182 litres/acre) as a systemic fungicide. Greenhouse experiments at the University of Minnesota have demonstrated that certain sulpha compounds, including sodium salts of sulphadiazine and sulphapyrazine, are effective as post- infection sprays.

Chemical control of rust has been receiving much more attention in recent years than in the past. A number of chemicals, such as RH-124 and Plantavax had given quite encouraging results. Von Schmelling and Kulka (1966), Edgington and Reinberg (1966) reported the effectiveness of Vitavax and Plantavax against rust pathogens. These oxathiin fungicides are effective as soil, seed and spray treatments. Plantavax was found more effective than Vitavax.

Triadimefon and Fenapenil were reported as good fungicides for the control of stem rust. Brahma and Asir (1988) found Propiconazole (Tilt) at 0.1% effective in controlling the three wheat rusts because it persists for 12 days and completely inhibits urediniospore germination.

Plant-disease forecasting, with special reference to rusts, forms an important and integral part of control. It may be added that in most countries in the northern hemisphere, the spring movement of black rust is from the south to the north, and the findings of Joshi *et al.* (1974) lend support to this pattern of movement of black stem rust. The determination of the source of inoculum in the south has an important bearing on disease control.

Genetic Resistance

Genetic resistance is the most commonly used and most effective means to control stem rust. For reasons that we do not fully understand, a few genes of race specific (vertical) resistance to stem rust in wheat remained highly effective for many years. The most successful among these was *Sr31*, a gene that is present on a segment of a chromosome from rye that was transferred into wheat by a complicated process of interspecific hybridization. Wheat with *Sr*31 quickly became popular worldwide, because in addition to *Sr31,* the rye chromosome segment also carried genes for increased grain yield as well as resistance to other rust diseases. Since the 1980s, wheat varieties with *Sr31* are being widely grown in nearly every major wheat-producing region throughout the world, other than Australia. The effectiveness of *Sr31* was so great that wheat stem rust declined to almost insignificant levels, nearly everywhere in the world by the mid 1990s.

In 1999, a new race of wheat stem rust fungus that is highly virulent to wheat varieties with *Sr31* was found in Uganda. The new race was named Ug 99 after the country and the year in which it was discovered. It rapidly spread in Uganda and further affected Kenya and Ethiopia, where it caused major epidemics. Within a few years, Ug 99 was identified in South Africa and in Yemen, from which it has spread to the North and East as far as Iran. It seems inevitable that Ug 99 will soon infest one of the world's richest wheat producing areas in Punjab, India. Previous examples of long distance dispersal of wheat fungi include spread of a unique race of wheat stem rust from South Africa to Australia, spread of coffee rust from Africa to South America and spread of Southern corn rust from Central America to Africa. To make matters worse, the Ug 99 lineage of the stem rust fungus has expanded its virulence through mutations that allow it to overcome the resistance of at least two other vertical resistance genes that wheat breeders have relied on for protection from stem rust in North America and many other parts of the world. However, Nagarajan (2012) believes that *P. graminis* f. sp. *tritici* virulence—Ug99, is not a threat to wheat production in the North West plain zone of India or to Indian food security and the concerned hype is a wrong signal.

For the long term, however, wheat breeders may rely more on minor genes with additional effects of partial resistance expressed primarily in adult plants (i.e. horizontal resistance). A number of high yielding wheat lines with moderate levels of horizontal resistance have been developed at the International Centre of Wheat and Maize Research (CIMMYT). These advanced lines are being intercrossed to produce wheat varieties combining as many as four to five horizontal resistance genes to effectively suppress stem rust epidemics. Jain *et al.* (2011) have reported a new pathotype of *Puccinia graminis tritici* in India and have discussed its implication in breeding programme. According to Jain *et al. (*2011), new sources of resistance and *Sr* genes, in combination, should be used in breeding programme to address the challenge posed by this new variant of stem rust.

Brown Rust of Wheat

This rust attacks leaves almost exclusively, rarely leaf sheaths, and very rarely stems. This rust, although more general in occurrence, is more common in the northern and eastern parts of India than in peninsular India. It is perhaps the earliest rust appear in certain parts of Bihar, Punjab, and Uttar Pradesh, and together with the yellow rust, causes great loss and damage. Epidemic of brown rust occurred in the north western part of the country in 1971-72 and 1972-73 causing a loss of 0.8 to 1 millions tons and 1.5 million tons, respectively (Govindu, 1977). Joshi *et al.* (1985) reported that Sonalika epidemic of leaf rust in U.P. and parts of Bihar caused a loss of one million tons of wheat.

Symptoms

As a rule, the urediopustules develop on the leaves, being rare on the sheath and stalks. They burst on the upper surface as points of a bright orange colour. They are never in rows like those of yellow rust but they may be gathered in small clusters or may be irregularly scattered. They are bigger in size than those of yellow rust. When old, the urediopustules of orange rust cannot generally be distinguished by colour from the yellow rust but for their irregular arrangement. The sori burst early and shed the urediniospores.

Telia are formed only rarely and in some ears may not be present. They are small, oval to linear, black and covered by the epidermis. Paraphyses, which are abundantly present, divide the sorus into numerous compartments (Fig. 16.4).

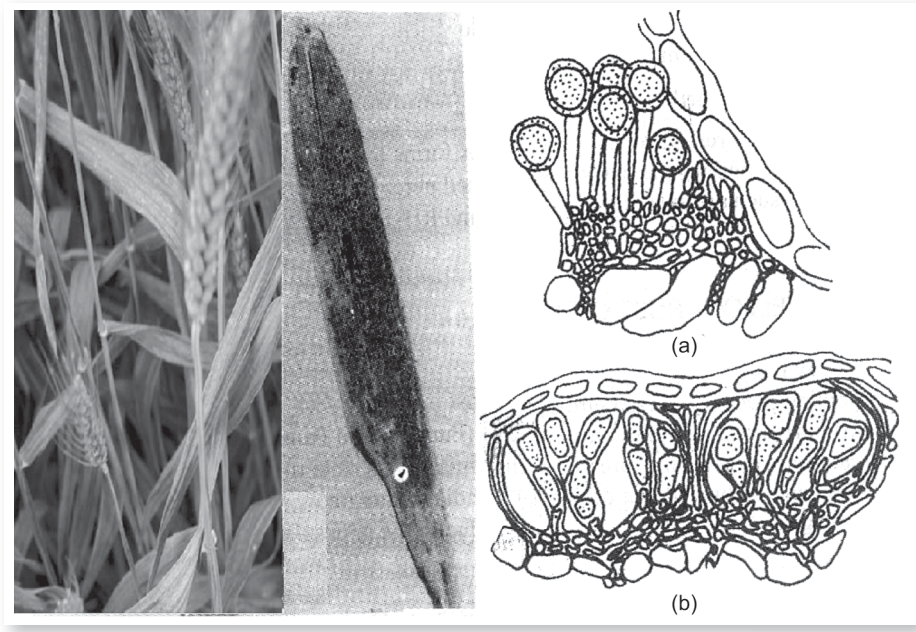


Figure 16.4 Brown rust of wheat *Puccinia tritiana* (*Puccinia recondita*): symptoms (a) section through a uredosorus (b) section through teleutosorus

Causal Organism

The pathogen responsible for brown rust of wheat is *Puccinia tritiana* Eriks. (*Puccinia recondita*). The pathogen is heteroecious. The aecial and pycnial stages are produced on *Thalictrum*.

The urediniospores of *P. tritiana* are brown and spherical 16-28 µm in diameter. The wall is minutely echinulate and furnished with 7-10 germ pores. Infection by germ tube from the urediniospores occurs through the stomata on either side of the leaf. The process of penetration is of the same type as in blank rust.

The teliospores are oblong-to-cuneiform, slightly constricted at the septum and the apex is rounded with prominent thickening (Fig. 16.4b & c).

Disease Cycle

In the case of brown rust of wheat, the only known functional host of the rust, *Isopyrum fumarioides,* does not occur in India, though susceptible species of *Thalictrum* are common in the hills. All the workers except D'Oliveira have always regarded *Thalictrum* as a non- functional host of *P. recondita.*

How far workers in other parts of the world will corroborate these findings is yet to be seen. It is doubtful that new species of brown rust appear due to hybridization. Around 100 physiologic races and biotypes of *P. tritiana* are known from the world. In all 30 races and biotypes of *P. recondita* have been reported so far from India. These are: 10, 11, 12, 12A, 12B, 16, 17, 20, 26, 61, 70, 77, 77A, 77B, 77A-1, 77-1, 77-2, 77-3, 77-4, 77-5, 77-7,77-8 and 104, 104A, 104B, 106, 107, 108, 131, 162,162A, 162B.

Brown rust has two foci of infection. If we take the whole of India, brown rust of wheat is the most widespread and important. It has been observed that in early January and sometimes even earlier, the rust gets established in the foothills of the Himalayas in the north and also in the plains of Tamil Nadu

and Karnataka in the south. The first buildup of inoculum takes place in the plains of Karnataka in the south. It moves northwards to Maharashtra and Madhya Pradesh. At the same time the inoculum of the foothills of Bihar and U.P. moves to the northern plains. Therefore, the brown rust appears slightly later in the western foothills of north India. The rust population of the north and south move in opposite directions, finally merging into each other, and cause epidemic in any part of the country under favourable climatic conditions.

Brown rust can withstand warm weather better than the yellow rust and has been found to survive during summer at altitudes of 1450 m to 2050m. Identification and validation of leaf rust resistance genes in Indian wheat genotype using molecular markers has been done recently by Gurjar *et al.* (2014).

Disease Management

As in the case of black rust, growing of resistant varieties forms the best control measure. Chemical control measures applied for the black rust can also be used here. There have been reported on the field control of this rust by fungicides, such as some of the Dithiocarbamates and RH-124. 1 or 2 foliar sprays have given good control.

Yellow Rust of Wheat

This is also known as stripe rust of wheat. This rust being susceptible to warm weather, does not oversummer below 1600 m. It dies in the open and under shade at Almorah (1450 m) every year during May and June. On the hills this rust has been found to appear on the new crop as early as November-December. It becomes fairly widespread at lower altitudes 1-2 months later. Yellow rust is mainly restricted to the north and northwestern parts and is also found in the Nilgiri and Pulney hills of south India. However, it is totally absent from peninsular India. In north India it normally appears in the foothills in December or in the first fortnight of January. The yellow rust is usually seen first in the northern foothills and adjoining places, such as Gurdaspur and Ropar (Punjab) and Pantnagar in the tarai region (Uttranchal). Stripe rust or yellow rust of wheat is as damaging as stem rust. The extensive cultivation of Kalyansona over a large area of northwestern India has aggravated the stripe rust problem during 1968-69 and its breakdown during 1970-71 was due to the appearance of new virulence of stripe rust pathogen.

Symptoms

In mild attacks the cereal pustules are formed chiefly on the leaves but in more severe attacks they appear on the sheath, stalk, and glumes also. The green colour of the leaves fades in long streaks on which rows of small urediosori appear. Each row consist of a series of oval, lemon-yellow pustules, arranged end to end and each distinct from the one above and below. In severe attack this serial arrangement seems to be lost. The urediosori do not break through the epidemism as quickly as in other rusts, but they do so eventually (Fig. 16.5).

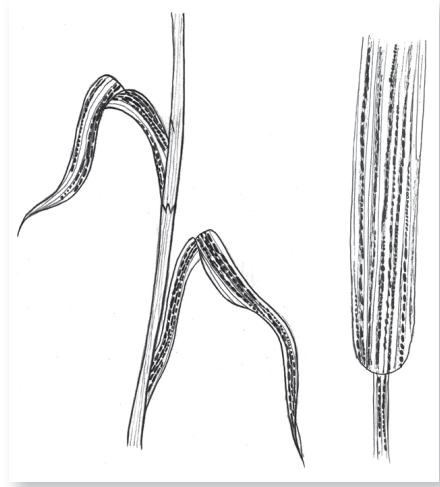


Figure 16.5 Yellow rust of wheat (*P. striiformis*) Symptoms

Causal Organism

Yellow rust of wheat is caused by *P. striiformis* West. [= *P. glumarum* (Schmidt) Erikss. And Henn.] (Fig. 16.6). The urediniospores are almost globose and not oval as in black rust. Their epispore is covered with fine spines. There are 6-11 germ pores.

The telia are hypophyllous or culmicolous and are long narrow lines, like the uredia. They are covered for a long time by the epidermis with brown paraphyses surrounding the spores and intermingling with them along the edges of the telium.

The teliospores are oblong to cuneiform, slightly constricted at the septum and the apex is less thickened and pointed than in *P. graminis*. The teliospores are capable of immediate germination when thickened and pointed than in *P. graminis*. The teliospores are capable of immediate germination when mature.

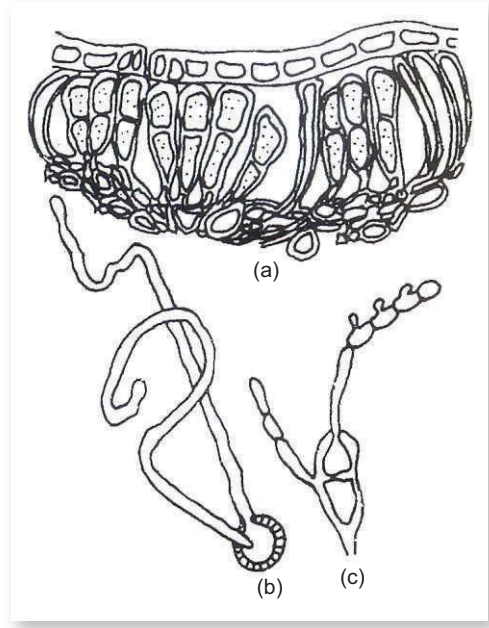


Figure 16.6 Yellow rust of wheat (*P. striiformis* (a) section through teleutosorus (b) a germinating urediniospore (c) a germinating teleutospore

Disease Cycle

The rust is heteroecious but the alternate host is not yet known. The inoculum responsible for starting annual epidemics comes from the hills. The black rust is able to oversummer at an elevation of 1375 m above sea level; *P. striiformis* requires still cooler temperature, and therefore, oversummers at 1900 m and above. The further sequence of events with regard to spread is the same as in black rust.

A grass, *Muhlenbergia hugelli*, growing wild in the Shimla hills, has been found to harbour yellow rust, but its role in the annual recurrence is yet to be determined. Some 90 weeds (e.g. *Agropyron semicostatum*, *Bromus catharticus*, *B. japonicus*, *Hordeum murinum*, etc), are known to serve as collateral hosts for the rust. There are 23 races and biotypes of yellow rust in our country reported so far, namely: 13, 14, 14A, 19, 20A, 24, 31, 38, 38A, 57, A, D, E, F, G, H, I, J, K, L, M, N.

Disease Management

Cultivation of resistant varieties is the best way of controlling this rust. The chemical control measures used for the black and brown rust can also be used here for controlling the rust.

Comparison of the Three Rusts of Wheat

Black rust *P. graminis tritici*	**Brown/leaf rust** *P. triticina* **(earlier known as** *P. recondita***)**	**Yellow rust** *P. striiformis* **(earlier known as** *P. glumarum* **)**
(1)	**(2)**	**(3)**
Appears first in plains in March-April	January	December-January
Stems more severely attacked than leaf sheaths, Leaves and ears	Attacks leaves almost exclusively, rarely leaf sheaths, very rarely stems	Leaves more severely attacked than leaf sheaths, stems and ears

(*Contd.*)

(*Contd.*)

Black rust *P. graminis tritici*	**Brown/leaf rust** *P. triticina* **(earlier known as** *P. recondita***)**	**Yellow rust** *P. striiformis* **(earlier known as** *P. glumarum* **)**
(1)	**(2)**	**(3)**
Urediniosori large, elongated coalescing and early breaking up large fragments of the epidermis	Urediniosori small, but often longer than yellow rust, oval or round. Scattered or aggregated, bursting with a fringe of broken epidermis around them. Never in long rows. Colour bright orange when fresh, becoming brown, chiefly on the upper surface of the leaf.	Urediniosori small, oval, not usually joined together, burst late with little displacement of the epidermis. Arranged in rows or stripes.
Teleutosori-like uredosori But black. Burst rather early. Found on all green parts of the plant but least on the leaf blades.	Teleutosori often absent, when present, resemble uredosori but more flattened and dull black. Do not burst through the epidermis. Chiefly on the under- surface of leaves, very rarely else where.	Teleutosori- like uredosori but more flattened, dull black and often run together. Do not burst through the epidermis. Arranged in rows chiefly on under surface of leaves but also on all green parts of the plants.
Urediniospores oval, 25-30 × 17-20 µm. Brown with a Thick cellulose wall having Four germ pores situated In an equatorial plane**.**	Urediniospores brown and spherical, 16-20 µm in diameter. The wall minutely echinulate, furnished with 7-10 germ pores.	Urediniospores are spherical to ovate in form, very variable in size from 23-35 by 20-35 µm. Spore wall is colourless, minutely echinulate and possesses 6-16 germ pores.
Teleutospore consists of two thick-walled, smooth, superimposed cells, the top cell being rounded or thickened at the apex. dark brown 40-60 × 15 -20 µm.	Teleutospores resemble those of yellow rust in size and shape. They are flattened at the top. The sorus is divided into small groups by paraphyses.	Teleutospores are dark brown and flattened at the top in contact with the epidermis. They are two celled, from 35-63 × 12-24 µm and are interspersed with brown unicellular paraphyses.
Alternate hosts are species of *Berberis* and *Mahonia*	Alternate hosts are *Isopyrum fumarioides* and species of *Thalictrum*.	Alternate host is not known.
About 250 races of the rust are known of which 34 races and biotypes occur in India.	About 100 physiologic races are known of which 32 races and biotypes are reported so far from India.	About 14 races are known however 23 races and biotype have been reported so far from India. (12 races are new races found only in India)
The annual recurrence is by urediniospores from hills	From urediniospores blown down the hills.	From urediniospores from hills.

Rust of Linseed (*Linum usitatissimum*)

The cultivation of linseed is greatly handicapped by the incidence of rust in most of the linseed (flax) growing areas of the world. In India the disease is of particular importance since linseed is one of the major oil seed crop. The crop is sown in October and November and harvested in March or April. The rust appears in February or so.

Injury to crop results from a reduced amount of foliage and utilization of plant food reserves by the obligate parasite. The disease causes reduction in seed yield and the weakening and disfiguring of fiber in flax. The pathogen occurs in Africa, Asia, Australasia, Europe, and North and South America. Saharan (1988) has reported 16 to 100% loss in yield.

Symptoms

Diseased plants become very conspicuous in the field because of the bright orange colour of the affected parts. This is due to the uredia, which occur in large number on both the surface of the leaves and other aerial parts of the plant. The leaves die prematurely and hence, telia, which appear late, are formed mostly on the stems. The brown to black telia appear as crusts covered by the epidermis (Fig. 16.7a).

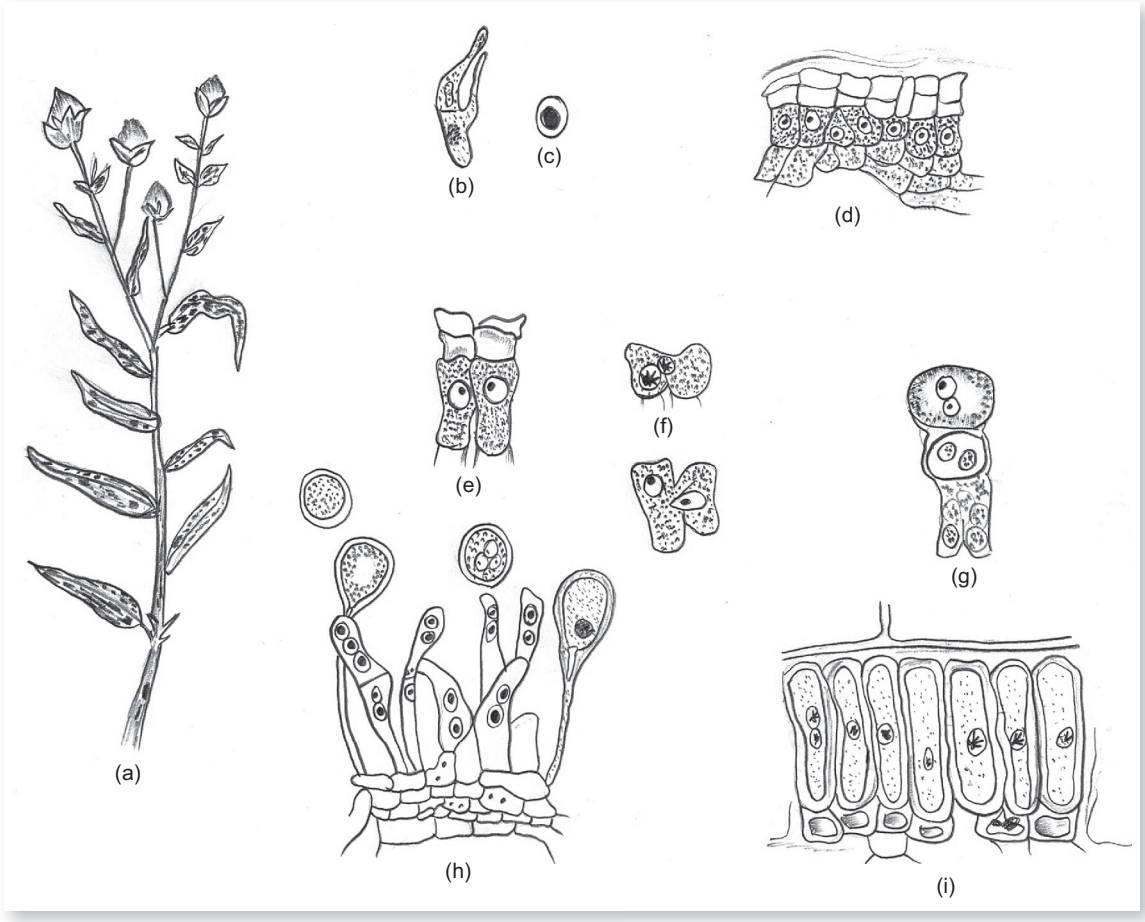


Figure 16.7 Linseed rust (*Melampsora lini*): (a) symptoms (b) Pycniospore (c) Pycniospores (d) Aecium (e) Paired cells at the base of aecium (f) fusion of cells (g) a chain of binucleate aeciospores in formation (h) Uredinium and urediniospores in section (i) Telium and teliospores in section

Causal Organism

The pathogen responsible for linseed rust is *Melampsora lini* (Ehrenb.) Lev. It is autoecious rust. Pycnia are amphigenous and about 5-10 are grouped closely together. They are usually flask-shaped but occasionally diffused, spermatiophores, without a definite structure, appear. Aecia are caemoid (i.e. lack peridium), hypophyllous associated with and often surrounding the pycnia, concentrically grouped

and often joining together to form a ring. Aeciospores are ellipsoidal, 15-25 µm diameter, uredinia are amphigenous and caulicolous, irregularly scattered, 0.5-1 µm diameter. Paraphyses intermixed with urediniospores, capitate, up to 80 µm long, head 20-30 µm diameter, hyaline. Urediniospores ellipsoidal or obovoidal, 15-25 × 15-22 µm, wall hyaline, finally echinulate, 1.5-2 µm thick. Telia are amphigenous and culmiculus circular or elongated along with stems, often fused and covering large areas, subepidermal, black. Teliospores cylindrical, 45-80 µm long × 8-12 µm broad, wall luteous, 1 µm thick at the side, sienna to chestnut, 2-4 µm thick above (Fig. 16.7 b, c, d, e, f, g, h, i).

It occurs on *Linum usitatissimum* and *L. catharticum* and other *Linum* spp.

Physiological Specialization

The rust has been shown to consist of different host species. It has been extensively studied by Flor (1954) for a period of about 25 years. He distinguished 179 races, mostly from the USA. Eighteen physiologic races of the fungus have been reported so far from India (Saharan, 1991). Flor (1956), by simultaneous studies on the inheritance of virulence in races of flax, put forward his concept of gene relationships between the host and pathogen. Person (1959) elaborated it further; he showed that more than one gene for rust resistance may exist in some of the differential flax varieties (Finchan and Day, 1971).

Disease Cycle

In temperate countries, soil-borne teliospores on crop refuge are the source of primary inoculum. These germinate to form the sporidia which infect young leaves. Prasada (1940) found that nearly 60 % of the telia of the fungus collected in March and stored at 5-10° C during summer, germinate after eight months. They die in the field at summer temperature in the Indian plains. However, in the hills (1080-1380 m), variable teleutospores have been recovered from plant debris collected with the seed and there is evidence of the active role of such teleutospores in initiating fresh outbreaks of the disease (Prasada, 1970). Volunteer flax plants, including some wild species, may also serve as important source of infection (Laundon and Waterston, 1965) and enable the overwintering of the uredinial stage in New Zealand. *Linum mysorens*, wild linseed, has been reported to harbour the fungus in India. However, it is not definite if the rust race on this plant is the same as on linseed (Prasada, 1976).

Disease management

The most promising method of control of linseed rust lies in the cultivation of resistant varieties. Another important control measure for this rust is the avoidance of excessively nitrogenous manures which favour lush growth.

Heggenes (1942) had reported that flax rust could be completely controlled by application of Borax at the rate 60 kg / ha. Since the source of primary inoculum lies in the hills, seed treatment to inactivate the teliospores and cultivation of resistant cultivars in the hills will go along way in controlling the rust of linseed. Some cultural practices such as destruction of diseased crop debris and weed hosts have also been recommended. The cultivars (RR) 9, 10, 56, Jawahar- 7, 17, 552, LC-54, K-2, LC-216, LC-255 had been cited as resistant to the disease.

Rust of Beans (*Phaseolus vulgaris*)

It occurs wherever been are grown. Several species of *Phaseolus*, *Dolichos* and *Vigna* are attached by this pathogen.

Symptoms

Rust sori are reddish-brown and are circular in shape. These may be surrounded by a yellow halo in some varieties. Sori are more frequent on the lower surface of the leaves. Telia are linear, dark brown and appear quite late in the season. Severe infections result in complete defoliation and total loss of the crop. It is autoecious rust but the pycnial and aecial stages are rarely found.

Causal Organism

The pathogens responsible for disease are *Uromyces phaseoli typica* Arth. on beans and *Uromyces phaseoli vignae* (Barel.) Arth. on cowpea. Earlier the fungus identified was *Uromyces appendiculatus* (Pers.) Fr. It is autoecious rust. Urediniospores are ellipsoidal to obovoidal, 20-30 × 20-26 µm, wall sienna, finely echinulate, 1-2 µm thick, pores 2, equatorial or super equatorial. Teliospores are ellipsoidal, umbonate at the apex, 28-39 × 20-36 µm, wall bay, umber to sienna, smooth or sparsely warted or striate, 25-35 µm thick, and pedicels fragile.

Disease Cycle

This is autoecious macrocyclic rust but the spermogonial and aecidial stages are rare. Soil-borne teliospores initiate the primary infection. They germinate to form the sporidia which infect young leaves. The urediniospores can also oversummer and perpetuate the fungus. Urediniospores are disseminated by wind and through contact with animals including man and his implements. The spread of the pathogen is favoured by cloudy, humid weather with heavy dews and temperatures of 21-26°C. More than 35 races have been identified so far.

Disease Management

Cultural Methods The following cultural practices should be adopted to avoid the incidence of disease

1. Removal and burning of crop debris
2. Suitable plant spacing to lower relative humidity
3. Long crop rotation
4. Removal of weeds.

Chemical Treatment The disease can be controlled by the application of Mancozeb, Maneb, Zineb or Daconil at the rate of 2 kg/ ha at 10-days intervals. Apart from this, sulphur dust is a good chemical means of control. Three sprays of 0.1% Triadimefon or Tridemorph fungicides 45, 60 and 75 days after sowing gave best control of the disease.

In the field of control by chemotherapy, effective systemic activity has been displayed by F-17, an anti-rust complex produced by *Streptomyces cinnamomeus* f. *azacoluta*, by Griseofulvin, especially in

the presence of glycerine and indolacetic acid, and by N-phenyl-N 1-3 sulphonyl-hydrazine. Garlic juice and Phleomycin have also been successfully used as antibiotics.

The use of resistant varieties has proved to be the most promising control measure.

Rust of Pea (*Pisum sativum* and *Lens esculenta*)

It is worldwide in distribution. The disease occurs in several parts of India. It is a macrocyclic and autoecious rust. The symptoms of this rust appear in the month of January. It attacks broad bean, pea, vetch and lentil, causing partial defoliation of susceptible varieties.

Two species of *Uromyces* occur on cultivated peas. *Uromyces pisi* (Pers.) Wint. is a heteroecious species having its aecial stage on *Euphorbia cyparissias* while *U. fabae* (Pers.) de Bary, is an autoecious rust and is the most common rust of pea and lentil in India.

Symptoms

Aecia are the earliest to appear around the month of February. Pycnia, mostly hypophyllous, are infrequent and rather inconspicuous. All the green parts of the host including the pods are affected. Aecia are hypophyllous cupulate and scattered over the entire leaf. The uredinial sori develop on both the surfaces of the leaf as well as on other parts of the plant. The teleuto pustules occur in the same sorus as the uredinia are developed from the same mycelium as the uredinia. These are most common on the stem and petiole and are almost black in colour (Fig. 16.8).

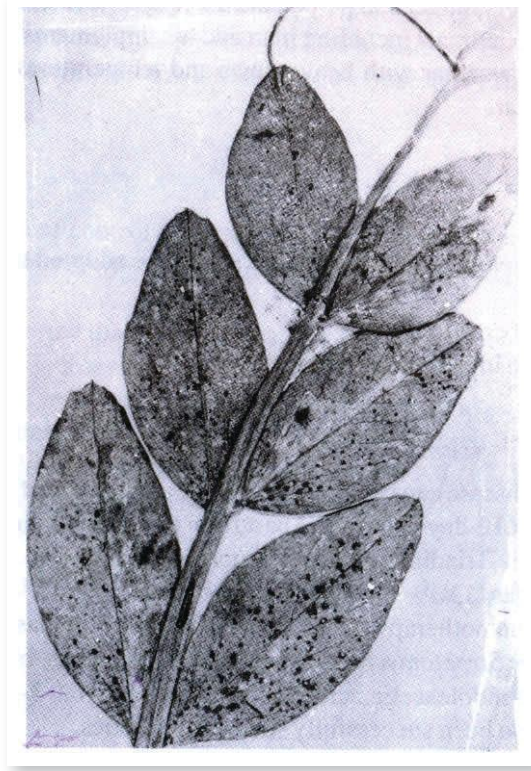


Figure 16.8 Rust of pea caused by *Uromyces fabae*

Causal Organism

Rust of pea and lentil is caused by *Uromyces fabae* (Persoon) de Bary. It is autoecious rust. Aeciospores are broadly ellipsoidal, 17-22 µm diameter; wall hyaline, finely verrucose, 1 µm thick. Urediniospores are broadly ellipsoidal, 22-28 × 19-22.4 µm wall luteous to sienna, very finely echinulate, 2.5 µm thick; pores 3-6 equatorial or scattered. Teleutospores broadly ellipsoidal, obovoidal or cylindrical, slightly bullate at the apex, 25-40 × 18-26 µm, wall chestnut smooth, 1-2 µm thick at the sides, 5-12 µm thick above. Pedicels are up to 100 µm long.

Disease Cycle

According to Prasada and Verma (1948) aeciospores play the major role in the dissemination of lentil rust during the active season. They form secondary aecia after infecting the leaves. Prasada and Verma

(1948) working with *U. fabae* from lentil found that at lower temperatures (17-22°C) infection with aeciospores results in the production of the secondary aecia while at 25°C the infection causes the development of uredia. Aeciospores do not survive for long during the off season for the crop. According to Prasada and Verma (1948) teleutospores resist high temperatures and remain viable for two years when stored at 3-18°C germinating in January to initiate fresh outbreaks of infection.

Disease Management

The use of resistant varieties forms the most important control measure. Chemical which have been given good control include sprays with Bordeaux, sulphur compounds, Zineb, Ziram, Thiram, Chloranil, and thiocarbamoyl thionitro paraffin compounds. Agrosan and Thiram have also been successfully used as seed treatment.

There is rust on pea. This is caused by *Uromyces pisi – sativi* (Pers.) Wint. It is heteroecious species having its aecial stages on *Euphorbia cyparissias* and is not common in India. Uredinia and telia are reported on *Lathyrus*, *Orobus*, *Pisum* and *Vicia*. It is worldwide in distribution. Pycnia mostly hypophyllous, scattered among the aecia, aecia hypophyllous over the entire leaf surface.

Uredinia are mostly hypophyllous, irregularly scattered, cinnamon, up to 1 μm in diameter. Telia are like the uredia but chestnut in colour.

Causal Organism

The causal organism of this rust is already indicated as being *Uromyces pisi***.** This is heteroecious rust. Urediniospores are broadly ellipsoidal, 22-28 × 19-24 μm; wall luteous to sienna in colour and very finely echinulate. The wall is 1.5 μm thick. There are 3-6 pores which are scattered. Teliospores are broadly ellipsoidal, slightly bullate at the apex and possess short pedicels.

Physiological Specialization

Various races occur.

Disease Management

Similar control measures as those of earlier described rust of pea are applicable.

Rust of Gram (*Cicer arietinum*)

This has been reported from Kenya, Morocco India, Europe and Mexico. Severe outbreaks have been reported in India where gram is an important pulse crop. In northern India it is common in Bihar, Uttar Pradesh, and Punjab. In the cooler northern districts of U.P. and Punjab, the appearance of the disease often coincides with the maturation periods of the pods. The damage is considerably high. Often the premature death of the leaves results in reduced yields. The rust appears as a rule by the end of February when the plants are about four months old.

Symptoms

The initial symptoms are small, round or oval cinnamon-brown pustules which coalesce on both the surfaces of the leaves. In severe attacks similar pustules appear on petioles and stems and sometime on pods also (Fig. 16.9).

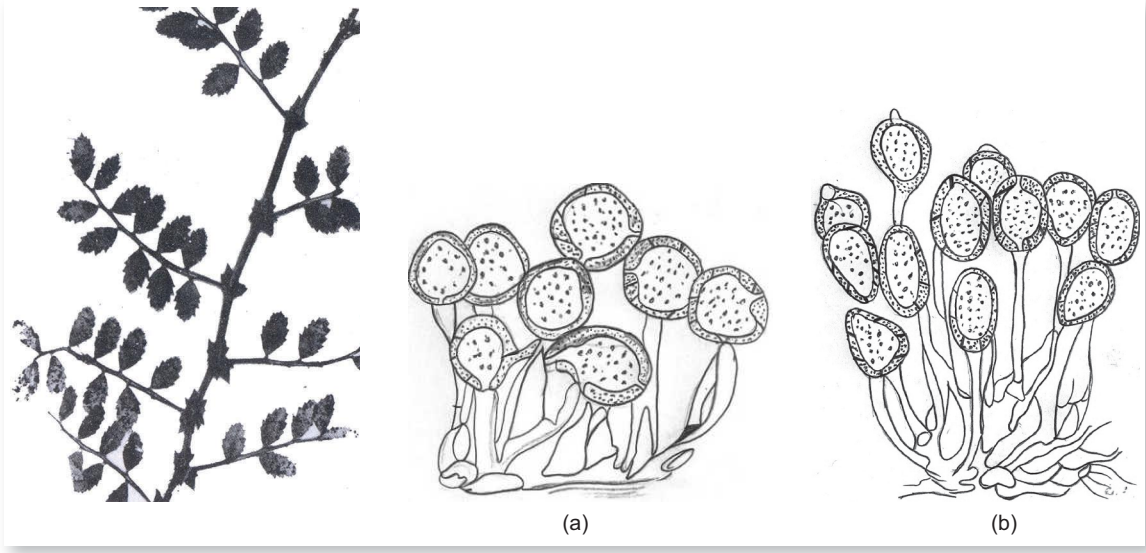


Figure 16.9 Rust of gram (*Uromyces ciceris arietini*): symptoms; (a) a section through uredosorus (b) a section through teleutosorus

Causal Organism

The pathogen responsible for gram rust is *Uromyces ciceris arietini* (Gragnon) Jacs. The rust seems to be a heteroecious one but the pycnial and aecidial stages of this rust are unknown so far.

The uredinia are hypophyllous; scattered, pulverulent, cinnamon, urediospores are globose to subglobose, 20-28 µm in diameter, wall 3-4 µm thick, yellow-brown, echinulate, 4-8 germs pores with hyaline papilla over the pores. Telia are like uremia but dark brown. Teliospores are variable in shape, round, ovate or angular, 18-30 × 18-24 µm, with an unthickened apex and paler over the pore, wall brown, 3-3.5 µm thick, verrucose, pedicel short (Fig. 16.9 a & b).

It occurs on *Cicer arietinum* and *Trigonella polycerata*.

Disease Cycle

The urediniospores are unable to survive the summer heat in the plains of northern India. Urediniospores survived for 2-4 weeks only at room temperature in soil in pots, but at 5-7°C for a period of 48 weeks (Saksena and Prasada, 1995). So far, attempts to germinate the teleutospores have failed. Their formation also does not take place at temperatures below 20-25°C.

The development of the telial stage depends upon a complex of climatic factors that are prevalent in the plains about the end of March and early April. But the teleutospores also do not survive the summer heat. A legume weed, *Trigonella polycerata*, has been found to harbour this rust in hills up to 182 m

(6000feet) where the fungus can survive in uredial stage. It is thought that the rust is reintroduced in the plains from this source of infection from the hills. Dissemination probably occurs from the hills to the plains where there is apparently no local source of infection.

Disease Management

So far no fungicides have provided satisfactory control measures in Mexico. Since chemical treatment is not practicable, the use of resistant varieties is recommended. In Lahaul valley, NRC34, NEC 249, JM 583, HPC 63, HPC136 and HPC 147 have been found resistant under natural condition.

Rust of Coffee

Coffee is probably originally an Ethiopian plant and its cultivation was monopolized by the Arabs who brought it to Yemen from Ethiopia around 1000 A.D. However, the Arabs were unaware that coffee had been introduced around 1600 A.D. in the hills of southern India. The Dutch people carried some of the plants to Sri Lanka. Coffee leaf rust is not only one of the most dangerous diseases of coffee but ranks as one of the classical plant disease of international importance. It appeared in Sri Lanka in 1868 and by 1875 the disease had assumed disastrous proportions and was responsible for the abandonment of the coffee plantations in Sri Lanka. The disease changed the entire economy of Sri Lanka. The disease also spread and caused devastation in south India, entered South Africa in 1878, Central Africa in 1913.

The main cultivated species of coffee are attacked in the field, but all possess strain resistant to the different races. In general, varieties of *C. arabica* appeared to be the most susceptible, while those of *C. liberica* are least susceptible. Varieties of *C. canephora* fall in the intermediate position.

Symptoms

Coffee leaf rust produce yellowish orange, powdery, rounded blotches on the lower surface of the leaves, which may coalesce with others to form an irregularly shaped lesion. The disease is restricted to the leaves, but on rare occasions has also been recorded on berries and young shoots (Fig. 16.10).

Causal Organism

The pathogen responsible for coffee rust is *Hemileia vastatrix* Berk. and Br. The rust is hypophyllous, densely scattered and gives a powdery appearance or yellowish orange, rounded blotches, about 3-25 µm or more in diameter. The pathogen consists of numerous narrow interwoven feeder hyphae and rounded cells below the stomata, bearing clavate filaments which emerge through the stomata and whose tips bear numerous small pedicels on which the spores are borne. New urediniospores arise from the same stalk lower down, each stalk thus producing several urediniospores and becoming something like a swollen basal cell. Urediniospores are not shed immediately, but remain attached to the top of the pedicels by short strigmata. In mature uredium the pedicel lose their individuality and form a compound column at the ends and sides of which a densely crowded mass of strigmata carries the spores. Urediniospores are reniform, 28-36 × 18-28 µm; wall hyaline, strongly warted on the convex surface, smooth on the straight or concave face, 1 µm thick. Teliospores arise in the same sorus. They were once thought to be rare but after a careful search have been collected abundantly from Karnataka and Sri Lanka. They are mixed up with the urediniospores. Teliospores are spherical or turnip shaped 20-28 µm in diameter; wall hyaline, smooth, 1 µm thick. These germinate without a rest period and *in situ* (Fig. 16.10) producing basidiospores but the latter do not infect coffee and no alternate host of the rust has been found so far.

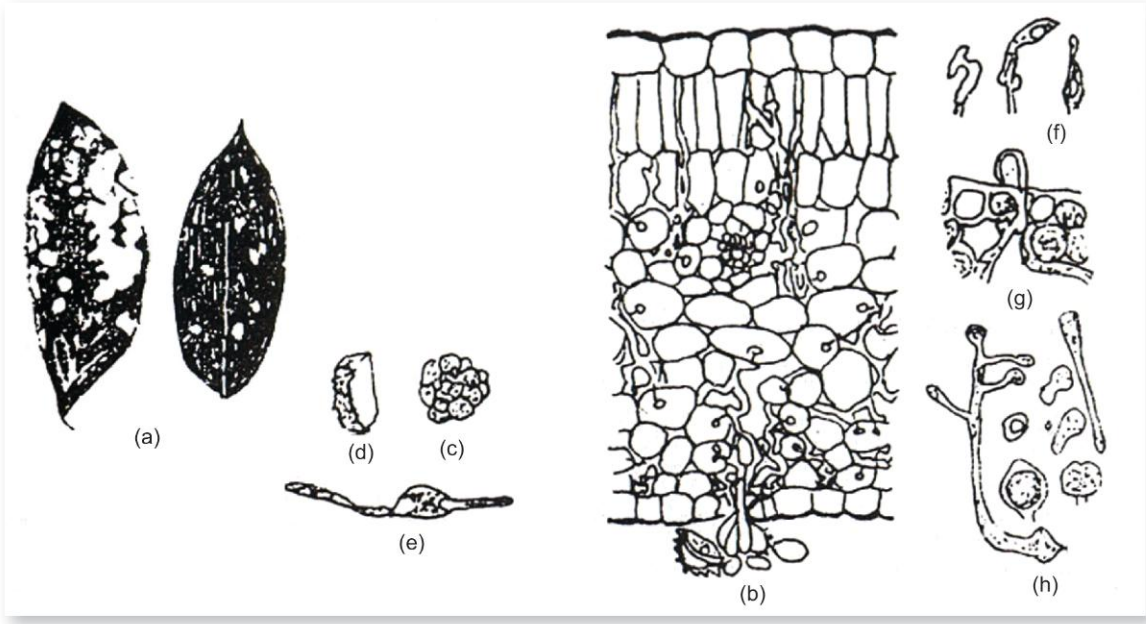


Figure 16.10 A coffee rust, *Hemileia vastatrix:* (a) symptoms (b) section through an affected leaf showing mycelium, haustoria and spore cluster (c) a spore cluster seen from below (d) and (e) urediniospores showing germination (f) appressoria (g) infection through stoma of leaf (h) teliospore and their germination

Physiological Specialization

Eleven races were distinguished by D' Oliveira and Rodrigues (1961), however 24 races have been encountered so far throughout the world.

Disease Cycle

A great deal of work has been done in Karnataka by the Department of Agriculture there and by the scientific officers of the Planters Association. It has been found that shelter from wind, intermittent rain, dew or moist, ample light, very light overhead shade, and moderately high temperature predisposes the plants to severe attacks. Earlier workers attributed spore dispersal to wind but more recent work has demonstrated the role played by rain-splash over short distances (Bock, 1962). Air current may be responsible for long range inter-continental dispersal. Two species of thrips have been found feeding on and aiding the dispersal of urediospores in India. Crowe (1963) has shown that two species of hymenopterous parasite of larvae of cecidomyid midges were found feeding on urediniospores in Kenya.

Disease Management

1. Efforts are now being made in Karnataka and Coorge to secure varieties of coffee resistant to leaf rust. As a rule, *Coffea robusta* appears to be less susceptible than *C. arabica*, but the former species does not yield good quality coffee. A variety, Coorg was quite resistant.

2. Control by spraying with copper fungicides has given good results when applied at the proper time. In south India two sprays of Bordeaux (2:2:40) one during April-June and other during September-November has been recommended. Muthappa and Kumari (1979, 1981) have reported that coffee rust could be controlled by the application of Carboxin 20EC and Oxycarboxin 20EC. The triazole fungicide, Triadimefon (Bayton) applied to coffee seedlings disrupts the rust hyphae in the host tissue.
3. Among the cultural practices, sanitation is essential- fallen leaves should be destroyed.
4. A possibility of biological control of the disease has been reported by using hyperparasites.

Rust of Groundnut

Groundnut rust is now of economic importance in most areas. In India losses in pod yield to the tune of 14-32% have been observed. McDonald and Subramanyam (1992) have reviewed the work done on this disease and have characterized it as a disease of international importance. Early and late spots caused by *Cercospora arachidicola* and *Cercosporidium personatum* (earlier called *Cercospora personata*) coincide with the appearance of the rust and hence the magnitude of loss is quite great (Kolte, 1984).

Orange coloured pustules (uredial) appear on lower surface of the leaves (leaflets), later on pustules may appear on the upper surface of leaf and on other aerial except flowers. Infected leaves become necrotic, dry up but tend to remain attached to the infected plants.

Causal Organism

Rust of groundnut is caused by *Puccinia arachidis* Speg. and only uredinial and telial stages are known. Spermogonia and aecial stages are not known. It is only in South America that the telial stage has been reported. There is no authentic report of the occurrence of the telial stage elsewhere.

Disease Cycle

Urediospores or urediniospores are short-lived in infected crop debris in the tropic and the fungus is unlikely to survive from season to season under post harvested conditions that include a break of more than one month or more. The practice of continuous cultivation of groundnut without any significant break in parts of India or other parts of the world may be instrumental in the perpetuation of the rust. It has been suggested that groundnut crops in the southern part of India may act as a reservoir for infection from which the urediniospores are carried northward with the wind during the monsoon period. The pathogen may also survive from season to season on groundskeepers and volunteer groundnut plants.

Disease Management

Based on several years of trials, it was noted that spraying of a mixture of Carbendazin (0.5%) and Mancozeb (0.25%) at 2-3 weeks interval starting from 4-5 weeks after planting of the crop was quite efficacious in controlling the rust disease.

Of course breeding for resistance is the best method of controlling the disease. At ICRISAT some progress has been made in this direction.

Mycoparasites such as *Verticillium lecanii, Penicillium islandicum* and several others have been reported. Mycophagous insects feeding on urediniospores of groundnut rust have been reported but no attempts have been made for the biological control of groundnut rust disease in India on the field scale so far (Shanmugham *et al*., 1975, Vaishnav *et al*., 1982)

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Questions

1. Name some outstanding workers in the area of plant rusts from different countries and list their main contributions.
2. In spite of the fact that the inoculum of wheat rust does not survive in the plains of India, the damage from wheat rusts is very high. Discuss this statement critically.
3. What new information do we have on the epidemiology of black stem rust of wheat in India?
4. What are the alternate hosts for the following rusts?
   (i) Black stem rust of wheat
   (ii) Brown rust of wheat
   (iii) Cedar rust of apple
   (iv) Rust of Gram
5. How will you identify three rusts of wheat in the field?
6. Give at least three examples of autoecious and heteroecious rusts.
7. What is an axenic culture?
8. Who cultured *Puccinia graminis tritici* for the first time in axenic culture?
9. How will you control the wheat rusts?
10. Breeding for disease resistance is the best method of controlling wheat rust. Discuss the statement critically.
11. What are multiline cultivars? What are the advantages and disadvantages of a multiline cultivar?
12. What are the important rust diseases in your locality?
13. What is Ug 99 and its possible role in the epidemic of *P. graminis tritici*?

Wilts, Root Rots and Head Blight

17

Vascular Wilts

Sadasivan (1961) states: "Despite the many approaches that have been made to the problem of the 'cause and effect' of wilt pathogenesis, we have a long way to go before we understand the many complex situations in the sequence of events that culminate in the syndrome". This holds true even now, despite the fact that many reviews have been written on the subject. The literature in the area includes review articles such as *The Physiology of the Wilt Disease,* by Sadasivan (1961) and books such as *The Nature of Wilt Diseases of Plants* of Mace and Beckman (1981), and Beckman (1987).

Typically, the wilt diseases are associated with plants that contain vessels in the xylem, that is, the angiosperms. While wilt or loss of turgidity is a common symptom of disease, it may result from one of the many possible causes. The vascular wilts are set off as a group because of the association of symptoms with the presence of the pathogen in the vascular system of the host, usually in the xylem tissue. The most common pathogens are vascular fusaria (life cycle of a typical vascular pathogen is depicted in Fig. 17.1), *Verticillum* spp. and bacteria, for example, the pathogens of cucurbit bacterial wilt and southern bacterial wilt. There are four genera of fungi that cause vascular wilts: *Ceratocystis, Ophiostoma, Fusarium* and *Verticillium.* Each of them causes diseases on several important crops, forests, and ornamental plants. *Ceratocystis* causes the vascular wilt of oak trees *(C. fagacearum)*, of cacao, and of eucalyptus. *Ophiostoma* causes the vascular wilt of elm trees, known as Dutch elm disease (*O. novo-ulmi* or *O. ulmi*). The wilt syndrome includes vein-clearing, epinasty, chlorosis, vascular discoloration, stunting, temporary and permanent loss of turgidity, and death. Apart from wilting, the syndrome of this group of diseases is not unique. The browning reaction occurs both in the local lesions and wilt diseases as a result of similar sequences of the biochemical reactions. Respiration is accelerated and auxins, phenols, and other products accumulate in a similar manner in the two cases.

Water relations are drastically changed in infected plants. The water content in diseased plants diminishes and eventually dries up altogether. Stress initially occurs in the tissues of individuals leaves and then throughout the plant. The water in the leaf blade is affected before the mycelium enters them. In infected plants, leaves lose turgour and eventually die and become desiccated. The mechanism by which this occurs is debatable. The debate centers around two theories put forward to explain the phenomenon of wilting. The first theory is called the "plugging theory" or "vascular occlusion theory" when there is very high resistance to the flow of water through the xylem, the leaves receives less water than they

transpire and as a consequence, they wilt. This is the basis of the plugging theory (Ludwig, 1952). The second theory is called "toxin theory" and is based on the fact that the permeability of parenchymatous cells in the leaves increases and as a result they lose their osmotic efficiency and this results in wilting (1951, 1954, 1957).

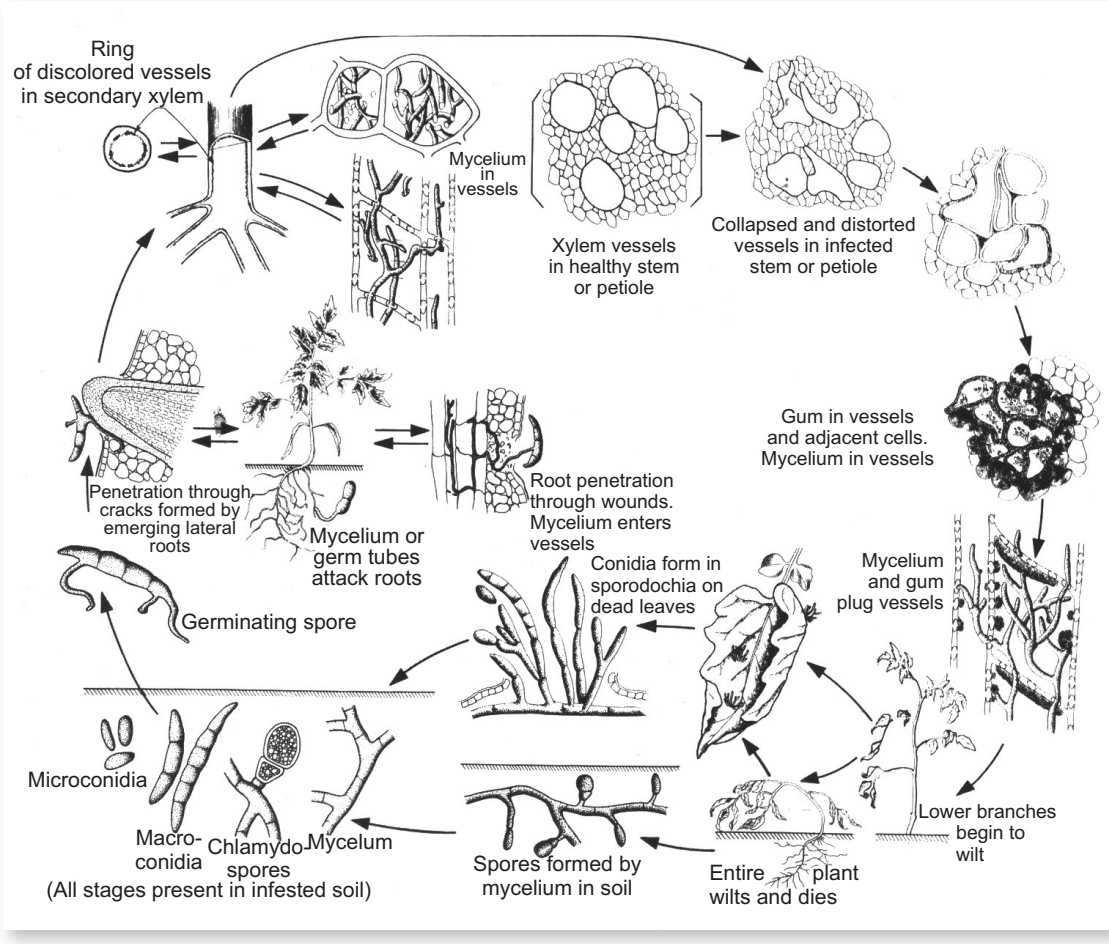


Figure 17.1 Life cycle of a typical vascular wilt pathogen *Fusarium oxysporum* f. *lycopersici* (From G.N. Agrios, *Plant Pathology,* Academic Press, London, p. 291).

Vascular Occlusion or Plugging Theory

Probably no single factor is responsible for vascular dysfunction in the wilt disease. High resistance to the flow of water through infected plants is probably the result of several factors. Some originate from the host as a result of response to the pathogens and others due to the pathogen itself. The infected plants may contribute to the altered resistance to flow by virtue of the changes in the vessel size that accompany infection and development of tyloses in conductive elements. These are: (i) collapse of vessels, (ii) formation of tyloses, (iii) the physical presence of the mycelium, production of bud cells, microconidia

and endoconidia within the vessels, (iv) altered viscosity of tracheal fluids, and (v) plugging by compounds of high molecular weight, such as polysaccharides, polymerized melanoid pigments, gums, gels, and pectins.

Collapse of Vessels Chambers and Corden (1963) have described the frequency with which vessels collapse, especially in petiolar bundles, in tomato plants infected with *F. oxysporum f. lycopersici.* IAA or culture filtrates of the pathogen also result in the collapse of vessels as in the infected plants. If a vessel's diameter has been reduced to half as a result of the collapse of the vessel, it will conduct the water at only one-eighth of its original rate.

Tyloses Tyloses are found more frequently in infected plants than in healthy plants. Development of tyloses in conductive elements reduces the effective radius available for transport, especially when tyloses are abundant. Some species of plants are more prone to form tyloses than others. New conducting elements may be formed in infected plants and partly compensate for the reduced efficiency of the blocked vessels, hence, the extent of wilting caused by tyloses is uncertain. Beckman (1964) suggests that tyloses and gel formation are basically a defensive reaction of the infected plants and that in susceptible plants, tyloses formation is inhibited or delayed. This inhibition is perhaps due to the pathogen. Metabolites, when present in sufficient quantities, reduce the plant's capacity to produce growth-regulating substances which bring about the tyloses and it may be that growth-promoting substances produced by the pathogen are involved. The pathogen moves through the xylem vessels by transported propagules and rapid tyloses formation reduces such transport. The rapidity with which tyloses are formed determines whether they will confer resistance or susceptibility to the host. If tyloses are formed rapidly well before the spread of the pathogen, they will confer resistance to the host, but if they are formed after the colonization of the pathogen, they will themselves be instrumental in bringing about more wilt symptoms.

Dixon and Pegg (1969) questioned the interpretation that tyloses are a mechanism for resistance. They found that in tomato plants attacked by *Verticillum albo–atrum,* tyloses appeared after colonization by the fungus of the roots, stem, and petiolar xylem.

The importance of the tyloses in reducing the efficiency of the transport system as a whole depends upon a number of factors. When no alternative unoccluded pathway of flow exists, tyloses may block the flow to distal tissues. The extent of interference with flow will also depend on the production of vessels containing tyloses, the extent of occlusion of the vessels even the area of development of tyloses along the vessels. Talboy (1958) had shown that wilting symptoms may be mild despite the frequent development of tyloses, in hops infected with *Verticillum albo–atrum*.

Mycelium Physical blockage in xylem vessels by the growth of fungal mycelium and/or spores produced in those vessels is perhaps the most obvious explanation of vascular occlusion. Grieve (1941) thought this to be the cause of bacterial wilt brought by *Ralstonia solanacearum* (*Pseudomonas solanacearum)*. However, this explanation was not accepted by Husain and Kelman (1958), who pointed out that although large masses of bacteria accumulate in infected plants even in completely wilted plants, many vessels are not blocked in this way. Also, the growth of a weakly pathogenic strain in the vessels does not cause plants to wilt.

Plugging of tracheal elements by mycelium has frequently been considered a cause of vascular dysfunction. Individual vessels may become densely packed with mycelium (Sadasivan, 1961) but in general, mycelium is not sufficiently abundant through all the vessels to be the sole cause. Waggoner and Dimond (1954) had examined interference with flow through a vessel caused by mycelium from a

hydrodynamic point of view and have concluded that mycelium can reduce the efficiency of flow in vessel elements

As is well known, the vascular fungi commonly produce bud cells, microconidia or endoconidia that break free from the mycelium and are transported through conductive elements. Conidia are carried upwards in the vascular stream and many accumulate at the end walls or the opening of the pits and thus cause impediments to the flow of water .The movement of conidia through xylem vessels leads to a more rapid colonization of the host and to the production of large quantities of the metabolic products of the pathogens, resulting in more severe symptoms. It has been suggested that the acute symptoms of Dutch elm disease, caused by *Ophiostoma* (*Ceratocystis ulmi*), may in part be caused by the plugging of the vessels of the smaller branches by the pathogen itself.

Altered Viscosity of Tracheal Fluids Husain and Kelman (1958), and Scheffer and Walker (1953), attributed the wilting of diseased plants to the altered viscosity of tracheal fluid. In some bacterial wilt diseases, the tracheal fluids become highly viscous as the plant is dying. When the stem is cut open and separated, white milky strands extend from one segment to the other. Although in moribund tissues viscosity of tracheal fluids may be high, this can be questioned as it could be a post –mortem change made possible after the flow has already ceased. Husain and Kelman (1958) demonstrated that the pathogen *Ralstonia solanacearum* provides an extracellular polysaccharide and that the pathogenicity of different strains of organisms is correlated with their ability to produce this polysaccharide. However, in *Fusarium* wilt of tomato, the viscosity of tracheal fluids is the same as that in healthy plants (Waggoner and Dimond, 1954). Polysaccharides are known to be produced by *X. campestris* pv. *phaseoli, R. solanacearum, C. ulmi, C. fagacearum, V. albo-atrum,* and species of *Fusarium,* and *Cephalosporium.* At least three species of *Corynebacterium* (now called as *Clavibacter*) produce glycopeptides (Rai and Strobel, 1967) and their presence in the diseased plants has been demonstrated. When polysaccharides and glycoproteins are introduced into vascular tissues, they introduce wilting. Wilting has been ascribed to increased viscosity of tracheal fluids but they cannot be the sole cause.

Plugging by Compounds of High Molecular Weight–Polysaccharides, Pectins, Gels, Gums Polymerized melanoid pigments are all compounds of high molecular weight. The walls of the conducting elements behave as ultrafilters and remove materials of high molecular weight from the transpiration stream. In the process, the channels become plugged and this impedes the flow of water. Polysaccharides and glycopeptides can affect the water economy of plants in two ways. A portion of these may accumulate in pit membrane of vessels which act as ultrafilters. Such entrapments will reduce the lateral and longitudinal flow of water through the system. Secondly, smaller molecules transported to leaves disrupt water transfer from veins and accumulate in pit membranes of parenchymatous cells, where they cause damage, which appears as marginal drying.

Although vascular browning is a regular symptom of wilt disease, it has never been regarded seriously as a cause of wilting. The browning originates in vascular parenchyma when cells are adjacent to invaded vessels. It is now established that vascular browning is the result of the oxidation and polymerization of phenolic compounds through the action of phenolases. Studies of the biochemical reactions involved in vascular browning point to the formation of melanoid pigments of high molecular weight. These result from the oxidation of phenols, polymerization of these products, and formation of products between oxidation products and proteins.

In *Fusarium*–infected tomato plants, especially in xylem parenchyma, phenols accumulate and phenolases become active. The phenols are oxidized to fungitoxic quinones and their polymerization leads

to fungitoxic products in these cells. Polymerization to dark-coloured melanins may then occur. In tomato wilt (*F. oxysporum f. sp. lycopersici*), conjugated phenols such as glucosides, tannins, and lignin may be hydrolysed by enzymes to polyphenols, which are in turn oxidized by PPO, possibly an induced enzyme, to toxic quinones which then polymerize to cause the characteristic vascular discoloration.

Winstead and Walker (1954) emphasized the role of pectic enzymes in vascular browning. Culture filtrates of several pathogens induced browning in the cuttings of a variety of plants. Browning occurred when both PG and PMG enzymes were present. This may mean that maceration caused by pectic enzymes results in the disorganization of host cells, and thus, phenolic oxidation can occur.

With the progress of wilt disease, the parenchyma cells are damaged and pigments leak into the vessels. When free in vessels, the large pigment molecules can cause plugging of the type observed by Chambers and Corden (1963), or they can become trapped in gels when the vessel is already plugged.

Gels and gums are also believed to be formed as a result of infection by several wilt pathogens. Beckman (1964) has discussed the frequency with which plants respond to infection, and wounding by forming gums and gels. Studies by him and his associates (Beckman and Halmos, 1962; Beckman *et al.,* 1962) have clearly indicated the formation of such materials in banana plants infected with *F. oxysporum f. cubens.* When liberated as colloidal solutions into the transpiration stream, they can cause plugging at cross walls.

It is doubtful whether these materials are the same as those responsible for plugs in vessels in moribund plants and this has been attributed to the accumulation of partially hydrolysed wall material resulting from the action of pectolytic enzymes.

The occluding plugs in elm infected by *C. ulmi* contain both pectin and lignin. The material in tomatoes infected by the pathogen *Fusarium* cannot be removed by phenol. In banana roots, the materials consist of a mixture of pectin, calcium pectate and hemicellulose.

Blockage Caused by the Action of Cell-wall Degrading Enzymes The wilt pathogens, in common with many microorganisms, excrete extracellular pectolytic and cellulolytic enzymes. The vascular wilt pathogens known to produce these enzymes are *Ralstonia solanacearum, V. albo atrum, V. dahliae, C. ulmi, C. fagacearum, F. oxysporum f. sp. lycopersici, F. oxysporum* f. sp. *cubense, F. oxysporum* f. sp. *vasinfectum*, and *F. oxysporum* f. sp. *niveum.*

The evidence of the role of these enzymes in pathogenesis is based upon their presence in diseased plants, the ability of the pathogen to produce them on host tissues, chemical evidence of disappearance of their substrates in diseased plants, microscopic evidence of degradation of middle lamellae and cell walls, and the ability of such products to produce vascular dysfunction.

Another piece of evidence implicates pectolytic enzymes in the pathogenesis of wilt diseases. Grossmann (1962) has shown that rufianic acid inhibits pectolytic enzymes activity, polygalactouronase being inhibited to some extent and pectin methylesterase more strongly, and also reduces the severity of vascular wilt of tomato when applied to infected plants.

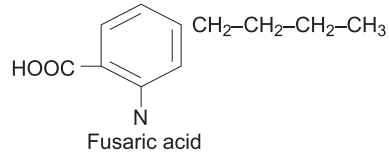
Toxin Theory

Toxins have been extensively investigated, especially in tomato wilt caused by *F. oxysporum f. sp. lycopersici,* in which Gottlieb (1943, 1944) showed that toxins might be involved; accounts are given by Dimond (1955), Ludwig (1960), Subramanian and Saraswathi Devi (1959), Sadasivan (1961), Beckman (1964) and others.

Gottlieb's pioneering work on vivotoxins was followed by an extensive series of investigations on the same disease by Gäumann, Kern and their associates in Zurich. They detected and identified toxins produced by pathogenic organisms *in vitro* and studied ways in which these substances affected the water relation of plant cells with a view to understanding their role in causing symptoms in the infected plants. The first wilt toxin to be studied was lycomarasmin, which was isolated in pure form culture filtrates by Clauson–Kass *et al.* (1944). Another important toxin thought to be involved in vascular wilt is fusaric acid (Gäumann 1957, 1958). Fusaric acid was first isolated as the metabolic product of *Fusarium heterosporum,* a non-specific parasite, by Yabuta *et al*. (1934). In 1952, it was recognized as an imported toxin of *Fusarium lycopersici, F. vasinfectum, Gibberella fujikuroi* (Gäumann *et al.*1952 a, b). It is now known to play a pathogenic role in the wilt of tomato and cotton and in the bakanae disease of rice. Page (1959) showed that fusaric acid is involved in the Panama disease of banana caused by *Fusarium cubense*.

Fusaric acid is called a wilt toxin as it is known to play a distinct role in the wilt of tomato and cotton plants. Even when the pathogen is restricted to the roots, symptoms like vein clearing and epinasty are seen in the leaves, along with reduction in respiration and growth, necrosis of cortical tissues overlying the vascular bundles (leading to the furrowing of stem), and epinasty of petioles (Gäumann, 1958). This seems to be due to the translocation of toxic metabolites to distant parts of the plant from the focus of infection. Pure fusaric acid stimulates some of the symptoms caused by pathogen.

Fusaric acid, 5-n butyl pyridine-2-carboxylic acid, has the empirical formula $C_{10}H_{13}O_2N$ and molecular weight 179 (Yabuta *et al.,* 1934). It has the following structural formula.



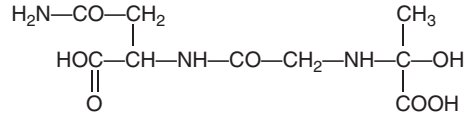
Fusaric acid

Stoll (1954) demonstrated the existence of dehydrofusaric acid in preparation of fusaric acid. This acid appears after the appearance of saturated fusaric acid. Fusaric acid impairs the permeability of plasma membranes. It also impairs energy metabolism and as a consequence, the non-osmotic water uptake of the plant protoplasts by two different mechanisms at higher and lower concentration.

Fusaric acid forms metal chelates. This toxin interferes with metal-containing enzymes, especially *Fe-porphyrin* oxidase, and consequently depresses respiration. On the basis of antagonism reported between *ferrioxamine* B and fusaric acid, it can be conjectured that fusaric acid specially interferes with the incorporation of Fe in the porphyrin skeleton.

Sanwal (1961) described the physiological effects of fusaric acid and suggested that it could cause a partial uncoupling of oxidative phosphorylation. Its role in pathogenesis remains problematical, and Deverall (1964) concluded that it plays no part in the development of wilt symptoms although it might act in conjugation with other metabolites. However, The Sadasivan School places great importance on the toxin being responsible for the wilt disease in cotton.

The toxin, lycomarasmin, is a polypeptide with a molecular weight of 277.3 and empirical formula $C_9H_{15}O_7N_3$. The following structural formula has been tentatively assigned to this toxin:



Lycomarasmin

The toxicity of this substance seems to arise from the fact that it can form metal chelates (such as with iron). The pathogenic effect of this toxin is partly due to the fact that its molecules, on their way through the infected plants, shoot chelate iron in the host, which otherwise would not be able to migrate and transport themselves into the leaves in the form of a lycomarasmin-iron complex. Here, part of the lycomarasmin molecules are regenerated as chelating agents and cause local deficiencies, while at other places there is excess of iron (i.e., iron plethora). Gäumann and Naef-Roth (1957) assume that the excessive transpiration occurring in consequence of lycomarasmin poisoning is mainly due to such iron injuries.

In addition to fusaric acids and lycomarasmin toxins, vasinfuscarin and phytonivein have been implicated in vascular wilts. Vasinfuscarin is apparently a protein with enzymic properties (Gäumann, 1954) and it is reported to produce the vascular browning typical of vascular wilts. Phytonivein is present in culture filtrates of *F. oxysporum f. sp. niveum.* Hiroe and Nishimura (1956) believe this to be involved in the wilt of water melons. Diffusible toxins may be implicated in several other wilt diseases including pea wilt.

It is however, the complexity of host-pathogen interaction, which is of interest. Physical blockages occur in the vascular system of infected plants as a result of integrated metabolic reactions between fungus and plant. In addition to the production of toxin and cell wall degrading enzymes mentioned earlier, the invading pathogen causes the plants to produce other additional compounds during infection. Ethylene and IAA are produced by both the pathogen and the susceptible host plants. Both these factors stimulate the production of tyloses in host plants, leading to increased phenol-oxidising and pectolytic enzymes. These phenolic compounds which are reported in the vascular elements, IAA and ethylene, also induce the formation of gels which accumulate at perforation plates and contribute to vascular blockage.

Wilt of Pigeon Pea (*Cajanus cajan*)

This is a very common disease. It is the worst disease of red gram in India, causing severe damage wherever the crop is grown, especially in Maharashtra, Uttar Pradesh, Bihar, and Tamil Nadu. The disease is not only common in India but also in many countries like Kenya (disease incidence, 15.9%), Malawi (36.3% disease) and Tanzania (20.4% disease). According to Kannaiyan *et al.* (1984), in a survey conducted in different parts of India during 1975-1980 by ICRISAT, the disease was found to be 0.1% in Rajasthan to 22.6% in Maharashtra. In Bihar and U.P., the average incidence of disease was 5-10% on standing crop, which is a common feature seen every year. Continuous cropping of pigeon pea in the same field results in 50% plant mortality. The disease is quite high during the flowering and pod formation stages.

Symptoms

The arhar plant is susceptible to the attack of wilt throughout the development. However, the symptoms are more pronounced and the damage greater when the plants develop after the rainy season. The main symptom of the disease is the wilting of seedlings and adult plants as if from water shortage, even when plenty of water may be present in the soil. The wilt is characterized by gradual, sometimes sudden yellowing, withering and drying of leaves followed by the drying of the entire plant or some of its branches. The main root and base of the stem tissues are blackened either uniformly or non-uniformly, especially

in the early stages in streaks (Fig. 17.2 a and b). If the stem is examined after it is broken and spilt, the black streaks may be traced up to a height of several feet on the stem. Partial wilting is also common, that is, often, only one side of the stem and root system is affected, in which case, the symptoms are found only on that side of the plant.

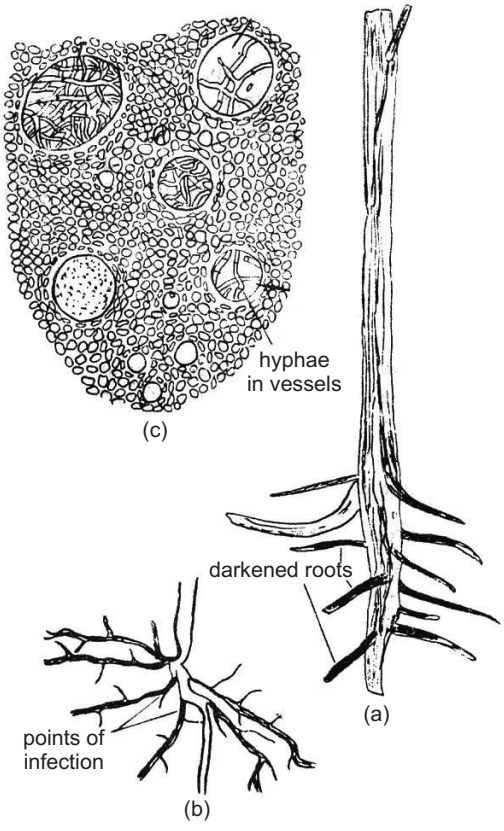


Figure 17.2 Pigeon pea wilt (*Fusarium udum*): (a) stem in early stages of attack with bark peeled off to show blackened streaks in the wood (b) roots of wilted seedlings showing points of infection (c) hyphae in vessels.

Causal Organism

The pathogen responsible for the wilt of pigeon pea is now named *Fusarium udum* Butler. The fungus is mainly confined to the vascular tissues and is both inter and intracellular. The septate hyphae run across the cells growing with great rapidity along the inside of the walls of the larger vessels. The vessels are partially plugged with these hyphae (Fig. 17.2 c). The profuse growth of the mycelium within the lumen of the xylem vessels and the consequent plugging by matted coils of hyphae interfere with free flow of water to the green parts of the plant, which results in drooping and wilting of the leaves. Toxins and enzymes have also been implicated in other *Fusarium* infections and it is probable that these may also be involved in the disease syndrome.

The fungus can be easily cultured on various synthetic media. The pathogen produced both micro and macroconidia. The macroconidia are linear, curved or fusoid, pointed at both ends and knotched at the

base, thin-walled, with 3-4 septate measuring 15-50 × 3-5 µm. The microconidia are small, elliptical or curved, thin-walled, unicellular with 1-2 septae and measure 5-15 × 2-4 µm. The chlamydospores are formed in culture as in host tissues. They are spherical to oval, thick-walled, single or in chains of two to three, terminal or intercalary .All the types of spores germinate readily in water. Chlamydospores are believed to survive for a number of years.

Rai and Upadhyay (1982) have reported *Gibberella indica* as the perfect stage of *F. udum* from the exposed roots and collar regions of the stem up to the height of 35 cm above the ground level. The mature perithecia are superficial, globose, sessile, dark violet, 350-550 microns in diameter, asci having 8 ascospores; asci are mostly sub–cylindrical, broader in middle while ascospores are ellipsoidal to ovate, hyaline, two-celled and rarely 3-4 celled.

Disease Cycle

F. udum is a soil-borne saprophyte, surviving in the soil in the absence of the living tissues of the roots. McRae and Shaw (1933) found the wilt pathogen to survive in the soil for 8-20 years, making it difficult to minimize infection even by crop rotation. However, the saprophytic survival of the fungus in the soil is limited by the fact that dead host roots and other parts have to be continually present. Infection occurs through the fine rootlets, which are penetrated by the hyphae and rapidly blacken and shrivell. From the laterals, the fungus passes into the larger roots, which are not directly penetrated by the hyphae, unless injured.

Reddy *et al.* (1993) reported that the resistance in pea to *Fusarium* wilt depends upon the extent of colonization of the plant by the fungus after infection. The plant shows wilt symptoms when infection is more than 50%. The incidence of the disease is more in fields where the disease had occurred in previous years. Upadhayaya and Rai (1989) have reported that enzymes like pectin–methyl esterase (PME), polygalactouronase (PG) and cellulase are involved in pathogenesis. In addition to these enzymes, fusaric acid has also been isolated from infected plants and it plays an imported role in pathogenesis.

Vasudeva and Govindaswami (1953) have isolated some of the antagonistic organisms like *Aspergillus niger, Rhizopus nigricans,* and *Bacillus subtilis,* from the rhizosphere. Later, Vasudeva and Chakrabarti (1954) demonstrated that *Bacillus subtilis* produced an antibiotic named bulbiformin, that inhibited the growth of *F. udum*. Mahmood (1964) further reported that cotton seedcake and groundnut amendments considerably enhanced the production of bulbiformin in soil. Adverse effects of oil cake amendment of soil on saprophytic survival of pathogen have been reported by many researchers (Singh and Singh, 1983 and Mukhopadhyaya and Sengupta, 1991). Agnihothrudu (1957) had reported that the rhizosphere of resistant varieties of pigeon pea had a greater proportion of *Actinomyces sp.,* which was antagonistic to the pathogen. This wilt is common in the areas where this crop is grown again and again, year after year. The fungus can withstand widely varying adverse conditions, including a pH range of 4.6-9.0 and soil temperatures as high as 35°C. Mundkur (1935) had reported that wilt of pigeon pea develops over a wide range of temperatures. He found that temperatures between 17 and 29°C are conducive to disease development. Sandy soils are also more conducive to wilt development (Shukla, 1975).

Disease Management

Since the disease is soil-borne, the treatment of soil on a large scale is not possible. The only method of wilt control is, therefore, through cultural practices. Several cultural methods are recommended for checking the severity of the disease. Crop rotation is the best way of eliminating soil-borne infection.

A 4-5 year rotation has been found to free the field completely of the wilt pathogen. Hot weather cultivation with deep ploughing in the summer is also recommended.

Soil solarisation has been found effective in controlling the pigeon pea and chick pea wilt by Chauhan *et al.* (1988). Solarization is done by covering the soil with transparent polythene sheeting (100 μm thick) for 6-8 weeks during summer (April-May). As a result of the polythene covering the soil, temperature rose by 6-10°C in the 0.50 cm soil profile. Increased mineralization of nitrogen to nitrate was noticed. A decline in population of *Fusarium* propagules and plant parasitic nematodes was noticed.

Mixed cropping with *Sorghum* (jowar) has been found to be quite efficacious in controlling the disease. Bose (1937) had recommended the rotation of pigeon pea with tobacco. It is believed that the adverse effect of the root exudates of tobacco is the main reason for the control of the pathogen.

Green manuring has been found to be efficacious in controlling the disease due to the antagonistic microbial population in the soil. Sarojini (1951) had reported that the addition of solutions of Boron, Manganese or Zinc provides protection to seedlings during emergence. Subramanian (1963) had reported that spraying or pre-soaking seeds in manganese salt solutions imparts resistance to the plants.

Inhibition of *F. udum* in soil amended with oil cakes, application of farmyard manure and oat crop residue have been reported. Seed treatment with systemic fungicides like Carbendazim suppresses early infection. It has been reported that *Trichoderma harzianum* was the best biocontrol agent against the pigeon pea wilt. Seed treatment with Thiram+ *T. harzianum* reduced wilt disease. Seed treatment or direct soil application of *Bacillus subtilis* and *Trichoderma harzianum* also reduces wilt disease. It has been demonstrated that population of *F. udum* is suppressed when the crop is pre-inoculated with fungi like *Penicillium citrinum*, *Aspergillus flavus*, *A. terreus*, *A. niger* and *Trichoderma* sp. and the bacteria like *Streptomyces griseus, Bacillus subtilis* and *Myrothecium.* The mode of action of these fungi may be due to the competition and antibiosis.

Application of trace elements like Zinc also suppresses the disease. Zinc inhibits the production of the phytotoxin Fusaric acid, the *Fusarium* pathogenicity factor. Vidyasekaran *et al*. (1997) reported that mycelial growth of *F. udum* was inhibited by *Pseudomonas fluorescens*. The application of Zinc Sulphate can promote efficiency of *Pseudomonas fluorescens* by suppressing *Fusarium* metabolites. Pre-inoculation of non-pathogenic strain of *Fusarium oxysporum* to pigeon pea provides cross protection and induced resistance against the disease (Chakraborty and Sen Gupta, 2001). At ICRISAT, Saifulla and Byre (2002) identified six lines i.e., ICPL 93001, 96047, 96061, 99055 and 87119, which were resistant to *Fusarium* wilt.

The research for the sources of resistance to wilt in pigeon pea began as early as 1905 at Poona (now Pune) in India (Butler 1908, 1910). Subsequently, screening has been conducted at many locations in India and quite a large number of resistant genotypes were found. Unfortunately, few resistant cultivars are popular with the farmers. The main reason for this seems to be lack of adequate efforts to breed varieties that are both wilt resistant and high yielding. One of the oldest wilt resistant, long duration varieties—NP (WR) 15, has very small seeds. The medium duration cultivars, C11 and BDN1 that have large seeds and yield well, do not have high level of resistance. There is need to develop high-yielding varieties with wilt resistance, and bold, white seeds in the short, medium, and long duration crops. Multilocational testing in India (Nene *et al.,* 1989*)* and earlier in Africa helped to identify several lines resistant to wilt at different locations. Some pigeon pea lines, ICP 4769, 7118, 7182, 8863, 9168, 10958, 11299 also showed broad-based resistances.

Wilt of Cotton (*Gossypium arboreum* and *G. herbaceum*)

Wilt is one of the major diseases of cotton and is found wherever this crop is grown. It is believed to have originated in Mexico or Central America, spreading to many African countries, parts of France, Italy, Yugoslavia, USSR, India and the West Indies. In India, the disease was first reported in Nagpur in 1908. The disease has now spread to almost all the black cotton soil tracts of the country (Maharashtra, Madhya Pradesh, Karnataka, and South Gujarat). The pathogen causes extensive damage in the heavy soils and is more or less absent in the loamy soils of the plains and the Indo-Gangetic plains. On the other hand, the disease is common and destructive in light sandy soil in the USA. The losses in the wilt are very high, to the extent of 40 to 60% because of reduced stand, stunted growth, small bolls, and poor lint quality.

Symptoms

The plants are affected during all stages of growth. The first symptoms appear on the young seedlings. These are vein-clearing of the leaves followed by interveinal tissue necrosis. There is yellowing and browning of the cotyledons and the formation of brown ring on the petiole. The seedling soon wilts and dies. If the plants are affected a little later, the leaves become flaccid, which result in the drooping and wilting, starting from the oldest leaves at the base and proceeding upwards finally involving the branches and the whole plant. Often, there may be complete defoliation, leaving the stem standing alone in the field. Discoloration of the stem may also be seen. The basal portion of the stem in young plants may turn black. In adult plants, the discoloration may be partial. Growth is retarded and the plant eventually wilts (Fig. 17.3).

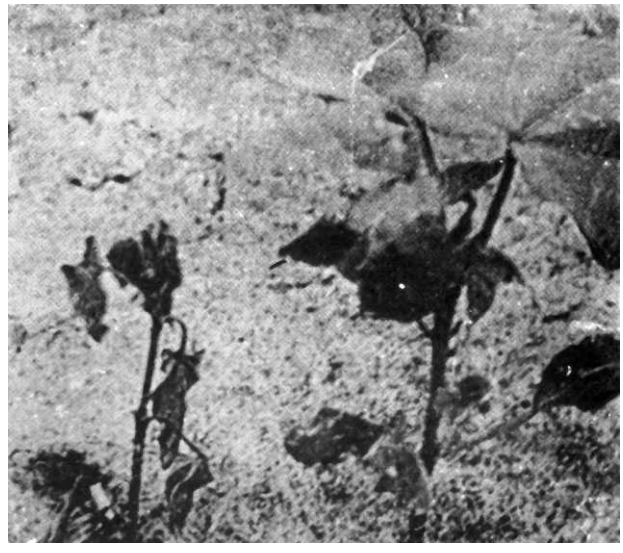


Figure 17.3 Wilt of cotton caused by *Fusarium oxysporum* f. sp. *vasinfectum:* wilted cotton plants.

Causal Organism

Fusarium oxysporum f. sp. *vasinfectum* (Atk.) Snyder and Hansen is the pathogen responsible for vascular wilt of cotton. The aerial mycelium of the fungus is white to greyish or white to bluish and often forms a mat on the collar region of the stem near the ground level. The mycelium is both inter and intracellular. On 2% potato sucrose agar at pH 6.5, the mycelium is white or pinkish to violet. Mycelium is sparse to floccose and the stroma becomes brownish white to violet and plectenchymatous. The pathogen produces both micro- as well as macroconidia. Macroconidia are formed on sporodochia and the pionnotes are light buff to salmon orange in mass and fusiform–falcate in shape. Macroconidia are curved inwards at both ends and have a pedicel at the base. Microconidia 0 septate, 6.4-10.6 × 2.3-3.2 µm, 1 septate 13.20 × 2.5-3.4 µm and the macroconidia are 3 septate 27-40 × 2.5-4 µm; 4-5 septate 27-40 × 2.5-4 µm; 4-5 septate 32-48 × 3.5-4.5 µm. Chlamydospores are formed both terminally as well as intercalarly. No perfect stage of the fungus has been reported as far.

Disease Cycle

The pathogen is soil-borne and survives on the stubble of the diseased plants. There is some evidence that it may be soil-borne also. The pathogen has been recovered from delinted seeds obtained from infected cotton plants in the Central African Republic, Congo, Tanganyika, and Brazil. The percentage of infection ranged from 0.2-5%.

Infection occurs through root system as in the wilt of pigeon pea. The pathogen enters the host roots when the plants are 1-3 weeks old and the symptoms of the wilt appear when the plants are 5-6 weeks old. The wilting occurs primarily due to the excretory toxic substances produced by the fungus in the vascular system, which are translocated throughout the plant. Fusaric acid is the wilt toxin which has been demonstrated to be produced in the plant tissues in the rhizosphere as well as in the soil around the roots. The pathogen, *Fusarium oxysporum* f. *vasinfectum* produces most of the necessary enzymes capable of attacking the cell wall substances. It is now known to produce either adaptively or non-adaptively various pectinolytic, cellulolytic, and proteolytic enzymes. Mahadevan (1967) had demonstrated the production of pectinolytic enzymes by the pathogen. The host-parasitic relationship in the case of *Fusarium* wilt of cotton had been intensively studied at the Botany Department, University of Madras, by Subramanian and his colleagues with special reference to the role of the trace elements and toxin (Subramanian, 1956; Subramanian and Saraswathi Devi, 1959). Cotton plants infected by *F. oxysporum f. vasinfectum* show reddish brown pigment in the vascular vessels. Raj (1974) reported the production of β-glucosidase by this pathogen and discussed the possible mechanism of the release of phenols by the pathogen and formation of reddish brown pigment. Wilt of cotton has an important relationship with the nematode injury to roots which helps in the entry of the pathogen into the host roots.

The disease is favoured by soil temperatures in the range of 20-30°C, the optimum temperature being 24-28 °C. Temperature above 35 °C is inhibitory to the development of the disease. Susceptible diploid plants, if inoculated at temperatures of 32.5 °C, 35.0 °C and 37.5 °C, result in an apparent recovery or masking of symptoms at 37.5 °C, whereas at the two other lower temperatures, wilt symptoms were prominent. Such recovered plants, however, showed higher quantities of fusaric acid and cysteine than low temperature ones, indicating that this excess cysteine chelated with some heavy metals *in vivo*, rendering them unavailable for fusaric acid potentiation (Kalyansundram and Subba Rao, 1957).

It has been reported that the amendment of the trace elements controls the disease considerably. Kalyansundram (1954, 1955) had ascribed the wilt resistance of cotton in zinc –amended soil to greater

reserves of carbohydrates, ascorbic acids, and reducing sugars in cotton plants. Lakshminarayan (1958), while working on the distribution and derangement of free amino acids in wilted cotton plants, found that cysteine was present in resistant cotton plants but absent in the susceptible ones. The amino acid cysteine has been correlated with the resistance of plants. Cotton wilt can prove to a serious disease, especially where soils are low in potash and other fertilizers and high in acidity.

Disease Management

Seed treatment Since the pathogen is seed-borne also, treatment of the seeds with fungicides such as Bavistin, Topsin M, and Thiram can effectively control the seed-borne infection. Treatment of the seeds with Chlorothalonil, Thiabendazole, and Carboxin also reduces the incidence of wilt by providing resistance to infection by inducing increased phenolic enzyme activity (Chopra 1998).

Chemical Treatment Drenching soil with Benlate and Bavistin can control wilt to some extent but it is not economical because of the cost of chemicals.

A systemic fungicide (Miejuncuzhangi) from China was reported to reduce disease infection upto 89% when used in combination with Carbendazim (Singh, 2007). High potash application in combination with salts containing nitrogen and phosphorus can lead to reduction in cotton wilt. Trace elements like zinc, molybdenum, lithium, aluminium, nickel, boron, and cobalt were inhibitory to conidial germination of the pathogen. The presence of these trace elements was beneficial for plant growth. Inoculations with non-pathogenic strains of *Fusarium* or dried biomass of *Penicillium chrysogenum* induces cross protection and systemic resistance in cotton seedlings against the pathogen.

Agronomic Practices The incidence of the wilt can be reduced up to some extent by manipulating the sowing date, application of potash, and amendments of zinc.

Resistance Varieties In India, tetraploid cotton varieties are immune to *F. oxysporum f. vasinfectum.* Numerous varieties/strains, which are either immune or resistant to wilt, have been developed. These are C-C-1-35, JLA-101, AKH-590, SM-143, LD327, and LD-254. These are being used in the hybridization programmes. All American cotton varieties, even though highly susceptible, were resistant to wilt in India and vice versa. The differences may be due to different races and their origin.

Wilt of Linseed (*Linum usitatissimum*)

The wilt disease of flax or linseed plant is very widespread and occurs in almost all the countries where linseed is grown.

Symptoms

The plants are attacked during all stages of growth. The tops of the plants droop and wilt, after which they die. The plants become yellowish before they wilt. The roots of the young seedlings may rot and the plants may drop off. The mature plants, if infected, remain stunted.

Transverse sections of the infected tissue show the browning and the fungus in the vascular tissues.

Causal Organism

Linseed wilt is caused by the fungus *Fusarium oxysporum* f. *lini* (Bolley) Snyder and Hansen. In addition to *Fusarium* from the infected tissue, *Rhizoctonia bataticola* was isolated from Punjab by Bedi *et al*. (1961). The morphology of the fungus is similar to other wilt Fusaria. The mycelium is septate, branched and intracellular. Both micro as well as macroconidia are produced by the pathogen. Microconidia measures 4.8-14.4 × 2.2-4.8 µm and macroconidia measures 21.0-53.0 × 2.4-5.6 µm.

Borlaug (1945) had distinguished several physiologic races of *F. oxysporum* f. sp. *lini*, a factor which complicated the breeding programme for resistance. When the fungus attacks the roots of resistant varieties, it is prevented from entering the vascular system by suberization of the cell walls and formation of cork layers in the cortex. The optimum temperature for the development of the disease is around 24 °C. The potassium content of the plants has been correlated with resistance.

Disease Cycle

The pathogen is known to survive in the soil for many years even in the absence of host roots. The pathogen is mainly soil-borne but it is also seed-borne. Optimum temperature for infection is 24 °C. Soil temperature has a profound effect on the incidence of disease. The disease is more prevalent in low moisture and light sandy soil.

Disease Management

Crop rotation may prove to be effective only when a long crop rotation is followed, as the pathogen is known to survive in the soil for a number of years. Seed-borne infection can be eliminated by treating the seeds with fungicides.

Potassium content of the plant is reported to be correlated with its resistance to wilt. Zinc application is reported to reduce fusaric acid production by the pathogen. Inoculation with non-pathogenic strain of *F. oxysporum* strain Fo 47 induces cross protection and the application of bacterial antagonist *Pseudomonas putida* enhances the action of non– pathogenic strain of *Fusarium*. The disease can be controlled by amending the soil with municipal solid waste compost as it suppresses the wilt pathogen and increases the soil microflora. The mode of action may be due to competition. The application of zinc abolishes the production of fusaric acid, the *Fusarium* pathogenicity factor.

Growing resistant varieties of linseed seems to be an effective method for the control of the disease. Some of the resistant varieties are NP12, 21,124, RPSB, PR80, PR82, 439, 440, 5, 10, 37, 38, B5128, NP(PR)5, NP(RR) 12, NP1, NP124, NP(RR)9, 10, 236, (Kulkarni *et al*., 1996 and Sharma *et al*., 1972).

Fusarium Head Blight of Wheat

Fusarium head blight of wheat, also called scab of wheat, is a destructive disease in humid and semi-humid wheat-growing areas of the world. Scab incidence epidemic ranged from 25 to 75%. Wheat scab is also becoming a greater threat to wheat production in many countries. Wheat scab can greatly reduce grain yield and quality, and may indirectly affect animal production. Scab causes indirect loss by reducing seed germination and causing seedling blight and poor stand. There is a growing concern over the potential of the disease in causing crop loss in parts of Punjab, the wheat bowl of the country. Direct yield reduction results from shrivelled grains, which may be blown off during winnowing. Since

it produces mycotoxins, storability of the grain and usage as feed is affected. This disease occurred in some areas of Amritsar and Gurdaspur districts of Punjab. It has been observed to occur when humid conditions prevail with a rise in temperature during antheses. Climate change due to changes in temperature, precipitation and increased ultraviolet B-radiation are distinct possibilities in the very near future. A large part of the scientific community is seriously worried about the effects of climate changes in Indian agriculture.

Symptoms

The first visible symptoms of infection usually occur on the first florets to flower, generally near the middle of the head. Under conditions highly favourable for infection, such as continuous wetness at 25 °C, visible lesions develop within 2 to 4 days. Lesions may be brown, purplish brown, or brown with a bleached centre. Under moist conditions, lesions may be water soaked initially, later turning purplish-brown. The presence of pink fungal spore masses is a reliable diagnostic feature of Fusarium head blight. In some cases, the first indication of infection may be the fungus itself, giving a pinkish tint to partially exposed anthers and slightly pinkish mycelium on floret surfaces. In more advanced infections, the fungus can produce macroconidia in association with a pink color on margin and surfaces of florets and glumes. As lesions enlarge and coalesce, entire florets become bleached or brown. As disease progresses in wheat, floret discoloration spreads apically and basally in the head from individual infected spikelets.



Figure 17.4 Symptoms of Fusarium head blight of wheat

Causal Organism

Many species of *Fusarium* cause wheat scab, and the symptoms caused by different species are almost the same. *Fusarium graminearum* Schw. is the principal pathogen responsible for head blight of wheat. Other related species such as *F. culmorum*, *F. avenaceum*, *F. moniliforme*, *F. oxysporum*, *F. poae* and *Microdochium nivale* may also contribute to the head blight complex but are generally less important than *F. graminearum*. The perfect stage of *F. graminearum* is *Gibberella zeae* Schw. & Petch. *F. graminearum* can survive as mycelium, ascospores, macroconidia and chlamydospores. Microconidia are not produced. Macroconidia, mycelia and chlamydospores are formed by the asexual stage while

ascospores are the propagules of sexual stage. In India, *F. avenaceum* and *F. graminearum* are causing ear blight and scab of wheat on varieties such as Sonalika, Kalyan Sona, Safed Lerma and Lerma Rajo in Arunachal Pradesh. *F. graminearum* causing head scab of wheat was also observed on varieties viz., Sonalika, Agra Local, HW 517, HW 741, HW 1042, E 9382 and C 306 at IARI, Regional station, Wellington. Several *Fusarium* spp. *M. nivale*, *F. compactum*, *F. moniliforme*, *F. subglutinans*, *F. oxysporum* and *F. pallidoroseum* were also found associated with head scab complex in the Gurdaspur area of Punjab (Singh and Aujla, 1994).

Disease Cycle

F. graminearum survives between wheat crops in living or dead host tissues. Ascospores, macroconidia, chlamydospores and hyphal fragments can all serve as inoculum. Ascospores and macroconidia may be the principal inoculum that initiate an epidemic of head scab, because aerial dispersal is necessary for the fungus to reach the infection site.

The source of primary inoculum for scab varies with cropping systems in different parts of the world, but in all cases, crop residue on the soil surface is the most important source of inoculum. Debris of wheat, corn or rice can be the principal reservoir of the fungus. Reduced tillage for soil conservation increases the amount of inoculum that can infect wheat crop. Airborne ascospores are deposited on or inside wheat spikelets, where they germinate and initiate infection. The fungus first infects the extruded anthers and then ramifies throughout the developing caryopsis, floral bracts and rachis. The fungus may also infect by direct penetration of the glume, palea or rachilla. Soon after infection, dark brown, water-soaked spots appear on the glumes of infected spikelets. Later, entire spikelets become infected or blighted. If the weather is warm and humid, conspicuous aerial mycelium spreads externally from the spikelets originally infected to adjacent spikelets. If the fungus spreads internally, brownish chlorotic symptoms extend down into the culm and up to the top of spike and as a result the entire spike may die and turn the color of straw. Visible pink mould appears on the spike when it is humid. Later in the season, perithecia of *G. zeae* may appear as raised purplish black spot. Infected florets often fail to produce grain, or the grain they produce is poorly filled.

Effect on Grain Quality

In addition to yield and quality loss, scab may also reduce the value of a crop because of the potent mycotoxins produced by the fungus. The three most important mycotoxins produced by *F. graminearum* are the estrogenic toxin Zearalenone (ZEA), the Trichothecenes Deoxynivalenol (DON) and Nivalenol (NIV). These mycotoxins have been linked with livestock toxicoses or feed refusal and thus reduce the value of the harvest either as grain or for feed. DON contaminated grain becomes less palatable to livestock and may cause emesis (vomiting), depressed feed intake and feed refusal in pigs. Humans consuming flour of wheat containing DON have been reported to suffer from nausea and headaches. *F. graminearum* mycotoxins are thus toxic to humans and animals (Agrios, 2005).

Management of Disease

Cultural Methods Because of the ubiquitous nature and availability of wide host range of *F. graminearum* and other *Fusarium* spp. capable of causing scab, adequate control by crop rotation alone is not effective. However, crop rotation coupled with deep ploughing to bury infected crop residue and weed hosts can be effective. Appropriate methods of land preparation, rotation with non-cereal crops,

timely harvest and proper storage help reduce disease by reducing primary inoculum and the likelihood of extensive infection. The adoption of minimum tillage for soil conservation greatly reduces options for this disease management technique.

Biological Control Antibiotic substances produced by *Bacillus subtilis* and *Pseudomonas putida* have inhibited spore germination and reduced the *in vitro* growth of *F. graminearum*. The activity of the gliding bacterium *Lysobacter* sp. against *Fusarium* head blight is believed to be due to induced resistance as well as chitinolysis.

Chemical Control Fungicide applications should not only reduce the disease to an acceptable level, but also control toxin contamination of the grain. A large number of fungicides like Benomyl, Mancozeb, Thiobendazole, Thiophanate-methyl and Triadimefon are quite effective for blight control. Folicur (Tebuconazole) application increased grain yield by 21% and decreased mycotoxin (deoxynivalenol – a trichothecene metabolite) contamination in grains. Benlate (50WP, Benomyl) increased yield by 10%. Benomyl and Benomyl-Mancozeb mixture were nearly as effective as Tebuconazole. Bavistin (4L/ha), Libero (1.5L/ha) also gave the best control with an efficacy of 60 and 61% respectively. The systemic Triazole fungicides and the strobilurins do not translocate from leaves to head, except in trace amounts. Therefore, an early application will protect only leaves whereas the later emerging heads are unprotected.

Control of wheat head blight by fungicide application or other practices is neither practical nor sustainable. It is said that the only effective method of controlling wheat scab is to grow resistant varieties and this is still true today. Recently, Chen *et al*. (1999) explored an alternative strategy for scab control in wheat through the deployment of pathogenesis related (PR) protein genes. A rice thaumatin-like protein (TLP) gene (tlp) and a rice chitinase gene (Chi 11) were introduced into the spring wheat cultivar Bobwhite by co-transformation of the plasmids PGL2 ubi-tlp and pAHG11. The transgenes *tlp* and *bar,* under the control of the ubiquitin promoter-intron were expressed. After inoculation with conidia with *F. graminearum* the symptoms of scab development were significantly slower in transgenic plants of the T_1, T_2 & T_3 generations expressing the *tlp* gene than in non-transformed control plants.

Panama Disease of Banana

The disease is widespread throughout Africa, Asia, Australia and Oceania, West Indies, and North, Central and South Africa. The disease was first discovered in Australia in 1880s but it reached epiphytotic proportions in 1950s in Panama, where it destroyed 40,000 hectares of the crop and was recognized as a devastating disease in Central America (Agrios, 2005).

Symptoms

Reactions to the clonal groups of cultivars of banana differ to this disease.

1. 'Inodoratum' or yellowing of erect leaves; this type is not found in Costa Rica, Panama, Columbia, and Equador.
2. 'Odoratum', which is found in the Carribean Islands, Honduras and Guatemala. Plants of this type, infected by Panama disease, do not turn yellow but suffer from the collapse of the petiole. The cut stem smells of rotten fish.

Symptoms are most clear on plants that are at least five months old. Sudden wilting of the plant or individual leaves is a characteristics symptom of the disease. Leaf symptoms appear after the fungus has spread through the corm. In young plants, the first signs are on the unfurling leaf which turns yellow and dies. In older plants, the leaves except the youngest ones collapse. Any new leaves which are produced are blotchy and yellow with the wrinkling of the lamina. The pseudostem later splits and the vascular symptoms extend up the pseudostem.

In Gros Michel, a very susceptible variety, the lower leaves become yellow and the petioles collapse around the pseudostem from the base to the top. If the base of the plant is cut open vertically, numerous brown and black lines can be seen running in all directions through the corm and upwards into the leaf bases and petioles.

The time between the rootlet infection and wilt is about two months. As a result of infection, the plant produces many suckers which are invariably infected with the fungus.

Causal Organism

The pathogen responsible for wilt of banana is *Fusarium oxysporum f.* sp. *cubense*. (E. F.S.) Snyder and Hansen. The hyphae of the pathogen are present both inter and intracellular in the host tissue. They are largely confined to the vascular bundles, often filling the cavity of the vessels. Once inside the vascular bundles, the pathogen spreads in a systemic manner and extends into the infected rhizomes and the suckers.

The fungus produces numerous micro and macroconidia and chlamydospores, which are characteristic of the genus. The sporodochium arises from a globose mass of pseudo- parenchymatous tissue and bears the conidiophores. Conidiophores measure $70 \times 4\mu m$. The macroconidia are sickle-shaped, hyaline, and thin-walled, with 2-5 septate and measure 22- 36×4-$5\mu m$. The chlamydospores are oval to spherical, often paired and measure 7-13×7-8 μm.

Several races of the pathogen have been reported. Race-1 attacks “Gros Michael” (AAA) and Silk (AAB), whereas Race-2 Bluggoe (ABB) and other cooking bananas are resistant. Cavendish varieties are resistant to Race-1 and mildly resistant to Race-2. This group is highly susceptible to Race- 4, which does not exist in our country.

Disease Cycle

The pathogen is soil-borne and invades the plant mainly through the roots or wounds in the rhizomes. The fungus colonizes the vascular bundles and produces masses of mycelium, which bear both macro and microconidia and the chlamydospores. The chlamydospores can survive in the soil for a long period. The pathogen can also survive in the diseased rhizomes and other plant parts saprophytically for a long period. The disease can be easily spread in infected banana trash and soil carried in surface flooded water. Suckers used for planting from diseased areas to other areas spread the disease easily. The entry of the pathogen into the host is helped by the incidental wounds and insect injuries on the rhizomes.

The disease is favoured by high soil moisture, light soils, bad drainage, and the strong development of new roots for example, after a nematode attack.

Disease Management

Flood fallowing has been found to be the most effective method of reclaiming infested soil. In flooded soils, the fungus is believed to be eliminated due to the effect of toxic substances (such as acetic acid)

produced in the soil and also due to the low oxygen availability in the soils. The carbon dioxide in submerged soil causes continued germination of the conidia but prevents the chlamydospores from developing. The amendment of the soil with carbohydrate sources and plant tissues reduces the population of the fungus by stimulating chlamydospore germination and the lysis of the germ tubes and the conidia, and by inhibiting further chlamydospores formation.

Soil fumigation has also been suggested but it is not economically feasible. A solution of 5% phenol or 5% formaldehyde is useful for sterilizing tools like machetes (knife), which can spread the disease. Infected plants should be eradicated by weed killers before their removal.

According to Lakshmanan and Mohan (1989), corm injection of 2% Carbendazim plus 0.1% Agallol has been found to be quite effective in controlling the disease. Aretan as soil treatment has been claimed as a successful method by Lakshmanan and Mohan (1989). Apart from this, some actinomycetes and soil bacteria capable of producing antibiotics Musarin and Monamycin and some lytic substances are found in the rhizosphere of resistant varieties of banana, which could be exploited for disease control.

Off late, Bavistin capsule application in the rhizome has been reported to be the most effective method. However, it is also known that dipping of suckers in Bavistin (0.1%) solution before planting, followed by bimonthly drenching of Bavistin (0.1%) from six months of planting onwards has given good results.

Application of antagonistic strains of *Pseudomonas fluorescens* is reported to give control of the disease because it activates the defence enzymes (peroxidase, chitinase and glucanases) and phenolic compounds. Suppressive soil against *F. oxysporum* f. sp *cubense* has been reported in Central America. Application of neem cake (margosa) + treatment of sucker with *Pseudomonas fluorescens* + soil application of *P. fluorescens* have been reported as the best control of wilt of banana. Quarantine procedures and micro- propagated planting material are quite effective in controlling the disease.

In addition to these chemical control methods, some cultural control methods like removal of diseased plants, use of healthy stock, control of nematodes, avoiding root injury during cultivation should be encouraged to avoid disease incidence. The most effective measure of control would be the cultivation of resistant varieties as is obvious that ‘Gros Michael’ has been replaced by ‘Giant Cavendish’. Efforts should be made not to import of suckers of cultivars from the countries where Race-4 persists. There is a good hope of getting resistant clones from the Central Banana Research Station, Adhuthurai (T.N.), where the existing commercial varieties are being hybridized with *Musa balbisiana*, *M. acuminata*, and *M. coccinea*. Resistant varieties should be cultivated. Cavendish is very resistant but other resistant varieties are Poovan, Moongil, Peyladen, Rajabale, and Vamankeli.

Dutch Elm Disease

The disease owes its name to the fact that it was discovered on elm in Holland in 1921. It was recorded in relatively rapid succession in many parts of Europe and Asia. The American elm (*Ulmus americana*) is threatened with extinction because of the ravages of the pathogen. The trees are being killed by the millions. The cost of cutting down dead elm trees is very substantial.

Symptoms

The first symptoms of the disease are sudden or prolonged wilting of the leaves of the individual branches or of the entire tree. When the foliage of the individually infected branches loses their turgour and

the wilt, the syndrome is called flagging. Once infection has occurred, the entire tree can be expected to die within several years. The diagnostic symptoms are the brown discoloration of tissues in the vascular region. The outer layer of wood may appear streaked, mottled or brown to dark grey. The xylem of the infected plant becomes brownish in color and brown, gummy materials accumulate.

Causal Organism

Ophiostoma (Ceratocystis) ulmi (Buisman) C. Moreau is the organism responsible for Dutch elm disease. *O. ulmi* produces two types of anamorphs: *Sporothrix*- type, directly on the mycelium, and a synnematous anamorph (*Graphium*) that is abundant in pupal chambers and provides much of the inoculum that is spread by the beetles, while *Ophiostoma* (*Ceratocystis*) *ulmi* is the ascigerous stage. Aggressive strains of the fungus causing Dutch elm disease pandemics have been placed in a new species, *O. nova- ulmi*. The life cycle of the pathogen is depicted in Fig. 17.5.

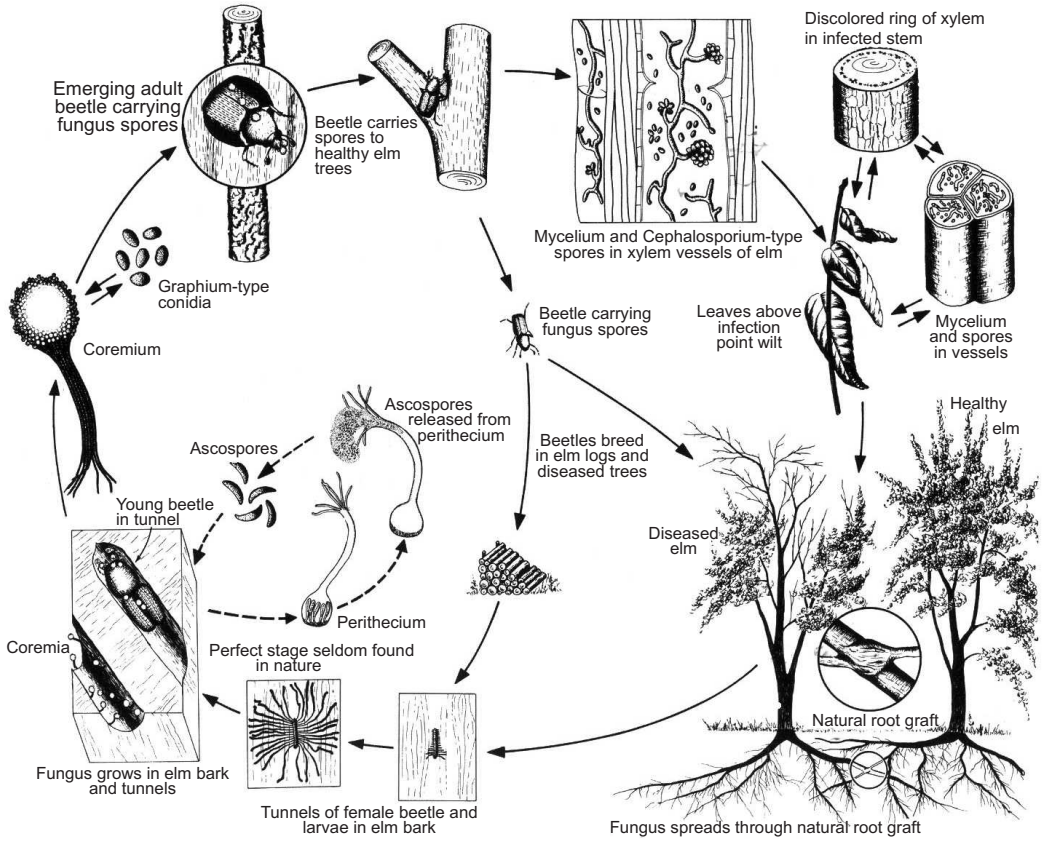


Figure 17.5 Life cycle of Dutch elm disease [*Ophiostoma (Ceratocystis) ulmi*]: From G.N. Agrios, *Plant Pathology,* Academic Press, London, p. 277.

The mycelium of the fungus is white in color, turning yellowish in mass with age and becoming covered with conidia, which give it a yellowish-white surface. Conidia are borne at the tips of short, simple or branched hyphae and may bud rapidly. Conidia are hyaline, elliptical, 2-3.5 × 4.7-10μm. Coremia (loosely bound bundles of conidiophores) may form on the surface of cultures as brown to black hair-like projections above the colony. They may also develop on the bark of affected branches as erect dark stalks with a colorless flaring head to which conidia adhere as a whitish to yellowish droplet. Quellette and Gagnon (1960) described the microendospores. They are the types found most frequently in the diseased tissues (Quellette, 1962). The fungus is heterothallic and requires the contact of two sexually compatible strains for sexual reproduction. The development of the perithecia follows a distinctive course designated by Luttrell (1951) as the *Ophiostoma* type.

The ascogonia are free upon the mycelium. Asci are produced irregularly throughout the centrum and never form a definite wall layer. Inside the perithecium, many asci develop slightly curved ascospores, each containing eight colorless. As the asci mature, they disintegrate, leaving the ascospores free in the perithecial cavity. The ascospores are discharged through the neck canal and accumulate in a sticky droplet which is supported by the fringe of hyphae surrounding the ostiole. Mitchell and Brasier (1994) had discussed information on mating compatibility and vegetative incompatibility in *O. ulmi* and *O. novo- ulmi.*

Disease Cycle

The disease is spread by the bark-boring beetles, especially *Scolytus multistriatus* and *Hylurgopinus rufipes,* which tunnel into wood and the bark to feed or to lay eggs. The adult female beetle tunnels through the bark and opens a gallery parallel with the grain of the wood with *Scolytus* and at a 45 degree angle with *Hylurgopinus rufipes*. The female lays eggs along the sides of the gallery; the eggs soon hatch, and the larvae open tunnels at the right angles to the maternal gallery. If the tree is already infected with the fungus, the latter produces mycelium and sticky *Graphium* type of spores in the beetle tunnel. When the adult beetles emerge, they carry thousands of fungus spores on and in their bodies. *Scolytus* beetles feed on the twigs of living, vigorous elm twigs. *Hylurgopinus* beetles feed on stems 5-30cm diameter. The beetles grow into the bark and wood; the spores are deposited in the wounded tissue of the tree, and germinate and grow rapidly into the injured bark and the wood. *Cephalosporium*-type spores are produced in the large xylem vessels of the spring wood. These spores reproduce by the yeast-like budding, germinate and start new infections. The extent of symptoms in the crown is correlated with the extent of vascular invasion.

Disease Management

Detection and eradication of diseased trees, especially in urban areas accompanied by sanitary measures, together with the spraying of trees with insecticides to eradicate the insect vector, form important measures to control the disease. The spread of disease to healthy trees by root grafts can be checked by the application of Vapam solution to the soil in 38cm holes, 15cm apart in one or more zones between diseased and healthy elm trees.

Smalley (1962) recommended the control of Dutch elm disease by treatments with 2, 3, and 6- trichlophenyl acetic acid, a systemic fungicide. Systemic insecticides which prevent the development and spread of vectors are also useful in controlling the disease. Some protection from Dutch elm disease has been obtained with certain *Pseudomonas* bacteria or with non-aggressive strain of the fungi

Ophiostoma or *Verticillium* (Agrios, 2005). Breeding of resistant varieties forms the best control measure. Resistant species are to be found in Asia. The American species are very susceptible. Attempts are being made to breed resistant varieties.

Root Rots

Root rots are essentially different from wilt diseases. In vascular wilts, the pathogen grows mainly in the vascular region of the plant and produces toxins and enzymes which bring about the wilt; while in the case of root rot, the pathogen invades the parenchymatous tissue of the root, cortex and pith and there is little invasion of the vascular tissues. Root rots are caused by a number of pathogens belonging to genera such as *Aphanomyces, Pythium, Phytophthora, Rhizoctonia, Sclerotium, Phymatotrichum, Thielavia, Helicobasidium, Ophiobolus, Armillaria*, and so on. Some of the diseases caused by these genera have been discussed in Chapter13. Here, we shall discuss diseases caused by *Rhizoctonia* species.

Rhizoctonia spp. cause diseases in plants of agricultural and horticultural importance and also of forest trees. Saksena (1971) had discussed the taxonomy and the parasitism of *Rhizoctonia* spp. and there is a monograph on *Rhizoctonia solani* by Parmeter (1970).

Rhizoctonia Root Rot of Cotton

Root rot is one of the most severe diseases of cotton in India. It also occurs in the USA, Africa and other counties of the world. The disease occurs in severe form in the province of Punjab, Haryana, Rajasthan and Gujarat in India, but it is of less importance in Karnataka, Tamil Nadu, Madhya Pradesh, and Western Uttar Pradesh. In contrast to wilt, which is mainly found in black cotton soils, root rot is confined to sandy soils. In the seventies, it was an important disease in Punjab but due to certain reasons, it is not a major problem now. In Rajasthan, this disease causes major losses up to 90%.

Symptoms

This disease is easily distinguished from the damping off disease. The stem of the infected plants remain erect and the diseased parts are not water soaked. The disease spreads in the fields in concentric circles. The pathogen attacks the seedlings as well as mature plants. If the affected plants are pulled out and examined, the entire root system may be found rotting. The lateral and thinner roots get completely rotted. The tap root is intact in the beginning while the lateral and thinner roots get shredded with yellow slime on the wood. Minute fungal sclerotia appear as minute black dots on the surface of woody tissues and on the rotting bark.

When young seedlings are infected, the cotyledonary leaves may be affected and they assume a 'pinched' look. Root rot of cotton is essentially different from the wilt of cotton. In the root rot, there is necrosis of the parenchymatous region of the root while the wilt infection is mainly confined to the vascular tract. The mechanism of wilt injury is also different to the root rot. *Rhizoctonia* root rot is also called the dry root rot.

Causal Organism

Root rot of cotton is caused by *Rhizoctonia bataticola* (Taub.) Butler and *R. solani* Kuhn in Punjab and Haryana and only by *R. bataticola* in Gujarat. The perfect stage of *R. bataticola* is *Thanatephorus*

cucumeris (Frank) Donk which is also known as *Corticium vagum* var. *solani* Bunt ex Rolfs or *C. solani* (Prill and Del) Sourd. Gaiz, or *Pellicularia filamentosa* (Pat) Rogens. *R. bataticola* produces pycnidia and this stage is known as *Macrophomina phaseolina* (Maubl) Ashby. The hyphae are present both intra and intercellularly in the host tissue. The hyphae are septate and thick, 1.5-2.5um and produce sclerotia 100um in diameter. The sclerotium produced by *R. bataticola* has its pycnidial stage in the fungus *Macrophomina phaseolina* while the perfect stage of *R. solani* is *Pellicularia filamentosa*.

A number of workers have carried out a series of comprehensive studies with reference to symptomatology, incidence and etiology and physiology of the causal fungi of root rot of cotton.

Disease Cycle

Both *R. solani* and *R. bataticola* are capable of living saprophytically in the soil on organic matter. Both these pathogens can also infect several other crops, such as groundnut, cowpea, and several leguminous plants, oil seed and vegetable crops on which they also pathogenic. The role played by the pycnidial and the perfect stage of the respective pathogen in the perpetuation of the disease is unknown. Soil temperature of 35^0C and above, soil moisture in the range of 15-20%, and alluvial or sandy soils make the plants susceptible to the disease.

Disease Management

Since the disease is found both in Indian and American cottons, attempts to evolve resistant varieties have not been successful. Varietal differences in susceptibility to root rot have been observed. Among *arboreum* race *indicum* cottons, the perennial type- Rohi of Gujarat- is known to be more or less immune to root rot (Balasubrahmanyam, 1950). Large number of cotton strains received from the USA and other countries have been tested under field and screen house conditions. Three lines viz. KH-33-146, 15-Kw-2(MB) and 9- Kw-2(MB) have been found to be resistant to both *R. solani* and *R. bataticola.*

Seed Treatment Treatment of seeds with Quintogene, Carbendazim and Oxathiin are quite effective in reducing the seed-borne and soil-borne infection.

Soil Drenching Pre and post sowing drenching of soil with Quintogene and Carbendazim provided maximum control. Seed treatment plus pre-sowing soil drench is most effective.

Agronomic Practices Certain cultural practices have been recommended for reducing the incidence of root rot of cotton such as adjusting the time of sowing of the crop when soil temperature are not high. Mixed cropping has been recommended for checking the disease. (Luthra and Vasudeva, 1940; Vasudeva, 1941). *Phaseolus aconitifolius* and Sorghum are commonly grown in mixed cropping.

Biological Control Kalyan and Verma (2002) identified *Penicillium petuletum*, *Aspergillus terreus*, *A. fumigatus* and *Chaetomium* sp. as antagonistic to *R. solani*. Seed coating with *Trichoderma* spp. reduced disease incidence upto 83%. The effectiveness of fluorescent Pseudomonads/ PGPR against seedling infection caused by *R. solani* has also been reported. The secondary metabolites of fluorescent Pseudomonads have received consideration attention because of their ability to produce a range of secondary metabolites that are inhibitory to the pathogen. Siderophores, metabolites with antimicrobial properties and other substances like cyanide are the main groups of metabolites known to be involved in the pathogen suppression. The biocontrol activity of a genetically manipulated strain of *Trichoderma virens* was enhanced against cotton seedling disease caused by *R. solani* due to the over expression of a chitinase gene (Back *et al*., 1999). A strain of *Pseudomonas fluorescens* is generally found in the

rhizosphere of cotton seedlings. Seed treatment with this bacterium increased seedling survival. The bacterium produces an antibiotic identified as Pyrrolnitrin. Seed treatment with the antibiotic also increased seedling survival.

Root and Stem Rot of Jute

Root and stem rot of jute caused by *Macrophomina phaseolina* is the most prevalent disease of jute in Assam, Bihar, West Bengal, Orissa, and the eastern districts of Uttar Pradesh.

Symptoms

The disease occurs during almost all stages of plant growth. In young plants, infection starts at the collar region and the cotyledons are also infected. The affected seedling shows damping off symptoms. Dark, thin streaks develop at the collar region which increase rapidly under humid conditions and rapidly kill the plant. On older plants the leaves are attacked. Lesions appear on the margins and apex of the leaves fall down leaving the jute plants with bare branches. The stems rot and the roots get shredded. Pycnidia are produced on the infected stem and the rotting tissue. When the pathogen infects the capsules they become black and the seeds become discolored and small. The sclerotia of the pathogen are often seen on the capsules.

Causal organism

The pathogen responsible for the root rot of jute is *Macrophomina phaseolina* (Tassi) Goid. The sclerotial stage of the pathogen is *Rhizoctonia bataticola* (Taub.) Butler.

Disease Cycle

The pathogen is both soil and seed-borne. Rajan and Patel (1946) established the seed transmission of the pathogen. The fungus is capable of growing saprophytically on dead organic matter in the soil. It has a very wide host range including cotton, tobacco, sesamum, potato, egg plant, and mulberry and so on.

Disease Management

The macronutrients play an important role in predisposing the plants to various diseases. Sawada (1916) found that the application of potash was effective in checking the stem rot incidence in Formosa. Ghosh (1963), and Ghosh and Basak (1965) have reported that the application of nitrogen alone incited the incidence of stem rot and root rot. It has been observed that low K_2O/CaO ratio in plants (Ghosh and Chakrabarti, 1966), potash deficiency in soil and application of nitrogen alone (about 60kg/ha) predispose the crop to severe stem rot incidence (Mukerjee, 1969). The influence of N.P.K. was investigated by Thakurji (1974) and it was found that stem rot on jute caused by *Macrophomina phaseolina* increased with increasing levels of nitrogen and was maximum at 80 kgN/ha. Since the disease is seed-borne (both externally as well as internally), treatment of seeds with suitable fungicides is recommended. Seed treatment with Bavistin at the rate of 2 gm/kg seed has been recommended.

Fungi like *Aspergillus aculeatus*, *Trichoderma lignorum*, *T.viride*, *Streptomyces albus*, *Bacillus cereus*, *and B. subtilis* have been found to inhibit the growth of *M. phaseolina in vitro*. Cultural practices

like more number of irrigation to reduce temperature and slower growth of fungus may be helpful. *M. phaseolina* is inhibited *in vitro* by Aureofungin at concentration below 1.5 µg/ml.

Jute Research Station at Kendrapara (Orissa) has reported five Capsularis Jutes, namely Halmhera, C- 58-9433, Bangkph, JRC- 9826 and Patchy albino and one Olitorius Jute (JRO-333I), to be field resistant. There is no absolute resistance available in existing jute germplasm. The influence of micronutrients on the incidence of stem rot of *Capsularis* was investigated by Thakurji *et al*. (1976). There was a positive decrease in the incidence of disease due to the application of micronutrients in combination with nitrogen, phosphorus, and potash or alone. It was observed that the incidence of stem rot increased in plots where no micronutrients or N.P.K. were added. The application of zinc, iron and boron has shown superiority in reducing the incidence of stem rot.

Black Scurf of Potato

Black scurf (Fig 17.6) is a very serious disease of potato and causes considerable losses to the crop. It is widespread both in the hills as well as in the plains of India.

According to Lakra (1992), 50% of seed tubers were affected on the surface, germination was reduced by 11% and yield up to 68%. As a result of post-emergence wilt, the number of necrotic stems is up to 90% and wilting is increased up to 27%. The disease causes loss as it decreases the marketability of tubers for food and for seeds.

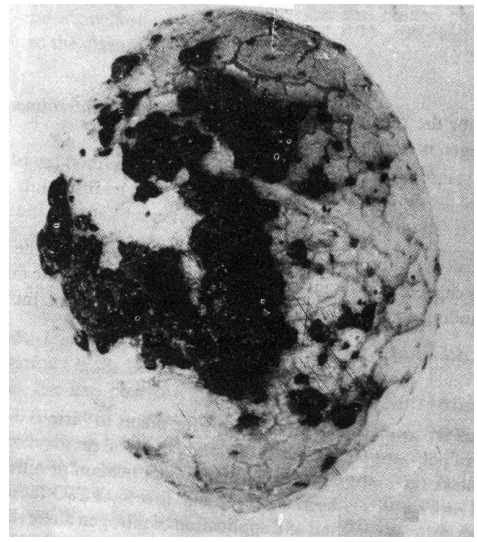


Figure 17.6 Black scurf of potato (*Rhizoctonia solani*): sclerotia on potato tuber.

Symptoms

Blight phase and the scurf phase are two distinct phases of the disease. In stem canker, the growing tip of the plant may be killed before emergence as it is very susceptible to the pathogen. The dormant bud of the tubers may be attacked and killed if the conditions are favourable for the growth of the pathogen. If the conditions are unfavourable, these dormant buds may produce sprouts and grow but the growth of the plant is stunted and yellowish which can be observed in the field. When stem cankers are serious they affect the carbohydrate metabolism of the plant resulting in stunted growth, resetting, and enhancement of purpling due to excessive accumulation of anthocyanins.

Causal Organism

The disease is caused by the imperfect stage of the fungus *Rhizoctonia solani* Kuhn. and its basidial stage is *Thanatephorus cucumeris* (Frank) Donk.

The mycelium of the fungus produces superficial, irregular scab-like, black sclerotia on the infected tissues. The basidial stage is mostly saprophytic, which is found on dead parts of the plant under conditions of high humidity. The basidia are produced in a cyme manner, barrel-shaped, size 9-25 × 5-12 µm, and bear 4 sterigmata. Basidiospores are ellipsoid or oblong - ellipsoid, flattened on one side and hyaline.

Disease Cycle

The pathogen is soil as well as tuber-borne. The infected tubers bear on their surface chocolate – colored, hard bodies called sclerotia. When the infected tubers are planted, the fungus attacks the growing sprouts or young shoots. These are killed which result in gaps in the stands. Other plants may also get diseased near the ground level, producing dark brown cankers at the collar. This girdling effect results in wilting and in the death of the plants. The downward movement of manufactured food material from the leaves is interfered with, which results in stunting and also purpling of the leaves.

Disease Management

Seed tubers should be taken from healthy areas. The seed tubers (after harvest and before sprouting) should be treated with an organomercurials, such as Agallol (0.5% solution) for about ten minutes before planting or before keeping in cold storage. Soil application of sawdust at 25 g/ha, with 20 kg of additional nitrogen at least 15 days before planting reduces the incidence of the disease. Soil application with Brassicol at 30 kg/ha at the time of sowing also keeps the disease in check. Dipping of infected tubers in a 1.75% solution of sulphuric acid for 20 minutes or 3% solution of boric acid has been found effective in controlling the disease.

Biological control of potato diseases caused by *Rhizoctonia solani* and *Fusarium* spp. was tried by Shrivastava and Saksena (1968). When the seed pieces were dipped in a heavy spore suspension of *Trichoderma viride*, the disease was controlled considerably. Among amendments, oatmeal and wheat straw were ineffective. Greater suppression of the disease was noted in soil amended with green organic manure (tops of maize plants and a legume). Singh *et al.* (1972) have reported increased yields by amending the soil with neem or margosa cake (25 kg/ha) or with sawdust (25 kg/ha) followed by application of 125 kg nitrogen/ ha. Another biological method to control the pathogen is by the introduction of hypo-virulent strains of the pathogen. Mulching of the soil with 2-3 cm thick layer of rice husk or with polythene sheets has proved effective as the pathogen is soil- borne and by this method soil temperature is increased, which results in the death of the pathogen.

Stem Rot of Rice

Stem rot of paddy causes severe damage to the crop. Although it occurs in all rice-growing areas of the world, it has not received sufficient attention from rice growers and research workers. The disease was reported from Bengal and Tamil Nadu as early as 1911-1912. Butler (1918) reported it from all the rice – growing areas of the country. Paracer and Luthra (1944) have estimated 50- 75% losses in yield in very badly infected fields while crop losses of 5-15% occur almost every year in Punjab. Sharma and Mehrotra (1985) observed that the yield components are adversely affected by the disease. The disease is of major importance in USA.

Symptoms

Stem rot of rice is detected in the field when the leaves begin to turn yellow indiscriminately followed by the stunting of the plants. The disease is characterized by sparse growth of the plant, and the stem becomes thin and leaves small, rigid, and pale. The culm of the affected plants is covered with black discoloration near the soil, which later spreads to the entire leaf sheath. Due to the pressure exerted by the

pathogen within the infected tissue, the epidermis of the plant bursts and the plants wither and die. In addition to the sterility, the plants show excessive tillering or the development of green sterile shoots from the basal nodes of stock. Young leaves of diseased plants are somewhat darker green and tend to become stiff and erect. If any panicle is borne in a severely- affected plant, it is poorly filled and the grains are extremely light. Two or three nodes from the base appear darker and show sclerotial infestation. The earliest manifestation of the disease is in the form of a small black lesion on the outer leaf sheath of the plants at the water line. The stem at this region shows considerable rotting as the lesion enlarges and finally the tillers that are produced normally at the base are killed and the whole plant succumbs to the disease even without producing any sclerotia internally.

Causal Organism

The causal organism is *Sclerotium oryzae* Cattaneo. The conidial stage of the pathogen has been described as *Nakataea sigmoidea* (Cavara) K. Hara (= *Helminthosporium sigmoideum* Cavara). It has its perfect stage in *Magnaporthe salvinii* (Catt.) Krause and Webster. It was earlier known as *Leptosphaeria salvinii* Catt. The conidiophores of *Nakataea* are dark, septate, simple or sparsely branched. The number of septa is 8-10. They measure 10-15 x 3 µm. The conidia are large, falcate-sigmoid, 3-septate and are borne singlely at the tip of sterigmata on the conidiophores. The middle cells of the conidia are dark brown in color while the apical cells are light green or hyaline with obtuse ends. They measure 55-65 x 11-14 µm. Natural occurrence of the asigerous stage is scarce. The perithecia are black, globose, clustered in the parenchyma of the leaf sheath. They are provided with short but broad beaks, flush with the epidermis of the sheath, and measure 350-400 µm without beak. The asci are clavate, stipitate, 120 µm long and contain 8 ascospores which are arranged in 2-3 series. Ascospores are oblong – fusiform, slightly curved, have 3 septate and measure 60.0 µm. Jain (1973) has reported parasitic specialization in the pathogen. The fungus is heterothallic.

Disease Cycle

Stem rot pathogen survives in the soil in the form of sclerotia in the absence of the host. These sclerotia are produced in enormous number in old rice straw and stubble left in the field and are disseminated by irrigation water or rain water. Survival in the soil is affected by depth (Mishra *et al*., 1966). The disease is increased in fields receiving increased levels of nitrogen. According to Mayee *et al.* (1976), thick stand of the crop and high doses of herbicide also favour disease development.

Disease Management

Cultural Methods Burning of rice stubble after harvest has been suggested by several experts. Patches of diseased plants should be marked out and should be uprooted and destroyed. Passage of irrigation water through or from infested fields to healthy fields should be checked. Since rice is an irrigated crop, the field should be flooded and then water should be drained off. The soil should be allowed to dry before the next irrigation is done. Sharma *et al*. (1984b) developed a sieve floatation technique which could quantitatively recover the sclerotia of *S. oryzae*. Mulching the soil with transparent polythene sheets during summer months has been tried with effective results (Usmani and Gaffar, 1982 Sharma *et al*., 1984).

Chemical Control There are a number of reports of laboratory screenings of fungicides for controlling the pathogen, but there are very few studies of controlling the disease under field conditions. Sharma and Mehrotra (1985a) have reported that 0.2% Bavistin or Topsin – M spray was effective in reducing the disease.

According to Kumar *et al*. (2003), Propiconazole was the most promising fungicide in reducing the disease followed by Isoprothiolane and Hexaconazole. Edifenphos, Carbendazim, Benomyl, Isoprothiolane have been found very effective in managing stem rot (Singh *et al*., 2002). The efficacy of resistance inducing chemicals like benzoic acid was found better over other chemicals in reducing the disease (Kumar *et al*., 2003). The growth of *S. oryzae* was found to be inhibited by *Trichoderma hamatum*, *T. harzianum*, *Stachybotrys atra*, *Aspergillus flavipes*, *Penicillium funiculosum* and *Bacillus subtilis* (Haroon Usmani, 1980).

Breeding for Resistance Most rice varieties are susceptible to the disease. The degree of susceptibility varies with the variety (Sharma and Mehrotra, 1985b). Gangopadhyay and Das (1983) tested a large number of varieties/ cultures of rice belonging to the long duration group against 8 isolates of the pathogen. MPN 234, Calaro, IET 563 3, ARC 12751 and Tadukar were found quite resistant to the stem rot pathogen. Ali and Singh (1994) have listed a large number of varieties showing different levels of resistance and susceptibility.

Sheath Blight of Rice (*Oryza sativa* L.)

Sheath blight of rice is a major biotic constraint of rice in most of rice growing countries of Asia. It is a destructive disease worldwide that causes significant loss and quality degradation. It was first reported from Japan by Miyake in 1910, which spread rapidly in the East and South-East Asian countries and therefore was popularly called as 'Oriental Sheath and Leaf Blight'. Losses upto 50%, depending on the severity of the disease, have been reported (Singh *et al.,* 2004). Roy (1979) reported yield loss up to 36% in Assam, depending on the growth stage of the plant when the disease occurs.



Figure 17.7 Symptoms of Sheath blight of rice caused by *Rhizoctonia solani* (Courtesy: Dr. Ram Singh, CCS HAU, Kaul, Kurukshetra)

Symptoms

Initial symptoms appear in the form of circular ellipsoid or oblong, green grey that occurs near the water line as water-soaked lesions. Plants are susceptible to infection at booting and flowering stage when the rice plant canopy is most dense, forming a microclimate favourable to pathogen growth. The pathogen can infect seeds of mature plants, causing moderate to significant yield losses. Visible plant disease symptoms include formation of lesions on leaves, plant lodging and presence of empty grains (Fig. 17.7).

The formation of lesions on infected sheath of lower rice leaves may lead to softness of the stem, thereby initiating stem

lodging (Wu *et al*., 2012). During rice sheath blight epidemics, severe lodging may occur which obstructs the transportation of water and nutrients assimilates through the xylem and phloem affecting grain filling (Wu *et al*., 2012). The disease intensity is also dependent on the amount of infectious inoculum present in the previous crop remaining in the field. The use of higher dozes of nitrogen fertilizers, double cropping, early maturing varieties, high plant densities, high tillering have been shown to enhance sheath blight severity. High nitrogen facilitates the sheath blight disease development and spread by increasing tiller density and moisture retention inside the rice canopy (Tang *et al*., 2007).

Causal Organism

It is caused by *Rhizoctonia solani* Kuhn. [Teleomorph: *Thanatephorus cucumeris (*Frank) Donk]. High temperature (22°C to 35°C), high relative humidity and pH 6-7 are favourable for mycelial growth and sclerotia formation.

Disease Cycle

Rhizoctonia solani is a universal soil saprotrophic and facultative plant pathogen. Disease cycle takes place predominantly through sclerotia in the soil. Sclerotia in soil can survive for 2 years and are spread during field preparation and flooding the field for irrigation (Webster and Gunnell, 1992). During flooding, the sclerotia may float and move within the field or to neighbouring fields through continuous flood irrigation. Sclerotia or hyphae attach to the plant, infecting and causing disease. Once infection occurs, secondary spread takes place through direct contact. The role of basidiospores is uncertain.

Management of Sheath Blight

1. Cultural Practices Burning the infected crop debris after harvest, keeping the field weed-free and bunds cleaning is necessary to control the diseases (Singh *et al*., 2004). Addition of neem and groundnut cake under flooded condition reduced the survival of the rice pathogen (Lakshmann, 1984). High density of seed, high rates of N fertilizers, early maturing, dwarf build, high tillering encourage the spread of the disease. The application of silicon and potash reduce disease severity. Crop rotation is another sound strategy and rotation of two years with soybean controls sheath blight. Controlling weeds by destroying grasses and other collateral host reduce infection.

2. Chemical Control Common fungicides used earlier against sheath blight were copper, organo-mercury and oragno-arsenic compounds (Ou, 1985). Benomyl, Edifenphos, Carbendazim, Propiconazole are the most effective fungicides reported by many Indian worker. Other fungicides found effective are Dithane M45, Demosan, Captafol, Topsin, and Cuman. Within the Strobilurins group, Azoxystrobin is used against sheath blight pathogen. The mode of action of Azoxystrobin is to inhibit electron transport and kill the fungal pathogen (Groth and Bond, 2006). Four antibiotics, two developed in Japan, viz., Validamycin and Polyoxin and two developed in China viz., Jingganmycin and Chingfengmeisu, have been found effective against sheath blight pathogen (Premalatha Dath, 1990). Acibenzolar-S-methyl has the unique property to protect plants from fungal, bacterial and viral infections. However, with the introduction of Pencycuron, a phenylurea-based fungicide, the control of seed-borne *Rhizoctonia* infections would become easy in many crops (Thind *et al*., 2002). Green house experiments revealed that spraying of antifungal metabolites SPM5C-1 obtained from *Streptomyces* sp PM5 on infected rice

plants decreased sheath blight development by 82% (Prabavthy *et al.,* 2006). Thifluzamide 24% SC, a new group of fungicide, was found effective as preventive and curative activity without any symptoms of phytotoxicity, reducing the disease severity and increasing the yield (Prasanna Kumar *et al.,* 2012).

3. Biological Control Antagonism between organisms is common in the ecosystem and the most common among them are soil microorganisms. Among the several varieties tried by various researchers, species of *Trichoderma, Gliocladium, Aspergillus* have been found effective in controlling the sheath blight and extensively explored for the control of soil-borne plant pathogens. Antagonistic effects of *Bacillus cereus, B. subtilis, Enterobacter sp. Pseudomonas putida, P. fluorescens, P. aureofaciens* on the growth of *R. solani in vitro* have been demonstrated by Gnanamanickam and Mew (1990), Lee *et al.* (1990), and Singh and Sinha (2009). Two fungal antagonists i.e., *Gliocladium virens* and *Trichoderma longibrachiatum* applied in soil as wheat bran dust preparation survived well in the soil and reduced the pathogen population effectively. Higher rates of *T. harzianum* were found effective in reducing disease severity and incidence and increasing grain yield over control. Chen *et al.* (2000) reported that fermented product of B-916 (*Bacillus subtilis*) and Jingganmycin are useful of controlling *Rhizoctonia solani.*

Pathogen is soil-borne but little information is available on the role of seed-borne inoculum in transmission of *Rhizoctonia solani* from seed, in disease development in the field. Seeds treated with *Trichoderma viride* and *T. harzianum* showed significant reduction in sheath blight infection. Seed treatment with *Trichoderma viride or* Carbendazim also reduced disease incidence on seedling. Seed treatment with *Trichoderma harzianum* and foliar spray with Bavistin was effective in reducing the disease severity of sheath blight (Biswas *et al*., 2008).

Plant extracts of *Ocimum basilicum, Citrus reticulata, Syzygium aromaticum, Jasminum officinale, Tagetes erecta* and 0.05% Palmarosa oil showed mycelial inhibition at higher concentration (Janki Kandhari *et al*., 2000).

Resistant Varieties

Two cultivars, Andrewsali and Manoharsali, show resistance against sheath blight disease**.**

Bakanae or Foot Rot Disease of Rice

Bakanae or foot rot disease of rice is an important disease across the world. The incidence of bakanae disease has increased steadily particularly on aromatic rice cultivars in all the rice growing states of India,% which causes severe qualitative and quantitative losses to rice crop (Bashyal *et al*., 2014; Gupta *et al.,* 2015). Yield losses ranging from 15-26% have been reported from Uttar Pradesh, Assam, Andhra Pradesh, Tamil Nadu, Haryana and Punjab states of India (Sunder et al., 2014).

Bakanae disease was first identified during 1828 in Japan. Hori (1898), for the first time reported the fungus *Fusarium heterosporium* induced bakanae symptoms in rice plants. It was later put in genus *Gibberella* under the name *Gibberella fujikuroi* (Ito and Kimura, 1931). In 1917, Sawada reported that elongation of rice seedlings might be due to some stimuli derived from Bakanae fungus hyphae. Kurosawa (1926) demonstrated the hypertrophic effect and elongation effect from culture filterate of dried rice seedlings, rice plants and other weeds which was also called as bakanae effect of fungus and owing to this, the disease is called as Bakanae disease. Kurosawa also reported that bakanae fungus secretes a chemical substance which suppresses the chlorophyll formation and encourages stem elongation. This

disease has been reported from South East Asia, Africa, America and European countries. It is also a serious problem in countries like Japan, Taiwan, Thailand, Turkey, California and Philippines.

Symptoms

Bakanae disease of rice is also known as 'foot rot' in India, 'white stalk' in China, 'man rice' in Philippines and 'bakanae byo' or 'elongation' disease in Japan. Bakanae is a Japanese word which means bad naughty seedling, referring to the abnormal elongation and then 'noodle seedlings', 'foolish seedling' or 'stupid rice crop' (Sun and Snyder, 1981). Bakanae disease is caused by one or more *Fusarium* species and complex of disease symptoms including seedling blight, root rot, stunting and crown rot. The most important symptoms of etiolation, excessive elongation of infected plants, foot rot, seedling rot, grain sterility, with ultimate effect on yield have been reported from different parts of world. The infected seedlings appeared taller, with chlorotic stems and leaves which turn yellowish green to pale; in later stages of growth, the disease can be noticed in nursery beds and fields from a distance (Fig. 17.8).



Figure 17.8 Symptoms of Bakanae disease of rice caused by *Fusarium moniliforme* (Courtesy: Dr. Ram Singh, CCS HAU, Kaul, Kurukshetra)

The pathogen attacks every part of rice plant *viz.*, roots, crown, stem, leaf sheath and panicles throughout the growing season. During crop growth, some of the infected plants die but some infected plants survive up to maturity and they have tall, thin, lanky tillers with white empty chafty panicles. Generally, panicles topple down due to their weight and eventually plants die prior to their maturity. Sporodochia of pathogen could be seen at the junction of lemma and palea of the damaged grain. In India, the development of adventitious roots from lower nodes of stem has been reported and the disease is called foot rot (Thomas, 1931).

Causal Organism

The disease was first described in 1828 in Japan by Ito and Kimura 1931, but it is not clear till today which *Fusarium* species is associated with multiple symptoms of the disease. Initially, the pathogen responsible for bakanae disease of rice was identified as *Fusarium moniliforme* Sheldon, later it was re-identified as *Gibberella fujikuroi* Nirenberg, the anamorph of *Gibberella fujikuroi* Sawada. The term *fujikuroi* was derived from the name of Japanese plant pathologist, Yosaburo Fujikuro. *Fusarium fujikuroi* is regarded as the most virulent species causing bakanae disease because it produces excessive gibberellin hormone, which is responsible for internode elongation of bakanae infected plants.

Morphologically, *F. fujikuroi* forms white mycelia on PDA at 25±1 °C that become violet or magenta with age. Sporodochia are normally absent but if present, they contain macroconidia. Microconidia are oval or club-shaped with a flattened base and 1-2 septa while, macroconidia are slender with tapered apex and 3-5 septa.

Disease Cycle

Bakanae is a monocyclic disease and the pathogen is both soil-borne and seed-borne. The seed-borne inoculum is a more significant source and soil-borne inoculum is reduced with passage of time. The pathogen survives under unfavourable conditions as spores on the coat of infected seed and as hyphae or macro conidia in infected crop residue in the soil (Saremi *et al.*, 2007). The fungus survives in infected stubbles for a long time. The secondary infection is by conidia which are disseminated by water and wind, causing new infections in the rice field. The seed-borne inoculum provides the primary infection. Under favourable conditions, the infected plant produces numerous conidia that subsequently infect new neighboring plants. Higher doses of nitrogen fertilizers reduce the population of pathogen in soil. The disease incidence is favoured by high temperature and high relative humidity.

Disease Management

Agronomic Practices Since the pathogen is mainly seed-borne, the effective means of management of this disease is the use of clean, non-infected seeds. Salt water (brine solution) is used to separate the light-weight seeds from the seed lot, which may reduce seed-borne infection (Cother and Lanoiselet, 2002). According to Gupta (2004), burning or destruction of crop residue may provide some benefits by limiting the amount of inoculum that may carry over to the next crop. Selection of proper geographical area, crop rotation, selection of resistant varieties, balanced fertilizers and irrigation management help in reducing the disease (Burgess *et al*., 1996). Bagga *et al.* (2007) found minimum disease incidence in late planted rice crop by the end of July which may be due to the low temperature during the infection period. A mixture of NPK, $ZnSO_4$, $FeSO_4$ reduced the survival of pathogen either through a direct toxic or stimulatory effect in the soil.

Sasaki (1987) observed that the pathogen can be destroyed by keeping the diseased seeds under snow or in the cold room at 5°C. Incorporation of neem cake and groundnut cake in soil has been reported to suppress the pathogen (Panneerselvam and Saravanamuthu, 1996).

Hot Water Treatment Thermal treatment is the best substitute for chemical fungicide in organic cultivation. Miyasaka *et al.* (2000) and Yamashita *et al.* (2000) recommended the soaking of seeds in hot water at 60^0C for 10 minutes before sowing, for reducing the seed infection.

Chemical Treatment According to Gupta *et al*. (2014), seed treatment with fungicides is the most common management practice for bakanae disease in India. The control of bakanae disease has been achieved by soaking the seeds in 0.39% sodium hypochlorite solution for two hours, as it reduces the inoculum load by 80 to 90% (Webster and Gunnell, 1992). Seed soaking in 0.1 % solution of organo-mercury compounds for 16-24 hours or 0.25% solution for 2 hours is recommended in Japan. However, longer soaking at higher concentration was phytotoxic (Ou, 1985). Fungicidal seed treatment with Benomyl, Thiram, Benomyl + Thiram, Carboxin + Thiram, Fludioxonil, Mancozeb, Iprodione + Triticonazole, Prochloraz at 1-2% of seed weight was found effective in different countries (Bagga and Sharma, 2006; Bagga *et al.,* 2007; Karov *et al.,* 2009; Ora *et al.,* 2011). Seed treatment followed by soil treatment with Derosal (Benzimidazole fungicide) was found effective in controlling the disease (Bhalli *et al*., 2011). Bagga and Sharma (2006) found seedling treatment with Carbendazim or Benlate (0.19) significantly reduced the disease incidence up to 92%. Treatment of seeds with Bavistin 50 WP (0.05%) plus Sterptocycline (0.019) and smearing the seeds with talc formulation of *Trichoderma harzianum* (15 g/Kg seed) was found effective against bakanae disease (Pannu *et al.,* 2009).

Biological Control Many workers from different countries found that fluorescent Pseudomonas is effective under *in vitro,* green house and field conditions. Soaking seeds with strains of *Bacillus* and *Pseudomonas* have protected the seedlings from infection owing to suppression of inoculum present on the seed. The mode of action is the detoxification of fusaric acid as *P. solanacearum* has a capacity to detoxify fusaric acid (Toyada *et al*., 1988). It is also possible that extracellular and heat-stable proteinases produced by *Pseudomonas* might be responsible for in inactivation of polysaccharide hydrolases of pathogenic strains of *Fusarium* spp. (Pietr, 1990). According to Luo *et al*. (2005), *Bacillus subtilis* and *B. megaterium* have also been found effective against bakanae disease. The most common biocontrol agents *Trichoderma strictipilis*, *T. atroviridae* and *T. neokoningii,* reduce the growth of bakanae pathogen (Nagamani *et al.,* 2012). In India, *P. fluorescens* and *T. viride* alone and in combination were found effective in controlling the disease. Compost and vermicompost tea produced with active aeration typically dominated by bacteria have been found effective for the suppression of bakanae disease of rice. Plant extracts having antifungal activity have been reported by several researchers against *Fusarium moniliforme.* Yasin *et al*. (2003) reported maximum inhibition of mycelial growth of *F. moniliforme* by the leaf extract of *Lawsonia inermis*, followed by root extract of *Asparagus racemosus*. The presence of antifungal agent lawsone (2-hydroxyl-1,4, napthoquinone) in the leaf extract of *L. inermis* might have been responsible for fungal growth inhibition. Antifungal properties of leaf extract of *Andrographis paniculata*, *Lagerstroemia speciosa*, bark extract of *Eucalyptus citricola*, essential oils from *Cymbopogon citratus*, *Thymus vulgaris* were found effective against *F. moniliforme* in controlling the seed infection by 95%. Sunder *et al.* (2014) identified highly resistant genotypes, namely HKR96-561, HKR96-565, HKR 07-40, HKR 07-53, MAUB 2009-1, PA 3456-46-6-1-1 and PNR 600 through seedling root dip method.

Quarantine is a reasonable path of action to prevent inadvertent introductions of dangerous plant pathogens or to attempt eradication of new incursions. A certificate issued by quarantine department indicating that a regulate article is not contaminated by pathogen may be allowed to move any destination. The Mississippi Department of Agriculture and Commerce, USA, has placed *G. fujikuroi* (bakanae strains) under quarantine regulations in the year 2009.

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Questions

1. How do wilts differ from root rots? Discuss in brief the physiology of hydromycotic wilting in plants.
2. Are toxins alone involved in vascular wilts?
3. How does browning occur in vascular wilts?
4. Do enzymes play a role in vascular wilts?
5. Are vascular wilt pathogens primitive or advanced? Discuss critically in brief.
6. What are form species?
7. Name some of the important genera involved in vascular wilts.
8. What is vascular occlusion?
9. How will you control vascular wilts?
10. American cottons are resistant to wilt when grown in India and Indian cotton varieties are resistant to wilt when grown in America. Why is this so?
11. Why are *Rhizoctonia* diseases more common in sandy soils and Fusarial wilt in black cotton soils in India?
12. What is soil solarization? How is this used in controlling stem rot of paddy?
13. Does Dutch elm disease occur in India? How is this controlled?
14. What is anamorph and teleomorph of the fungal pathogen of stem rot of rice?
15. What causes the Bakanae disease of rice? Discuss in brief symptoms, causal organism, disease cycle and management of the disease.

Leaf Spots, Leaf Blights and Anthracnoses

18

Leaf Spots and Leaf Blights

'Blight' implies sudden and extensive damage to leaves, but in many leaf diseases the area of necrosis is limited, which gives the appearance of a spot. These spots may be big or small, depending upon the organism responsible for them and the environmental conditions. Leaf spots can also be due to unfavourable water relationships or temperatures, mineral deficiencies or excesses, or be caused by insects. Viruses can also cause leaf spots, particularly "ring spots".

Species of *Erwinia*, *Pseudomonas* and *Xanthomonas* may induce leaf spots and blights of plants. Fungal leaf infections are of two types, depending upon the activity of the pathogen. Some leaf-infecting pathogens are actually saprophytic, killing susceptible cells by enzymatic and toxic actions well in advance of themselves, while others are truly parasitic, obtaining food from living tissues, which die as a result of attack by the pathogens.

Fungal leaf spots are caused by a large number of fungi mainly belonging to Fungi Imperfecti, such as species of *Gloeosporium*, *Colletotrichum*, *Sphaceloma*, *Phomopsis*, *Phoma*, *Phyllosticta*, *Ascochyta*, *Diplodia*, *Botryodiplodia*, *Septoria*, *Alternaria*, *Helminthosporium*, *Cercospora*, etc.

Leaf spots and leaf blights resemble each other considerably in the expression of symptoms as well as mechanism of pathogenesis. In the leaf blight, a larger area of leaf or the whole leaf is involved. In certain cases, it may involve the whole plant. The limited development of lesions caused by many leaf spot pathogens has been attributed to several causes, hypersensitivity being a common cause. Akai (1951) showed the formation of abscission cells around necrotic areas in peach leaf spots, induced by *Xanthomonas pruni.* Infection by *Cladosporium carpophilum* leads to perforation of spots in the leaves of *Prunus amygdalus.* Formation of an effective cork barrier around the lesion prevents its further development, or limitation of the lesion may mark the point at which the defensive reactions of the parasitized tissue are able to halt the pathogen. In unspecialized leaf spot fungal pathogens, the fungus generally produces its reproductive structures in the center of the lesion in the dead areas, while in specialized ones, such as *Phytophthora infestans*, the sporangia are produced mostly at the margins of the necrotic spots.

Leaf spot diseases of crop plants have not received much attention. Bilgrami (1963) and Tandon (1967) gave a comprehensive survey of leaf spot diseases of ornamental plants and fruit trees respectively. Suzuki (1965) discussed the histochemistry of foliage diseases.

Bacterial and viral leaf spot pathogens have been discussed under bacterial and viral diseases. Some of the fungal leaf spots or blights have been discussed under downy mildews, while some others are discussed in this chapter.

Leaf Spot of Turmeric (*Curcuma longa*)

The disease is common along the coastal areas of Orissa and Andhra Pradesh and in the turmeric growing areas of Gujarat and Tamil Nadu, where it causes considerable damage. The disease is also quite common in northern India, where it causes considerable damage and in certain areas, almost all the leaves on the plant are infected.

Symptoms

The spots are produced on both surfaces of the leaves but are more prominent on the upper surface. Individual spots are comparatively small and are light yellow to dark yellow. Spots, however, form larger patches causing the drying up of the entire lamina (Fig 18.1a) Attacked plants are not killed, but due to excessive spotting and destruction of the green tissue of the leaf, there is a heavy reduction in yields. It may be emphasized that the pathogen does not stimulate the hyperplasia and hypertrophy of tissues in contrast to peach leaf curl caused by *Taphrina deformans.*

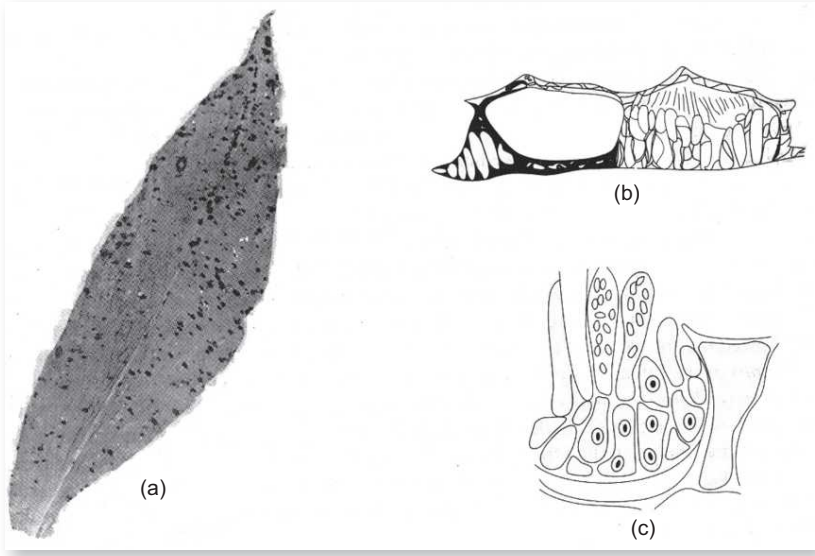


Figure 18.1 Leaf spot of turmeric caused by *Taphrina maculans:* (a) symptoms (b) mycelium in the walls of epidermal cells (c) asci and ascospores

Causal Organism

Taphrina maculans Butler is the causal organism. The mycelium is formed between the cuticle and the epidermis or inside the epidermal cells. Branched or lobed haustoria are sent within the host tissues. The

hyphae are confined to the epidermis and may grow down in the vertical walls of the next layer of cells but do not penetrate further. When the spots are fully formed, an almost continuous layer of hyphae occupies their central portions. The outermost cells of this layer grow into cylindrical or club-shaped, thin-walled projections which rupture the epidermis and become asci (Fig. 18.1b and c). All the outer cells of the fungus are ascogenous but the asci mature at different intervals, so that ripe asci are formed in little groups. Basal cells are also seen below each ascus. The asci measure 20-30 × 6-10 µm and normally contain eight ovoid, hyaline, unicellular ascospores, which measure 4-7 × 2-3 µm. Ascospores multiply within the ascus by budding, to form sprout conidia.

Disease Cycle

The exact mode of transmission of the fungus is not yet known. The duration for which the ascospores remain viable in plant debris has not yet been ascertained. The possibility that the mycelium reaches the rhizomes to remain there in a dormant state and then gives rise to the disease when the rhizomes are sown the following season is remote. It is just possible that the inoculum might be surviving on the dried infected leaves in the soil in the fields from which the asci might be the chief source of the subsequent year's infection.

Disease Management

Some of the cultural methods like removal of diseased leaves should be adopted. Timely spray of Bordeaux mixture (5:5:50), Perenox, Fytolan, Blitox 50, and Dithane Z-78 checks the spread of the disease. Upadhyay and Pavgi (1967) reported some of the resistant varieties like 'China' and 'Jaweli'. According to Rathaiah (1982), cultivars Ca 69 and Shillong are resistant to leaf spot of turmeric.

Brown Spot of Maize

Shaw first noted this disease in Bihar in 1910. Since then, it has been reported from other parts of the world, such as China, Japan and the USA. All types of maize are susceptible. *Euchlena mexicana* (teosinte) is the only other known host. *Euchlena mexicana* is used as a cereal and fodder, like *Zea*, and is similar to maize but the female spikelets are free from one another and do not form a cob. The brown spot disease of maize is of minor significance in India but becomes severe under favourable environmental conditions. Lal and Chakravarti (1975, 1976) assessed the loss due to brown spot of maize in Rajasthan and under natural conditions there was a loss of 16 to 21%.

Symptoms

The disease occurs on leaf blade, leaf sheath and culms but rarely on the outer husk of the ear. The spots are more common and severe on the lower portions of the plant than on the upper parts. The first incidence of the disease is the appearance of slightly bleached or yellowish spots about 1mm in diameter which turn darker and finally brown to reddish brown with a lighter margin. Numerous spots may develop in a small area, giving the leaf blade or sheath a rusty brown appearance (Fig. 18.2). Sometimes, the spots coalesce to form larger spots. Spots formed on the midrib and leaf sheath are larger than those on the blade and are irregular in shape. The browning of the host tissue is due to necrosis of the host cells and accumulation of the spores of the fungus. Affected leaves may die prematurely. Spots on culms are generally produced on the nodes, which weaken the plant, causing them to lodge in certain cases.



Figure 18.2 Brown spot of corn (*Physoderma zea maydis*): symptoms on the leaf

Causal Organism

The pathogen responsible for brown spot of maize is *Physoderma zea maydis* Shaw. This is an obligate parasite. The brown spot that appears in the older lesions consists of a large number of separated, thick-walled, spore-like bodies, which are called the resting sporangia or resting spores. These are smooth, brown and flattened at one side where a lid or cap-like structure can be seen. A large number of these sporangia are produced in the infected tissues. The sporangia perennate from one season to the next in the diseased debris or in the soil and germinate in the next season. The sporangia germinate by the opening of the lid like structure and a thin-walled vesicle extends through the opening. The vesicle ruptures at the apex and liberates uniflagellate zoospores (3-4 × 5-7 μm). These zoospores, when come in contact with the leaves, germinate and infect the host cells and on the outside give rise to a slipper-shaped sporangium, which is anchored to the surface of the host cell by means of a rhizoidal system. These are the thin-walled zoosporangia, which on maturity produce about 300 zoospores. Zoospores produced in these sporangia are much smaller; these are the secondary zoospores. The secondary zoospores are supposed to function as planogametes, but fusion in pairs has not been seen.

The infection hyphae expand within the host cell to form several enlarged vegetative cells called "sammelzellen". These cells are intercellular and give rise to other thin hyphae, which in turn give rise to more enlarged cells. These are either directly converted into sporangia or produce special hyphae, which bear terminal spores. When sporangial formation is complete, the mycelium entirely disappears and in the infected host tissues, only the thick-walled sporangia remain. Lal and Chakravarti (1975) have

studied the survival of the pathogen. Sporangia of *P. maydis* survived for seven years under laboratory conditions as well as in soil having moisture content of 15%. Sporangia survived in the host tissues for four years in the field.

High temperature (28-29°C) and abundant moisture during the early growth period of the host are ideal for the occurrence of the disease. The disease is common in low-lying and ill-drained fields. Primary infection is through the soil-borne sporangia and secondary dissemination is by spore dust carried by wind.

Disease Management

Field sanitation and crop rotation reduce the intensity of disease. Development of resistant varieties is the only possible control measure.

Early Blight of Potato

The disease is common wherever potato is grown and under favourable conditions, may cause serious damage to the crop. In areas where both late and early blight diseases occur, the early blight comes earlier than the late blight in the season. While late blight epidemics are predominant in cooler areas, the early blight is free from inhibitions caused by weather conditions and occurs in cool as well as warm areas. The disease also occurs on tomato, chilies, eggplant and related wild hosts. Among the fungal diseases, early blight is the most destructive.

Symptoms

The disease first becomes visible as small, isolated, scattered, pale brown spots on the leaflets. These spots become covered with a deep growth of the fungus. The lowest leaves are attacked first and the disease progresses upwards. In the necrotic spots, concentric rings appear on the older leaves and darkened areas on the stem. This gives a target board-like effect (Fig. 18.3a). There is usually a narrow chlorotic zone around the spots, which fades into normal green and increases with an increase in the size of the spots. There may be a few spots or a large number of them may appear, occupying major portions of the leaf. When the leaf lesions involve larger veins, chlorosis commonly extends well beyond the necrotic spots. This is due to the toxin - alternaric acid, produced by fungus and translocated through the veins. In dry weather, the spots become hard and the leaves curl. In humid weather, the affected area increase and large rotting patches may appear. In severe attacks, leaves shrivel and fall down. In severe infections, all the aerial portions of the plant have a blighted appearance.

Causal Organism

The causal organism for the early blight of potatoes is *Alternaria solani* (Ell. and Martin) Jones and Grout (Fig. 18.3b). The mycelium consists of septate, branched, light brown hyphae, which become darker with age. The hyphae are at first intercellular, later penetrating the cells of the invaded tissues. Conidiophores emerge through the stomata from the dead center of the spots. These are 50-90 × 9 µm, dark colored and borne singly. In culture, they form chains of conidia (the chains are of 2-3 conidia only); the conidia are beaked, muriform, dark colored and 120-296 × 12-20 µm. There are both transverse and longitudinal septa in the mature conidia. *Alternaria solani* produces a non-specific toxin

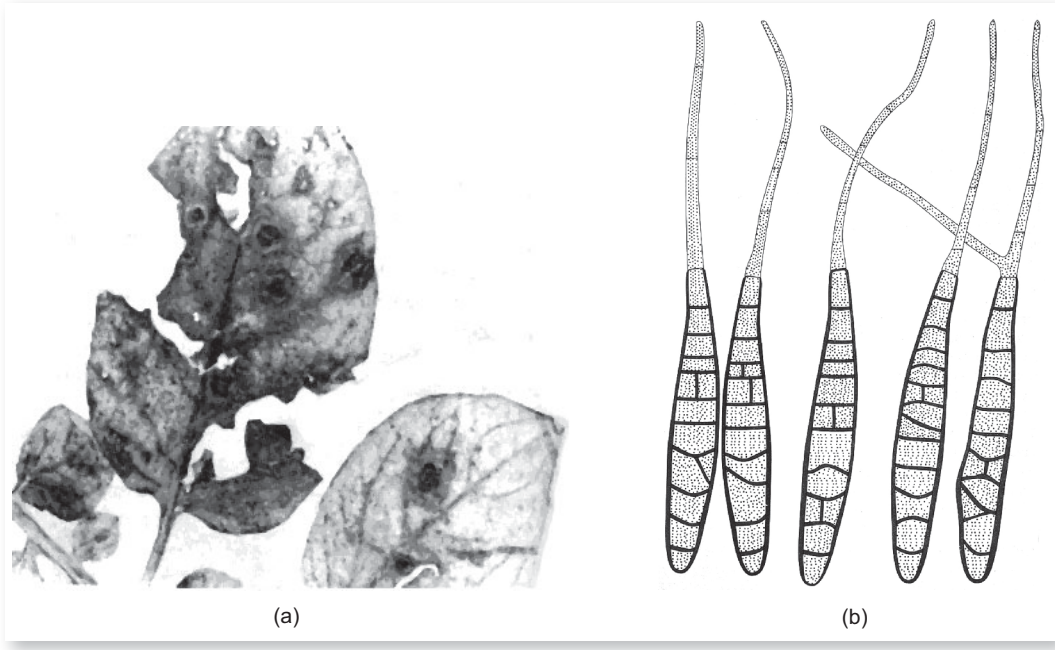


Figure 18.3 Early blight of potato caused by *Alternaria solani* (a) symptoms (b) conidia with long beaks

known as alternaric acid. The role of this acid in disease is doubtful. The causal agent of the early blight of potato usually does not sporulate in pure culture. Padhi and Rath (1973) demonstrated that if cultures are exposed to sunlight, sporulation is induced. Isolation of saltants producing abundant conidia is another method by which the capacity of sporulation can be increased.

Disease Cycle

The pathogen can survive in soil and plant debris, particularly in temperate climate. The primary infection may be through tubers. The mycelium of the fungus remains dormant in dry infected leaves for a year or more. Conidia have been found to retain viability for 17 months at room temperature. Mycelium and conidia thus survive in the soil in diseased plant debris to cause primary infection in the next year's crop. Collateral hosts such as tomato play an important role in the perpetuation and dissemination of the pathogen. The climate and soil exert a considerable influence on the development of the disease. It becomes serious when the season begins with abundant moisture followed by low temperature. Bhatia *et al.* (1972) studied the phenolic contents of resistant and suscept varieties of tomato in relation to early blight disease. The disease is favoured by moderate temperature (17-25°C) and high humidity around 75%. Intermittent dry and wet weather is more conducive for early blight. Madden *et al*. (1978) developed the FAST (Forecaster of *Alternaria solani* on Tomato) predictive system for initiating and timing fungicide sprays on tomato in Pennsylvania. The FAST system uses leaf wetness, air temperature, relative humidity and rainfall to calculate daily severity, and rating values that quantitatively represent conditions favourable for development of early blight.

Disease Management

Since the disease is soil-borne, crop rotation is helpful. Dead haulms should be raked together and burnt. Use only disease free tubers for raising the crop. The crop must be given only balanced doses of fertilizers, especially nitrogen, as the incidence and severity of early blight is generally high in crops receiving imbalanced doses of NPK; particularly, low nitrogen with significant yield losses. The crop, if sprayed with 1% urea at 45 days and given one more subsequent spray after 8-10 days, can easily escape the severe onslaught of early blight (Prasad, 1999). Copper oxychloride (0.30%) sprays have been recommended for the control of early blight of potato. Azariah *et al.* (1962) found Bordeaux mixture (1.0%) to be effective. Apparao *et al.* (1966) from Andhra Pradesh advocated the application of 0.312% Zineb for the field control of this disease. Mathur *et al.* (1971) recommended controlling the disease with Brestan 60, Dithane M-45 and Zineb. Fungicidal sprays, preferably with copper fungicides are quite effective. Zineb, Brestan 60 or Dithane M-45, given at 15-day intervals, effectively controls the disease.

Dahmen and Staub (1992) recommended the application of Difenoconazole (Score), a protectant, curative, and eradicant fungicide for the control of early blight. Some of the moderately resistant varieties against early blight are Kufri Naveen, Kufri Sindhuri and Kufri Jeevan.

Leaf Blight of Wheat

It is a common and destructive disease especially in the northern parts of India. Although the disease was noticed as early as 1924 (Prabhu and Prasada, 1966), it did not cause much concern till very recently when it became widespread. Many of the wheat varieties that are resistant to rust have been found highly susceptible to this disease; the younger seedlings are not affected. It is only when the crop is 7-8 weeks old that the disease first makes its appearance in the field. According to Prasada and Prabhu (1962 a,b), Durum wheats are most susceptible.

Symptoms

The disease first appears as small, discolored lesions, which are irregularly scattered on the leaves. These spots become irregular in shape and then increase in size and appear brown to grey in color (Fig. 18.4). A light yellow zone surrounds the spots. Several lesions coalesce to cover large areas of the leaf and in severe infections the whole leaf dies. Black powdery masses of conidia are produced on the surface of the leaves. Kumar et al (1974) investigated the nature of disease resistance and susceptibility in leaf blight of wheat caused by *Alternaria triticina in vivo* and *in vitro.* The fungus failed to penetrate the leaves of highly resistant varieties, such as *Triticum sphaerococcum.* In highly susceptible varieties, penetration was stomatal and the fungus formed an appressorium before penetration.

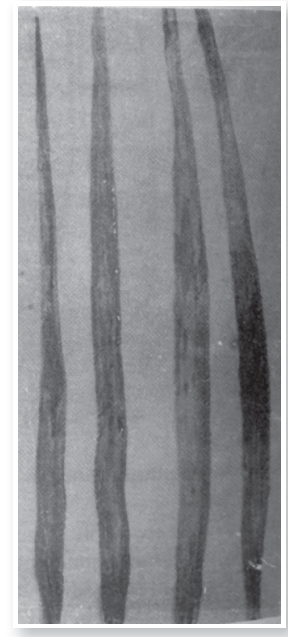


Figure. 18.4 Leaf blight of wheat caused by *Alternaria triticina:* symptoms

Sunil Kumar (1974) investigated the quantitative and qualitative variations in leaf surface wax in three varieties of wheat, varying in their susceptibility to blight caused by *Alternaria triticina.* The wax content was high in the resistant variety (NP 4), low in the susceptible one (NP 830) and intermediate in the moderately susceptible variety (HD 1925). Among the constituents, acetate of n-w hydroxy acid was found in the wax of the resistant variety only.

Causal Organism

The disease is caused by the fungus *Alternaria triticina.* Prasada and Prabhu described it in 1962 for the first time. The mycelium of the pathogen is deep-seated in the embryo of the seed. The pathogen produces conidiophores, which are usually unbranched. However, sometimes they are branched, erect, single or in fascicles and emerge through the stomata. These measure 17-28 × 3-6 µm. Conidia are borne singly or in chains of 2-4. They are oval, with both the ends rounded or ellipsoid and gradually taper into a beak. Conidia measure 15-89 × 7-30 µm. There are 1-10 transverse septa and 0-5 longitudinal septa.

Sokhi and Joshi (1972) demonstrated physiological specialization in *A. triticina* and they distinguished at least two races. Garg *et al.* (1972) had reported the existence of at least six physiologic races in our country. The effect of different sources of nitrogen and carbon on the growth and sporulation of *A. triticina* was studied by Singh and Tyagi (1978a). Asparagine and sucrose supported highest growth among nitrogen and carbon sources, respectively. Singh and Tyagi (1978b) studied the production of polygalactouronase by the pathogen and showed that the enzyme activity was not influenced by divalent cations, such as Mg^{++} and Ca^{++}.

Disease Cycle

The pathogen is both externally as well as internally seed-borne. It also survives in diseased plant debris in soil. Prabhu and Prasada (1967) put forward the possibility of infected seeds being a potential carrier of the disease. Kumar and Arya (1976) studied the role of soil, plant debris and seed on the development of the disease and found that the pathogen perpetuated on the infested seed caused local infections only and was air-borne. Sokhi and Joshi (1976) evaluated the seed-borne infection caused by *A. triticina* in different wheat varieties. Maximum infection of up to 13% was observed in variety NP. 830 and there was no infection in variety Agra local. In the samples received from Punjab, the infection was minimum. *A. tenuis* infection was comparatively more in all the samples in comparison to *A. triticina.*

Sokhi and Joshi (1973b) investigated the relationship between the degree of susceptibility of different varieties of wheat to *A. triticina* and the sugar and phenol contents of their leaves. No correlation between these properties could be established. Kumar *et al.* (1974) investigated the total sugar, nitrogen, protein contents and free amino-acid composition in the leaves of highly resistant, moderately resistant and highly susceptible varieties of wheat. Glutamic acid was found in higher concentration in the leaves of highly susceptible varieties. Sugar contents were lowest in the leaves of highly resistant varieties. Sugars became nearly double in moderately resistant varieties and as high as seven times in highly susceptible varieties. Nitrogen, and consequently protein contents, was maximum in highly susceptible varieties.

Internal infection is deep-seated. As already indicated, 15 days-old seedlings are not susceptible to infection. The virulence of the pathogen increases with an increase in the age of the plant.

The optimum temperature for the germination of the conidia is 15-27°C. A 100% relative humidity is required for maximum germination. The disease develops most at 25°C and this temperature favours maximum growth of the fungus in pure culture.

Disease Management

As the disease is deep-seated, seed-borne infection cannot be removed by ordinary organomercurial seed-dressing (Prabhu and Prasada, 1967). Pre-soaking the seed in water for four hours followed by a ten-minute dip in hot water at 52°C gives satisfactory control. Since the pathogen can also survive on diseased plant debris in the soil, it is essential that suitable sanitary precautions should be observed. Sankhla *et al.* (1972) reported that Cuman was the most effective chemical for checking the vegetative growth of the pathogen and reducing disease incidence. Dithane M-45 and Bisdithane could control the disease significantly at 1% level and Miltox and Vitavax at 5% level. Singh and Singh (1971) have reported effective control by six sprayings of Dithane Z-78 or Dithane M-45, each at 0.25% concentration. Basant Ram and Joshi (1977) reported that Thiram, Dithane M-45 and Zineb proved effective in checking the disease as well as in reducing the loss in grain weight. Sokhi and Joshi (1973a) studied the reaction of wheat varieties to leaf blight.

Tilt 25 EC at the rate of 500 ml/ha has been found very effective in reducing the blight incidence when applied as foliar spray at fortnightly intervals, twice in the crop season (Goel and Sinha, 1996).

Fourteen *Triticum* species were evaluated against *H. sativum* and it was found that diploids such as *T. aegiolopoides* and *T. monococcum* are fairly resistant, while among the tetraploids *T. timopheevi* was found resistant. Some of the resistant varieties reported by many researchers are NP4, NP52, NP200, NP809, Arnautka, E6160, K7340, HP1163, HD1941, Janak, M-134, K8904, K8908 and UP262.

Stripe Disease of Barley

This disease occurs worldwide. Damage to the crop is from the seedling stage to maturity. Losses up to 60% in individual fields may occur.

Long, parallel, brownish streaks appear in leaf sheaths and leaves, and on the latter, the streaks are continuous from the base to the tip of the leaf (Fig. 18.5a). The infection is systemic and the symptoms can, therefore, be seen on all the tillers of a plant and on all the leaves. The yellow stripes soon become brown as tissue necrosis progresses, and finally the tissues dry out and the leaf blade may become shredded. A grey to olive grey mass of conidiophores and conidia develops on the diseased tissues. The spikes fail to emerge in many diseased plants. Those that emerge are blighted, twisted, compressed and brown in color.

Singh and Siddiqui (1986) estimated that there are 22% less grains in diseased ears but Mathur and Bhatnagar (1991) had estimated losses up to 31.9% when 24.2% tillers are infected.

Causal Organism

The disease is caused by *Drechslera graminea (*Rabenh. *ex* Schlecht) Shoemaker and was earlier known as *Helminthosporium gramineum* Rabenh ex Schlecht (Fig. 18.5 b). The perfect stage of the fungus is *Pyrenophora graminea* Ito and Kuribay. The mycelium in the tissues is abundant and sub-hyaline to light yellow. In culture, the mycelium varies greatly in color from grey through olive to black. In culture, the pathogen does not produce conidia unless it is exposed to cyclic changes of light and darkness. The conidiophores and conidia are produced on the plants at the time of heading of the susceptible plants. Conidiophores are borne in fascicles of 3-5. The basal segment is enlarged, the distal portion is slender and the color is grey to olivaceous. The conidia are sub-hyaline to yellowish brown, straight, sub-cylindrical

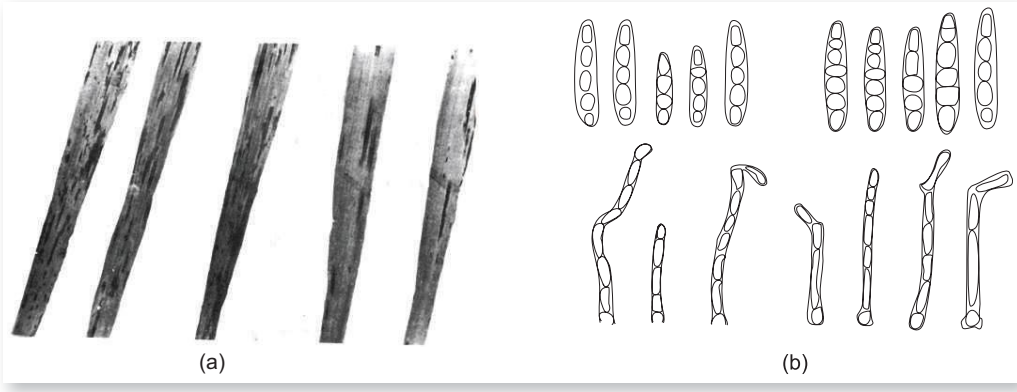


Figure 18.5 Stripe diseases of barley caused by *Drechslera graminea* (*Helminthosporium gramineum*): (a) symptoms (b) conidiophores and conidia

to slightly taper, having rounded ends, 1-7 septate without any constrictions at the septa. Conidia, on an average, are 105 × 20 μm in size. Germination takes place generally from both the end cells.

Disease Cycle

The natural infection from conidia of *Drechslera graminea* occurs at or soon after the flowering of the barley spikelet. The mycelium is established on or in the pericarp or in embryo tissues before the kernel matures. The mycelium remains viable in the seed for an indefinite length of time. The seed-borne inoculum is the major source of perennation of the disease from season to season. The pathogen can also survive in the form of mycelium in the soil on stubbles of the previous year's crop. Infection usually takes place on the meristem of the germinating seedlings. This seems to be the only species in the genus *Drechslera* in which a systemic type of infection occurs. Conidial production is synchronized with the heading, blossoming and early stages of kernel development of the suscept. The conidial formation is a direct response to the physiology of the suscept tissues.

Environmental conditions influence floral infection and disease development in the seedlings. Sufficient moisture to wet the spores is necessary for floral infection. The disease is, therefore, more common in areas where there is heavy dew or rainfall during flowering. A cool, moist, fertile soil favours the stripe development in the seedling and developing plant. The disease is favoured by deep placement of seeds in soil (Mathur and Bhatnagar 1990 b).

Disease Management

Amongst the cultural methods recommended, field sanitation is best to reduce the soil-borne inoculum. Seed treatment with organomercurials or soaking of seeds in 0.3% solution of copper sulphate or 0.5% ferrous sulphate or 0.5% zinc sulphate has been found effective against seed-borne inoculum (Mathur and Bhatnagar, 1990a).

According to Paul and Sharma (2002), the aqueous extract of neem leaves (*Azadirachta indica*) provides good control of the disease through indirect means. The extract induces high activity of enzyme phenyl ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) along with rapid accumulation of fungi-toxic phenolic compounds. Some of the resistant varieties are K12, CN292, K40, K69, K30,

K572-22-64/69, K24, K125, K129, K131, K133, K441-7, Morocco, Algerian, VL10, VL11, HBL107 and HBL 108.

Brown Leaf Spot (*Helminthosporiosis*) Disease of Rice

Like blast, brown leaf spot (also known as Sesame spot, helminthosporiosis, seedling and leaf blight) of rice is a widespread disease in almost all the rice growing areas of the world. In India, the disease is prevalent in all rice growing areas, especially in heavy monsoon areas in West Bengal, eastern parts of U.P., Assam, Tamil Nadu and parts of Kerala. The disease is seed-borne and rice seeds have been indiscriminately taken in the past from India to other countries without any precautions.

The disease is of great importance in several countries and has been reported to cause enormous losses in the leaf-spotting phase when it can assume epiphytotic proportions. Two major epidemics have been recorded in India. The most notorious of these outbreaks was in Bengal in 1942, when loss in yield of up to 90% was recorded (Ghosh *et al.*, 1960). Nothing more devastating than the Bengal epiphytotic of 1942 has been recorded in plant pathological literature. Padmanabhan (1973) discussed the great Bengal famine. Bedi and Gill (1960) reported losses in the weight of grains ranging from 4.6-29%.

Symptoms

All the aerial parts show symptoms of the disease; irregular, rectangular brown spots with a dark center appear on the coleoptiles, leaf blades, leaf sheaths and glumes, but are most commonly seen on the leaves. Spots first appear as minute brown dots, later becoming ellipsoidal or oval to circular and when fully developed, have a light brown, tan or grey center and a dark or reddish-brown margin (Fig. 18.6 a). Seedlings are often heavily attacked with numerous spots, about 2.5 mm in diameter; in such cases, leaves may dry out and die. Badly affected nurseries can be very easily distinguished from a distance by their brownish scorched appearance, but seedlings are not usually killed. Spots also appear on the older leaves where they become larger than those on the seedlings. These may be as much as 1 cm in length but are usually smaller. On the coleoptiles, the spots are brown and small. On the glumes, the disease is indicated by dark brown or black oval spots; when the attack is more severe, the whole surface of the grain may be blackened and the seeds may be shriveled and discolored. Thus, there are three types of infections, resulting in losses. The first is due to poor germination of infected seeds, the second is leaf infection resulting in the reduction of the effective leaf surface and the third is the attack on the grains themselves.

Causal Organism

The pathogen responsible for the brown spot disease of rice is *Drechslera oryzae* (Breda de Haan) Subramanian & Jain, earlier called as *Helminthosporium oryzae* Breda de Haan [= *Cochliobolus miyabeanus* (Ito and Kuribayashi) Drechsler ex Dastur]. The genus *Drechslera* was erected by Ito in 1930 for those species of *Helminthosporium* with cylindrical conidia that germinate from any cell. *Drechslera* was accepted and reviewed by Shoemaker (1959, 1962), who redefined the genus and transferred some of the species that germinate only from the end cells into the genus *Bipolaris*. In *Helminthosporium*, the conidiophores are determinate ceasing growth with the production of the apical conidia. The fungus grows both intra and intercellular within the tissues. Conidiophores arise as lateral branches from the hyphae, usually in tufts, and emerge through stomata or wounds.

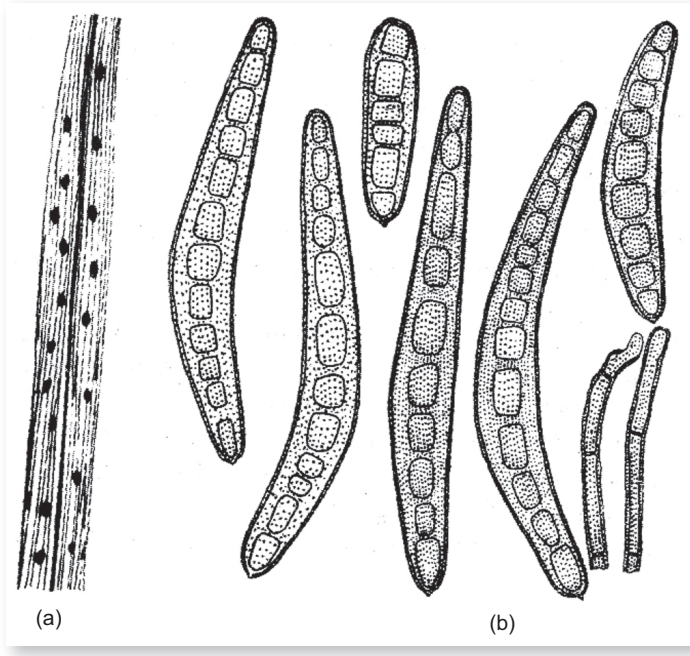


Figure 18.6 Leaf spot of rice caused by *Drechslera oryzae:* (a) symptoms (b) conidiophores and conidia

Conidiophores are short, erect, branched only at bases, short, segment, and dark brown to olivaceous at the base and somewhat paler at the growing tip. Scars mark the points of attachment of successive conidia at regular intervals in the conidiophores. Conidia are olivaceous, brown, slightly curved, tapering towards the rounded ends and vary greatly both in size as well as in the number of septa (Fig. 18.6b).

The perfect stage of the fungus is *Cochliobolus miyabeanus*, which has been obtained in culture as well as in diseased stubble in Japan and Mexico. Perithecia in culture are globose, pseudoparenchymatous, and black, with an ostiolar beak. Asci are cylindrical to long, fusiform, slightly curved and usually contain 4-6 ascospores. The ascospores are filiform and are formed in a close helix in the ascus.

The fungus produces two toxins viz., Cochliobolin and Ergosterol, the former (Cochliobolin toxin) is toxic to rice seedlings. The toxin also affects respiration of the plant by disturbing the equilibrium of the protoplasm. The fungus also produces proteolytic enzymes, which are responsible for the breakdown of protein fragments of the cell walls (Vidhyasekharan *et al*., 1986).

Disease Cycle

The disease is soil and seed-borne. The fungus may live for two to three years in infected plant parts, particularly the seeds, and the disease often starts from infected seeds. In India, it has been found that the conidia are present in the air near rice fields throughout the off-season (Ghosh *et al.*, 1960), and in many parts of the subtropics, such conidia could form another source of primary infection. Several grasses can be infected by *C. miyabeanus* artificially and such grasses (e.g. *Echinochloa colorum* and *Leersia hexandra*), which grow around rice fields have been reported to be natural hosts of the fungus.

Gangopadhyay and Chattopadhyay (1976) studied the carbohydrate contents of rice leaves under varying levels of nitrogen. Reducing sugars (RS) were positively correlated while non-reducing sugars (NRS) and acid hydrolysable polysaccharides (AHP) were negatively correlated with the brown spot disease development. It was not the total carbohydrates but the relative preponderance of RS, NRS and AHP, which determined the susceptibility of rice leaves to the brown leaf spot. Polyphenol oxidase activity in infected tissue has been found to be higher than in non-infected one (Chattopadhyay and Sammadar, 1980 b). Peroxidase activity was found to be higher in resistant rice cultivars (Chattopadhyay and Bera, 1978). According to Sridhar and Mahadevan (1979), prohibitins were the first chemical barriers of resistance to *Drechslera oryzae.*

Gangopadhyay and Chattopadhyay (1974) studied the correlation between the leaf angle and the incidence of brown spot disease of rice. The disease incidence increased in rice with an increase in the leaf angle. The narrower angle and topmost leaves always had lower infection values. High yielding and high nitrogen responsive varieties were associated with narrower angles and lesser brown spot development.

Disease Management

Cultural Methods

1. After the harvesting, the stubbles should be burnt so that there is no soil-borne inoculum present in the soil.
2. All the alternate/ collateral hosts like *Setaria*, *Leersia*, *and Echinochloa* etc. that harbour the pathogen in off-season should be removed.
3. During irrigation, the water from infested fields should not pass into healthy fields.
4. Leaching down of the important elements like K, Fe, and Mn during rainy season should be checked, as leaching of these elements from the soil makes the plants more susceptible to the disease.
5. Dry conditions of the soil during plant growth should be avoided. It has been observed that flooding the field when soil temperature is 20-28°C will prevent conidial germination.
6. Severity of the disease decreases with increase in the potassium level of the soil during growth period of the plant. Vidhyasekharan *et al.* (1983) observed that while excess of nitrogen predisposes plants to infection. Kaur *et al.* (1986) reported that 30 ppm Calcium reduces disease while 50 ppm increased it. It was concluded that brown spot symptoms are the product of complex nutritional stress.

Chemical Control Since the disease is seed-borne in many regions, the seeds should be disinfected with a suitable fungicide such as Ceresan or Agrosan GN. In Japan, seed treatment with mercuric chloride, silver nitrate, copper sulphate, calcium hypochlorite, formaldehyde and phenol has been found quite effective in controlling the seed-borne inoculum. Seed treatment may ward off the seedling blight phase. Spraying copper fungicides or Dithane M-45 (0.3%) at regular intervals can control the disease. Several other fungicides like Chlorothalonil, Iprodione, Vinclozolin and Maneb can be applied at the onset of the disease and then repeated at 10-15 day intervals helps in controlling the disease considerably. Some of the antibiotics like Aureofungin (200 ppm) in combination with copper sulphate gave good control of the disease. Apart from antibiotics, some sulpha drugs like sulphanilamide and antibiotics like Nystatin and Griseofulvin have also been used for seed treatment.

Hot water treatment at 55°C for 10 minutes gives better control than chemical seed treatment.

Secondary air-borne spread of the disease can be checked by chemical treatment. Two to three sprays with Bordeaux mixture (5:5:50) or Dithane Z-78 (0.2%), Hinosan (0.1%), Aureofungin (25 ppm), Blitox (0.3%), Benlate (0.2%) had been found effective in controlling the disease. Some of the systemic fungicides like Brestanol, Brestan, Tricyclazole (0.08%) have been found to give good control of the disease.

Resistant Varieties

Growing resistant varieties is the best way to control the disease to under fungicidal umbrella (Chaube and Pundhir, 2005). Some varieties have been bred in different centers in the country.

According to Singh and Sharma (1975), the varieties Padma and IR-24 are comparatively resistant and should be grown in areas where epiphytotics occur.

Blast Disease of Rice

Blast disease has been reported from almost 70 rice-cultivating countries of the world. It has, for a long time, been recognized as a major problem of rice production in Japan, Taiwan, the USA and many other countries. Losses due to blast may range up to 90%, depending upon the part of the plant infected. It is a major disease of rice and is most destructive in South India, particularly from Andhra Pradesh, Kerala, Maharashtra, Gujarat, Orissa and Tamil Nadu along with Uttar Pradesh, Jammu and Kashmir and other rice-cultivating areas of the country. Padmanabhan (1963) has critically reviewed the methods that could be adopted for estimating the loss caused by blast. Bhat and Singh (1992) have recognized this disease of international importance and had reviewed the work done on this disease till 1992.

Symptoms

The disease produces brownish lesions and spots on leaves, leaf sheaths, culms and panicles and finally the foliage may be completely blasted. Lesions on the neck of the culm and panicle branch bases result in typical “rotten necks”. There are also nodal infections (Fig. 18.7a, b, c, d).

The leaf spots are typically elliptical with more or less pointed ends. The color of the spots is usually grey or whitish and the margin is usually brown or reddish brown. However, both the shape and color of the spots vary, depending upon environmental conditions, the age of the spots and the degree of susceptibility of the rice variety. The spots are usually small, water-soaked, whitish, greyish or bluish dots. They enlarge quickly under moist conditions; they are 1-15 cm long 0.3-0.5 cm broad, and usually develop a brown margin. Spots on susceptible varieties growing under moist, shaded conditions show very little brown margins but instead sometimes have a yellow halo around the spot. On highly resistant varieties, only minute, brown specks, each of the size of a pinhead may be observed. Varieties with intermediate reaction show small, round or short elliptic lesions, a few millimeters long, with a brown margin.

Brown to black spots or rings are formed on the rachis of the maturing inflorescence. Ears may also show similar small spots. The most characteristic symptoms appear on the culm. The neck becomes shriveled and covered with a grey fluffy mycelium. Examining the bluish patches on the neck or the stem can help in easily identifying the affected plants. If the infection has occurred before grain formation, the latter are not formed and the panicle hangs down. However, due to necrosis of neck tissues, the ears tend to break and fall off. This stage of the disease causes maximum damage.



Figure 18.7 Blast of rice (*Pyricularia grisea*): (a) symptoms on inflorescence and leaf (b) symptoms (spindle shaped spots) on the leaves (c) node blast (d) neck blast

Causal Organism

The causal organism of blast disease is *Pyricularia grisea* (Cooke) Sacc. (*Pyricularia oryzae* Cav.). The perfect stage of the fungus is *Magnaporthe grisea* (Herbert) Yaegashi and Udagawa. Luttrell *et al.* (1960) opined that the morphological similarities of the graminicolous strains of *Pyricularia* were so great that they should all be regarded as specialized forms of *P. grisea*, the oldest accepted binomial,

accompanied by an appropriate forma specialis designation. Rossman *et al.* (1990) suggested that the correct name of the blast pathogen should be *P. grisea.* Thirumalachar *et al.* (1956) suggested the inclusion of *P.oryzae* under the family Dematiaceae on account of the dark color of the mycelium of the pathogen.

The mycelium of *P.grisea* consists of septate, multinucleate, branched hyphae. Conidiophores are single or in fascicles, simple, rarely branched, showing sympodial growth. Conidia formed singly at the tip of the conidiophore at points arising sympodially and in succession, pyriform to obclavate, narrowed towards tip, rounded at the base, 2-septate, rarely 1 or 3-septate, hyaline to pale olive, 14-40 × 6-13 µm with a distinctly protruding basal hilum (Fig. 18. 7e). Chlamydospores are often produced in culture, thick-walled, 5-12 µm in diameter.

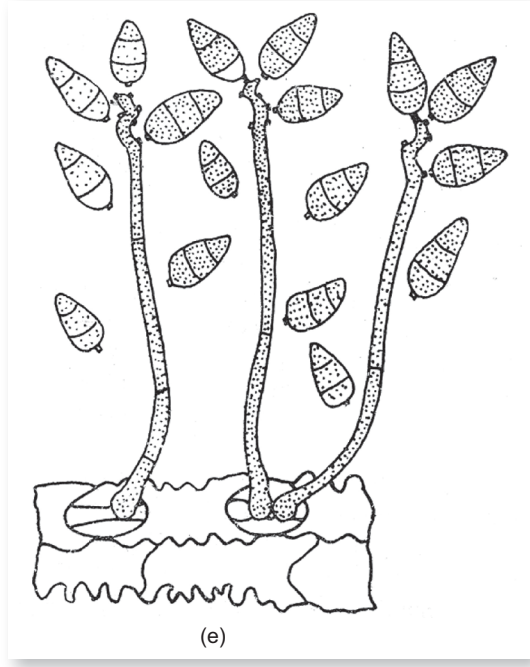


Figure 18.7 (e) *Pyricularia grisea:* conidiophores and conidia

The conidia of *P. grisea* form appressoria at the tips of the germ tubes when they germinate on the host plant, or on a glass slide or a cellophane sheet. Appressoria vary in size and shape. They are smooth, thick-walled, ranging from 5-15 µm in diameter, globose, ovoid, or oblong. *P.grisea* consists of many physiologic races, which differ in their ability to infect rice varieties. Thirteen races are reported from Japan, ten from Korea, nineteen from Taiwan, thirty-two from India (Chakrabarti *et al.*, 1966, Padmanabhan *et al.*, 1970), and fourteen from Columbia and twenty-five from the USA. Pathologists at the International Rice Research Institute identified eighty-one races in the Philippines. The pathogen produced two toxic substances, pyricularin and a-picolinic acid – in culture filtrates as well as from severely diseased plant tissues. Pyricularin is stable in water, especially on the acidic side. The toxin inhibits the growth of the rice seedlings as well as germination of the conidia of the fungus. Pyricularin may be detoxified by chlorogenic acid and ferulic acid, the two naturally occurring phenolics in rice plants. Varieties of paddy with more chlorogenic acid and ferulic acid are more resistant to blast infections.

Disease Cycle and Predisposing Factors

The blast disease is common where rice is grown from 9^0-45^0 NL. The fungus survives through hot dry months in the tropics and through the cold winter in the sub-tropics and in temperate rice areas by very different means. One common method of survival is through the infection of collateral hosts, such as sugarcane (De Gutirrez, 1954), *Setaria intermedia* (Pawar and Kulkarni, 1954), *Digitaria marginata*, *Dinebra retroflexa*, *Panicum repens*, *P. proliferum*, *Brachiaria mutica*, *Arundo donax*, *Leersia hexandra* (Padmanabhan, 1965), *Echinochloa crusgalli*, *Digitaria sanguinalis*, *Stenotapharum secundatum*, *Eremochloa ophiuroides* (Johnson, 1954).

Epidemiology

The carry-over of the blast inoculum from one season to the next has not been fully understood, except perhaps in the northern hilly regions, where the pathogen can survive the winter in infected plant parts under snow. However, it is doubtful whether the pathogen can survive in the infected plant debris or in the soil through the hot and dry summer months in the principal rice growing regions in the plains of India. Kapoor and Singh (1977) have demonstrated that *P. grisea* can overwinter in mycelial form in lesions on leaves, nodes, within straw piles, or indoors under the climatic conditions of Himachal Pradesh.

Misra and Misra (1965) reported that conidia from cultures could remain viable for 4-6 months, when harvested from the host surface. The fungus could be easily isolated from the infected seeds at the time of sowing in the months of June or July. Soil temperature above 32°C during June or July is not favourable for the development of the seedling blight.

According to Padmanabhan (1974), on the basis of cross-inoculation studies, the chances of survival of the pathogen on hosts other than rice in summer are remote, and its survival of through summer is most probably through an ascomycetous stage, with the ascospore discharge taking place under conditions favourable to infection, just after the cessation of the monsoons.

Apparao (1965) had demonstrated that the fungus survives in unsterilized soil for only a limited period due to immense competition with, and antagonism of soil microflora. The seed-borne inoculum is not of much consequence in India due to high soil temperature in June and July when the paddy crop is sown. The most probable sources of perennation and initiation of the disease in the plains of India, therefore, appear to be the grass hosts and early sown paddy crop. However, more studies are needed to understand the overwintering of the fungus and the epidemiology of the disease.

The effect of temperature on the infection and development of blast has been studied by many researchers. Suzuki (1975) reviewed the meteorological factors in the epidemiology of rice blast. It is now well established that low nyctotemperatures during the growth of rice plants are the most critical of the environmental factors that predispose the host to infection by *P. grisea* (Suryanarayana, 1958a; Sadasivan *et al.*, 1965; Suryanarayana, 1966). Subramanian (1967) and Suryanarayana (1967) further stressed the role of night temperature and humidity in blast incidence. According to them, the resistance to blast is governed not only by genetic factors but also, to a larger extent, by a set of very critical environmental factors including nyctotemperature (20°C), which influences the metabolic pattern of the host. At low temperature, the absorbed nitrogen tends to accumulate as soluble nitrogen in the leaf. Further, at low temperature under high levels of nitrogen fertilization, the plant absorbs very little silicon. The shift in favour of nitrogen metabolism, resulting in better nitrate reduction and accumulation of soluble nitrogen such as glutamine, causes exhaustion of carbohydrates, which could otherwise help in the production of secondary metabolic products imparting resistance. Conversely, high night temperatures favour secondary

metabolism leading to increased mechanical resistance and formation of phenolics associated with active resistance. The accumulation of nitrate in rice leaves grown at high night temperatures due to a failure of nitrate reductase activity would entail the shunting of the carbon compounds to structural materials, such as cutin and waxes. Indeed, the nitrate reductase system of the susceptible rice variety, CO 13, when grown at 20°C night temperature, is stimulated, resulting in the incorporation of nitrogen in the amide. However, such a situation is not noticed in the resistant variety, CO 29, where the glutamine content is low, as compared to the susceptible CO 13. The determining host resistance factors seem to operate through the nitrate reductase system, which in turn is dependent on genetic inheritance. This has its repercussions in amide and amino-acid synthesis, which are again known to be sensitive to environment, especially temperature (Sadasivan, 1968).

With an increase in nyctotemperatures from 20 to 25 and 30°C, the susceptible CO 13 showed more and more resistance, and consequently, the frequency, size and nature of lesions manifested less. However, CO 29 remained resistant at all the three temperatures. The percentage of spore forming appressoria was less on plants grown under high night temperatures and this was more significant in CO 13. This has confirmed the observations of the Japanese workers that epidemics of rice grown in soils at low temperature contained a greater number of appressoria and the number of cells penetrated was greater than in plants grown at high soil temperatures. While the fungus successfully penetrated and grew freely in CO 13 plants grown at low night temperature (20°C), it either failed to penetrate, or if it did penetrate, was arrested in the epidermis or in the cells lower down in the high night temperature treatments (25-30°C) and also in the resistant CO 29 (Ramakrishnan, 1966). Conidia that are lodged in leaves or necks require free water (dew, etc.) for germination before penetrating directly into the host tissue. It takes only 4-5 days for a spore to develop a visible lesion after infection, and the cycle of the disease repeats very quickly. High humidity favours the development of lesions; at low humidity, broken margins develop on the lesions and their expansion is limited.

Numerous conidia are produced under humid conditions, usually during the night. A typical lesion produces as many as 4-5000 conidia each night and continues to do so for 10-14 days under laboratory conditions. In extreme cases, 15,000,000 conidia may be deposited in a square meter of rice field in a day. The humidity of the air and the soil moisture greatly affect the susceptibility of plants to attack, as well as disease development. Rice plants become susceptible when grown in dry soil, moderately resistant in moist soil and resistant under flooded conditions. Irrespective of varietal resistance, age, whether the variety is upland or lowland one, or the part of the plant affected (leaf blast or neck blast), susceptibility to blast is related to soil moisture. Silicification of the epidermis of rice leaves as well as other anatomical characteristics associated with resistance is favoured by low soil moisture, which predisposes the plants to infection (Suzuki, 1934 a, b).

High air humidity favours lesion development. In the tropics, since variation in temperature is small, the air humidity in the macro and microclimate and dew formation are the major controlling factors for disease development. Many experiments over a long period of time have shown that a high nitrogen supply always induces heavy incidence of blast, regardless of the phosphorus and potassium supply.

Many investigations have been made to explain the increased susceptibility induced by a high nitrogen supply. Ito and Sakamoto (1939-1943) and Sakamoto (1948) demonstrated that resistance decreased with increased water permeability of the epidermal cells. They interpreted this to be due to the injurious effects of ammonium accumulation in the cells of plants treated with high nitrogen. Some workers demonstrated that there was a marked increase of soluble nitrogen, particularly amino acids and amines, in plants receiving high quantities of nitrogen. A close correlation exists between the severity of the

disease and the soluble nitrogen content of plants under various environmental conditions. Plants receiving larger amounts of nitrogen are found to have fewer silicated epidermal cells and thus lower resistance. The influence of potassium and phosphorus fertilizers on blast incidence is usually not very great.

Information on the influence of meteorological conditions on the incidence of blast has been used in attempts to develop a method of forecasting outbreaks of the disease. At the Central Rice Research Institute, Cuttack, India, it has been found that an outbreak of blast can be expected at a minimum night temperature of 20-24°C and relative humidity above 90% during the early morning period for 2-4 days (Padmanabhan, 1966).

Disease Management

(a) Culture Methods Field sanitation and destruction of weeds are precautionary methods that should be followed. Apart from this, early planting usually shows less disease than late-planted crop. Application of lesser amount of nitrogenous fertilizer in split doses generally reduces the disease severity.

(b) Chemical Methods Among the fungicides recommended for the control of blast disease are the copper fungicides (Blitox, Bordeaux) and organomercurials (Ceresan, Agrosan). Seed treatment with Agrosan GN (organomercurial) is effective in eliminating externally seed-borne inoculum. Copper fungicides are not effective in severe epidemics because it is phytotoxic to rice and reduces yield. But according to Thirumalachar (1967), when a mixture 200 ppm copper sulphate is mixed with 20 ppm of Aureofungin, it is effective in controlling seed-borne inoculum.

Among the other fungicides found highly effective against rice blast were Hinosan (1.5 ml/L water), Brestanol (0.44 g/L water), Pyroquilon (Coratop 2.5 G), Hexaconazole (Contaf 5EC) at 2 ml/L, Triflumizole (Rilan 70 WP) at 1 g/L, Fungorene (8 g/kg seed), Kasumin 2% WP (1g/L water, Di-Ter (2.5g/L).

There are some systemic fungicides that display strong systemic action against the rice blast disease. Kapoor and Singh (1982) have reported that seed treatment with Benomyl (1:400 W/W) gives protection to seedlings, because it inhibits spore germination and appressorium formation of the pathogen. But Venkata Rao and Muralidharan (1983) reported Benlate, Bavistin, MBC (all 0.1%) and Dithane M 45 (0.25%), a non-systemic fungicide to be effective against the disease. Tewari and Kameshwar Rao (1983), Saikia (1991) have confirmed the same findings.

Apart from these fungicides, some of antibiotics developed and used in Japan were Cephalothecin, Antiblastin, Antimycin A, Blasmycin, Blasticidin A and Blasticidin–S; but none of them became popular due to chemical instability and toxicity to fish.

Thus, there is a great deal of interest in non-fungicidal disease controlling agents since they are supposedly specific to target organisms and are less likely to lead to resistance problems. Actually, two groups of non-fungicidal rice blast chemicals are currently on the market; melanin biosynthesis inhibitors (MBIs), e.g., Fthalide, Tricyclazole, Pyroquilon, Carpropamid, Diclocymet and Fenoxanil, and the so called priming effectors or plant defense activators such as Probenazole, Acibenzolar-S-methyl and Tiadinil, which induce host resistance against the pathogen's attack (Yamaguchi, 2004).

Resistant Varieties

Ito (1963) had summarized work done in Japan. He mentions the following lines of approach being used: hybridization of japonica types, deriving resistant lowland varieties from upland varieties, using Chinese varieties and incorporation of indica strains. Nagai (1966) had reviewed the origin of blast resistant

varieties in Japan with special reference to the role of foreign varieties. In India, Padmanabhan (1963) summarized the work. Taichung Native I and other similar varieties introduced in India for their high yield have been found to show susceptible reaction to blast. Studies at CRRI, Cuttack, indicate that the source of resistance to the disease had been identified. Three varieties—Tetep, Tadukan and Zenith—have been found to be resistant to all the physiological races of the pathogen occurring in the country (Chakrabarti, 1976). According to John (1977), resistant varieties such as IET, 1444, RPC 2B 849, CR 12-178, RP5-2 and Jaya are available among the dwarfs for cultivation in the plains (Govindu, 1977).

Varieties T-603, T-141 (in Orissa) and A-67, A-90, A-200, and A-249 (in Maharashtra) GS-397, GS-480, ADT-20, CH-20, Suchi, Alkulu, Kukulu, Gennibera, Kamala (in Bihar), Co-4, TKM-1 (in Tamil Nadu), IR-579, Bala Lata-6 (in Himachal Pradesh), Jaya, Pankaj, Jayanti (in Raipur, M.P.) have been found resistant to blast of rice.

Tikka Disease of Groundnuts

One or both of the imperfect stages of the two species, *Cercospora arachidicola* and *Cercosporidium personatum*, occur in all the main groundnut cultivating areas of the world, depending on the type of the groundnuts cultivated and the weather conditions. The perfect stages, *Mycosphaerella arachidicola* and *M. berkeleyii*, which play an important part in the survival of the pathogen and in establishing primary infection in the North American crop, are not found in other groundnut cultivating regions.

Losses from the disease have been reported from 20 to 50% but can be up to 70% if the tikka disease is associated with rust (*Puccinia arachidis*). Smith *et al.* (1992) have considered these leaf spot diseases of international importance.

Symptoms

Symptoms appear when the plants are one or two months old. Due to excessive spotting on the leaves, there is gradual weakening of the foliage, which results in defoliation. Consequently, fewer and smaller nuts are formed. According to Sundaram (1965), severe intensity of infection may lead to up to 22% reduction in yield. Woodruff (1933) designated the disease caused by *Cercospora arachidicola* as early spot and that caused by *Cercosporidium personatum* as late spot, based on the relative time of their appearance in the USA. The first symptom of both leaf spots is the appearance of pale areas on the upper surface of older leaves. As the lesions develop, the two species can be distinguished. *Cercospora arachidicola* forms circular or irregular reddish-brown to brown lesions, which are bigger on the lower surface. The lesions are approximately1-10 mm in diameter and are surrounded on the upper leaf surface by a narrow yellow halo. Haloes are indistinct on the lower surface. Their presence or absence may be due to varietal or nutritional effects. *Cercosporidium personatum* forms darker brown to black leaf spots, which are smaller (1-6 mm), almost circular and with a less diffuse margin than those of *Cercospora arachidicola.* They rarely have haloes and are abundant on leaves, stipules, petioles, stems and pegs. Ramakrishnan and Appa Rao (1968) studied the symptoms caused by two species of *Cercospora* on groundnut and they found that neither the shape of the lesion nor the period of appearance of the halo is a reliable criterion for distinguishing the spots produced by *Cercosporidium personatum* and *Cercospora arachidicola.* A more reliable criterion that serves to easily identify the spots produced by the two pathogens is the color of the spot in the lower surface, which is light brown in the case of *Cercospora arachidicola* and carbon black in the case of *Cercosporidium personatum* (Fig. 18.8).

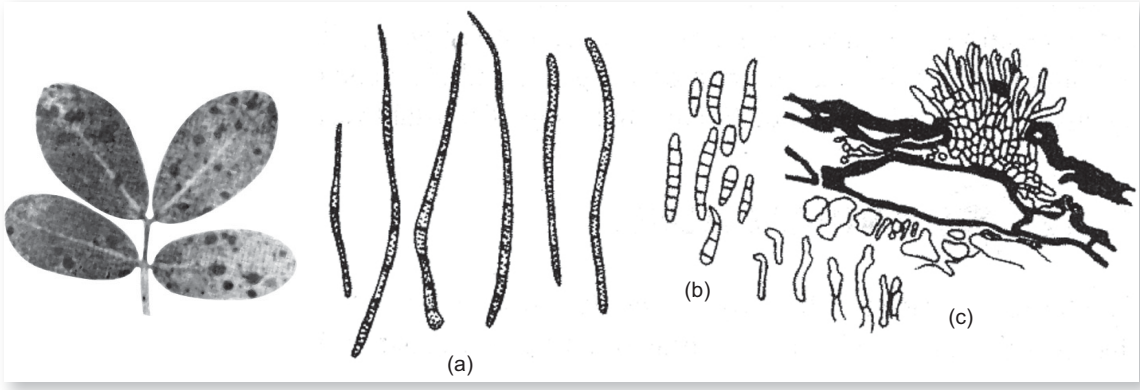


Figure 18.8 Tikka disease of groundnut caused by *Cercospora arachidicola* and *Cercosporidium personatum* symptoms (a) conidia of *C. arachidicola* (b) conidia of *C. personatum* (c) conidiophores of *Cercosporidium personatum.*

Causal Organisms

The causal organisms for the tikka disease of groundnut are *Cercosporidium personatum* (Berk. and Curt.) Deighton earlier known as *Cercospora personata* (perfect stage *Mycosphaerella berkeleyii)* and *Cercospora arachidicola* perfect stage (*Mycosphaerella arachidicola* W.A.Zenkins) (Fig. 18.8). The latter does not produce haustoria but host cells are killed in advance of the mycelium, which is initially intercellular. When the mycelium has killed the host cells, it becomes intracellular. *Cercosporidium personatum* produces an intercellular mycelium. It does not kill host cells in advance and haustoria can be seen in the living cells. In *Cercospora arachidicola* mycelium is both internal and external.

C. arachidicola appears earlier in the life of the plant, usually 3-4 weeks after planting. *C. personatum* lesions develop 2-4 weeks later when both the pathogens are present under favourable conditions. *C.personatum* is potentially more damaging because it produces more spots, spreads faster and causes earlier defoliation.

Conidiophores of *C. personatum* are 25-54 µm long and 5-8 µm broad, continuous or 1-2 septate and geniculate (shouldered), the points at which the conidia were attached being clearly visible. They emerge by rupturing the epidermis in tufts, their base being densely stromatic. Conidia are obclavate or cylindrical, measure 18-60 µm in length and 6-10 µm in breadth, are 1-7 µm septate.

Conidiophores of *Cercospora arachidicola* are amphigenous, but on the younger spots, they are produced exclusively on the upper surface. They are yellowish-brown, and as the conidia are formed and adjointed apically, they continue to grow past them and become geniculate. Conidia leave definite scars as they fall off. Conidiophores are 22-44 µm long and 3-5 µm broad, continuous or 1-2 septate. Conidia are hyaline or pale yellow to slightly olivaceous, obclavate, 38-108 µm long and 3-6 µm broad. They are 4-12 septate.

Species of *Cercospora* produce a phytotoxin, cercosporin. Rajgopal and Vittal Rai (1983, 1984) have isolated two water soluble, non-specific phytotoxic glycopeptides from *in vitro* and *in vivo* conditions. The toxins inhibit germination of seeds of several crop plants.

Disease Cycle

The diseases caused by both, *Cercospora arachidicola* and *Cercosporidium personatum*, are disseminated by wind, which blows the spores from leaf to leaf. Attacks on the new crop are apparently initiated

by the spores, which lie in the soil. The spores remain viable in the soil for a long time and attack the succeeding crop, as the conditions become favourable. There is evidence that they are seed-borne, especially in the shells. The disease is perpetuated through conidia lying in the soil on diseased plant debris and through conidia carried in the shell. The fallen leaves provide an excellent medium for the growth of *Sclerotium rolfsii* – another serious parasite of the crop. The perfect stages of *C. arachidicola* and *C. personatum* have been found on fallen leaves and stems late in autumn or in early winter in the USA.

Levin *et al.* (1973) studied the correlation between sowing time and weather factors on the intensity of groundnut disease. The early planting of groundnut decreased damage by *C. arachidicola* and *C. personatum*, which became more severe with later plantings. Nath and Kulkarni (1967) had stated that the intensity of spotting due to *Cercospora* spp. decreased as the sowing was delayed.

It has been observed in the central part of India that the climatic factors in July are not favourable for the disease. The minimum conditions for the development of the disease are obtained in August. The maximum predisposing factors for disease development occur in September (Sulaiman and Agashe, 1965). Relative humidity is the most important factor for infection (Shanta, 1960). A period of three days of high humidity is essential for maximum infection in the case of *C. arachidicola* and *C. personatum* (Ramakrishnan and Apparao, 1968). Prolonged low temperature and dew also favour severe infection. According to Shanta (1960), the application of nitrogen and phosphatic fertilizers increases disease incidence, while potash decreases it only slightly. Magnesium deficient plants are particularly susceptible to infection. Leaf spot development is minimal with full NPK fertilization as well as when gypsum is applied as a source of calcium.

According to Kaur and Dhillon (1988) and Mayee and Suryawanshi (1995), resistant varieties have thick epidermis-cum-cuticle, palisade layer, high frequency of trichomes and calcium oxalate crystals, fewer and smaller stomata. Some researchers have reported that the infected leaves produce some gaseous substance, which induces leaf abscission and pectic deposits around infection site, inducing resistance to colonization by the fungus.

Disease Management

(a) Cultural Methods Crop residues should be removed as they may harbour infection and provide a congenial medium for the growth of other disease such as *Sclerotium rolfsii.* Burning and burying crops together with crop rotation is recommended for the control of the disease. Early planting helps to reduce infection. Mineral deficiencies such as deficiency of magnesium should be corrected by soil or spray application as these deficiencies may favour the development of the disease. Change in the date of sowing can help in avoiding the incidence of the disease. Early maturing varieties usually escape the losses caused by the disease. Varieties that have more foliage are less liable to the damage than erect and less foliaged varieties.

(b) Chemical Control Chemical spraying or dusting is used in some areas as a routine practice. Some of the protectant fungicides used are Bordeaux mixture (4:4:50), Dithane Z-78 (0.2%), Dithane M-45 (0.2%), Fycol 8E, Cosan, Brestan (0.1%), and copper sulphate mixture (15-25 kg/ha). Apart from these, some systemic fungicides like Benomyl, Bavistin, Brestanol, Cercobin are highly effective against *Cercospora* leaf spot of groundnut. Dubey *et al.* (1995) reported that Bavistin (Carbendazim) is better than copper fungicides in controlling the disease. Among protectant fungicides, Du-Ter (Fenlin hydroxide, sold as **Du-Ter** is a broad-spectrum fungicide) gave better result than dithiocarbamates. Other recent fungicides like Tilt 25EC (Propiconazole), SAN-619 (Cyproconazole) both at the rate of 1.2 g/L and Hexaconazole (0.2%) are reported to be effective against leaf spot of groundnut.

The efficacy of three botanicals—neem leaf (*Azadirachta indica*), debdaru leaf (*Polyalthia longifolia*), and datura leaf (*Datura metel*)—and BAU-Biofungicide, along with a check (Bavistin) and untreated control, were evaluated as foliar spray in the field; among the treatments, BAU-Biofungicide and extracts of neem, datura and debdaru leaves showed excellent performance in controlling leaf spot and in increasing pod yield by 53.61, 51.91, 40.85 and 38.72%, respectively (Mahedi Hasan *et al.*, 2014). Finally, breeding of resistant varieties forms an important control measure.

Anthracnoses

The term 'anthracnose' literally means – like coal – and was first used by Fabre and Dunal, to describe a disease of grapes in which blackening of tissue was a characteristic feature. The term is now commonly used for plant diseases characterized by black lesions, usually sunken, caused by certain imperfect fungi that produce conidia in acervuli, that is, *Colletotrichum*, *Gloeosporium* and some closely related *Sphaceloma* spp.

A feature that distinguishes the fungi is that the conidia are borne on acervuli, which are erumpent, cushion-like masses of conidiophores. The conidia are hyaline, one-celled, ovoid to oblong. There are three important genera: *Gloeosporium*, *Colletotrichum* and *Sphaceloma.* The distinction between *Gloeosporium* and *Colletotrichum* is that while the latter has long sterile hair or setae, *Gloeosporium* does not possess setae in the acervulus. However, this distinction is not clear because many *Colletotrichum* spp. either produce setae rarely or only under certain conditions (Frost, 1964). The distinction between these two genera and *Sphaceloma* is also confusing. The conidiophores arise from a stroma in *Sphaceloma*, whereas in *Gloeosporium* and *Colletotrichum*, they do not. However, in natural infections, gradation between the stromatic and non-stromatic type is not uncommon.

Red-Rot of Sugarcane

Red-rot was first identified in Java in1893 under the name red-smut and it soon became a major cause of the decline of several varieties of sugarcane in the USA, Australia, India, and Hawaii. In 1906, Butler reported that the disease caused severe damage in certain cane varieties in India and renamed it as red-rot. Butler and Khan (1913) later reported it at Pusa. Epiphytotics of red-rot disease have been reported in 1939 and 1942, when a virulent form appeared in North Bihar and the Eastern United Provinces of India (now Uttar Pradesh) that threatened the very existence of the sugar industry in India. Some of the very promising Coimbatore canes became highly susceptible and had to be withdrawn. A similar outbreak was reported from Nigeria in 1951. Significant losses due to the disease occur almost every year in Haryana, U.P. and Bihar. It has been recognized as a disease of international importance (Singh *et al.*, 1992).

Symptoms

It is difficult to recognize the disease in its early stages in the field. The first symptoms of the disease are seen after the rainy season, when the plant stops growing and sucrose starts forming. The first external symptom of the disease is that the upper leaves of a shoot, usually the one approaching maturity, begin to lose color and droop slightly. The entire tip withers and the withering progresses down the margins.

At this stage, there is no visible alteration in the cane stem, except that the withered and green shoots may occur in the same stool. This condition may be assumed to be due to drought. In later stages, the canes themselves showed these effects. They become shriveled, the rind shrinks and becomes longitudinally wrinkled. On the midrib of the leaves, infection originates as a dark reddish area, which elongates rapidly, forming blood red lesions whose margin becomes darker (Fig. 18.9). The center becomes straw colored in older lesions. When the fructifications of the pathogen are formed, the lesions are covered with a powdery mass of conidia.

If the affected canes are split open, the tissue of the internode, which is normally white or yellowish-white, will be longitudinally reddened in one or more internodes (Fig. 18.9), usually towards the base. The reddening is most conspicuous in the vascular bundles and progresses towards the pith. The red color is interrupted transversely and this symptom is very characteristic of disease. It may be emphasized that in sugarcane any injury leads to the reddening of tissues and the characteristic cross bars in the reddened areas give an indication that the disease is due to red-rot pathogen.

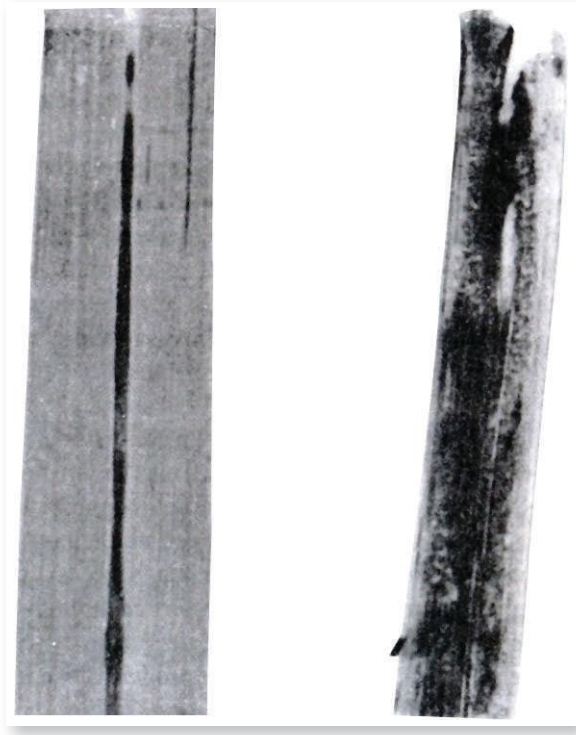


Figure 18.9 Red rot of sugarcane caused by *Colletotrichum falcatum*: Spots on the mid-rib and reddening of the pith with white transverse bands

Causal Organism

Red-rot of sugarcane is caused by the pathogen *Colletotrichum falcatum* Went. Its teleomorph is *Glomerella tucumanensis* (Speg.) von Arx and Muller.

The fungus, after entering into the host tissue, grows rapidly, producing inter and intracellular septate mycelium. As a result of host-pathogen interaction, the host protoplasm changes its color and a gummy,

dark-red material oozes out and fills the intercellular spaces of the cells. The soluble pigment present in this ooze is absorbed by the cell walls, resulting in the typical red rot appearance. The hyphae finally produce a large number of chlamydospores and they can persist in soil for a long time.

Conidial Stage The pathogen produces its conidia from conidiophores arranged in acervuli. These appear as minute, black dot-like bodies on the surface of the host under favourable environmental conditions. Acervuli are rounded or elongated and 70-300 µm across. Brown-colored setae are interspered between conidiophores and are about 150 µm long, 4-6 µm wide, 1-4 septate, swollen at the base with a tapered to rounded tip. Conidia are borne on small conidiophores, and are falcate (sickle-shaped), hyaline and aseptate, Conidia are 20-38 × 5-7 µm in size and possess a large oil globule in the center.

Perfect Stage Carvajal and Edgerton (1944) first reported the perfect stage. Chona and Srivastava (1952) first reported the perithecial stage of the fungus in culture at IARI New Delhi. Chona and Bajaj (1953) reported the perfect stage of the fungus in nature on sugarcane leaves in New Delhi. Perithecia are globose and are produced on various parts of the host but are abundant on leaf sheaths and blades and measure 150-300 µm in diameter. Asci are numerous, hyaline, eight-spored, clavate, thickened at the apex and 50-60 × 7-10.5 µm. Ascospores are aseptate, hyaline, straight or slightly fusoid, ellipsoid or ovoid when mature and 12-30 × 5-11 µm. Paraphyses are numerous, septate, unbranched, with granular contents extending along the ostiole.

Physiological Specialization

New physiological races differing in their pathogenicity to sugarcane cultivars have been frequently reported from different parts of the world. In India, a light-colored, heavily sporulating, virulent form appeared in 1935 and 1936, and was responsible for the epiphytotics reported in northern India.

Sugarcane red rot incited by *G. tucumanensis* is characterized by the reddening of the vascular tissue with transverse bars of uncolored tissue occurring at intervals and by the formation of lysigenous cavities. The pathogen infects the host mainly through the leaf scars at abscission or immediately thereafter, enters the parenchyma and grows intracellular in the early stages of disease development. It forms an intercellular mycelium in the later stages (Srinivasan and Alexander, 1966). Srinivasan (1970) had studies the hydrolyting and polyphenol oxidase enzymes in sugarcane red rot to know the relationship of hydrolyting enzymes to hydrolysis and that of PPO activity to resistance and its influence on the hydrolyting enzymes. Srinivasan (1970) found considerable differences among the different strains of *G. tucumanensis* in the production of hydrolyting enzymes *in vitro* as well as *in vivo.* PG, PME and cellulase were not reported in the early stages of disease development when the pathogen spreads through the tissues. It was observed that the fungal hyphae penetrate the host cell wall during the progressive stage of the disease by forming a minute penetration peg, seen as a constriction in the hypha, which expands to the normal hyphal diameter immediately after reaching the other side of the cell wall. This suggests the agency of mechanical pressure rather than enzymatic dissolution. The hydrolyting enzymes are not produced in the presence of sugar, but they are produced at a later stage when the tissues begin to die and the pathogen grows on the dead cells of the host. Therefore, contrary to the appearance of soft-rot fungi or even of other anthracnose fungi such as *Colletotrichum lagenarium* and *C.trifolii*, hydrolytic enzymes do not play a part in pathogenesis in sugarcane red-rot but have a role in the saprophytic phase of the fungus which follows the parasitic phase.

Disease Cycle

According to Chona (1950), and Chona and Nariani (1952), *C. falcatum* from sugarcane is capable of growing saprophytically in soil and producing acervuli. The fungus, however, does not survive in the active stage beyond 3-4 months. This has been ascribed to the competitive and antagonistic effects of associated soil microflora. Sugarcane has no dead season and this limited survival for a few months may be sufficient for the carry-over of the disease from the crop being harvested to the new crop. The pathogen also produced chlamydospores and these can survive for a long period in the soil. There is not sufficient evidence to indicate that the perithecial stage is very common in India. However, if this stage does occur, it may be one of the major means of survival of the pathogen and also for the sudden appearance of newer races of the pathogen. Diseased seed-setts are the chief means of survival and spread of the pathogen. When such diseased seed-setts are sown, they give rise to infected shoots. When such infected shoots appear in the field, secondary infection is caused by conidia, which are produced in acervuli and transmitted through insects, wind, and water. Initial penetration by conidia is facilitated by mechanical injury to the stems and buds, and infestation by the cane borer, *Diatraea saccharalis*, can be associated with a high percentage of infection. Growth cracks, leaf scars, root primordia, growth-rings and the region between the leaf-sheath and stalk are also susceptible (Edgerton, 1959). The mycelium grows in parenchyma tissue but spores can migrate through large ducts in the vascular tissue of the stem and leaf midrib.

High humidity, waterlogged conditions and lack of proper cultural operations result in weak growth. Continuous cultivation of the crop year after year and the cultivation of susceptible varieties of the crop and the build-up of inoculum, result in epiphytotics. Agnihotri and Sinha (1996) have discussed the epidemics and pathotype scenario in India.

Disease Management

Cultural Methods Rotation of crops after two to three years is also useful. Field sanitation is one of the important means of checking inoculum build up. In the field, fallen diseased leaves should be collected and burnt. Ratooning should be discouraged. Cultivation of crop in low-lying areas should be avoided and finally same variety should not be cultivated for several years in a particular field.

Heat Therapy With a view to eliminate the inoculum present in the seed material, heat therapy is practiced. Divergent views have been put forward on the success of this therapy. Good control of the disease was claimed by Joshi (1954), when setts were treated with hot water at 52°C for 18 minutes. Off-late, aerated steam and moist hot air have been observed to be effective in eradicating red rot inoculum for seed material. By aerated steam, canes are heated at 52°C for 4-5 hours and by hot air at 54°C for 2 hours.

Chemical Control Seed treatment (seed setts) with organomercurials such as Aretan, Agallol (0.25%) for 5-10 minutes dip helps only in eradication of superficial inoculum and not deep-seated mycelium. Waraitch (1983) and Singh *et al.* (1984) reported that Bavistin (0.5%) and Thiram (0.1%) could reduce the incidence of infection. Apart from chemical treatment, to eliminate seed (setts) borne infection, hot air treatment is recommended by various workers.

Resistant Varieties The best means to control the disease is to use resistant varieties. *Saccharum spontaneum* is the chief source of infection and several disease-resistant varieties have been bred at Coimbatore from time to time. Some of the resistant varieties recommended are: Co244, 62399, 62101, 1336, 1261, 1148, 1007, 975, 951, 846, 568, 562, 561, 513, 508, 475, 449, 393, 385, 370, 356, 349, 301, CoK32, 30, CoS510, 109, 443, BO32, 24, 3, 7, 10,11 and 22.

Ripe Fruit-Rot and Die-Back of Chillies

Chili (*Capsicum annuum L.*) crop has been observed to suffer due to two very serious diseases: ripe rot and die-back caused by anthracnose fungus, *Colletotrichum capsici* (Syd.) Butler and Bisby. The disease is capable of causing considerable damage to chilies in the years when there is continuous rain or high humidity in the latter half of September and the beginning of October. The diseased fruits appear at the end of September or in the first half of October when the plants are mature and have started flowering. It spreads rapidly from field to field. Ripe fruit-rot is also very conspicuous as it causes severe damage to mature fruits in the fields as well as some times in storage and transport. According to Chowdhury (1957), this disease in Assam affects about 12 to 32% of the fruits.

Symptoms

Fruit – Rot The disease affects only ripe fruits. The first outward sign of infection is the appearance of a small, black, circular spot, generally sharply defined or at times diffused, or a spot, black, greenish black or dirty grey, or one that is markedly delimited by a thick and sharp black outline enclosing a lighter black of straw colored area. Badly diseased fruits lose their normal red color and turn straw-colored, or in some cases, pale white. On the diseased fruits, numerous black-colored acervuli are found in concentric zones (Fig. 18.10). When the diseased fruit is cut open, the lower surface of the skin is found covered with minute, elevated, spherical, black, stromatic masses or sclerotia of the fungus. In advanced cases of infections, even the seeds are infected. Such seeds turn rusty in color.



Figure 18.10 Ripe fruit-rot of chilies (*Colletotrichum capsici*): symptoms on fruit

Die-Back The attack commences from the growing point of the flower bud; the tops of the affected branches wither and turn brown. The plant dies back as the attack spreads downwards; when it reaches a fork, the infection runs up around the limb. In certain cases, the attack starts not from the growing point but from a wound on the stem. As the disease progresses, the infected part of the stem takes on an enamel-white color and is sharply demarcated from the healthy green bark by a black line running all along the whitened area. The white of the diseased portion is punctuated by scattered, black, bristly and minute elevations called acervuli.

Die-back usually appears after the rains have stopped and there is prolonged deposition of dew on the plants. Partially affected plants bear fruits which are few and of low quality.

Causal Organism

The pathogen responsible for ripe fruit-rot and die-back is *Colletotrichum capsici* (Syd.) Butler and Bisby. The mycelium of the pathogen is septate, inter and intracellular. Acervuli and stroma in the stem are hemispherical and 70-120 µm in diameter. Setae are scattered and dark brown. The tips are light brown and several septate and up to 150 µm in length. Conidiophores are aseptate and unbranched. Conidia in mass appear light pinkish in color. Conidia are borne singly at the tips of the conidiophores. Individually, they are hyaline, unicellular and curved with narrowed ends. This measures 17-28 × 3-4 µm. There is a characteristic oil globule in the center of each conidium. Conidia germinate in water within four hours. The germ tube soon forms an appressorium.

The optimum temperature for the growth of the fungus is around 28°C and best growth occurs at 92% relative humidity (Chowdhury, 1957). According to Misra and Mahmood (1960a), the optimum temperature for the germination of conidia is 30°C and no germination occurs at less than 100% relative humidity. Maximum disease development takes place at 28°C and 95.7% relative humidity. According to Misra and Dutta (1963), the physiology and the virulence of the pathogen vary with the isolates, indicating the existence of strains within the species. Misra and Mahmood (1970a, 1960b, 1961), Thind and Randhawa (1957), and Chaturvedi (1964) have studied the nutrition and growth requirements of the pathogen.

The pathogen has also been reported to cause leaf spots and blight of marigold, pothos, coriander, turmeric, and *Cucumis melo* var. *momordica.* Narain and Das (1970) have reported that the pathogen produced a thermostable toxin. The toxic metabolite, unlike enzymes, is thermostable and causes injury, particularly to the protoplasmic content of the host tissue cells. The cell walls, however, remain unaffected.

Dastur (1921) has reported that the disease (die-back) is closely dependent on heavy and prolonged dew deposits after the rainy season.

Disease Cycle

The fungus is believed to survive in the field and plant debris and secondary spread takes place by windborne conidia. Seeds from badly diseased fruits may also carry the primary inoculum.

Grover and Bansal (1968) reported the occurrence of *Colletotrichum piperatum* (E. and E.) on *Capsicum fruitescens* in India. According to them, *C. piperatum* can be distinguished from *C. capsici* on the basis of the symptoms on the fruits, both in the field and under laboratory conditions. In the former, the conidia are single-celled, short, hyaline, rod-shaped with rounded margins and measure 5.5-6.5 × 15.0-19.24 µm in size, setae have not been observed in any of the media tried, nor did they appear when the cultures were subjected to different light temperature treatments.

The conidia from all the diseased fruits remain viable from July to May of the next year (ten months), though in the case of chilly fruits buried in the soil and subjected to weathering in the field, conidial germination is reduced from 96% to around 30-38% at the end of the ten-month period. The mycelium also remains viable in the diseased tissues of the whole fruits kept under different conditions. The seeds from the diseased fruits also carry the infection.

Grewal and Grover (1974) investigated the changes in amino acids, carbohydrates and capsicin content of red fruits of chilies infected with *C. piperatum.*

Narain and Mahapatra (1973) investigated the factors controlling the resistance of chili leaves to *C.capsici* and found that the higher phenolic content of the leaf was positively correlated with its resistant reaction to *C. capsici.*

Panigrahi and Avadesh Narain (1971) analyzed the pectin content of the fruits at different stages of ripening. An examination of the carbohydrates available in two stages of ripening of the chilly fruits revealed some interesting qualitative and quantitative differences. The ripe fruits have a higher quantity of sucrose than the green fruits in addition to the presence of glucose and fructose, whereas in the green stage, only glucose and sucrose are present. An examination of the suitability of these sugars, in the presence of pectin, for the production of pectic enzymes by *C. capsici* indicated the superiority of fructose whereas the presence of glucose markedly reduced pectic enzyme production.

Disease Management

Seeds for sowing should be taken from healthy and spotless fruits. Grover and Bansal (1970) reported Thiram and Vitavax to be most effective *in vitro* studies against *C. capsici*, but in pots, only Thiram, Brassicol and Bisdithane have been found effective in eliminating seed-borne inoculum. Good control of the fruit-rot disease has been obtained in Assam by spraying the crop with Perenox (0.2%). In all, 3-4 sprayings were given, starting when the fruits began ripening and repeated at 15-21 day intervals (Chowdhury, 1957). Narain and Panigrahi (1971) observed Ziram to be most effective against *C. capsici*. However, Jharia *et al.* reported that four sprays of Zineb (0.25%) were most effective against dieback and fruit-rot.

Recently, Datar *et al.* (1990) obtained maximum reduction in disease incidence by 3 sprays of Mancozeb (0.25%) at 15-day intervals after transplanting. Following varieties have been recommended as resistant to the disease from time to time: Bengal Green, Lorai, Perennial, H-1, H-4, H-6.

Mango Anthracnose

Anthracnose disease has been found to infect mango in Punjab, Uttar Pradesh, Bihar, Assam, and Kerala and thus covers all the parts of India. The pathogen attacks young as well as mature fruits. Mature fruits become blemished and their market value is reduced.

Symptoms

The symptoms vary according to the plant parts infected. On the leaves, dark brown necrotic areas develop; on the twigs, they are in the form of black necrotic patches; and on the main and secondary rachis of the panicle, the spots are small and dark; while black spots appear on the older fruits. If younger fruits are infected, they drop down from the trees. Dying back of young shoots is not uncommon. The affected fruits rot during storage. Anthracnose hampers the photosynthetic activity of the leaves. The pathogen also causes the blossom blight (Ramkrishnan and Srivastava, 1967).

Causal Organism

The pathogen responsible is *Colletotrichum gloeosporioides* Penz. the conidial stage of ascomycetous fungus *Glomerella cingulata* (Ston.) Spauld and Schrenk. The mycelium of the fungus consists of rather numerous, sparsely septate hyphae, which are at first hyaline but later take on a slightly darker color. Acervuli are abundantly formed on the affected host surface. Setae are common on twigs but not on fruits. The conidia of the pathogen remain in viscid fluid, which swells in moist conditions, ruptures the epidermis and exposes the conidial mass, which is disseminated by rain drop splashes and insects. Conidia in mass are pinkish but hyaline individually, oval to oblong with rounded tips, non-septate, but sometimes contain 1-2 oil-globules and measure 12-16 × 4-6 μm.

In addition to *C. gloeosporioides*, many other fungi have been reported to cause tip blight and die-back and these include *Diplodia* sp., *Botryosphaeria ribis*, *B. theobrome*, *Physalospora rhodina*, *Ceratocystis fimbriata* and *Hendersonia toruloidea.*

Disease Cycle

The pathogen survives in diseased twigs and leaves, which fall on the ground. It has the ability to survive saprophytically for a long period in dead twigs. Most of the infection in the fruits takes place from the start of the blossoming period until the fruits are more than half-grown. It has been observed that the fungus enters through the pores of the fruits while it is still green and develops further in the flesh during the ripening period. Latent infection of mature fruits has also been reported through the lenticels. Humid and moist conditions are favourable for infection. The fungus does not grow below 95% relative humidity.

Disease Management

Over-crowding of the orchards should be avoided. Pruning of the infected plant parts and then burning, along with other sanitary measures, should be adopted. Sattar (1946) suggested three sprays of 3: 3: 5 Bordeaux mixture in February, April and September under north Indian climatic conditions.

According to Tandon and Singh (1968), spraying the trees with Zineb or Bordeaux mixture can effectively control mango anthracnose. Aaragaki and Ishi (1960), trying various fungicides, found that the application of 4lb Captan (50% WP) in 100 gallons of water along the spreader proved best. Dipping mature fruits in hot water at 51°C for 15 minutes saves them from damage during storage (Pennock and Maldonaldo, 1962). Organic sulfur group fungicide Mancozeb was found to be effective in controlling anthracnose, but cannot be used because of ethylene produced as a by-product. In field experiment, Azoxystrobin at 1, 2, and 4 ml/L significantly suppressed the development of both panicle and leaf anthracnose. Mango trees treated with Azoxystrobin produced more fruits compared to control and showed no phytotoxicity (Sundravadana *et al.*, 2006).

Anthracnose of Grapes

Anthracnose of grapes is a widespread disease and causes considerable losses wherever grapes are grown. In the states of Punjab and Haryana, it causes a loss of 15-20% every year.

Symptoms

The disease infects different parts of the vine, starting from the young shoot to the mature berries (Fig. 18.11) on the stem and twigs. Depressed dark brown cankers are formed which become crater-like during the later stages of infection. If young shoots are infected, their growth is arrested and they finally dry up. Tendrils also do not develop properly and become curled and dry up on the leaves. Spots are formed on the petiole and fruit stock. Infection on the berries causes maximum damage to the yield. Dark brown, roundish, sunken spots develop on the fruits of different ages, which enlarge in size, resulting in the shriveling and drooping down of the fruits.



Figure 18.11 Anthracnose of grapes (*Gloeosporium ampelophagum):* symptoms on berries, twigs and leaves (Courtesy Dr. T.S. Thind, P.A.U. Ludhiana)

Causal Organism

The disease is caused by *Gloeosporium ampelophagum* (Pass.) Sacc. (*Sphaceloma ampelina* de Bary). The fungus is known to produce the perfect stage, viz., *Elsinoe ampelina* Shear. The ascus stage was first described in the USA. It has not been reported in India so far. The conidia produced by the pathogen are unicellular, hyaline, 5-6 $\times$ 2.5-3.5 µm.

Disease Cycle and Disease Management

In view of the fact that the pathogen remains deep-seated in the infected portions and is able to perpetuate throughout the year in old canes, disease control is very difficult unless proper control methods are

undertaken in a very systematic manner. All diseased portions should be pruned and destroyed, either by burning or by burying. All parts of the vines, after pruning, should be painted with a paste of Ferrous Sulphate in commercial H_2SO_4. The solution is prepared by dissolving 5 lb of commercial $FeSO_4$, in half pint of sulphuric acid added to one gallon of water. The treatment kills the deep-seated mycelium in the shoots. The new shoots formed after pruning should be protected with Thiram or Ziram at 1.5 lb of 80% dispersible powder per 100 gallons of water. The first spray should start at the bud-burst stage at a rate of 10 to 15 gallons per acre and this should be repeated after 10-12 days, increasing the volume to 20-30 to 40 gallons per acre. If this practice is meticulously followed the disease could be effectively controlled. Bedi *et al.* (1969) tested nine different fungicides and out of them, Blitox, Ziram and Aureofungin gave better results than did the others. Bordeaux mixture was toxic to foliage under the prevailing conditions in Gurdaspur, especially when applied during or just preceding the period of high temperature. Splitting of berries, which is very common in Punjab, was comparatively less in Captan sprayed plants.

Ascochyta Blight of Chickpea (*Cicer arietinum L.*)

India produces about 70% of chickpea grown in the world. More than 50 pathogens have been reported so far on chickpea from different parts of the world (Nene *et al.*, 1989; Mahendra Pal, 1998). Blight caused by *Ascochyta rabiei* (Pass.) Lab. (Teleomorph: *Didymella rabiei* synonym *Mycosphaerella rabiei*) is considered to be the most important disease of chickpea. In North Indian states, it appeared in epidemic form during 1981-83 and chickpea crop was almost wiped out (Nene, 1984). Chaube and Misra (1992) have reviewed the work on this disease and have considered this disease to be of international importance.

Symptoms

All the above ground parts of the plants are attacked (Fig. 18.12) and symptoms recalling those of anthracnose of beans. Dark spots appear on the stem and leaves first, then on the pods. On the stem, they are oval or elongated; on leaves and pods, round and up to half an inch across. When well developed, the margin is brown and the center is yellowish, withered, and studded with the tiny, dark fructification of the pathogen, which are often concentrically arranged. When lesions girdle the stem, the portion above the point of attack rapidly dies. If the main stem is girdled at the collar region, the whole plant dies.

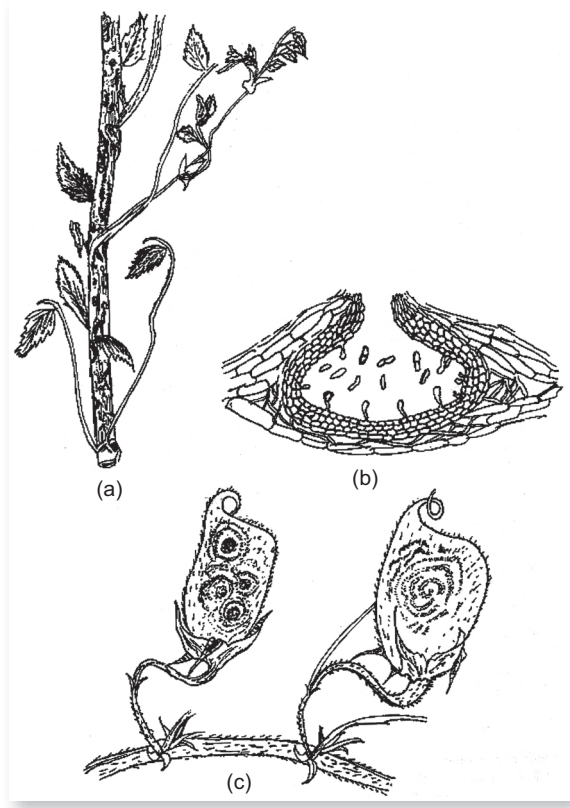


Figure 18.12 Blight of gram (*Ascochyta rabiei*): (a) disease plant showing symptoms (b) pycnidium showing conidiophores and conidia (c) diseased pots showing lesions

Causal Organism

The pathogen responsible for this disease is *Ascochyta rabiei* (Pass.) Labrouse. There is some dispute with regard to the naming of the organism. Some call it *Phytllosticta rabiei.* The name *P. rabiei* is based on the concept that the genus *Phomes* contains 5% bi-celled spores in the pycnidium. Singh *et al.* (1997) have suggested that the fungus is better placed in *Phoma rabiei* on the basis of ultra-structural studies on the conidiogenesis of this organism. According to Singh *et al.* (1997), the condiogenous cells of this pathogen resembles phialides and not annelidic as in *Ascochyta* in most of their features. The phialides are generally simple, but occasionally they proliferate in a percurrent fashion.

The mycelium of the pathogen is hyaline to brownish and septate. The pycnidia formed on stems, leaves and pods are immersed becoming erumpent, globose and dark brown and 140-200 µm in diameter. The wall of the perithecia is composed of 1-2 layers of elongated pseudoparenchymatous cells. The color of the pycnidia is reddish brown, and there is a round mouth to the opening to the surface. The spores are hyaline (pale pink in mass), oblong, obtuse at the ends, with a median septum, and measuring 9-20 µm by 3-6 µm. Occasionally, there are two septa. When young, there is no narrowing at the septum, but as the spores begin to germinate, it swells up and is deeply constricted in the middle. There is a mucilaginous substance around the spores which assist in their expulsion from the pycnidium by swelling up when wetted and fixes them in a crust around the mouth as they dry. The ascigerous stage of the pathogen is *Mycosphaerella rabiei* Kov. It was later named as *Didymella rabiei* (Kov.) von Arx. Both these genera belong to the family Dothidiaceae of the order Dothidiales, class Loculoascomycetes. The perfect stage has not been reported from India so far. Many workers have reported pathogenic races in the fungus and their criterion was the pathogenicity of the fungus on different host variables as parameters for identifying physiologic races. At IARI, New Delhi researchers have used isozyme varability, protein profiling and serology to estimate the amount of variation in *A.rabiei* (Uma Devi, 1995, Uma Devi *et al.*, 1997).

Epidemiology and Disease Cycle

The pathogen survives as pycnidia on diseased plant debris left in the field and also in the seed. Under Indian conditions, the main source of gram blight is seed. Singh and Mahendra Pal (1995) have reported that infected seeds always do not give rise to infected seedlings. Transmission of disease from seeds to seedlings is around 6% but this much of infection is sufficient to cause epidemic if weather conditions are conducive for the development of the disease. Long distance dispersal of the conidia (pycnidiospores) is not uncommon. The disease spreads fast if wet weather with strong winds prevails in February and March and the temperatures are between 22-26°C. The spread of the disease is along the direction of the prevailing wind. Thind *et al.* (2007) developed computer-based software for *Ascochyta* blight prediction.

Disease Management

Mahendra Pal (1998) has recommended integrated disease management approach for managing the disease in pulse crops such as change in the date of sowing (Tripathi and Chaube 1988), healthy seed material, need-based pesticides, cultural methods (Tripathi *et al.* 1987a, 1987b).

Since infected and/or infested seeds are the main sources of infection (seeds both externally and internally containing the pathogen) so seed treatment is an important measure in the absence of resistant

varieties. Sattar (1933) recommended immersion of seeds for 10 minutes in 0.5 % copper sulphate, or presoaking the seeds in water at 20 °C for 6 hours followed by immersion in hot water (53°C) for 15 minutes. This treatment affects seed germination. Several fungicides have been reported to control seed-borne infection. Seeds can be treated with organomercurials, Thiram, Benomyl or Calixin M. Calixin is reported to completely eradicate the seed-borne inoculum. Tripathi *et al.* (1987a) have reported control of the disease by seed treatment with Bavistin +Thiram in 1:3 ratio at the rate of 2.5 g/kg seed followed by 3 sprays of Bavistin (0.5 kg/ha) at intervals of 10 days at the time of disease appearance.

Several other fungicides have been recommended for getting rid of seed-borne infection. For example, the combination of Bavistin and Thiram (1:1 or 1:3) at the rate of 3 g/kg seed gives almost 100% reduction in the disease incidence. In mild attacks, sprays of Zineb, Maneb, Captan and Daconil are recommended.

Breeding for disease resistance is the best method for controlling this disease. Screening work done in India (ICRISAT) and many centers for screening trails sources of resistance has been located in cultivars/ germplasm. Kabuli type of germplasm is resistant. Singh *et al.* (1984) have summarized the work on the identification of sources of resistance to blight in chickpea. Another aspect, which is of concern, is that Kabuli gram, which is resistant to *Ascochyta*, is susceptible to Fusarium wilt and vice versa. Thus, breeding for resistance becomes more complicated.

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Questions

1. Name any *Helminthosporium* disease, which is internally seed borne.
2. How will you differentiate the following:
   (i) *Alternaria* and *Helminthosporium*
   (ii) *Cercospora* and *Pyricularia*
   (iii) *Colletotrichum* and *Gloeosporium*
   (iv) *Drechslera* and *Bipolaris*
3. How will you control the following?
   (i) Leaf blight of wheat
   (ii) Blast of rice
   (iii) Tikka disease of groundnut
   (iv) Anthracnose of grapes
   (v) Ripe fruit-rot and die-back of chilies
   (vi) Sheath blight of rice
4. What are anthracnoses?
5. How will you differentiate the genera *Helminthosporium*, *Drechslera* and *Bipolaris*?

Galls and Abnormal Growths

19

Infection often leads to abnormal growth. This may result in the hypertrophy of organs and in the formation of witches' broom, galls, and tumors. Hypertrophy is usually accompanied by some degree of distortion and is often due to highly specialized parasites. In the case of *Plasmodiophora brassicae*, for example, which causes the well-known clubroot or 'finger and toes' disease of crucifers, the infected roots become greatly swollen and develop numerous spindle-shaped knobs or club-shaped galls. This is due to enlargement and increased division of parenchymatous cells, chiefly in the cortex. Leaf curl, thickening, and distortion are produced by *Taphrina spp*. due to stimulated division and enlargement of the mesophyll cells.

In addition to fungi, bacteria, nematodes, and insects produce gall. Bacterial and nematodal galls have been discussed in Chapters 24 and 26 respectively. Uma Kant (1999) has very ably discussed and reviewed plant teratomas, their causes and consequences.

Clubroot Disease of Cabbage (*Brassica oleracea var. capitata*)

The records of this disease go back to the thirteenth century in Western Europe. By 1872, it was widespread in Russia. Woronin (1874-1878) was the first to undertake an intensive study on this disease. Clubroot is now known to exist in many parts of the world. Colhoun published a monograph on the disease in 1958.

Clubroot is the most dangerous disease of the mustard family. The disease is not prevalent in India but is quite common in Sri Lanka. Clubroot of rapeseed mustard has been reported from India (Laha *et al*., 1985; Chattopadhyay *et al.,* 1991). Once the disease appears in a particular field, there is complete loss of the crop. Bhattacharya and Pramanik (1998) reported that this disease is spreading at an alarming rate on rape and mustard (yellow sarson variety) in Bengal.

Symptoms

The disease may progress to a considerable extent before signs are noticeable above the ground. Infected roots enlarge relatively rapidly to form "clubs" which assume a variety of shapes (Fig. 19.1). The clubbing of roots can take many forms, such as: (i) clubbing is completely on main and lateral roots, (ii) clubs are present only on main roots, (iii) clubs are present on lateral roots, (iv) rootlets are free from

clubs, (v) clubs appear as tumors on the roots, and (vi) sometimes, dark spots are present on the root. These varying types of symptoms are due to specific roots infected by the pathogen.

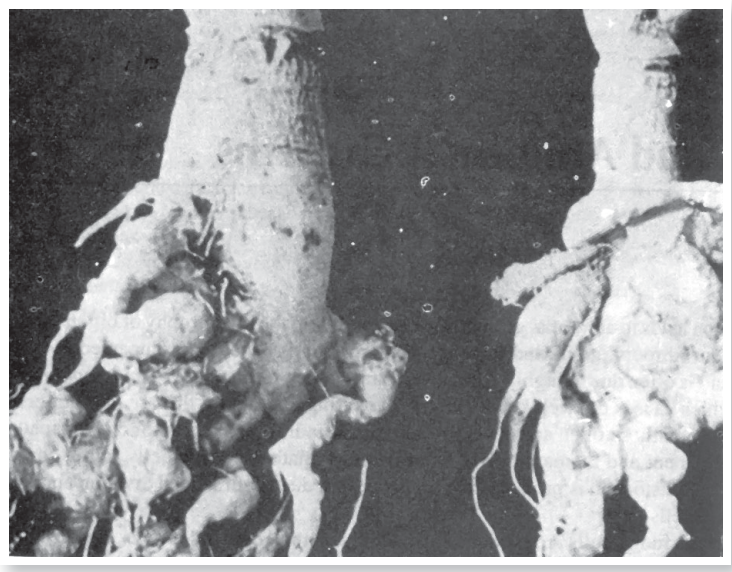


Figure 19.1 Clubroot disease of crucifers (*Plasmodiophora brassicae*), Courtesy: BASF India Ltd.

Seedlings, when infected, show flagging and symptoms of chronic water deficiency. The leaves assume a pale green or yellowish color and after infection, the diameter of the roots swells up to 10-12 times the diameter of normal plants, resulting in their death. In the case of late infections, the disease does not assume such a severe form. An affected plant, when pulled up, shows a grossly distorted mass of large and small, single or many, irregular galls. Translocation of food materials and water is interrupted, which results in the starvation of the plants. Heads are normally not formed but if they are, they remain very small.

Causal Organism

Plasmodiophora brassicae Wor. is the pathogen. Woronin (1878) described, in detail, the life cycle of the causal organism. The pathogen produces resting spores inside the host cells. The spores are hyaline, spherical and measure about 4 μm in diameter. Electron micrographs show that the resting spores have spiny walls (Williams and McNabola, 1967).

The resting spore germinates to produce a single zoospore with two flagella of unequal lengths, both of the whiplash type. Substances diffusing from cabbage roots stimulate germination.

The primary zoospores infect the root hair or young epidermal cells of the host. Before infecting the host the zoospores become amoeboid. The amoeba attaches to a root hair and penetration occurs. Aist and Williams (1971) described the mechanism of zoospore infection and entry of the pathogen with the help of electron micrographs. The entire body of the amoeba enters the host cell. Within the root hair or epidermal cell, a small uninucleate stage is first seen. Later, the nucleus divides and a small plasmodium is formed. The plasmodium divides to form zoosporangia. Each zoosporangium finally produces 4-8 uninucleate zoospores. The mature zoosporangium latches on to the host cell-wall and a pore develops

at this point, through which the zoospores escape. Sometimes, the zoospores are released into the lumen of the host cells. The behavior of the released zoospores is not properly known but it is possible that these may function as gametes.

Hypertrophy of the host cells is apparently brought about by the blocking of the mechanism for cell division and is accompanied by enhanced DNA synthesis. Starch accumulates in the infected cells.

According to Heim (1955), the young plasmodia in the cortical and medullar parenchyma form pseudopodia, which actively penetrate from the infected cells to a neighboring cell. Most of the nuclei in such plasmodia become associated in pairs and then fuse to form diploid nuclei around which the cytoplasm condenses. Such plasmodia no longer put out pseudopodia. The diploid plasmodia produce haploid spores following reduction division and these spores can germinate inside the host plant.

Disease Cycle

The fungus persists in the soil as resting spores in diseased host tissues. The pathogen is also carried in cattle and other manures used in the nurseries. It was also observed that clubroot incidence increased rapidly after irrigation or rain (Bhattacharya *et al*., 2014). When a susceptible cruciferous host is planted in the soil, it infects the roots. It multiplies and spreads in the plasmodia stage, causing the hypertrophy and hyperplasia of infected cells. Later, resting spores are formed. Thus, the disease cycle is continued.

Temperature, moisture, aeration, soil reaction, and other factors have pronounced effects on the development of the disease. The disease can occur within a temperature range of 12-27°C, the optimum being 25°C. Disease intensity is directly correlated with the increasing level of moisture in the soil. The disease occurs in neutral to acidic soils with a pH of 5.0-7.0.

Disease Management

Control of club rot of crucifers is difficult because of the 15-year life period of resting spores in soil and the ineffectiveness of most chemical treatments. Eradication by partial soil sterilization is expensive and difficult, and liming the soil and crop rotation are less effective and often impractical. Fungicidal treatment can be effective for transplanted crops. However, the following cultural methods have been recommended.

Cultural Method The disease can be controlled by the following methods:

1. Long rotation for crop.
2. Use of pathogen-free plots.
3. Eradication of crucifer weeds from the vicinity of the plots.
4. Seedlings should be raised in pathogen-free soil.
5. Yellow sarson and flax seed, when cultivated as an inter-crop in clubroot-infested plots, reduced clubroot incidence and enhanced yield. Possibly, hydrogen cyanide present in root exudates of the flax seed (Poulton, 1990) acts as an inhibitor to resting spore germination and club formation on yellow sarson.

Disease Management with Soil Amendments Treatment of soil with standard doses of fertilizers (N:P:K, 80:40:40 kg/ ha) along with lime (CaO, calcium oxide, quicklime), applied at 3 ton/ ha increased the soil pH from 5.7 to 7.2 and reduced clubroot disease incidence significantly. Suppression of clubroot disease using molybdenum in coarse sandy soil (pH 5.6) in acidic conditions has been demonstrated (Sen, 2005).

Chemical Control As the disease is severe in soil with an acidic pH, addition of hydrated lime to raise the pH slightly above neutral (usually about pH 7.2) is quite effective in containing the disease. At that pH level, spores of the clubroot pathogen germinate poorly or not at all.

Although a large number of chemicals such as Chloropicrin, Methyl bromide or Metam sodium or PCNB have been recommended to eradicate the soil-borne inoculum, these chemicals are hazardous and pollute the environment; as a result, they are on the banned list now.

Bhattacharya and Pramanik (1998) isolated a number of rhizobacteria, namely, *Pseudomonas fluorescens* H 237, *Bacillus subtilis* T 99 and *Streptomyces graminofaciens* G11 from the rhizosphere of diseased crucifers and tested against *Plasmodiophora brassicae,* both *in vitro* and *in vivo* conditions. It was found that *P. fluorescens* and *S. graminofaciens* reduced the growth of club formation as well as of root hair infection, while *B. subtilis* stimulated club formation and root hair formation. It was further found that Neem (Margosa) oil suspension, when applied in soil, was phytotoxic, but neem cake and nemin—a gelatinous neem product produced by Godrej India Ltd. —reduced disease severity under laboratory and green house conditions.

Bhattacharya and Pramanik (1998) studied the effect of plant products and biocontrol agents on two phases of the life cycle of *P. brassicae*. Indian neem tree oil emulsion in water (2% v/v) emulsified with 0.1% Tween 80, when applied as a soil drench, resulted in maximum inhibition (80%) of root hair infection of yellow sarson during the primary phase of colonization in a laboratory study. Only a few small clubs were produced following this treatment. Field trials conducted with different formulations of the neem product (granular and suspension) revealed significant reductions in severity of clubroot disease relative to other control methods (Bhattacharya and Pramanik 1998).

Resistant Varieties According to Chattopadhyay *et al*. (1991), all exotic cultivars belonging to *Brassica napus*, *B. nigra* and *B. carinata* are either free from the disease or are resistant. Resistance to rapeseed-mustard isolate of the pathogen in West Bengal has been found in cabbage cultivars KK Cross, Drumhead, Cauliflower cultivars Dania, Snow ball and Knol-Khol cultivar White Vienna (Chattopadhyay *et al*., 2001)

Wart Disease of Potato (*Solanum tuberosum* L.)

Potato wart is a quarantined disease of cultivated potato, caused by the obligate biotrophic, soil-borne fungus *Synchytrium endobioticum*. It is a very destructive disease. It was first discovered in Hungary in 1895. It is found in most European countries, South Africa, India, Peru, Bolivia and Mexico. In India, it is prevalent only in the North-East Himalayas. Potato wart is known by various names like black wart, black scab, potato tumor, potato cancer or canker, cauliflower disease, warty disease and many other descriptive terms in diverse languages and cultural backgrounds where potato is grown and the disease is present (Frank, 2007). Ganguly and Paul (1953) reported the disease for the first time in India from Rangbul in Darjeeling district (WB). The disease is endemic in that area and about 60% of the crop was affected. Phadtare *et al.* (1990) have provided a comprehensive review of the problem in India. Singh and Shekhawat (2000) have given brief, useful information on this disease.

Symptoms

All underground parts exhibit symptoms of the disease, except the roots and basal part of the stem. The parts that show abnormal activity and the formation of wart symptoms are buds of underground stems,

stolons and tuber but not on the roots. The size of the warts also varies depending on the intensity of the disease. Diseased tubers, when lifted, bear dark brown, warty, cauliflower-like excrescences. In severe infections, the galls may also be produced on the aerial shoots and they are green with convoluted, leaf-like masses of tissue. As the galls become confluent, enormous, warty outgrowths and tumors are formed. Tumors may turn brown to black with age. The yield of saleable potatoes from a heavily infected crop may be less than the actual weight of the seed potatoes planted (Fig. 19.2).



Figure 19.2 Wart disease of potato caused by *Synchytrium endobioticum*: symptoms

Causal Organism

The pathogen responsible for wart disease of potato is *Synchytrium endobioticum* (Schilb.) Perc. The pathogen produces thin-walled sporangia or summer spores and the resting sporangia are also known as winter sporangia. The resting sporangia are released by the decay of wastes and these may remain alive in the soil for many years (30-40 years). The resting sporangia function as prosporangium on germination and the endospore balloons out to from a vesicle within which a single sporangium differentiates (Kole, 1965). Infection of the tubers takes place by uniflagellate zoospores. A zoospore on the surface of a host cell loses its flagellum and forms a wall around itself. The naked protoplast enters the host tissue through a pore developed on the surface. Once inside the host cell, it reaches the lower part of the cell. The host tissue surrounding the infected cell and the adjacent epidermal cells divide repeatedly to form a characteristic tumorous tissue. A golden-brown, double-layered wall is secreted around the thallus and at this stage, the thallus is termed a prosorus or summer spore. The summer spore or prosorus germinates to form a sorus of 5-9 sporangia and then ultimately forms zoospores. Zoospores are liberated outside the soil and the cycle of infection could continue if environmental conditions remain suitable for infection. According to Curtis (1921), Kohler (1956) and others, the resting sporangia of *S. endobioticum* are formed after copulation. When a zygote infects a host cell, the infected cell undergoes hyperplasia, that is, repeated cell division. As a result of repeated divisions of the host cells, the fungus lies buried under several layers of the host tissue. The resting sporangia ultimately produce zoospores.

Disease Cycle

The pathogen survives in the soil in the form of resting sporangia for many years (three to four decades). Viable resting spores were found in air-dry soil after 15 months and in dry-waste material after 5 years. Contaminated manure and infested soil, transported in various ways, are means of spreading the pathogen. Short periods of saturation of the soil are essential for the germination of resting spores and sporangia, as well as for the liberation and movement of zoospores. Infection is limited to a temperature of 12-24°C. Infection can occur over a wide range of soil pH (3.9-8.5), but neutral to slightly acidic soils are most favourable.

Physiologic Specialization

Blattny (1942) recognized three pathogenic races in central Europe. Since then, another ten races have been reported from Germany. The occurrence of numerous races complicates the breeding programme. The most common biotype of the pathogen in Darjeeling has been identified as biotype 1.

Disease Management

Various degrees of quarantine procedures have helped in keeping the disease under check. In India, the disease has been restricted to the Northeastern Himalayan region (Darjeeling hills), by prohibiting the export of potatoes from that region.

Some other preventive measures are: practicing long crop rotation (5 years or more); roguing out plants of susceptible varieties by burning and destroying warty tubers; avoiding using diseased potatoes as seed potatoes. However, diseased potatoes may be consumed or fed to cattle only after boiling.

The application of lime to the soil to raise the pH level and the use of soil fungicides has met with partial success.

There are some other soil treatments like steam sterilization of the soil and application of mercuric chloride, ammonium sulphocyanate, copper sulphate and formalin (5%). The only drawback with these treatments is that they are costly and could not be implemented on a large scale. Resistant varieties are being bred but the occurrence of virulent races and too many biotypes of the pathogen have complicated the whole programme. But there are varieties that are immune to wart disease; they are: Kufri Jyoti, Kufri Sherpa, Kufri Kanchan (SC/1-1307), Kufri Sheetman and Kufri Khasi

Stem Gall of Coriander (*Coriandrum sativum*)

This is a common and widespread disease of coriander causing heavy damage, especially when associated with wilt. Tubaki (1957) has studied *Protomyces* species in culture. There have been reports of losses of up to 23% with this disease.

Symptoms

The disease appears in the form of tumor-like swellings on leaf veins, leaf stalks, peduncles, stems, and fruits. The swellings on the veins give a hanging appearance to the leaves. The tumors are at first glossy but later rupture and become rough. They are about 5 mm in diameter (Fig. 19.3a).

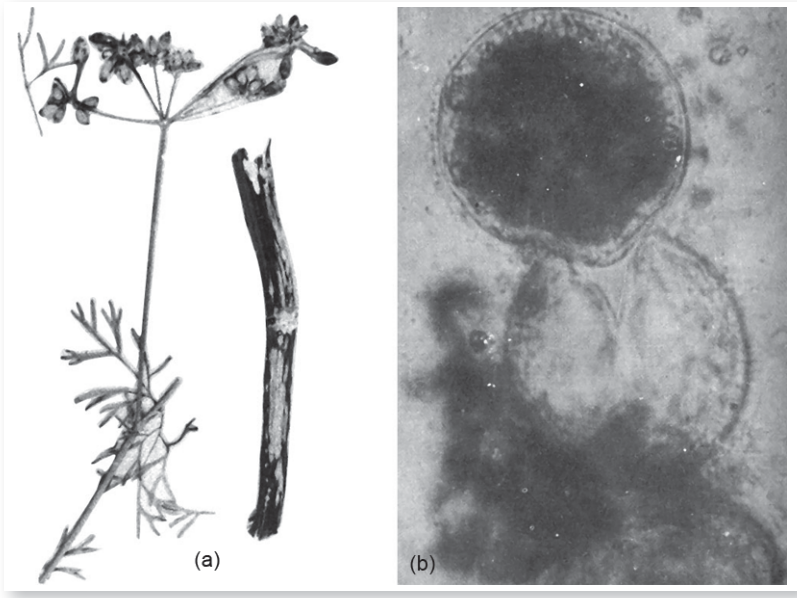


Figure 19.3 Stem gall of coriander caused by *Protomyces macrosporus* (a) symptoms and (b) germinating chlamydospore

Causal Organism

The disease is caused by *Protomyces macrosporus* Unger. The mycelium of the fungus is found only in the tumors. Attempts to trace the mycelium in tumor-free regions have failed. It is believed that the resting spores of the fungus cause the infection. The infection becomes systemic. The hyphae are intercellular and closely septate and branching is irregular. Scattered cells in the hyphae swell to form ellipsoidal or globose bodies, which later develop into chlamydospores (Fig. 19.4).

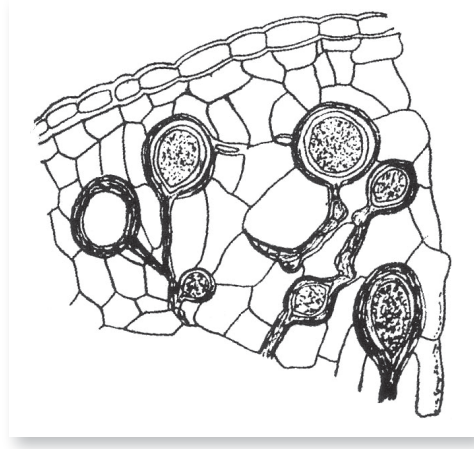


Figure 19.4 Stem gall of coriander (*Protomyces macrosporus*): A section through the gall showing intercellular septate mycelium with terminal and intercalary chlamydospores.

Mature chlamydospores with smooth, three-layered walls have a diameter of 50-60 microns. The chlamydospores function as the resting spores of the fungus. Resting spores germinate in water by rupturing the outer wall (exospore). The spore sac is formed by the emergence of cell membrane through the ruptured outer wall of the spore. The protoplasm from the spore passes into this vesicle and gathers towards the periphery (Fig. 19.3b). The nucleus divides several times, forming 100-200 daughter nuclei. The multinucleate protoplast of the spore sac becomes oriented in a peripheral layer, while the nuclei undergo reduction division, resulting in the production of four endospores per nucleus. On maturity, these spores separate and collect in the centre of the vesicle. The latter bursts and the spores are set free. These are capable of multiplying further by

budding in a yeast-like fashion and cause infection of the host. The detailed lifecycle of the organism is not known. The pathogen is now placed under the class Ascomycetes, in the order Protomycetales, (Kramer, 1973). According to Kirk *et al.* (2008), the pathogen *Protomyces* is placed in the class Taphrinomycetes of the division or phylum Ascomycota under the order Protomycetales.

Histopathology

Gupta (1962) studied the pathological anatomy of the malformed fruits of coriander. Rao (1972) made detailed histopathological studies of the abnormal growth caused by *Protomyces macrosporus* in *Coriandrum sativum*. Jain *et al.* (1995) studied the histopathology, histochemistry, and physiology of this gall. Maheshwari *et al.* (1984) observed changes in carbohydrates and protein content in the stem gall of coriander. The mycelium of the fungus occurs only in the tumors. Attempts to trace the fungus in tumor-free portions of the host have not been successful. The galls are produced as a result of hypertrophy and hyperplasia of the host cells. The galled tissue possesses stele, derived from the stelar region.

The flower-infected parts become massive. Pollen grains are amassed and become unhealthy. In the infected host parts, the oil canals are severely affected and collapse. The fungus does not invade the xylem.

Disease Cycle and Predisposing Factors

The pathogen survives in the soil in the form of chlamydospores and this seems to act as the primary source of infection. However, there are some who believe that the disease is both soil and seed-borne (Gupta, 1954, 1958). High soil moisture and shade predispose the plants to infection. Minimum infection occurs at pH 4.6 and maximum at pH 7.4. Loss of sugars, fats, proteins, carbohydrates, and so on has been reported from infected fruits. Gupta (1975) studied the mineral matter content in coriander leaves and fruits after they became infected by stem gall disease. A chemical analysis of healthy and diseased plant parts of coriander showed that mineral matter, magnesium, sodium, and silica contents increased, while calcium, manganese, phosphorus, potassium and iron contents decreased in the infected fruits.

Disease Management

Cultural Methods Following are some cultural practices that can avoid the disease incidence:

1. Use of healthy and clean seeds.
2. Proper field sanitation.
3. Destruction of the diseased plants.
4. Rotation of crop.

Chemical Methods Nene *et al.* (1966) have reported very effective control by combined seed and soil treatment with Thiram. For seed treatment, the dose recommended is 250 g/100 kg seed and for soil treatment, 20 kg/ha. Four doses of Thiram, Captafol or Carboxin, when sprayed at fortnightly intervals, from the time of disease manifestation, was found far more effective than seed treatment or combined seed and soil treatment in controlling stem gall and increasing the yield of coriander. According to Kumar *et al.(*2014), the effect of seed treatment and foliar spray of bioagents like *Trichoderma viride*, *Pseudomonas fluorescens,* and fungicides like Carbendazim, Ridomil, Blitox-50, Hexaconazole and Propiconazole on the incidence of stem gall disease of coriander showed better disease control.

Leaf Curl of Peach (*Prunus persica*)

This disease occurs throughout the world, wherever peaches are grown. In India, the disease is prevalent in the peach orchards of Kumaon, Kulu and other parts of the sub-Himalayan range. It also occurs in West Pakistan (N.W.F.P.). The disease also occurs on apricots and other *Prunus* species in addition to peaches.

Symptoms

The disease first becomes manifested in early spring. The leaf blades thicken and pucker along the mid-rib and the leaves curl downwards. The leaves are pale green or yellowish, but later on become reddish, thick and fleshy. The upper surface is covered with a whitish bloom as the fungus begins to produce spores. Affected leaves die and drop prematurely. The dormant buds become active to produce new leaves. Twigs may also be affected. Young shoots, when attacked, become swollen and distorted. Even flowers and fruits may be infected and they drop quickly (Fig. 19.5).



Figure 19.5 Peach leaf curl (*Taphrina deformans*): symptoms

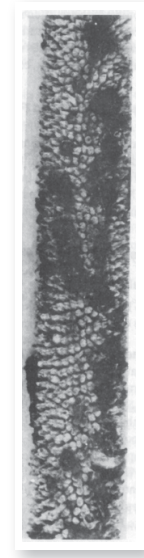


Figure 19.6 Ergot of bajra (*Claviceps fusiformis*): symptoms

Causal Organism

Taphrina deformans (Berk.) Tul. is the causal organism. The mycelium of the pathogen forms a compact, subcuticular layer of hyphae that fragment into individual ascogenous cells, each of which develops into an ascus. The mycelium is intercellular and grows in the tissues, obtaining its nutrition from the host. The host reacts by increasing its growth. The growth is uneven and is more towards the upper surface of the leaves causing puckering and downward curling. Asci are formed in a palisade-like layer above the epidermis. Each ascus contains ascospores but these may bud and give rise to secondary

spores, which are often referred to as conidia. Yarwood (1941) has shown that there is a diurnal cycle of ascus development and discharge in *T. deformans*. Raggi (1967) showed that respiration in host tissues increases, as does the free amino acid content in diseased leaves. Peach leaves affected by the leaf curl fungus *T. deformans* exhibit higher cytokinin activity than healthy leaves.

Disease Cycle and Predisposing Factors

The pathogen overwinters in the form of ascospores and sprouts conidia, which lodge on the surface of trees and cause infection in the following spring season. If they are washed down and lodged on the young unfolding leaves and buds, they infect the young meristematic tissues in the early spring when the buds are opening.

Mycelium of the pathogen can also persist in the cortex of the infected twigs. Towards the end of winter, the mycelium penetrates the buds beneath the infected area.

Disease Management

Cultural Method

1. All fallen and diseased leaves should be burned, away from the orchard.
2. Orchard and tree sanitation is important.

Chemical method Spraying with a suitable fungicide like Bordeaux mixture (6:6:50), Perenox or Fytolan (0.3%) or Blitox 50 (0.25%) can control peach leaf curl. The fungicide should be sprayed before bud-break in spring.

Sharma *et al.* (1990) recommended that Bavistin (0.05%) or Dithane M-45 (0.2%) should be sprayed during the dormant stage (January) rather than the flowering stage (February). Mehdi and Shah (1994) have reported good control of the disease by fungicides such as Hexacap (Captan), Dithane M-45, Bavistin Topsin-M, Baycor, Bayleton, and Calixin; out of the fungicides tested, Captan (1500 ppm) gave 90% control, Dithane M-45 (2500 ppm) gave 87.9% control, and Calixin gave 71.6% control. Chlorothalonil (Bravo or Daconil) controls the diseases, if applied twice in late fall and in early spring before the leaf bud swells. Two sprays of Captafol, Ziram given prior to green tip stage provided best control of leaf curl, followed by Captan and Chlorothalonil. Difenoconazole-a sterol inhibitor fungicide, when applied twice during bloom, showed eradicational activity and was as effective as Chlorothalonil.

Resistant Varieties Late-blooming varieties are generally resistant to the disease. Tolerant cultivars like Bed Will's Early and July Elberta have been found.

Ergot of Bajra (Pearl Millet)

The disease has assumed great importance recently due to its occurrence on new, high-yielding bajra hybrids. The disease was first reported from South India.

This disease, in its epiphytotic form, was first reported in India in 1956 from the South Satara area of Maharashtra (Bhide and Hegde, 1957; Shinde and Bride, 1958). Sundaram *et al.* (1969) related the increased importance of the disease to the introduction of susceptible hybrids, HB-1 and HB-2. The All India Coordinated Millets Improvement Programme Report of 1967-68 mentions severe epiphytotics of ergot in several states of India, such as Delhi, Rajasthan, Maharashtra, Mysore (now Karnataka) and Madras (now Tamil Nadu). Since then, it has been reported from other states as well.

In addition to India, there are reports of the incidence of ergot from several countries in Africa, for example, Gambia, Ghana, Nigeria, Rhodesia Senegal, Tanzania, and Zambia (Ramakrishnan, 1963; Loveless, 1967).

Although several workers have stated that the losses due to this disease are enormous, only Natarajan *et al*. (1974) provided a detailed report. They assessed ergot incidence at the seed maturity stage by counting the diseased ear heads, which were harvested and thrashed separately. They calculated the average incidence to be 62.4% with 58.4% grain loss. HB-4 showed 49.3% incidence and 41.7% loss and a hybrid derived from 23D2 AXJ 104 showed 76.2% incidence with 70.5% loss.

There are several reports of human beings and cattle being poisoned in India by ergot. Shinde and Bhide (1958) reported that the mature sclerotia contain 0.42% total alkaloid as ergotoxin. The alkaloids present in honeydew and sclerotia from 20 millet varieties were identified as setoclavine, agroclavine, penniclavine, elymoclavine, an unidentified alkaloid and chanoclavine. Symptoms of ergot poisoning in animals and humans (giddiness, diarrhoea, nausea and vomiting, followed by dehydration) from contaminated millet grain have been reported in Rajasthan and Maharashtra in India (Kumar and Arya, 1978). Loveless (1967) stated that the pearl millet ergot sclerotia contain groups of water-soluble alkaloids, which are different from rye ergot (i.e. ergotoxin, ergotamine and ergometrine). Ergot of pearl millet has been recognized as a disease of international importance (Thakur, R.P. 1992).

Symptoms

As a result of infection, each infected spikelet starts exuding small droplets of pinkish or light honey-colored fluid (commonly called the honeydew stage). As the disease progresses, these droplets become darker and ooze out to form the glumes. These dark structures are the sclerotia of the fungus (Fig. 19.6), which contain alkaloids like ergotoxin, ergotamine, and ergometrine that are responsible for poisoning the cattle. Assimilation of these alkaloids in the blood stream of animals and humans causes the disease 'ergotism'.

Causal Organism

There is some confusion regarding the identity of the organism responsible for ergot of bajra. Shinde and Bhide (1958) stated that the morphology and measurements of conidia, their mode of germination, the color of the stromata and germination of sclerotia of the ergot fungus are similar to *Claviceps microcephala,* which was described as *P. hohenackeri*. Loveless (1967), after examining several specimens from Africa, proposed to name the ergot on *Pennisetum americanum* (*P. typhoides*) as a new species, *Claviceps fusiformis* Loveless. He further stated that Petch considers the identity of the fungus, *C. microcephala*, synonymous with *C. purpurea.*

Loveless (1967) and Siddiqui and Khan (1973a) investigated the ergot fungus occurring in India and agreed that it should be called *C. fusiformis* and not *C. microcephala*. These researchers described the conidia as hyaline, fusiform and broadly falcate, measuring 13-18 × 3-4 μm.

The lifecycle of the fungus is similar to *Claviceps purpurea*. The honeydew stage is followed by the development of sclerotia (Fig. 19.6). The sclerotia measure 3.6-6.1 × 1.3-1.8 mm, germinate by producing 1-3 or upto 16 fleshy, purplish, 6.26 mm-long stipes. These stipes bear perithecial stroma or capitulum at the tip. Cross-section of the capitulum reveals numerous pyriform perithecia embedded in stroma. In each perithecium, there are several asci, which are long and cylindrical, tapering towards the base. Each mature ascus contains 8 ascospores, which are long, filiform, hyaline, aseptate and measure

103-176 × 0.4-0.5 µm (Thakur *et al*., 1984). These uninucleate ascospores germinate to produce primary and secondary conidia. These are small, dark grey, and whitish inside. Shinde and Bhide (1958) reported the germination of a few sclerotia after 45 days, when kept in a mixture of sterile sand and soil.

Each produced 1-3 stipes. Asci were produced in mature stromata. Rama Sastry (1973) has described the detailed procedure of sclerotial germination.

Disease Cycle

Ramakrishnan (1963) stated that the conidia retain their viability upto 13 months but this observation has not been verified. Sundaram (1969) stated that the ergot disease spreads from one region to another mainly by the admixture of sclerotial bodies with the seeds. According to him, the sclerotia get mixed with the soil along with the seeds and take about 30-45 days to germinate. This coincides with the time taken by the plants to flower and receive the air-borne spores.

Secondary Spread

Under favourable conditions, ergot spreads very rapidly. It appears that the secondary spread occurs mainly through conidia produced in large numbers in honeydew and are presumably either picked up from the honeydew by insects or scattered by rain.

Sundaram (1969) states that infection takes place mainly through the stigma and occasionally by the piercing of the thin ovary wall before fertilization. Reddy et al. (1969), however, observed infection through the ovary. It takes about 5-6 days to develop honeydew and thus 2-3 generations may be completed within the anthesis period. Kannaiyan et al. (1973a) reported that the ergot fungus could not infect the spikelet, once fertilization is completed. Puranik *et al.* (1973) observed that even the fertilized ovaries of *Sorghum* are susceptible for at least five days by *Sphacelia sorghi* McRae. Ramaswamy (1968) tried to analyze the influence of meteorological factors in the pearl millet ergot epidemic of 1967 in Delhi. Small (1922), Ramakrishnan (1963), Sundaram *et al.* (1969) and Reddy *et al.* (1969) have reported various hosts of *C. microcephala* (now *C. fusiformis*).

Disease Management

Cultural methods

1. Since the pathogen is soil-borne, long crop rotation is recommended.
2. Use of sclerotia-free seed is another important precautionary measure.
3. As the pathogen can survive in soil for a long time, deep-ploughing and adjustment of sowing dates during dry summer may reduce its viability.
4. Mixed cropping with legumes, especially mungbean is reported to reduce the disease incidence.
5. Dipping the seeds in 20-32% salt solution floats the sclerotia and sclerotial fragments, which can be further removed by hand.
6. Deep-plowing buries sclerotia in the soil. Ascospores are not formed or they cannot be released into the free air in spring. When cereals are grown with "no-till" soil management, *i.e.*, new crops are grown directly in the residues of the previous crop, crop rotation is even more important.
7. Wild and weedy grasses within or outside the fields are the first source of ergot inoculum in early spring from overwintered sclerotia, or as a source of honeydew if produced prior to crop flowering. They should be controlled by herbicides along fences and hedges or by simply cutting grasses at the boundary ridges before flowering.

Chemical Method Since pearl millet is a food crop of resource for poor farmers in the arid and semi-arid tropics and with ergot being strictly air-borne, the control of this disease by chemicals is neither economical nor feasible. However, there are reports of various degrees of success in reducing ergot at experiment stations using chemical sprays. Several sprays of fungicides have been used.

Sundaram (1967) recommended sprays with Ziram or a mixture of Copper oxychloride and Zineb (1:2, 375-450 g.a.i/ha) applied 2-3 times at 5-7 day intervals, starting just prior to ear head emergence. Reddy *et al*. (1969) carried out a greenhouse trial where the fungus was inoculated 24 hours after a single spray of seven fungicides was tried and an inorganic sulphur preparation (80% wettable sulphur) was found to be most effective. Bavistin, Benlate and Brestan have been reported to provide partial to complete control.

Prom and Isakeit (2003) tested 14 fungicides and have reported reduction in spore germination of *Claviceps africana*. Application of Propiconazole, Tebuconzole, Myclobutanil or Azoxystrobin (25 µg/L) on full-bloom panicles suppressed ergot severity. Captan and Mancozeb completely inhibited germination of conidia on seeds (Bhuiyan *et al.,* 2000). Even the spray of garlic extract (12%) has shown disease control (Singh and Navi, 2000).

Resistant Varieties Thakur *et al.* (1988) reported that ICRISAT had developed some resistant lines against ergot and smut of bajra, which are quite effective.

There is a definite correlation between the stages of flowering and infection. Pollination of flowers before or at the time of inoculation has been shown to reduce infection through rapid withering of stigma (Thakur and Williams, 1980). Planting early-flowering pollen donor lines may provide good protection against hybrid varieties that are cross-pollinated (Thakur *et al*., 1983).

Biological Control Studies with antagonistic organisms have continued to show promise. *Fusarium semitectum* var. *majus* was effective in controlling ergot by reducing infection and the formation of sclerotia in infected florets and by disintegrating the sclerotia (Rao and Thakur, 1988). *Fusarium chlamydosporum* colonizes sclerotia in the field, and a culture filtrate of this fungus inhibited *C. fusiformis* growth *in vitro*, causing cell discoloration and the formation of chlamydospores instead of normal mycelial growth and conidial formation (Gill and Chahal, 1988). Three antagonists, *Trichoderma harzianum*, *Trichoderma viride* and *Gliocladium virens*, significantly reduced sclerotial germination (Mohan and Jeyarajan, 1990). Treatment with *Aspergillus niger* or *T. viride* completely inhibited the germination of sclerotia (Mahadevamurthy *et al.,* 1990). Under field conditions, spore suspensions and culture filtrates of a number of fungi and bacteria [*F. chlamydosporum*, *Fusarium heterosporum* (*Gibberella gordonii)*, *T. harzianum*, *T. viride*, *A. niger*, *Epicoccum andropogonis*, *Bacillus subtilis*] reduced the incidence of ergot when applied to flowering heads (Mahadevamurthy *et al.,* 2006).

Mower *et al.* (1975) have reported *Fusarium roseum* as a potential biological control agent against *Claviceps purpurea*. Nene and Singh (1976) reported that *Fusarium sambucinum* might possibly be utilized for biological control. Tripathi *et al.* (1981) reported *F. sambucinum* and *Dactylium fusarioides* as mycoparasites of sclerotia. Application of these biological agents reduced about 50% sclerotia formation.

Bhuiyan *et al.* (2003) reported *Pseudomonas aeruginosa*, *Burkholideria cepacia*, *Penicillium citrinum* and *Trichoderma* spp. as biocontrol agents against the pathogen in *in vitro* condition. However, bacterial antagonists fail to inhibit infection of sorghum *in vivo* and in glass house conditions but all the fungal isolates, including *Epicoccum nigrum,* reduce the percentage of infected spikelets per plant and at times completely suppress ergot development.

Increased pollen availability in hybrids during the early stages of flowering protects them from ergot infection. Significant reductions in infection and considerable increases in grain yield occurred in pearl millet hybrids grown with the pollen donor line (Thakur *et al*., 1983). A field experiment was conducted in Hisar, Haryana, India during the Kharif seasons of 1993-1994 to determine the efficacy of controlling pearl millet ergot by pollen management. The treatments comprised of no pollination-no inoculation, inoculation-no pollination, pollination-no inoculation, pollination followed by inoculation the next day, simultaneous inoculation and pollination, and inoculation followed by pollination the next day. The lowest disease incidence was recorded for pollination (3.72%) followed by bagging and the no pollination-no inoculation treatment (5.50%) (Kumar *et al.,* 2002).

Malformation of Mango

Watt (1981) first noted the disease in Bihar. Since then, it has been reported from other parts of the world such as Egypt, South Africa, Sudan, Switzerland, Brazil, Bangladesh, Central America, Mexico, Cuba, Australia, USA, Israel, Malayasia, Pakistan, and United Arab Emirates. In India, the disease is most prevalent in the northwestern region of the country. However, the disease is reported to be increasing in the southern and eastern parts of India, although the manifestation of disease symptoms in these areas is less severe (Chakrabarti and Kumar 1999). In severely affected areas, the disease causes 50 to 86% losses in yield. Mango malformation has been reviewed by a number of researchers. Important ones are those of Kumar and Beniwal (1992), Chakrabarti (1996), Chakrabarti and Kumar (1998), Kumar (1998), Kumar *et al.* (1993), and Prakash and Misra (2001).

Symptoms

Malformation is of two types: (i) vegetative, and (ii) floral. The characteristic symptoms of vegetative malformation are the occurrence of a large number of small leaves and stems which are substantially reduced and crowded together into a compact head, either at the apex of the shoots (bunchy top) or in leaf axils (Fig. 19.7A). In case of floral malformation, the length of the axis and secondary branches of panicles are reduced, thus the flowers appear in a cluster (Fig. 19.7D). Malformation panicles ranged from small and compact (heavy) to loose (light) type, and assume the shape of a witches' broom. Light type of floral malformation is common in Tamil Nadu and West Bengal, where the disease has been recently identified. Other distinctive features are: (i) increase in number of staminate flowers (Fig. 19.7C), (ii) poor viability of pollen and (iii) development of hyperplastic and hypertrophy of cells in the cortex and stele.

Causal Organisms

There are four types of causal agents investigated by various workers: physiological, nutritional, entomological and pathological (Prakash and Misra, 2001). Recent findings have demonstrated that the disease may be of fungal origin.

Due to the uniqueness of the disease symptoms and the confusion over its occasional inability to reproduce was created about the causal agent of the disorder. Initially, the disease was attributed to viruses and mites on the basis of resemblance of the symptoms to a physiological disorder. In 1966, Summanwar *et al.* demonstrated *Fusarium moniliforme* (basionym *F. moniliforme* Sheldon *var. subglutinans*

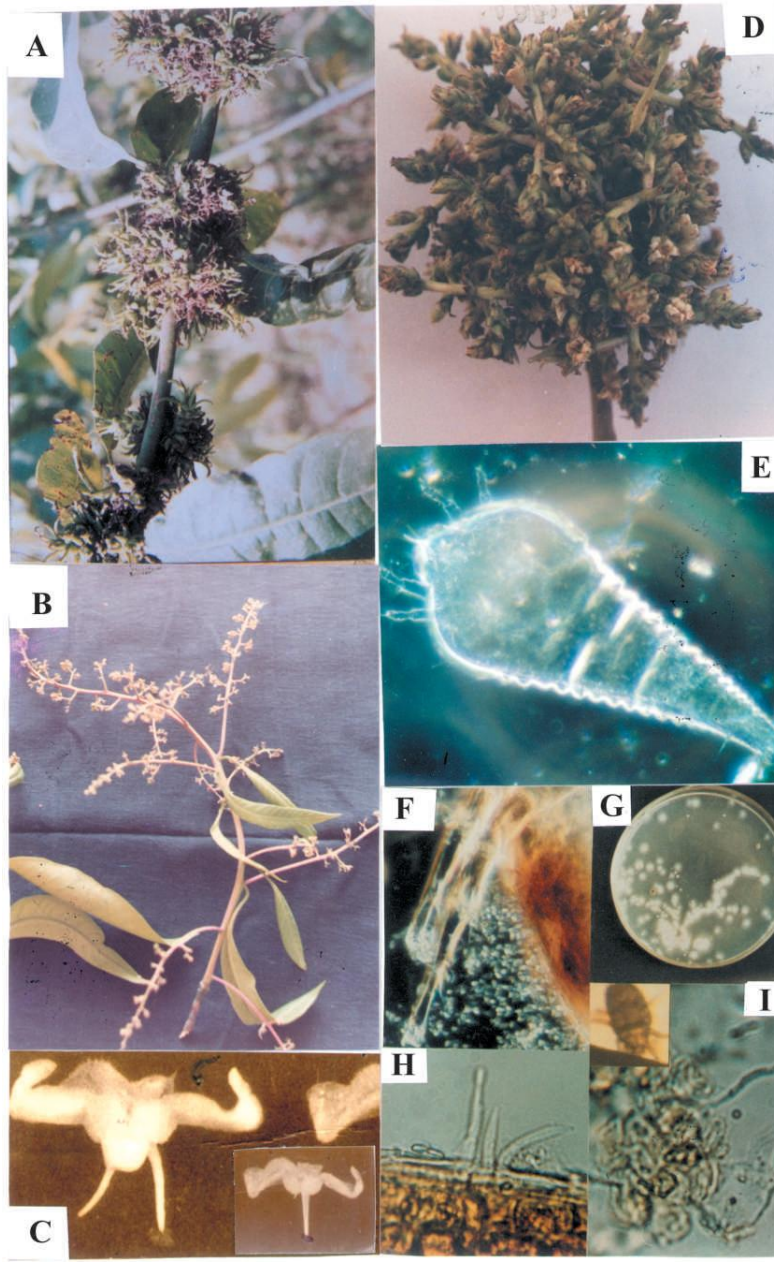


Figure 19.7 (A) vegetative malformation at the leaf axis, (B) normal inflorescence, (C) mango florets- staminate and hermaphrodite (D) malformed panicle (floral malformation), (E) eriophyid mite (*Aceria mangiferae*) (F) conidia of *F. moniliforme* adhered on the city and body of *Tyrolichus casei* (G) trail of the fungus colonies was found along the pathway of *T. casei* on PDA, (H) ingress of the fungal hyphae deep into the host cells being prevented due to accumulation of plant defence chemical (mangiferin) brown colored in the cortical cells, (I) *T. casei* causing injury on the surface of mango bud and the infection peg from the germinated conidia reached inside the host cells through injury points.

Wollenweb and Reinking) to be the causal organism for this disease. It has been corroborated in the USA, Israel, Egypt, and South Africa. On potato-dextrose-agar (PDA), the fungus produces white aerial mycelium, purple-tinged but dark blue at a later stage. Microconidia are abundant, oval, and usually single-celled but may have as many as three septa. Microconidia are formed on polyphialides in false heads but never in chains (Fig. 19.8). Macroconidia are fusiform, having a conspicuous foot-shaped basal cell (Fig. 19.8). Chlamydospores are not formed. The pathogen induces many biochemical and physiological changes in the host cells. The combined effects of the aberrant host metabolites and the fusarial toxins manifest the disease symptoms (Ghosal *et al*., 1979; Kumar *et al*., 1995).

Physiological Specialization

F. moniliforme, isolated from malformed mango shoots and panicles, shows host specificity and differs distinctly from other strains of the fungus (Person, 1984; Kumar and Chakrabarti, 1992). The host metabolite, mangiferin, induces considerable changes in the strain of *F. moniliforme,* developing a new physiological race (Chakrabarti and Kumar, 1996).

Disease Cycle and Predisposing Factors

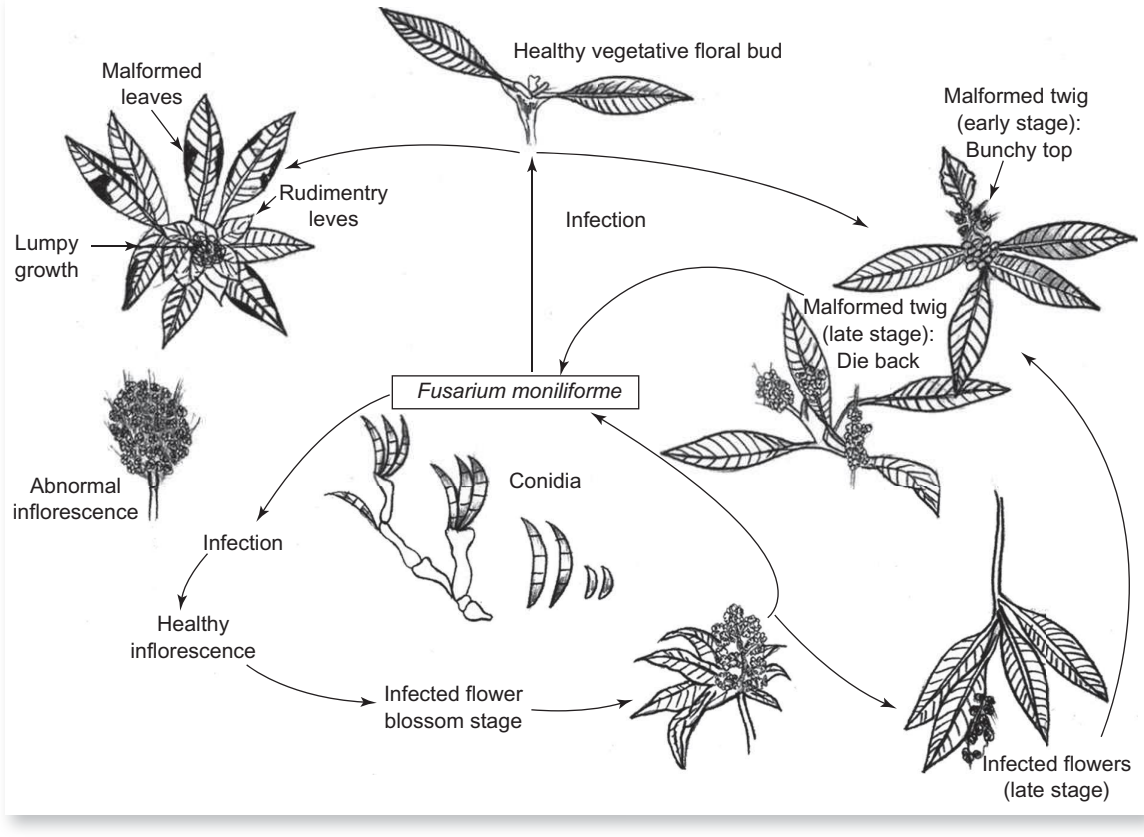


Figure 19.8 Disease cycle of mango malformation

The disease cycle is given in (Fig. 19.8). The fungal propagules that survive the intense summer heat in North India grow profusely over dead, necrotic, malformed shoots and panicles and produce new crops of conidia that initiate fresh infection. Vegetative and floral buds are the primary sites of infection. The pathogen may also invade through florets. The infected buds are ultimately transformed into malformed shoots or panicles. Later, the malformed plant parts undergo necrosis due to the cytotoxic effects of the fusarial toxins. Mites like *Aceria mangiferae* (Fig. 19.7 E) and *Tyrolichus casei* (Fig. 19.7F) carry the propagules on their body surface to the infection site. Injuries sustained from feeding of mites provide an entry point. The use of infected scions for propagation and distribution of malformed saplings results in wide and erratic distribution of the disease.

The highest incidence of the disease is associated with spring flush. The summer growth, even of diseased plants escapes the infection. The disease is most severe where cooler conditions precede flowering. The fungal density reaches a maximum in February when minimum and maximum temperatures range from 8-27°C and humidity is high (85%) (Kumar *et al*., 1993). Hotter and drier periods coincide with a decline in the fungal population. Disease severity is related with the age of the tree. The vegetative malformation is very high on young seedlings. Chadha *et al*. (1979) observed an increasing trend in floral malformation in 1-10 year old plants and a decreasing trend thereafter. The spread of the disease is faster on regular bearing cultivars.

Disease Management

Imposition of a domestic quarantine barrier to check the movement of infected scions and saplings is urgently needed. Increasing intra and inter-line plant distances and avoiding monoculture can impede rapid build up of the epidemic. Pruning proved to be successful (Narasimhan, 1959) when the disease severity was low. Majumdar *et al.* (1970) suggested spraying with Napthyl acetic acid (NAA) (200 ppm) in October, followed by deblossoming at bud-burst stage. Deblossoming is done on fruit trees in order to increase the size and quality of the fruit crop.

Varma *et al.* (1971) screened a large number of fungicides, insecticides and four growth regulators for their fungicidal and fungistatic action and found Benlate, Brestan, Busan, Captan, Dithane MK-45, Panogen, and Thiram to be most effective. Among the insecticides, Aphiden and Phosphamidon were fungicidal at 1000 ppm but not at lower concentrations. Other insecticides and growth regulators were only fungistatic.

It is evident from the literature that at present, no definite foolproof method is available to control mango malformation disease. However, Prakash and Mishra (2001) recommended the following measures:

1. It is advisable to avoid scion stock from trees bearing malformed inflorescences for propagation. Also, indexing of healthy trees should be done, which should provide material for future propagation.
2. Only certified saplings should be used for plantation and for propagation.
3. As soon as the disease is observed in an orchard, the affected terminal of the suspected trees should be pruned along with the basal 15-18 cm-healthy portion and be burnt.
4. As a prophylactic measure, healthy orchards located in disease-prone areas should be sprayed with fungicides/insecticides as a precautionary measure to avoid the occurrence of the disease. Spraying of 200 ppm NAA in the first week of October is advocated, followed by deblossoming at bud-burst stage.
5. Early flowers should be deblossomed.

An integrated management strategy comprising of pruning, spraying with Phosphamidon (0.05 %) and mangiferin metal (copper and zinc) chelates (40 ppm) thrice (pre bud-burst, after emergence and pre-blooming stage), proved effective in controlling floral malformation (Chakrabarti and Ghosal, 1989; Chakrabarti *et al*., 1997; Kumar and Chakrabarti, 1997).

Use of Plant Hormone and Other Growth Regulators Reduction in the malformation of panicles was observed by the use of GA3 of flower bud differentiation stage. It was reported that treatment with 200 or 500 ppm of Chlormequat—the first plant growth retardant (PGR) discovered in the late 1950s by Dr. N.E. Tolbert—mixed with 100 ml Bayfolan, reduced the percentage of malformed panicles (El-Beltagy *et al*., 1980). Spraying 200 ppm NAA in the first week of October, followed by spraying of 500 ppm ethrel at bud inception stage during February was highly effective in reducing floral malformation (Singh and Dhillon, 1986).

Treatment with anti-malformins like glutathione (560 ppm) ascorbic acid (1055 ppm), $K_2S_2O_5$ (560 ppm), silver nitrate (2400 ppm) and NAA (200 ppm) resulted in 87, 93, 80 and 40% conversion to healthy panicles respectively (Ram and Bist, 1984).

Deblossoming at the bud-burst stage alone or in combination with spraying of 200 ppm NAA was reported to be effective in controlling malformation.

Use of Nutrients In some cases, nutrient application has improved the nutritional status, thus improving yield. The combined effect of potassium sulphate as soil applicant and Monocrotrophos as trunk injection cured the malady. Partial control of the disease has been achieved in India by spraying the malformed parts with mangiferin- ZN^{+2} and mangiferin - Cu^2 chelates (Chakrabarti and Ghosal, 1989).

Pruning of Malformed Parts Pruning, followed by spraying a mixture of fungicides (Captan 0.1%), miticide (Akar 338-0.1%) and sticker (Tenae) considerably helped in controlling the spread of disease (Summanwar, 1967).

Use of Insecticides Pruning, followed by the spray of insecticides viz., Follidol and/or Metasystox as a control measure was recommended (Giani, 1965).

Use of Fungicides It was found that copper fungicides were superior to organic fungicides due to excellent tenacity under monsoon conditions. In view of experimental evidences and economy of Fytolan, it may be recommended for controlling malformation of mango trees in which the disease is a serious problem. Benomyl failed to control the problem in South Africa and in India, but spray application of Benomyl has been reported to reduce severity of the disease in India.

Other Measure Veneer grafting is an effective control measure by way of propagation. The seedling trees exhibited malformation earlier than the grafted ones.

Hydrogen Cyanide Cyanide derived from ethylene biosynthesis could contribute to the development of tissue-necrosis during hypersensitive response to plants. Increased levels of cyanide due to stress ethylene may result in the accumulation of toxic levels of cyanide, resulting in the necrosis and death of malformed tissues of mango (Kukreja and Pant, 2000).

Biopesticides Usha *et al.* (2009) clearly demonstrated strong antifungal activity of a concoction brewed from *Datura stramonium, Calotropis gigantea, Azadirachta indica* and cow manure against *Fusarium moniliforme*. It was proved that the concoction brewed from compost is effective, inexpensive, easy to prepare and constitutes a sustainable and ecofriendly approach to control floral malformation in mango.

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Questions

1. What are hyperplasia and hypertrophy?
2. How does *Plasmodiophora* cause infection in crucifers? How does liming of the soil control the disease?
3. What are obligate parasites? Mention a few examples.
4. Does wart of potato occur in India?
5. Mention the seed-borne pathogens of the following:
   (i) Stem gall of coriander
   (ii) Ergot of bajra
   (iii) Clubroot disease of cabbage
   (iv) Wart disease of potato
6. What are the organisms involved in the mango malformation disease? What control measures you will adopt?

Post-Harvest Diseases of Fruits, Vegetables and Post-Harvest Decay of Grains and Legumes

20

Post-harvest loss due to fungal organisms is a serious problem for the producer, distributor, and the consumer. It escalates 'non-essential' overhead cost, reduces market supply, affects the family food cost, imposes serious problems in countries where fruits get heavily damaged, drastically cuts down the quantum of export, and influences the consumer price, leading to the failure of post-harvest technology and management (Singh and Sumbali, 2009). Different diseases arise after crops are harvested because the seeds, fruits or other storage organs are essentially dormant structures and their cells are physiologically unlike those of the growing plant. Therefore, the bulking of these structures in transit and storage pose problems quite different from those encountered in the field. In India, huge losses are being incurred on account of different types of post-harvest diseases. The post-harvest microbial deterioration and spoilage of fruits, vegetables, grains, etc. has been grouped under the field of Market Pathology. This specialized area of study deals with problems encountered during picking, packing, transportation and storage of produce. It is for this reason that the study of post-harvest diseases is conveniently treated as a special aspect of plant pathology.

Economic losses caused by post-harvest diseases are more considerable than often realized because fruits and vegetables increase manifold in unit value while passing from the field at harvest to the consumer. According to the U.S. Department of Agricultural Research Survey in 1965, the annual loss of fresh fruits and vegetables from deterioration during shipment and retail marketing in the USA was approximately 200 million dollars. Post-harvest diseases destroy 10-30% of the total yield of the crops, and in some perishable crops, especially in developing countries, they destroy more than 30% of the crop yields (Agrios, 2005). A substantial portion of these losses is due to diseases caused by microorganisms. In India also, there is considerable loss due to post-harvest diseases. The data collected from some studies carried out in India on post-harvest diseases of fruits and vegetables put the average loss at 20-30% (Sumbali *et al.,* 2001). The National Committee appointed by the Government of India had estimated food grain losses during the post-harvest period to be 9.3% and part of it could be due to fungal deterioration.

Post-harvest losses of fruits and vegetables in developing countries have been estimated to be in the range of 5-20% or more. In addition to the loss of food calories and nutritional elements, mycotoxins and toxic stress metabolites found in vegetables and fruits that are colonized by post-harvest pathogens are emerging as a major dietary problem.

The numerous diseases that occur in transit and storage result mainly from the activity of fungi and bacteria or are of non-parasitic origin. Stakman and Harrar (1957) divided the pathogenic storage diseases into two categories:

1. Diseases of dry bulk material, such as grains.
2. Diseases of succulent storage organs, such as fruits, rhizomes, bulbs and tubers.

Further important consequences that are often overlooked include toxic metabolites produced by diseased plant tissues in response to fungal attack/exposure to ethylene, unacceptable taste of produce or products associated with diseased materials and disintegration of processed fruits by heat-tolerant pectolytic enzymes of post-harvest pathogens.

Harvested fruits and vegetables are vulnerable to attacks by microorganisms because of their high moisture content and rich nutrients. Injuries of various kinds caused during harvesting, packing and transportation facilitate the entry of certain pathogens. A single infected fruit can also be the source of infection to other fruits and vegetables during storage and transit.

Soil-infesting bacteria or fungi that cause loss of fleshy tissues infect plants at the time of, or just before, harvesting. Infection may occur during post-harvest handling or storage as well. Common airborne molds such as *Penicillum* spp. may gain entry into susceptible tissues and cause loss during packing and shipping operations.

All important rot pathogens initiate disease in the same general way. They all produce extracellular enzymes and start the degenerative process in advance of the fungal hyphae or bacterial cells of the attacking pathogens. In fact, these organisms are all saprophytic pathogens and obtain nourishment from dead or dying cells. According to Simmonds (1963), the ripened fruits are infected easily, while the green fruits show resistance to infection. He attributed the following reasons to this phenomenon:

1. Green fruits do not meet the nutritional requirements of the fungus.
2. The enzyme-potential necessary for invading green fruits is greater for ripe ones and is temporarily beyond the capability of the fungus.
3. Some toxin present in the green fruits disappears or becomes inactive in the ripe ones.
4. The energy requirement of the fungus can only be met when the metabolism of the host has passed from the green to the ripening phase.

As a result of infection, the market and nutritive value of the fruit is lowered, either due to its ugly appearance or due to the changes in the stored products of the fruits, vegetables and seeds.

Bacterial Rots

Soft rot bacteria attack vegetables such as carrots, potatoes, onions and celery. The soft rot is brought about by the action of enzymes (pectolytic and celluolytic) on the pectic substances and cellulose of the cell walls. As a result of these enzymes, the host tissues disintegrate and their protoplasts die. *Erwinia* spp. are mainly responsible for bacterial rots in storage. In the Bergey's *Manual of Systematic Bacteriology Vol I*, edited by N.R. Kreig and J.G.Holt (1984), the soft rot bacteria are *Erwinia carotovora pv. carotovora, E. carotovora* pv.*atroseptica, E. aroidea* and *E. chrysanthemi*. The factors that predispose the fruits and vegetables to soft rots under stored condition are humidity, temperature, presence of bruises or lesions or attack by other pathogens. Some other bacteria also cause soft rot at high temperatures and high humidity. For example, *Bacillus polymyxa* reduces potatoes to a yellow sticky mass with a characteristic fishy odour. Some anaerobic clostridia are associated with soft rot bacteria.

Soft rot bacteria may survive in infected tissues in the soil and in contaminated equipments and containers. Some of them overwinter in insects. They enter plants or plant tissues primarily through wounds within the tissues, multiply profusely in the intercellular spaces where they produce several kinds of pectolytic and cellulolytic enzymes, which ultimately result in softening of tissues and their disintegration.

Fungi

A large number of fungi are responsible for storage diseases of fruits and vegetables. *Rhizopus* spp. cause considerable loss to peaches, grapes, strawberries, sweet potatoes, cucurbits, crucifers, tomatoes and eggplants. *Rhizopus* spp. produce soft rot of the fleshy parts, which proceeds rapidly at high temperatures. There is often leakage of juices from the affected parts of fleshy fruits and vegetables. Under humid conditions, the typical cottony, coarse and stringy mycelium of *Rhizopus,* with characteristic black sporangia, covers the diseased tissue. In temperate regions, stone and pome fruits are affected by brown rot fungi such a *Sclerotinia fructigena*, *S. laxa* and *S. fructicola*. The fruits get shriveled and mummified and become a mass of dried fruit tissue, completely penetrated by the mycelium of the fungus and covered with conidia. Bananas, in storage and transit, are subjected to various fungal rots. Rotting is generally brought about by *Gloeosporium musarum. Penicillium* spp. cause considerable damage to citrus fruits in storage and transit. The diseases are popularly called green mould (*P. digitatum*) and blue mould (*P. italicum*). Potato tubers are commonly attacked by *Fusarium caeruleum.* Species of *Aspergilli* are very often responsible for the deterioration of stored grains. *Aspergillus flavus* produces a fungal toxin known as aflatoxin in groundnut kernels. Several other fungi, for example, *Alternaria, Helminthosporium,* etc., bring about the deterioration of grains in storage. A list of major post-harvest diseases of fruits and vegetables is given in Table 20.1.

Table 20.1 Major Post-Harvest Diseases of Fruits and Vegetables

Apple and Pear	Lenticel rot	*Cryptosporiopsis malicorticis* (= *Gloeosporium perennans*)
	Eye rot	*Nectria galligena*
	Blue mold rot	*Penicillium expansum* (Fig. 20.1a)
	Gray mold rot	*Botrytis cinerea*
	Black mold rot	*Aspergillus niger*
	Soft rot	*Rhizopus arrhizus*
	Bitter rot	*Gloeosporium fructigenum* (Fig. 20.1b)
	Pink rot	*Trichothecium roseum* (Fig. 20.1c)
	Phoma rot	*Phoma violacea* (Fig. 20.1d)
Banana	Crown rot	*Colletotrichum musae* (=*Gloeosporium musarum*) *Fusarium roseum, Verticillium theobromae* (Fig. 20.3b) *Ceratocystis paradoxa* (=*Thielaviopsis paradoxa*)
	Anthracnose	*Colletotrichum musae* (=*Gloeosporium musarum*)
Cabbage	Bacterial soft rot	*Erwinia carotovora*
	Black rot	*Xanthomonas campestris*
	Watery soft rot	*Sclerotinia sclerotiorum*
	Grey mold rot	*Botrytis cinerea*

(*Contd.*)

(*Contd.*)

Carrot	Centrospora rot Watery soft rot	*Centrospora acerina* *Sclerotinia sclerotiorum*
Citrus fruits	Stem-end rot Green mold rot Ceratocystis rot Blue mould rot	*Phomopsis citri* *Diplodia natalensis* *Alternaria citri* *Sclerotium rolfsii* (Fig. 20.2c) *Penicillium digitatum* *Ceratocystis* sp. (Fig. 20.2d) *Penicillium italicum*
Grape	Grey mould rot Cladosporium rot Black rot Gloeosporium rot Blue mould rot Alternaria rot	*Botrytis cinerea* *Cladosporium herbarum* *Guignardia bidwelli* *Gloeosporium* sp. (Fig. 20.3 d) *Penicillium* spp. *Alternaria* and *Stemphylium* spp.
Cauliflower	Brown rot Ring rot	*Alternaria brassicae* *Mycosphaerella brassicicola*
Cucumber	Bacterial spot Anthracnose Cottony leak Scab Soft rot	*Pseudomonas lachrymans* *Colletotrichum lagenarium* *Pythium aphanidermatum* *Cladosporium cucumerinum* *Pellicularia filamentosa*
Papaya	Anthracnose Fruit rot	*Colletotrichum gloeosporioides* *Phytophthora* spp. *Ascochyta caricae Phomopsis* spp., *Botryodiplodia* sp. *Cladosporium herbarum* (Fig. 20.3 a)
Peach and Cherry	Black rot	*Monilinia fructicola* =(*Sclerotinia fructicola)*
Pineapple	Black rot	*Ceratocystis paradoxa* (=*Thielaviopsis paradoxa*)
Potato	Soft rot Dry rot Gangrene Skin spot Silver scurf	*Erwinia carotovora* and other species *Fusarium* spp. *Phoma exigua var. foveata* *Oospora pustulans* *Helminthosporium solani*
Mango	Anthracnose Fruit rot Diplodia stem-end rot Black mould rot Soft rot	*Gloeosporium mangiferae* (Fig. 20.2a) *Diplodia natalensis* (Fig. 20.2b) *Botryodiplodia theobromae* (Fig. 20.2b) *Aspergillus niger* *Rhizopus arrhizus*

(*Contd.*)

(*Contd.*)

Muskmelons	Alternaria rot Bacterial soft rot Bacterial spot Blue mould rot Charcoal rot Rhizopus rot Fusarium rot Pink mould rot	*Alternaria tenuis* *Erwinia* spp. *Pseudomonas lachrymans* *Penicillium* spp. *Macrophomina phaseolina* *Rhizopus stolonifer* *Fusarium* spp. *Cephalothecium roseum*
Tomato	Alternaria rot Anthracnose Canker Soft rot Bacterial spot Cladosporium rot Early blight rot Fusarium rot Ghost spot Helminthosporium rot Late blight rot Phoma rot	*Alternaria tenuis* *Colletotrichum* spp. *Corynebacterium michiganense* *Erwinia carotovora* *Xanthomonas vesicatoria* *Cladosporium herbarum* *Alternaria solani* *Fusarium* spp. *Botrytis cinerea* *Helminthosporium* spp. *Phytophthora infestans* *Phoma destructive*
Strawberry	Grey mould rot Rhizopus rot	*Botrytis cinerea* *Rhizopus stolonifer*
Sweet potato	Black rot	*Ceratocystis fimbriata* (*Endoconidiophora fimbriata*)
Guava	Rhizopus soft rot Gloeosporium rot Phytophthora fruit rot Botryodiplodia rot Pestalotia rot	*Rhizopus stolonifer* *Gloeosporium psidii* (Fig. 20.3c) *Phytophthora nicotianae var. parasitica* *Botryodiplodia theobromae* *Pestalotia psidii*

Non-Pathogenic Diseases

As a result of bulking together of a large number of fruits or other vegetative parts, some rots and other diseases appear. These are not directly due to the pathogens; for example, when apples and potatoes are stored in a poorly ventilated room, the level of CO_2 may become excessively high in the atmosphere, leading to a corresponding decrease in the O_2 levels. This results in a condition known as black heart. Externally, the tubers look normal, but when they are cut open, the central portion appears black. Black heart is caused by lack of oxygen, which kills the tissues. High temperatures of about 32°C and poor ventilation favour it during transit. Apples suffer from brown heart in transit in a similar fashion. Various

types of discolorations and scalds may appear on the skin of various fruits as a result of poor storage conditions. Factors responsible for bringing about discoloration are thought to be volatile substances, probably esters, and precursors of volatile substances to materials of low volatility or to toxic substances accumulating beneath the cuticle.

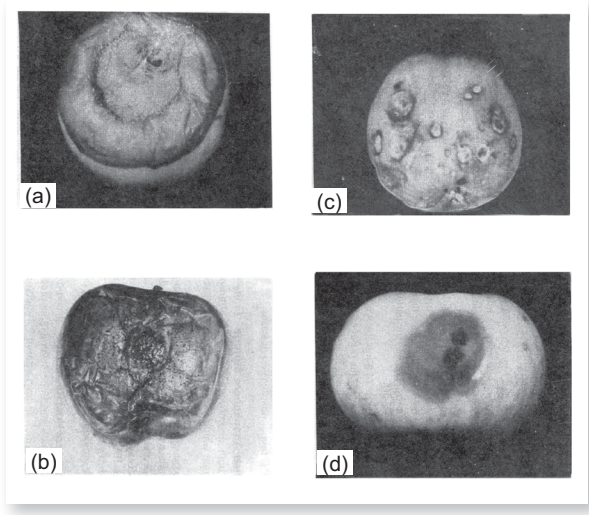


Figure 20.1 (a) blue mold rot of apple caused by *Penicillium expansum* (b) bitter rot of apple caused by *Gloeosporium fructigenum* (c) pink rot of apple caused by *Trichothecium roseum* (d) phoma rot of apple caused by *Phoma violacea* (Courtesy of Prof. Geeta Sumbali, Jammu University, Jammu)

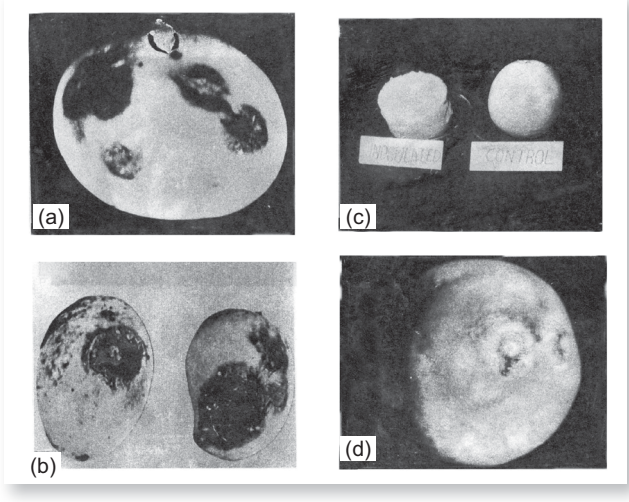


Figure 20.2 (a) anthracnose of mango caused by *Gloeosporium mangiferae* (b) fruit rot of mango incited by *Diplodia natalensis* (c) rot of orange caused by *Sclerotium rolfsii* (Courtesy Late Dr. Ranjana Dhingra) (d) rot of orange caused by *Ceratocystis* sp.

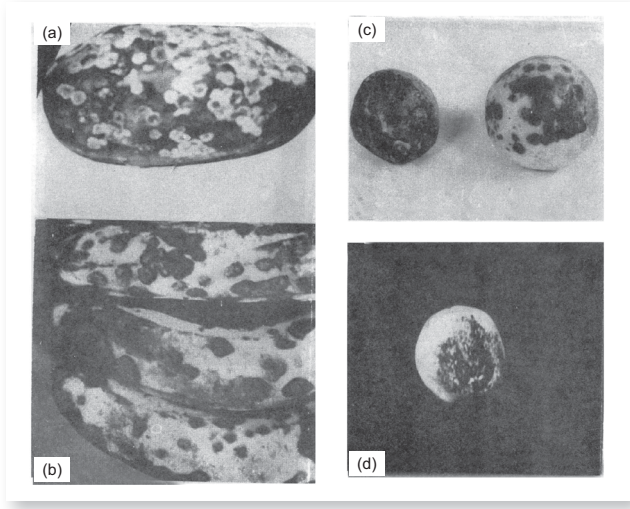


Figure 20.3 (a) rot of papaya caused by *Cladosporium herbarum* (b) storage rot of banana caused by *Verticillium theobromae* (Courtesy Late Dr. Ranjana Dhingra) (c) fruit rot of guava by *Gloeosporium psidii* (d) fruit rot of grape caused by *Gloeosporium* sp.

Mode of Infection and Factors Influencing Post-Harvest Diseases

In many post-harvest diseases, the pathogen responsible for the spoilage enters the fruits, vegetables or seeds before harvesting. For example, spores of *Colletotrichum gloeosporioides* germinate in moisture on the surface of avocado, banana, citrus, mango and papaya fruits at any time during the development of the fruit on the plant. The infection remains latent for months and then gives rise to disease after harvesting, when the fruit ripens. *Diplodia* and *Phomopsis* spp., which are responsible for stem-end rots of citrus fruits, enter the fruits at any stage of development. The infection lies in the quiescent stage in the stem button (calyx plus disc) of the fruit. Brown rot of citrus fruits caused by *Phytophthora* spp. develops after harvest, during storage. The disease develops from incipient infection caused by zoospores of *Phytophthora* spp., which splash on to low-hanging fruits.

Lenticels of potato tubers are a common site of initiation of bacterial soft rot after harvest. The lenticels of most tubers are contaminated with the cells of *Erwinia carotovora* at the time of harvest. *Phlyctema vagabunda* (*Gloeosporium album*) and *Cryptosporiopsis curvispora* (*G. perennans*) survive as latent infections in the lenticels of apple fruits. The injury created by severing the fruit from the plant is a frequent point of initiation of post-harvest diseases by wound pathogens. Some examples are crown rot of banana, pedicel rot of pineapple, stem-end rot of mango, papaya and green pepper.

Propagules of pathogenic fungi and bacteria are abundant in the atmosphere and on the surfaces of fruits and vegetables, as they approach maturity in the fields. *Penicillium, Rhizopus, Geotrichum* and *Thielaviopsis* are not capable of penetrating the intact surfaces of the host, but they readily gain entry through injuries or natural openings and thus cause devastating rots of the mature produce. In addition to pre-harvest contamination, harvested produce may become infected by contact with decayed fruits or contaminated equipment in the packaging house. Physiological injuries caused by cold, heat, oxygen deficiency and other environmental agents predispose fresh agricultural products to post-harvest

diseases. Alternaria black rot and bacterial soft rot of tomatoes have been reported to increase due to such storage conditions. This is also true for Nigrospora rot of bananas and surface rot of yams.

Moderately high temperatures cause fresh produce to decay. Tuber crops, if exposed to direct sunlight for more than a few hours, are liable to be infected by severe storage rots. Injuries can also be caused by hot water treatment undertaken to eliminate infections; for example, in the case of lemons, *Penicillium* decay sets in during storage when lemons are soaked in water at 48°C for four minutes to get rid of incipient infection of *Phytophthora* sp. Post-harvest diseases are often influenced by the manner in which the crop is grown; for example, the mineral nutrition of the apple tree affects the infection of bitter pit in storage. The amount of brown rot on apples, pears and plums is to a certain extent determined by the level of field inoculum. In the case of Fusarium storage rot of potato tubers, the presence of field inoculum is important. *Fusarium caeruleum* is frequently present in field soils and in soil, adhering to tubers and this subsequently contaminates the seed boxes, bags and stores.

Control of Post-Harvest Diseases

Harvesting and Handing

The way the produce is harvested is critical because many pathogens involved in storage diseases enter through wounds. Insects may also cause wounds. Rotting of citrus fruits by *P. digitatum* and *P. italicum* is closely associated with the punctures made in the skin by the fruit fly, and *Sclerotinia laxa* with those made by the plum curculio *(Conotrachelus nenuphar).*

Harvesting of vegetables and fruits should be done at the proper stage, keeping the near or distant market in mind. It should be done preferably in the early morning hours to avoid heat. Proper harvesting is essential, otherwise, the quality of the harvested produce cannot be improved but it can only be preserved. Curing of roots, bulbs and tubers is an effective means of reducing post-harvest decay and water loss, right at the farm level.

Packaging

In our country, gunny bags, bamboo baskets and wooden crates lined with newspaper sheets are mostly used for packing, which ensures ease of transportation and handling. In foreign countries, the vegetables are cleaned, trimmed, pre-treated, pre-cooled and packed in bags of transparent film, trays or cartons wrapped with transparent film etc.

Transportation

The system mainly adopted in India to supply vegetable produce is road transportation. The poor road conditions, coupled with speedy driving, also cause much harm to the produce. The use of railway wagons will continue to be difficult unless there is a comprehensive redesigning of the wagons to carry mere perishable produce across the country. It is necessary to modify the long-distance transport vehicles by introducing more ventilation, reducing temperature, increasing relative humidity, improving loading and unloading systems.

Storage

It is one of the most important aspects of the post-harvest handling as the storage life of almost all the vegetables is short due to dehydration, losses by respiration and rotting etc. This causes deterioration in quality due to wilting, shrinking and weight loss. Refrigeration is one of the useful methods for long-term storage of fresh vegetables. Various sprout inhibitors like maleic hydrazide (MH) and naphthalene acetic acid (NAA) have been found to check sprouting, rotting and weight loss.

Temperature and Relative Humidity

To a very large extent, temperature, humidity and ventilation determine the rate of disease development during transit and storage. Fruits and vegetables continue to respire after they are harvested and as a result, heat is produced. This is known as vital heat. The fruits and vegetables that have a high rate of respiration perish most rapidly. High temperature accelerates respiration and ageing; hence, these fruits must be cooled quickly to remove their field heat. This is achieved in several ways: by spraying or immersing in water kept at a temperature of about 0°C, by vacuum-treatment, which induces rapid evaporation of water from the produce or by placing in pre-cooled rooms or railcars. The produce is then transported in vehicles at approximately 0°C. Green peas and strawberries fall in this category. Some produce can be transported safely at higher temperatures. For other fruits and vegetables, especially those with low respiration rates, higher temperatures during transit are essential because low temperatures lead to damage. Lemons become pitted and develop internal browning if kept at 4.4°C or at a lower temperature.

Near-freezing temperature prevents most bacterial and fungal rots. The development of some non-parasitic diseases such as bitter pit and scar of apples is also inhibited. Humidity during transit and storage should be generally high; otherwise, the produce dries and loses its prime market quality.

Control of Pre-Harvest Infection

The principal way to control pre-harvest infection is by the application of protectants or eradicant fungicides to the crop in the fields. In the case of Botrytis rot of strawberries and raspberries, the application of a fungicidal spray at intervals of 7-14 days during the period of flowering has given good control. Field sprays of Thiram and Captan have given good control of Botrytis rot of strawberries after harvest. But Chlorothalonil, Dichlofluanid and Benomyl are now the most effective materials.

Several tropical fruits are susceptible to infection by *Colletotrichum gloeosporioides* at all stages of development, and therefore, it is essential that these fruits be protected continuously with fungicidal sprays from the time of fruit-set until harvest.

Lenticel rotting of apples during storage has been controlled in England and France by late summer sprays of Captan, Benomyl and Thiabendazole, which protect plants against or eradicate the lenticel infection at this time of year. Before 1960, the control of latent infection of fruits was limited to provide the protective coating on the fruit during the period when infection was likely to occur. In recent years, it has been shown that systemic fungicides such as Benomyl and Thiabendazole can eradicate latent infections as well as protect plants against their establishment.

In India, Aureofungin has given promising results in the control of a number of post-harvest diseases of fruits and vegetables. *Alternaria cucumerina* considerably reduced the fruit rot of summer squash. Different oils have also effectively controlled fruit rot. Grover and Aulakh (1968) reported the control of *Oospora* tomato fruit rot by the application of castor and coconut oils.

Pre-harvest sprays with Carbendazim, Benomyl and Thiophanate methyl prevented blue mould rot of apples stored both at ambient temperature and in air-cooled storage. Calcium sprays to control bitter pit disorder also confer resistance to *Penicillium expansum* during storage. Pre-harvest sprays of Captafol and Carbendazim enhanced the shelf-life quality of apples by protecting them from storage rots. Two or three sprays of Carbendazim, Benomyl or Thiabendazole provided control of storage decay of pears caused by *Penicillium expansum* and *Botrytis cinerea.*

Pre-harvest application of calcium chloride in peaches reduced post-harvest rots (Berton *et al.,* 1992). Multiple pre-harvest applications of calcium chloride reduces the ripening of black berries during post-harvest holding and thus can help in reducing post-harvest losses. Benomyl, Chlorothalonil, Triforine, Triarimol, Dichlozline and Thiophanate methyl, when used as orchard sprays, were as good or better than Captan for controlling brown rot of prunes. Strawberry plants sprayed weekly with Thiabendazole, Benomyl or Captan showed reduced brown rot during storage while *Rhizopus stolonifer* remained unaffected. Application of Iprodione, Vinclozolin, Meclozolin, Anilazine, Carbendazim and Captan several times during the season have given good control of major post-harvest diseases of strawberry and raspberry. Field sprays of fungicides such as Carbendazim, Benomyl, Thiabendazole, Captan, Dichloran, Iprodione and Vinclozolin prevented the establishment of *Botrytis cinerea* and *Penicillium* in grapes.

Thus, pre-harvest sprays of systemic fungicides can eradicate quiescent infections as well as protect against their establishment before harvest and reduce disease pressure.

Strategies for Post-Harvest Disease Control

(A) Orchard Management

- Dead wood, leaves and other parts of fruit tree harbour a number of post-harvest fruit pathogens. These infected parts should be removed from the vicinity of the plant.
- Time of harvesting can influence the disease incidence.
- Fruit resistance in die-back of mango caused by *Botryosphaeria ribis* is influenced by nutrient deficiencies.
- Certain diseases are more severe in some parts than in other parts. Fruits should be cultivated in those regions, which are unfavourable for infection. Mango production is most successful in regions where flowering and fruiting occur in the dry season, which does not favour anthracnose.
- Cultivars that are resistant should be cultivated. It is known that polyembryonic varieties of mango suffer fewer diseases than the monoembryonic varieties.
- During harvesting, handling and packaging efforts should be made to minimize the fruit damage and place wounded fruits in an environment that is conducive to reactions like lignifications, periderm formation and phytoalexin formation.

(B) Disinfection and Washing Formaldehyde and nitrogen trichloride have been used to disinfect lemon storage boxes and SO_2 has been recommended for disinfecting packing boxes used for figs. These treatments have the disadvantage of being corrosive to the box materials. The current practice is to disinfect these with a hot Borax solution before the boxes are refilled with a new lot of lemons for storage. When fruits and vegetables are cleaned and cooled with water or treated with aqueous formulation with chemicals for purposes other than disease control, broad-spectrum antibiotics are added in water. Hypochlorous acid, added to water as chlorine gas or a hypochlorite salt, rapidly kills microbial cells in the processed water and reduces the hazards of inoculating the products with pathogenic microbes during operations involving the recycling of water. Sodium-O-phenyl phenate has been used occasionally for

this purpose. There are certain advantages of sodium-O-phenyl phenate over hypochlorite because it is noncorrosive, stable and compatible with the chemicals, which react with chlorine.

Sodium-O-phenyl phenate has been added to hydrocooler water to prevent the inoculation of peaches with brown rot pathogen and 100 ppm has been added to a solution of sec-butyl ammonium phosphate used to disinfect oranges before ethylene degreening. The population of pathogenic fungi on the surface of citrus fruits and peaches arriving from the orchard may be greatly reduced by surface treatment with a solution of Sodium hypochlorite or Sodium-O-phenyl phenate. Washing with water alone reduces the incidence of Fusarium rot in potatoes and decay of carrots in long-stage storage. 1-3% formaldehyde solution is atomized each night into the atmosphere of a packaging house having spores of *P. digitatum* and *P. italicum*. Fruit conveying and treating equipment is generally treated regularly with quaternary ammonium compounds, Sodium-O-phenyl phenate, formaldehyde or hypochlorite.

(C) Post-harvest Chemical Treatment Two major strategies have been developed for the treatment of produce to combat infection by pathogens:

- Cover the surface of the produce with a fungicide deposit before harvest so that any superficial injuries created during or subsequent to the harvest contain an inhibitory concentrate of a fungicide.
- Treat the produce after harvest with the intention of depositing an inhibitory quantity of the fungicide in those superficial injuries, which were formed during the harvesting and handling of the crop.

After an injury, if the pathogen gets entry, it is still possible to prevent disease by interrupting the infection process with fungicide treatment, provided, the development of the pathogen in the host is still quite limited. It is not necessary to kill a pathogen present in an injury in order to prevent disease development. The fungicidal chemical is applied at concentrations that arrest the development of the fungus. Sodium-O-phenyl phenate, Sodium tetra borate, Sodium-carbonate, Sec-butyl amine, Biphenyl and Thiabendazole are the chemicals which are only fungistatic under usual conditions, while Sodium hypochlorite is lethal to fungal propagules and fungi *in vitro*. Most post-harvest treatments result in the accumulation of fungistatic residues in superficial injuries, which prevent infection at these sites.

Apart from these fungicides, some novel chemicals like homoeopathic drugs have been reported to prevent infection when applied on harvested fruits. Some of the growth regulators like IAA and MH were effective against Aspergillus and Rhizopus rots of papaya fruits. Several workers have reported that leaf extracts of *Eucalyptus globulus, Punica granatum, Lawsonia inermis* and *Datura stramonium* were effective in checking fruit rot of lemon. Similarly, leaf extract of Tulsi reduces spore germination, growth, total proteins, pectinolytic and cellulolytic enzymes of various rot pathogens. Gupta and Pathak (1990) found groundnut, mustard, castor and paraffin oils effective against Rhizopus rot of mango and Aspergillus rots of papaya.

Heated Fungicide Suspension A three-minute dip in hot Benomyl and Dichloran gives more effective control of Rhizopus rot of prunes than unheated dip. Fungicide-enriched hot water treatment and heated wax emulsions are more effective than either chemical or heat alone.

Sugar Analogues as Potential Fungicides A 1% solution of 2-deoxy-D-glucose showed promise in treatment of *Penicillium expansum* and *Botrytis cinerea*.

Salt Solutions Spore germination of *P. expansum*, *B. cinerea* and *Mucor piriformis* was reduced with a 40-minute exposure to sodium lignosulfonate. In commercial packaging houses, decay caused by *Phialophora malorum* was less in pear boxes floated in sodium lignosulfonate with Sodium-O-phenyl phenate.

Inorganic Salts Application of calcium salts during growing season or as a post-harvest dip is often used to enhance the storage life of apples. McLaughlin *et al*. (1990) tested the efficacy of ferrous sulphate, potassium chloride, magnesium chloride, manganese chloride, calcium carbonate, calcium chloride and sodium chloride to control *B. cinerea* and *P. expansum* on apple fruits and found that calcium carbonate was the most effective salt.

Management of Fungicide Resistant Strains Use of some or closely-related fungicides before and after harvest is not advisable because it can lead to rapid development of resistant strains of the pathogen. Most of the newer groups of fungicides have been seriously affected by the development of resistance in the pathogen. Resistant strains of *Penicillium expansum* to Benzimidazoles and some other fungicides have been reported (Prusky *et al.*, 1985). Strategies recommended for the control of fungicide-resistant strains of *P. expansum* are:

1. Uniform treatment with a mixture of fungicide having different modes of action.
2. Heterogeneous treatment in which two fungicides are applied separately to different lots of fruits placed in the same store room.
3. Combination of treatments in which a mixture of fungicides and single fungicides are applied to various lots of fruits placed in the same store room.

(D) Fumigation Some volatile fumigants are ideal for controlling post-harvest diseases, particularly lenticel rots. Sulphur dioxide fumigation of grapes eliminates most of the pathogen inocula and particularly *Botrytis cinerea*. Post-harvest rots of grapes caused by *B. cinerea* and *Aspergillus niger* were reduced by using Quick Release Grape Guard i.e., sulphur dioxide-generating paper sheets impregnated with 1.5% sodium bisulphite. Sulphur dioxide also eliminated *Trichothecicem roseum* and effectively checked *Glomerella cingulata*, *Penicillium expansum* and *Monilinia* sp. in apple and pear (Sharma and Kaul, 1998).

(E) Fruit Wraps, Waxing, Coating and Packing Various antifungal chemicals have been used for impregnating fruit wrappers and box liners. Wrapping grape clusters in tissue papers impregnated with O-phenyl butyrate and sodium metabisulphate reduces post-harvest decay of grapes caused by *A. niger* and *P. canescens*. Potassium iodide wraps provided effective control of *Gliocladium roseum* on apples (Sumbali and Mehrotra, 1983). Fruits can be individually wrapped in a heat-shrinkable polyethylene film that prevents the spread of diseases as well as the transfer of spores and debris from fruit to fruit in the same container. Keeping fruits in newspaper impregnated with Carbendazim and Thiabendazole checked the blue mould rot when stored in cold storage. El-Ghaouth *et al.* (1992) reported that chitosan, an ingredient of some fruit waxes, reduced the growth of *R. stolonifer*, *B. cinerea* and *Alternaria alternata in vitro*.

Coating skin of fruits with oils, waxes and colloidal solution of carboxymethyl cellulose can improve the quality of fruits by decreasing water loss, retard ripening and inhibit rotting by various pathogens.

Apples coated with mustard oil, paraffin wax and castor oil checked the infection of a large number of pathogens (Sumbali and Mehrotra, 1983). Another treatment of extending post-harvest life of apple, pear and plum is by coating skin with 'Prolong', a mixture of sucrose esters of fatty acids and polysaccharide. 'Prolong' changes the permeability of fruits to gases in such a way that oxygen permeability is considerably reduced while carbon dioxide permeability is little affected.

(F) Heat Treatments Heat treatments have the advantage of being low cost, have relatively simple equipment requirement and no chemical residues on the treated product. Rosenbaum (1920) reported

that incipient infection due to species of *Phytophthora* could be removed by immersing the fruits in water at 60°C for 1.5 minutes.

The utility of heat treatments in reducing decay of peaches and other stone fruits has been investigated. An effective and practical treatment consists of submerging peaches in water at 51.5°C for 2-3 minutes or at 46°C for 5 minutes. Hot water treatment may cause injury to peaches when stored at a low temperature after treatment but not when stored at the room temperature. This is true for *Penicillium, Alternaria* and *Botrytis,* which infect fruits stored at low temperatures after treatment. Hot water treatment has been used for the control of *Penicillium* and *Diplodia* on mangoes, *Colletotrichum* on papayas, crown rot of banana, stem scar and rind mold on cantaloupes and *Colletotrichum* on mangoes.

Hot water dip at 50°C for 5 minutes controlled various post-harvest rots caused by *Trichothecium roseum, Monilinia laxa, Glomerella cingulata, Penicillium expansum* and *Rhizopus stolonifer.* Aerated steam has been found effective against post-harvest diseases of papaya, mango, guava, and citrus. Aerated steam treatment method has been found better than hot water treatment. Hot-water treatment has a number of advantages which include relative ease of use, short treatment time, reliable monitoring of fruits and water temperatures and the killing of skin-born decay-causing agents. Heat treatment eliminates incipient infection. Heat may also inhibit pathogen spread by inducing defense mechanism in the outer layer of epicarp. Heat treatment can also be used to inhibit ripening process, induce resistance to chilling injury and external skin damage during storage, thus extending storability and marketing. Natural openings and barely visible cracks in the epidermis of treated fruits were partially or entirely sealed with rearranged natural wax components present on the cuticle, thus limiting sites of fungal penetration into the fruits.

(G) Irradiation Gamma rays are capable of penetrating fruits and vegetables and destroying pathogens in established lesions, deep in the host tissues. However, there is a serious drawback in irradiation treatment of deep-seated infection. Most of the fungi, which are deep-seated, are relatively insensitive to radiations at dosages which are not lethal to the host. Irradiation is quite effective in superficial infections.

Germination of *Aspergillus niger* conidia was reduced by gamma (γ) irradiation. Inoculated apples with *A. niger,* exposed to 150-200 Krad, could be stored for 5-7 days at 20°C without rotting. Johnson *et al.* (1990) observed that irradiation of strawberry reduced the incidence of grey mould but increased the incidence of Alternaria rot during storage.

Radiation has lethal effects on spore germination in several fungi. Low doses of ultraviolet irradiation (254 nm UV-C) reduced post-harvest brown rot of peaches and Penicillium rot of Indian gooseberry. In this case, the low-dose ultraviolet light treatment had two effects on disease development: reduction in the inoculum of the pathogen and induced resistance in the host. When the fruits are exposed to low doses of UV, a number of changes are induced, including the production of antifungal compounds and delay in ripening. This introduced a new terminology called 'hormesis'. Hormesis is the application of potentially harmful agents at low doses to living organisms in order to induce stress responses. Low doses of UV-C hormesis were shown to induce resistance in post-harvest commodities to harvest decay and to extend shelf life of fruits by delaying the ripening and senescence process (Sharma and Alderson, 2005).

(H) Low Temperature Storage In certain cases, post–harvest decay of fruits and vegetables can be delayed by storing them at low temperatures. Pathogens with relatively high temperature optima, such as *Geotrichum* and *Erwinia*, may be inhibited almost indefinitely by storage at 0°C. But these pathogens become active as soon as the produce is brought to higher temperatures for ripening or marketing.

Pathogens such as *Botrytis, Penicillium, Sclerotinia, Centrospora* grow slowly at near-freezing temperatures and limit the storage life of the crop if they are not controlled by some other treatment. Low temperature delays the development of the disease in different ways: (1) it delays ripening of the fruits and thereby the period of resistance to the pathogens is prolonged; (2) there is direct inhibition of pathogens at low temperature.

Lowering the temperature of non-climacteric fruits only reduces spoilage, while in climacteric fruits, lower temperature also delays the onset of ripening. The low storage temperature is most effective for apples and grapes, which tolerate near-freezing temperature that checks the development of pathogenic microbes. Greater the reduction in respiration and general metabolism, longer the storage life of fruits held just above its freezing point. Fruits that are chilling-sensitive must be stored at 3-5°C. The effects of temperature and relative humidity are often inseparable and interrelated because the capacity of air to hold moisture varies with the temperature. However, climatic conditions like relative humidity of above 90% and a temperature of about 5°C favour post-harvest diseases.

(I) Controlled Atmosphere Storage Fruits stored in conditions low in oxygen, high in carbon dioxide, or both are known as controlled atmosphere storage. Such storage conditions also suppress respiration rate, which ultimately reduces diseases in storage and transportation by suppressing fungal growth.

Avocados stored for 45 and 60 days at 10°C in an atmosphere of 2% oxygen and 10% carbon dioxide show tremendous reduction in anthracnose (Spalding and Reeder, 1975). The growth of *Botrytis cinerea* in apple is checked markedly only if oxygen level is reduced 1%. Adding 10% carbon monoxide to air retards decay of grapes caused by *B. cinerea* for the first two months at 0°C. A combination of 2% oxygen and 10% carbon monoxide controlled decay in grapes stored at 0°C up to 4 months and caused less browning and bleaching than sulphur dioxide. Storage and transportation under low oxygen or hyperbaric atmosphere also helps in reducing post-harvest diseases.

(J) Maintenance of Host Resistance In addition to storing fruits and vegetables at low temperatures to retard ripening and senescence, several other practical methods have been followed. In California, lemons are treated with isopropyl ester of 2, 4-dichlorophenoxyacetic acid (2-4-D) before storage to delay senescence of the button (clays plus disc), which is the usual point of attack by the fungus *Alternaria citri*. Application of gibberellic acid reduces *Penicillium* decay of oranges by delaying the senescence of the skin of the fruit.

Modifying the storage atmospheres by changing the levels of oxygen and carbon dioxide influences the development of post-harvest diseases by either directly inhibiting the pathogen or by altering host resistance.

(K) Moderation of Disease Symptoms The value of fresh fruits and vegetables is reduced significantly by superficial fungal growth or by visible contamination with spores or other debris arising from an adjacent disease. Sooty moulds on citrus fruits or on other surfaces of the crowns of bananas at the cut surfaces are examples of this type of disease. Fungicidal treatment after harvesting controls these conditions.

(L) Inhibiting Growth and Sporulation of Pathogen on Surface of Diseased Host Vapours of biphenyl inhibit the sporulation of *Penicillium* on the surface of decayed oranges. Lemons may be fumigated with nitrogen trichloride during storage to control *Penicillium* sporulation. Dibromotetrachloroethane controls saprophytic growth of Alternaria on the buttons of oranges. Superficial growth of *Alternaria* on melon fruits is checked by post-harvest treatment with Dithiocarbamate, Tetraiodethylene, Sodium-O-phenylphenate and heat.

(M) Preventing Spread of Pathogen from Diseased to Healthy Produce The spread of disease by contact frequently accounts for the major losses caused by these pathogens. Oiled paper wraps containing copper sulphate can prevent the spread of *Botrytis* on pears. Wraps impregnated with copper sulphate could not control Rhizopus rot on tomatoes. However, wraps impregnated with organic compounds that generate active chlorine and active bromine retard the spread of *Rhizopus* in tomato packs. Similarly, paper wraps impregnated with 2, 6 - dichloro-4-nitroaniline are highly effective in preventing the spread of *Rhizopus* in peaches. Trichoderma rot of lemons could be confined to initially diseased fruits by wrapping the fruits in paper impregnated with O-phenyl-phenol. Fungicide-treated wraps check the secondary spread of the pathogens. These do not control the disease in the fruits that are already infected. Grapes in storage are fumigated with sulphur dioxide at 10-day intervals to prevent the spread of *Botrytis* infections from diseased fruits. Sodium disulphate is added to shipping containers to generate sulphur dioxide, which prevents the spread of *Botrytis* during storage and long-distance shipment of grapes.

(N) Chemical Treatments The first treatments employed to control post-harvest decays of citrus fruits were alkaline solutions of sodium tetra borate (borax) and sodium carbonate. Early experiments with oranges showed that 6-8% of borax solution was required for effective control of *Penicillium* molds and stem-end rots due to *Diplodia natalensis* and *Phomopsis citri*. Since the active constituent of borax treatment is the borate ion, alternatives to borax treatment have been suggested, such as sodium metaborate, sodium penta borate, lactic acid complexes of boric acid and mixtures of borax and boric acid. The most widely used treatment has been a combination of 4% borax and 2% boric acid at a solution temperature of 43.5°C. The treated fruits are always rinsed with fresh water to remove most of the borax residue from the surface of the fruit, since no tolerance has been established for the residues of this compound in fruits. The borax treatment of citrus fruits is now being discontinued because of the difficulty in disposing of the large volumes of water containing phytotoxic borate salts. Since the 1960s, borax has been replaced by O-phenyl phenate, which is more effective.

Solutions of 3-5% sodium carbonate at 38-48°C are commonly used in California to control *P. digitatum* and *P. italicum.*

(O) Inorganic Sulphur Compounds Elemental sulphur dust has been used commercially for the post-harvest treatment of peaches to protect them against infection by *Monilinia fructicola* during marketing. Sulphur dust is presumably a protectant fungicide.

(P) Dithiocarbamates and Related Compounds The heavy metal dithiocarbamates are a major class of pre-harvest fungicides. They have found comparatively little use as post-harvest fungicides. A paste of Sodium dimethyldithiocarbamates and sodium 2-Mercaptobezothioazole in synthetic latex was applied to the cut end of a banana main stem to prevent the entry of pathogenic fungi at this site (Meredith, 1961). This practice became redundant when the Central American banana industry started the shipment of individual fruits severed from the main stem. In the early 1960s, the standard practice was to dip the cut hand stems (cushions) in a suspension of Maneb. This treatment is moderately effective but it could not prevent infection when the inoculum-potential of the pathogen was high and the shipment conditions were not ideal.

An emulsion wax containing Sodium dimethyldithiocarbamate is a standard commercial treatment for the control of post-harvest diseases of melons. Sodium dimethyldithiocarbamate applied in hot water controls stem-end rot, anthracnose and Aspergillus rot on mango fruits. Maneb and Thiram have provided some degree of protection against fungi, which attack tomatoes after they are harvested.

(Q) Soft Fungicides Due to the development of resistance in pathogens towards traditional fungicides and concern for public safety, the need arose for the search of compounds that are effective, natural in origin, safe for humans and are compatible with other control methods. Chitosan, the deacetylated form of chitin, is soluble in dilute organic acid and has been used as a preservative coating material for fruits. Chitosan treatments lead to the induction of chitinase, a defense enzyme with great potential to destroy chitinaceous cell wall of fungi. Chitosan reduces decay by inhibiting growth of several fungi. Recently, Sharma *et al*. (2006) reported chitosan as a soft fungicide that successfully inhibited spore germination of *Aspergillus niger* on mango.

Biological Control

According to Janisiewicz and Korsten (2002), the application of fungicides to fruits after harvest to reduce decay has been increasingly curtailed by the development of pathogen resistance to many key fungicides, lack of replacement fungicides, negative public perception regarding the safety of fungicides and consequent restrictions on fungicidal use. Biological control of post-harvest diseases has emerged as an effective alternative. There is an interesting article on control of post-harvest diseases: "Old issues and innovative approaches" authored by Mari *et al.* (2014).

Trichoderma spp. reduce severity of a number of fruit rots. The antagonistic effect may result from the production by *Trichoderma* spp. of antifungal antibiotics, lytic enzymes, volatile metabolites, which inhibit sporulation or any other mechanism. *Bacillus subtilis* and *Pseudomonas cepacia,* when used to control post-harvest diseases, have also been found to be pathogenic to their hosts under certain circumstances. But *B. subtilis* has been patented for the control of brown rot of stone fruits and yeast (*Pichia guilliermondii* strain US-7 and *Hanseniaspora uvarum,* a yeast strain 138) for the control of citrus stone fruits and pome fruits. It has been reported that bacterization of seed potatoes before harvest and of the tubers after harvest with *Pseudomonas putida* isolate antagonistic to *Erwinia* spp. resulted in 50% and 75% reduction of rotting respectively. This was possibly due to better colonization of the antagonist following the post-harvest treatment.

Two new products, whose main components consist of yeast antagonist, *Candida saitoana*, and either a derivative of chitosan (Biocoat) or lysozyme (Biocure), have been tested worldwide and shown to have strong eradicant tendencies (El-Ghaouth *et al*., 2000). The antagonistic tendency can be expressed in a number of ways: the most common are production of metabolites, competition and direct parasitism, but other mechanisms are also involved, for example, induced resistance sometimes associated with reduction of pathogen enzyme activity. Some of the commercial biocontrol agents like Aspire (containing *Candida oleophila*), Yield Plus (containing *Cryptococcus albidus*), Biosave (containing *Pseudomonas syringe*) were registered for the control of of post-harvest rots of pomes and citrus fruits (Janisiewicz and Korsten, 2002).

Botanicals

Many researchers have investigated the antifungal and antibacterial efficacy of botanicals. Several plant extracts and essential oils have been found to be effective in inhibiting the growth of several fungi and bacteria. The advantage of essential oils is their bioactivity in vapor phase, a characteristic that makes them attractive as possible fumigants for stored product protection. Recent studies have shown that especially vapour-extracted essential oils of many different tropical and temperate plant species are highly potent biocides that in very low concentration can control or kill fungi and/or bacteria at least by same efficiency as synthetic antimicrobial compounds (Singh and Tripathi, 2012).

Integrated Pest Management (I.P.M)

In India, Patel (1991) first applied the concept of IPM for prevention of guava fruit rots and found that the integrated treatment was better than individual treatment. Patil (1992) obtained the lowest severity of both Rhizopus rot and Botryodiplodia stem-end rot of mango, after subjection to an integrated treatment that combined aerated steam, Bavistin, D-Arginine, Ocimum leaf extract, and *Trichoderma harzianum,* than in fruits subjected to individual treatments. Compatibility of *Bacillus subtilis* for post-harvest control of peach brown rot with fruit waxes, Dichloran and cold storage conditions have indicated the potential for commercial application. Post-harvest dip treatment of peaches with Iprodione reduced *Monilinia* sp., *Penicillium* sp. and *Alternaria* sp., particularly when calcium chloride was applied before harvest. Effective and consistent control of storage diseases is dependent upon integration of the following practices (Sharma and Prabha, 2012):

- Select disease-resistant cultivars wherever possible.
- Maintain correct crop nutrition by use of leaf and soil analysis.
- Irrigation based on crop requirements and avoid overhead irrigation.
- Initiate pre-harvest treatments to control insects and diseases.
- Harvest the crop at the correct maturity for storage.
- Initiate post-harvest treatments to disinfect and control diseases and disorders.
- Maintain good sanitation in packaging areas and keep dump-water free of contamination.
- Store produce under conditions least conducive to growth of pathogens.

Post-Harvest Decay of Grains and Legume Seeds

Several ascomycetes and mitosporic fungi attack grains and legumes. These fungi require high moisture content in the seeds (25%) in order to grow and are therefore unable to grow much in grain after harvest when the moisture content in the seeds is usually 12-14%. But, when such fungi grow on these seeds, they discolor seeds, kill ovules, embryos and may produce mycotoxins i.e., fungal compounds toxic to humans and animals. One of the most important effects of post-harvest decays of seeds is the induction of diseases in humans and animals caused by the consumption of feeds and foods infected by common fungi. These fungi produce toxic substances called mycotoxins. The diseases they cause are called mycotoxicoses.

Mycotoxins

In 1960, a large number of young turkeys died in England after they were fed contaminated peanut feed. This led to extensive research on mycotoxins, which established that they are a global problem. When they are present in high concentration, they cause acute diseases. Most mycotoxins are produced by *Aspergillus*, *Penicillium* and *Fusarium* species.

Some of the mycotoxins that commonly contaminate various foods and feeds are given below.

Aflatoxins They are a group of potent liver toxins produced by strains of *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, which coexist with and grow on almost any crop of food. They are four main aflatoxins: B1, B2, G1 and G2; and two additional aflatoxins that are of significance: M1 and M2. They frequently cause injury to liver and are therefore, called as heptotoxic mycotoxins.

Sterigmatocystin It is produced by several species of *Aspergillus*, *Bipolaris* and *Chaetomium*. The main concern is that it is carcinogenic.

Ochratoxins It is produced by *Aspergillus ochraceus* and *Penicillium viridicatum*. Ochratoxin A is a toxin that causes kidney and urinary tract cancers. This toxin has been detected during storage of wheat, barley and corn.

Cyclopiazonic Acid It is produced by *Penicillium cyclopium*, *Aspergillus flavus*, *A. tamari* and *A. versicolor*. It has been detected from corn and peanut. It is the only mycotoxin that affects muscle tissue in chickens and other animals.

Fumonisins It is produced by *Fusarium moniliforme* and *F. proliferatum* on field and stored food-stuff including corn, wheat, rice, sorghum. It causes oesophageal cancer in humans.

Trichothecenes They are a group of closely-related naturally occurring *Fusarium* toxins which include deoxynivalenol (DON), nivalenol, and diacetoxyscirpenol. Toxicological characteristics of Trichothecenes include skin inflammation, digestive disorder and haemorrhagic syndrome.

Citrinin It is produced by several *Aspergillus* and *Penicillum* species. It causes kidney damage.

Patulin It is produced by several species of *Penicillium*, *Aspergillus* and *Byssochlamys nivea*. Patulin is of public concern because it has been found in commercial apple juices.

Zearalenone This mycotoxin is produced by strains of *F. graminearum*, *F. tricinctum*, *F. oxysporum*, *F. sporotrichioides* and *F. moniliforme*. It has been detected in maize and other cereals. It reduces the fertility of cattle.

Penicillic Acids They is produced by several *Penicillium* species and *Aspergillus ochraceus*. Mixture of penicillic acid and Ochratoxin A are synergistic and may cause death in animals.

Ergotism It is caused by several toxic substances that are contaminated in sclerotia (ergots) of the ergot fungus (*Claviceps*), when they contaminate grains such as rye, barley, sorghum, millet, wheat. Ergotism is identified with symptoms like convulsions and limb swellings.

Prevention of Mould Contamination and Growth

Some of the practices recommended are to create unfavourable conditions for the growth of mycotoxin producing fungi:

1. Development of fungal-resistant varieties of plants.
2. Control of field infection by fungi of the crops.
3. Making a schedule for the suitable pre-harvest, harvest, and post-harvest conditions.
4. Lowering moisture content of seeds post harvest and during storage.
5. Store commodities at low temperature wherever possible.
6. Use fungicides and food preservatives against fungal growth.
7. Control of insect infestation in bulk stored grains with approved insecticides.

Methods of Prevention

Treatment of Mould Damage

Mould damage of stored grain may be prevented by three kinds of treatments: (1) drying of grain, (2) the use of natural products and (3) chemical treatment.

Drying of Grains It is an established fact that dry grain stores have a longer shelf life and are safe from insects and moulds because the requirement of moisture for their growth and development are not met. Exposure of aflatoxins-contaminated groundnut oil to sunlight has given very promising results as it destroyed about 99% of aflatoxins. Other methods of grain-drying include mechanical drying, in-bin drying, infrared, microwave or sonic and solar energy drying.

The Use of Natural Products Constituents of numerous plants have been found to exhibit antifungal activity against storage fungi. Oregano (*Origanum vulgare*), Clove, Cinnamon, Garlic and Thyme (*Thymus vulgaris*) are some of such plants, which were reported to inhibit different mycotoxins.

Chemical Treatment Phenol and 2, 4-D inhibited the cyclopiazonic acid production by *Penicillium griseofulvum*. Similarly, p-nitrophenol and alpha-naphthol are effective inhibitors of satratoxin-production by *Stachybotrys atra*. Seed treatment with Bavistin and TMTD (Trimethyl thiuram disulphide) at 0.25% concentration gave 100% protection to wheat grain during a storage period of one year against species of *Aspergillus* and *Penicillium*. Certain mild phenolics like ferulic acid and O-Vanillin have also been reported to prevent aflatoxin production on rice, wheat, maize, groundnut and mustard seeds. O-nitrophenol, p-nitrophenol, p-chloronitrophenol and pentachlorophenol were effective in checking the growth of *P. griseofulvum*.

Some of the organic acids such as acetic acid, propionic acid and butyric acid, malonic acid, benzoic acid, sorbic acid, lactic acid, citric acid and their sodium salts are reported to be effective in the control of mycotoxigenic fungal infestation and mycotoxin contamination.

Potassium sulphite and potassium fluoride, dichlorvos and fumigants like ammonia and phosphine are reported to be effective in the management of mycotoxin problem. Allicin and related substances from garlic and onion extracts are effective in mycotoxin control. Chitosan or derivatives of chitin isolated from crustacean cells were also effective in checking mycotoxin contamination. Cinnamon extracts containing trans-cinnamic acid, trans-cinnamalaldehyde and ferulic acid (p-hydroxy-3-methyl cinnamic acid) was found to be useful in controlling mycotoxin contamination.

Improved Farm Management

Studies of Bilgrami *et al.* (1991) have revealed that agronomic practices such as the planting date, plant densities and irrigation level of crops are reported to influence mycotoxin contamination in general and aflatoxin in particular. Therefore, good farm management practices such as using fungus-free seeds for planting, controlling insects and plant diseases, and improved irrigation practices should be employed. In addition, harvesting the crop at maturity and proper moisture, operation of harvesting equipment to avoid damage to seeds should also be employed. Good storage facilities and proper environmental conditions can be created to prevent mould growth and mycotoxin contamination. Strict control of food and feed and appropriate public health measures are, therefore, of considerable importance in reducing the risk of human and animal health.

Separation of Infested Grains

Physical screening and subsequent removal of damaged kernels by air-blowing, washing with water or use of specific gravity method have shown to be effective by some mycotoxins like fumonisins and aflatoxins.

Volatile Compounds

Volatile compounds, which move fast, leave no residues, and are highly active at low concentrations, are being advocated in the protection of food grains at post-harvest stage. Vapours of cyclohexane and ethyl acetate inhibited the patulin production by *A. terreus* while vapours of n-butanol inhibited the production of terreic acid. *Penicillium griseofulvum* was inhibited for the production of cyclopiazonic acid by vapours of acetic anhydride, n-butanol, formic acid and formaldehyde.

Food Preservatives

Use of food preservatives against mycotoxigenic fungi has also been advocated. Sodium metabisulphite and propionic acid could suppress production of aflatoxin, cyclopiazonic acid, patulin, terreic acid, penitren B and satratoxin by respective fungi.

Antioxidants

Nicotinic acid, methylxanthine, kaemperol and flavonoids, polyphenols and parabenzoquinone, vitamin A, C, E, B, carotene, butylated hydroxyl-anisol (BHA) and propyl gallate (PG) are important antioxidants that can effectively check the growth of mycotoxigenic fungi. Antioxidant-rich cabbage seed extract and garlic extract have a profound protective effect against fumonisins.

Antagonists

Many microorganisms such as bacteria, actinomycetes including fungi inhibit the growth of mycotoxigenic fungi or mycotoxin production. *Absidia repens* can convert toxic aflatoxin to non-toxic aflatoxicol within 3-4 days. Similarly, *Lactobacillus acidophilus, L. bulgaricus* and *L. plantarum* could prevent the mould growth, which in turn may pave the way for inhibition of mycotoxin production. The possible mechanism by which fungal growth and mycotoxin inhibition in a competititive environment include competition for nutrients and space, induction of defense mechanisms, hyperparasitic interactions and antibiosis.

Several microorganisms such as *Trichoderma viride*, *Cladosporium fulvum*, *Lactobacillus* sp., *Flavobacterium aurantiacum* were reported to inhibit aflatoxin production by *A. flavus*. *Saccharomyces cerevisiae* inhibited the fumonisin production by *F. moniliforme*. *Aspergillus parasiticus* and *Bacillus subtilis* could suppress alternariol and alternariol monomethylether and tenuazoic acid by *A. alternata.*

Control of Environmental Conditions

Environmental conditions of storage place are of paramount importance in keeping the food commodities free from mould infestation and mycotoxin contamination. Among various factors, moisture, temperature, presence of contaminating microorganisms and the nature and physiological state of commodity are important in minimizing the mycotoxin contamination. The practice of underground storage

sometimes may prove to be safe, which results in grain cooling and depleting the oxygen to the desired level whereby the microbes and the insects cannot grow.

1. Moisture Moisture is a critical factor for keeping quality of stored products. Drying of seeds and other agricultural commodities to the safe moisture level is a critical factor (9% for kernels and 13.5% for corn) in the control of mycotoxins.

2. Temperature Temperature is again an important factor for mould growth and mycotoxin production. *A. flavus* produces aflatoxins over a wide range of temperatures ranging from 7.5-35°C. If commodities are stored at 4°C, one can prevent *A. flavus* growth and aflatoxin production. Air-tight storage is not desirable as it may provide ideal moisture content for encouraging mould growth.

3. Modified Gaseous Atmosphere If the normal composition of gasses of the atmosphere is changed, the mould gets discouraged. Generally, oxygen level is reduced and in its place, CO_2 and N_2 are increased, resulting in an anaerobic condition. This atmosphere is called modified atmosphere packing (MAP) and controlled atmosphere storage (CAS). CAS is generally used for bulk storage for a period of 6-9 months and by this method it was possible to control the growth of *A. flavus* and aflatoxins elaboration in peanuts.

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Questions

1. What is latent infection?
2. List some important post-harvest diseases of your area and the control measures you would suggest for them.
3. Name some important chemicals used for controlling post-harvest diseases of fruits and vegetables.
4. How will you control post-harvest diseases of fruits and vegetables?
5. How will you control post-harvest diseases of cereals and legumes?
6. What is biological control? Why is it being preferred to other conventional fungicidal control now?
7. Describe some mycotoxins that commonly contaminate various foods and feeds.

Root Diseases

21

Since the beginning of the study of microbiology, it has been recognized that soil is a great reservoir of microorganisms; the important ones being fungi, bacteria, actinomycetes, algae, protozoans and small animals. These organisms live in a constant state of competition and struggle for survival, as does the macro-vegetation on the surface of the earth. In this struggle, some of the species become dominant and some remain subdominant and a sort of dynamic equilibrium is attained as a result of the conditions prevailing in a particular place and time. In the case of plant pathogens, there is a wealth of information on such interaction (Baker and Snyder, 1965; Toussoun. *et al.,* 1970; Baker and Cook 1974). Some recent contributions in this area are those of Hornby (1990), Hall (1996), Bagyaraj and Govindan (1996), Mehrotra *et al.* (1997) and Manoharachary and Padma (2001). Hoitink and Boehm (1999) have recently reviewed the biocontrol within the context of soil microbial communities. Kerry (2000) has reviewed "rhizosphere interactions and exploitation of microbial agents for the biological control of plant parasitic nematodes".

It is worthwhile to quote Baker and Cook (1974): "Man does not know the total roster of microorganisms in a single tiny lump of soil, let alone their complex interactions with each other and with their physical and chemical environment."

In 1904, Hiltner announced the discovery of rhizosphere, a zone of intensified microbial activity around the growing roots of higher plants. It is now well know that the rhizosphere flora is not only quantitatively but also qualitatively different from that of the flora found in soil far from the roots. These differences in flora increase as we come nearer to the root surface. Although a number of suggestions have been offered of explain this intensified microbial activity, it seems likely that it is chiefly due to the production of substrates by the growing root in the form of root exudates (containing sugars, organic acids, amino acids, nucleotides, vitamins, lipids and other substances), dead cells and tissues sloughed off during growth.

The idea of rhizosphere was not fully appreciated. After a quarter of a century, R.L. Starkey (1929) of the Microbiological Research Institute, Canada, understood the full implications of these phenomena. The presence of plant roots in the soil provides nourishment to organic matter. The result of this additional nourishment is stimulated growth and multiplication of the organisms, which result in their greater population in the rhizosphere as compared to the population in the normal soil. This change in the microflora is often peculiar to the roots of a particular species, probably due to the specific products exuded. Thus, a plant species usually has its own specific flora around its roots. The degree of increase in flora is often expressed by a ratio R/S in which 'R' is the population of the rhizospere and 'S' is that

of the soil. Clark (1949) suggested that the root surface itself—the rhizoplane—be used in studying the rhizosphere phenomenon, since it may be a more sensitive index of the specific qualitative effects of roots on soil microorganisms. Katznelson (1965), and Sadasivan and Subramanian (1960) discussed the nature and importance of the rhizosphere and the rhizoplane. Mitchell (1976) discussed the effect of roots on the activity of soil-borne plant pathogens.

It is well-known that root diseases cause considerable economic losses to crops. Root diseases are in several ways different from the diseases of the aerial parts. Koch's postulates, which helped to elucidate the pathogenesis in aerial diseases, virtually proved to be a handicap in the study of root diseases. Knowledge of the complex microbial interactions occurring in the soil and of the ecology of soil microoraganisms, saprophytic and parasitic, is essential for a proper understanding of root diseases and for a rational approach to their control.

Root Exudates and Soil-Borne Pathogens

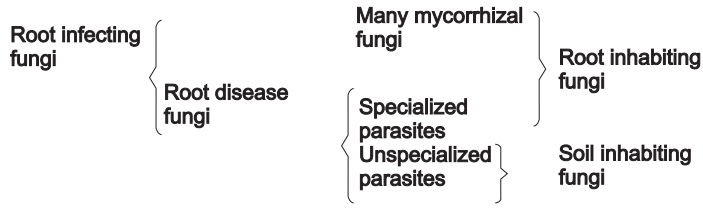
One of the major factors determining the behaviour of plant pathogens in the soil is the availability of root exudates, which has great relevance to the biological control of root diseases. Rovira (1965a, b) and Hale *et al.* (1971) have reviewed plant root exudates and their influence on soil microorganisms. There is a vast amount of literature on the various types of compounds exuded by plant roots under different environmental and physiological conditions. Schroth and Hildebrand (1964) discussed the influence of plant exudates on root-infecting fungi. Schroth and Synder (1961) demonstrated the effects of roots on the germination of chlamydospores of *F.solani* f.sp *phaseoli*. Buxton (1957a, 1957b, 1962) found that the effects of exudates from the roots of pea cultivars, growing under sterile conditions on germination of conidia of *F. oxysporum* f. sp. *pisi,* depend upon the variey of the host and the physiological race of the parasite. Exudates from the resistant varieties depress the germination of conidia of avirulent races but have far less effect on the conidia of virulent races. Surprisingly, there is no corresponding reduction in the hyphal growth of different races. However, Kommedahl (1966), working with near isogenic lines of peas, was unable to confirm Buxton's observations with peas. Banihashemi (1968), working with two strains of *F.oxysporum melonis,* found that both isolates colonized equally in the roots of resistant and susceptible melons. This was in conformity with the result obtained by Armstrong and Armstorng (1948). Coley-Smith and Hickman (1957) have carefully investigated the germination of sclerotia of *Sclerotium cepivorum,* which is related to stimulation of a pathogen propagule in the presence of the host root tissue. The stimulation was host specific. The behaviour of *R. solani* in the soil and the root surface has been reviewed in 1965 in a symposium on the fungus. There are numerous reports of the stimulation of growth of *R. solani* due to the root exudates of host plants. The work of Kerr (1956), Flentje *et al. (*1963), De Silva and Wood (1964) demonstrated that host exudates could stimulate growth of the pathogen before penetration of the host tissue. Flentje *et al*. (1963) demonstrated that the natural constituents of radish stems and cotyledons stimulated infection and cushion formation on collodion membranes. In the case of pathogens producing zoospores in the soil, the phenomenon of chemotaxis of zoospores and their accumulation on root tips has been demostrated by a number of workers. Mehrotra first demonstrated this accumulation of zoospores in soil or plant roots in 1970.

Accumulation of zoospores in the soil is generally a non-specific phenomenon, but it definitely plays an important role in the build up of inoculum potential on roots in the soil.

Ectotrophic growth of *Gaumannomyces graminis* on wheat and barley roots provides direct evidence that the fungus obtains factors essential for growth in this microhabitat (Garrett, 1934). Flentje (1947,

1959) has shown that exudation of sucrose is a major factor governing susceptibility. Garrett (1970) discussed pathogenic root-infecting fungi in detail.

Garrett (1956) had classified soil-inhabiting fungi as follows:



Obligate Soil Saprophytes

The first are the obligate sparophytes, which include many species of Mucorales, Aspergillus and Penicillium species, *Agaricus campestris* and other basidiomycetous fungi. Then, there are the unspecialized parasites, which can live as saprophytes on plant materials and can also infect roots under favourable condition, for example, *Corticium solani, Macrophomina phaseoli, Pythium* spp. and *Fusarium culmorum*. These are mainly soil-inhabiting fungi, which have the capacity to parasitize. Root-infecting fungi also include more highly specialized parasites with little or no ability to survive as free-living saprophytes in soil, although they often survive in the form of resistant propagules of various roots. *Gaeumannomyces* (*Ophiobolus) graminis* and *Phytophthora infestans* are parasites that fall in this category. A few of these specialized parasites are obligate in nature and some appear to have attained a near mutualism with their host roots (as perhaps in some form of mycorrhiza). Some of the root disease pathogens in this category are *Synchytrium endobioticum, Plasmodiophora brassicae, Spongospora subterranea, etc.*

The more highly specialized root pathogens are more sensitive to antibiotics than soil saprophytes. They are also known as root-inhabiting fungi. The specialized parasites have comparatively poor capacity to survive and grow in soil from the living tissues. Garrett (1956, 1963, 1970) had placed great emphasis on competition and has termed this capacity as 'competitive saprophytic ability'. This is an intrinsic and genetically governed quality of a fungus by which it competes with saprophytes living in the soil. The success of a fungus in competitive saprophytic colonization is dependent on:

1. Competitive saprophytic ability of a particular fungus.
2. Its inoculum potential.
3. Environmental conditions such as soil pH, temperature aeration, moisture, and soil population, including the population fungi and other soil microorganisms.

Associative and Antagonistic Effects

Microorganisms, when they grow in proximity, exert various kinds of influences on one another, some of which are favourable or associative and others are antagonistic. An extreme example of beneficial effects may be cited here. *Polystictus audus* is unable to synthesize its own thiamine supply though it is able to form the biotin required by it. The reverse is the case with *Nematospora gossypii,* which can form thiamine but not biotin. These two fungi, if grown separately, are not able to survive, but if grown together, they supply the missing vitamins to each other and grow well. Competitive saprophytic ability

is a cumbersome phrase that was coined by Garrett (1950). According to him, it is "the summation of physiological characteristics that make for success in competitive colonization of dead organic substrates". Garrett has postulated that this competitive saprophytic ability depends on four factors:

1. High growth-rate of hyphae and rapid germination of spores.
2. Good enzyme-producing equipment.
3. Production of antibiotic toxins.
4. Tolerance of antibiotics produced by other microorganisms.

A sum of these attributes determines the growth and survival of an organism in the soil. Another factor conditioning the success of competitive saprophytic colonization is the inoculum potential. Various plant pathologists, in connection with different types of disease situations, have used this term. However, Garrett (1956) defined inoculum potential as the energy of growth of a fungus available for the colonization of a substrate at the surface of the substrate to be colonized.

Root Diseases

Root diseases can be divided into the following five groups:

Pre-emergence Killing, Damping off and Seedling Blight The important fungal pathogens are *Pythium, Phytophthora, Fusarium, Sclerotium* and *Rhizoctonia*, which generally affect the soft, immature parenchymatous tissues. Considerable tissue disintegration occurs and the infected seedlings are often completely killed or severely disabled. The pathogen responsible for pre-emergence killing, damping off and seedling blight has an armoury of pectolytic, cellulolytic, and other cell-wall degrading enzymes. They often produce toxins. The pathogens of this group possess high competitive saprophytic ability.

Root Rots In this group, it is mainly the extravascular tissue that is invaded. Extravascular sometimes leads to foot rot, crown rot, or stem rot. The antagonistic action is well known among microorganisms. This is accomplished by the production of various kinds of toxins, antibiotics, and other inhibitory substances.

Recent knowledge of the role of microflora has revolutionized our ideas about root diseases. Earlier, root pathologists did not appreciate the role of soil microflora and a root disease was considered as an interaction between the root and the pathogen. Study of root infection was done under sterilized soil conditions and reactions were noted. Recent knowledge has revealed that this does not give a correct idea of the actual happenings in the soil where soil microflora plays an important role in the development or arrest of the disease. This gave a new orientation to the study of root diseases.

Fungi attacking the roots or underground parts of plants show varying degrees of parasitic specialization. There is rotting of the parenchymatous cortical tissues of the roots. Some of the fungal pathogens of this group are *Macrophomina phaseoli*, *Pyrenochaeta terrestris, Armillaria mellea,* some *Fusarium* species that are not vascular pathogens, etc. Toxins and enzymes, particularly those degrading cell walls are doubtless involved, although we do not have much information on this subject. The root rots are to be distinguished from vascular wilt diseases.

Vascular Wilt Disease In this disease, the pathogen resides in the vascular tract and produces toxins and enzymes that bring about wilt. Vascular wilts have been discussed in detail in Chapter 17.

Diseases Involving Hypertrophy Some of the root pathogens stimulate excessive cell division or enlagement, producing galls or overgrowths. Some of the pathogens in this group are *Plasmodiophora*

brassicae (club root of crucifers), *Synchytrium endobioticum* (wart disease of potato), *Spongospora subterranea* (powdery scab of potato), etc. All these are obligate parasites.

Non-Parasitic Root Pathogens In certain root diseases, although a pathogen may not be growing on or inside the host tissue, it may still cause the disease. *Periconia circinata* fungus causes milo disease of sorghum, although it may not invade the living roots. This is due to the toxin periconin, produced by the pathogen *P. circinata* around the roots in soil.

Some other diseases in this category are frenching of tobacco caused by certain metabolites and soil bacteria.

Predisposing Factors

Soil conditions, including moisture content, temperature, pH levels, physical and chemical structure and content of organic matter and nutrients, play an important role in the development of root diseases. There can be many other predisposing factors. *Plasmodiophora, Spongospora, Synchytrium, Aphanomyces,* and *Phytophthora,* which produce zoospores, generally favour wet soil. However, *Streptomyces scabies* and many cereal smuts prefer drier soils. High temperatures are favoured by diseases such as wilt of cabbage and tomato, caused by *F. oxysporum,* whereas the reverse is true for stem canker of potato (*Corticium solani*) and root rot of tobacco (*Thielaviopsis basicola*). Tomato wilt is found to be more intense at 28°C while the optimum temperature for Verticillium wilt is 20°C.

Rhizoctonia diseases favour soils of light texture, while wilt of cotton is severe in black cotton soils which are rich in organic matter and do not have a light texture (heavy clays). Certain disease are severe in acidic soils, for example, root rot of cotton (*Phymatotrichum omnivorum*), take all of cereals (*Gaeumannomyces graminis*), root rot of pines [*Heterobasidion (Fomes) annosus*] and potato scab (*Streptomyces scabies*). Nutrient status of the soils, content of organic matter and fertilizer treatment play a significant role in predisposing the roots of peas to many root diseases, such as pre-emergence damping off caused by *P. debaryanum*. Hussain and McKeen (1963) showed that in the case of strawberry root rot caused by *Rhizoctonia fragariae*, exudates from plants grown at 5°C or 10°C stimulated both spore germination and mycelial growth of the pathogen, whereas the exudates from plants grown at 20°C and 30°C did not. The resistance of flax to *F. oxysporum* f. sp. *lini* has been ascribed to the production of HCN in the roots of resistant varieties (Timonin, 1941). HCN is known to inhibit the growth of rhizosphere fungi but *Trichoderma viride* is not affected. Buxton (1962) reported that root exudates from Lactin banana plant (3-6 months old) inhibited spore germination, whereas root exudates from Gros Michel banana (susceptible variety) did not. Oort (1972) discussed the ecology of soil-borne plant pathogens.

In contrast to foliar pathogens or air-borne diseases which are mainly dependent on the climate, the physical and chemical characteristics of the soil, especially the soil biota (fauna and flora) play a predominat role in soil-borne diseases, in addition to the climate. Figure 21.1 shows the complexity of the interrelationships of various environmental factors, which may affect the host, the pathogen and consequently the host-pathogen relationship. According to Oort (1972): “each change in the environment may bring about a chain reaction, each step of which may branch off a side chain of reactions, so that the consequences of a change are manifold and are generally not predictable.” The subject of the ecology of soil-borne pathogens is too vast to be discussed in detail here. Only the pre-disposing factors, the dispersal, survival and control will be discussed here.

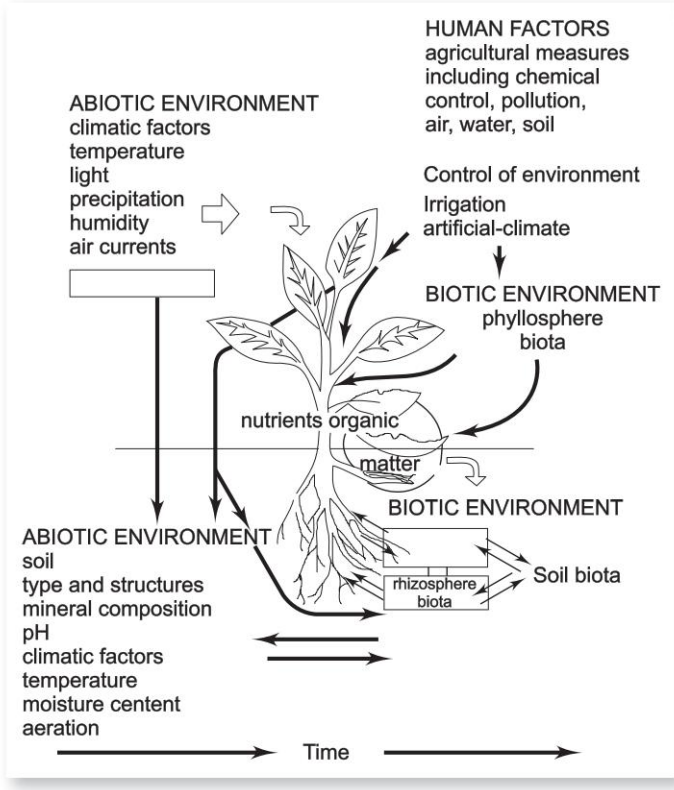


Figure 21.1 Plant, soil-borne pathogens and environment (Redrawn from A.J.P. Oort, 1972)

Survival

There are three ways in which soil-borne pathogens can survive:

1. Dormant survival by resting or latent stage.
2. Saprophytic survival on dead tissues of the host or on dead organic substrates.
3. Parasitic survival on or in the host.

Many pathogenic soil-borne fungi have resting spores, sporangia or sclerotia (*Pythium* and *Phytophthora* species, *Fusarium* species, *Sclerotium rolfsii* and *Sclerotinia* species). Root infecting nematodes (*Heterodera* species) survive as egg cysts or stay as adults. Saprophytic survival is less common. *Gaumannomyces graminis* remains alive in the roots and the stubble as long as these parts (roots and stubble) serve as a source of food.

Dispersal of Soil-Borne Pathogens

Dissemination of soil-borne pathogens can take place by wind, water, living vectors and by humans. Dispersal of soil-borne pathogens is less common. In the case of the fungus *Gaumannomyces graminis,* which produces its perithecia on the stubble of cereal plants, the ascospores are violently discharged into the air and are then carried away by air currents. Basidiospores of *Heterobasidion (Fomes) annosus*, a root rot fungus of conifers, can be carried over distances of several kilometers, infecting stumps of

freshly felled trees. Cysts of *Heterodera* spp. are spread over distances of at least a few kilometers by dust storms. Dispersal of zoospores of phytopathogenic soil-borne fungi can easily take place by irrigation water or by their own active movements. It is not clear whether soil-borne viruses are dispersed by wind or water. However, the vectors—the nematodes and some fungi may be spread in this way.

Control of Root Diseases

Control of root diseases is closely bound with agricultural practices, which keep the soil in good condition and thus encourage vigorous growth of the crop. Root diseases can be controlled by crop rotation, crop sanitation, and palliative measures to hinder the spread of infection, soil treatment, biological control and control by breeding disease-resistant varieties.

Crop Rotations

Curl (1963) has reviewed the control of plant diseases by crop rotation. Crop rotation may be defined as the growing of economic plants in recurring succession and in definite sequence on the same land, as distinguished from a one-crop system, which usually lacks a definite plan. Pammel suggested crop rotation in 1888 for the control of cotton root rot caused by *Phymatotrichum omnivorum*. In 1890, he suggested a way of control by rotating small grain crops, with a note of precaution that wheat and oats are not desirable in southern Texas because of their susceptibility to rust. Very often, continued cultivation of the same crop or related crops leads to the perpetuation of a pathogenic soil-borne fungus and the gradual increase in the intensity of the disease. Such soils are called sick soils. Soil-borne diseases are common where crop rotation is not followed. Some of these diseases are wheat mosaic, wilt of arhar (*F.oxysporum* f. sp *vasinfectum*), wilt of linseed (*F. oxysporum* f sp. *lini*), club-root disease of crucifers (*Plasmodiophora brassicae*), wilt of pea (*F. solani* f. sp. *pisi* and *F. oxysporum* f. sp. *pisi*), wilt and root rot of gram (*F. oxysporum* f. sp. *ciceri* and *Rhizoctonia* spp.), red rot and wilt of sugarcne (*Colletortrichum falcatum* and *Cephalosporium sacchari*), ergot and smut of bajra (*Claviceps microcephaia* and *Moesziomyces penicillariae* respectively), Karnal bunt of wheat (*Tilletia indica*), leaf smut of rice (*Entyloma oryzae* and *Neovossia horrida*, respectively), Molya disease of wheat (*Heterodera avenae*) and root rot of vegetable crops (*Sclerotium rolfsii* and *Rhizoctonia* spp).

Crop rotation is generally recommended with botanically unrelated crops. Rotation of alfa-alfa-corn-wheat and corn cow-peas-wheat was equally effective in reducing root rot of wheat in Kansas. *Ralstonia* (*Pseudomonas) solanacearum* is controlled by crop rotation of varying lengths.

Crop Sanitation

Like plant sanitation, field sanitation is very essential for the control of soil-borne diseases such as red rot of sugarcane, Panama disease of banana, Verticillium wilt of cotton and arhar wilt, foot rot of *Piper betle*, take all of wheat, root rot of beans, root rots of vegetable crops, etc. There are many other ways in which the farmer can take action to reduce disease in the field. Inoculum from crop residues may be reduced by ploughing or by adjusting planting time, and from the soil by solarization, the addition of amendments, and in some instances, by flooding. Some pathogens require specific wavelengths of light in order to sporulate (Reveni *et al*., 1989). Their prevalence in greenhouses may be reduced by coating the glass with materials that do not allow the penetration of such wavelengths while still allowing the wavelengths that are crucial for photosynthesis (Reveni *et al*., 1989).

Some control of wind-borne pathogens may be achieved by planting mixtures of varieties or multilines. For example, an experiment on the control of rice blast caused by *Magnaporthe grisea* was carried out over a large area in Yunnan Province, China. Susceptible sticky rice was planted as single rows interspersed between four or sometimes six rows of resistant hybrid rice. Grain production of sticky rice was 89% greater and blast 94% less when planted in mixtures compared with monocultures (Zhu *et al*., 2000). This principle may be extended to intercropping whereby different species of plants are grown in close proximity. However, care must be taken to ensure that no two or more members of the mixture are susceptible to the same organism, a condition that is not easy to fulfill with pathogens that have a wide host range. Vectors transmit diseases caused by pathogens such as mycoplasmas, viruses, and viroids. Control of vector mobility, for example by means of reflective mulches or interference with their feeding habits on plants by the use of genotypes with sufficient concentrations of antifeedants, would reduce infection by these agents.

Palliative Measures

The planting date and depth, application of fertilizers and manures, adjustment of soil pH, control of soil tilth, aeration by ploughing and other means, use of mature sowing materials, spacing between plants and the number of plants per hectare can all be manipulated in such a way that they are advantageous to the host and detrimental to the growth of pathogen. These practices have been discussed in detail in Chapter 11. Some examples are given here.

Rhizoctonia root rot of gram is severe if gram is sown immediately after the rains. This is due to the fact that the pathogen develops rapidly under high temperature and moisture. A slight delay in the time of sowing is helpful in this case. In the case of foot rot of *Piper betle* and rhizome rot of ginger (caused by *Phytophthora parasitica* var. *piperina* and *Pythium myriotylum* respectively), it is advisable to obtain seeds from disease-free areas. Ploughing under the surface refuse before sowing can control *Sclerotium rolfsii* of peanuts. This deprives the fungus of a food base since it can only grow on or near the soil surface. Root rot of cotton, caused by *Rhizoctonia bataticola,* has been successfully controlled in Punjab by mixed cropping. Mixed cultivation of cotton and moth (*Phaseolus aconitifolius*) reduces the incidence of root rot of cotton.

Soil Treatment

Plant pathogens can be eradicated from the soil by dry heat, stem or hot water flooding and fumigation with chemicals. Heat and chemical treatments have been used with much success in the eradication of plant pathogens from greenhouse soils and soils in plant beds. Bliss (1951) has successfully controlled *Armillaria mellea* by fumigating the citrus orchard soils with carbon disulphide (CS_2). Newhall (1955) discussed the subject of soil disinfestation. Nematodes are generally eradicated from the soil by fumigating the soil with DD mixture or with other nematocides.

Application of fertilizers or other agrochemicals could reduce the incidence of several diseases. Diseases caused by *Fusarium, Rhizoctonia* and *Aphanomyces* increased in severity by ammonium nitrogen and decreased by the nitrate form.

Tillage

The method of tillage modifies soil structure, crumb size, aeration, temperature and water potential. It can change the soil environment and biological balance to control root-rot pathogen. Fungal root diseases like take all, *Rhizoctonia* root rot and *Pythium* root favour direct drilling (no tillage).

Disease-Suppressive Soils

Certain soils have the natural ability to suppress diseases. There are several theories on the mechanism of disease suppression, which include: (i) antagonistic effects of associate microorganisms; (ii) faster multiplication of bacteria, which reduce the germination of inocula; (iii) hyphal lysis; (iv) ethylene production; (v) elevated fungistasis to fungal propagules; (vi) microbial competition and (vii) unidentified bilogical factors (Bagyaraj and Govindan, 1996). Disease-suppressive soils have been reported for *G. graminis tritici, Phytophthora cinnamomi, R. solani* and *Pythium* spp.

Biological Control by Addition of Antagonists and Organic Amendments

This process involves the control of a disease through some biological agency. Garrett (1956) defined biological control as any condition or practice under which or whereby survival or activity of a pathogen is reduced through the agency of another living organism (except humans), which results in a reduction in the incidence of the disease caused by the pathogen. There is an enormous amount of literature available on biological control of plant pathogens.

Biological control of soil-borne diseases is particularly complex because the pathogens occur in a dynamic environment at the rhizosphere interface. The rhizosphere is typified by intense microbial activity involving, firstly, a high population of microorganisms, and secondly, a rapid change in pH, in salt concentrations, and in osmotic and water potential (Handelsman *et al.,* 1996). Microorganisms indigenous to the rhizosphere are ideal for biological control, since the rhizosphere provides a first-line defense for roots against attacks by plant pathogens (Weller, 1988). Microorganisms can protect the plants from fungal attacks through the production of antifungal metabolites, competition with the pathogen for nutrients, niche exclusion, parasitism or lysis of the pathogen, or through induction of plant-resistance mechanisms (Whipps, 2001). Beneficial microorganisms of interest for biological control of plant pathogenic *Pythium* spp. have been identified among fungi and bacteria. Isolates of *Trichoderma* spp. and *Gliocladium* spp. are antagonists of *Pythium*-induced soil-borne diseases and several strains are already commercially available for the biological control of *Pythium* root rots (Howell et al., 1993; Fravel, 2005). Various actinomycete species, including *Streptomyces*, *Actinoplanes*, and *Micromonospora,* have the potential to inhibit *Pythium coloratum* cavity-spot on carrots (El-Tarabily et al., 1997). Other bacteria effective against *Pythium* are found in various genera, including *Enterobacter*, *Erwinia*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, and *Rhizobium,* but the most extensively studied group of bacterial biological control agents are *Pseudomonas* spp. (Chin-A-Woeng *et al*., 2003; Bardin *et al.,* 2004). Competition for organic carbon and iron is one of the mechanisms through which some biocontrol agents suppress *Pythium* spp. (Hoitink et al., 1999). Sensitivity of *Pythium* spp. to competition and antagonism during its saprophytic phase of growth is one of the key factors in managing *Pythium* diseases through biological control (Martin et al., 1999). In contrast to this view, it is commonly known that *Pythium* spp. propagules germinate rapidly in response to seed or root exudates and quickly infect seeds or roots, and this complicates the application of biological control (Whipps *et al.*, 1991). It is, therefore, of great importance that the activity of the biological control agent coincides with the period of host susceptibility and it should persist as long as the plant remains susceptible. Insufficient survival of the antagonists may lead to inadequate or partial control of the pathogen.

However, some examples of successful biological control are given here. The first successful control of a root disease by biological means was by Millard and Taylor (1927), who reported control of scab in potatoes caused by *Streptomyces scabies* with green manuring. A saprophytic species of *Streptomyces*, that is, *S.praecox* develops rapidly on the dead green manure and inhibits the pathogen.

Bliss (1951) successfully controlled *Armillaria mellea* root rot of citrus by fumigating the soil with carbon disulphide. Fumigation of soils with carbon disulphide helped in boosting the population of *Trichoderma viride,* an antagonistic fungus. The fumigant kills directly and the fungus, *Trichoderma viride,* indirectly affects the pathogen (Garrett, 1958). A very interesting example of biological control is provided by the work of Rishbeth in England on the control of *Heterobasidion (Fomes) annosus* root disease of pines, by a weakly parasitic basidiomycetous fungus, *Peniophora gigantea.* The usual practice in pine plantations is to fell pine trees and the cut tree-stump surfaces are painted with Creosote oil. This brought about only partial control because the stump tissues cracked with progressive drying, causing fissures, through which fungal spores of *Heterobasidion* (*Fomes*) *annosus* could enter and cause infections. Rishbeth showed that if the paste of oidia of *Peniophora gigantea* is applied on the stump, it parasitizes rapidly and kills the tissues, leading the way for the saprophytes to colonize and decompose the stump tissues. These become unfit for any attack by *H. annosus,* which is a specialized parasite.

Wood and Tveit (1955) obtained good control of *Fusarium nivale* on oats in England with *Chaetomium cochliodes* isolates, growing on oat straw and amending the same in the field soil.

Organic amendments have been tried for the control of quite a few soil-borne pathogens. Amending the soil with organic materials that stimulate the germination of sclerotia controls *Phymatotrichum* root rot of cotton and thus exhausts the inoculum. In the case of *Gaeumannomyces graminis*, it has been suggested that the organic amendments rich in carbon and deficient in nitrogen control the take all of wheat (Garrett, 1970). This is explained as follows:

Organic amendments having a high C/N ratio result in an increase in the microbial number and activity in the soil. Hence, there is a considerable liberation of CO_2 by soil saprophytes, which suppress the pathogenic activity of this fungus. Low nitrogen content in the soil reduces the longevity of *Gaeumannomyces graminis* in the soil. There are several other examples where soil amendments have been successfully utilized for the control of root-disease pathogens. Synder *et al*. (1959) have showed that the bean root rot was controlled by barley amendment, that is, the amendment having greater C/N ratios; but if nitrogen was added to the soil, control was nullified. *Fusarium solani* requires nitrogen for germination and penetration and if there is deficiency of nitrogen in the soil, it cannot develop. Maurer and Baker (1965) showed that C/N ratio greater than 25 significantly suppressed symptoms of bean root rot.

Papavizas and Davey (1960), Papavizas (1963) and Davey and Papavizas (1960, 1963) controlled *Rhizoctonia* root rot of snapbeans by amending the soil adjusted to C/N ratio of 100-40.

Mitchel and Alexander (1962) reported the control of root rot of bean by manuring the soil naturally infested by *F. solani* f. *phaseoli* with chitin at the rate of 225 kg/acre. Mitchell and Alexander (1963) showed that only chitinous-walled fungi are affected by this treatment.

Some other examples of biological control include *Phytophthora* root rot of Avocado by amending the soil with alfa-alfa meal of low C/N ratio and root rot of *Piper betel* (Mehrotra and Tiwari, 1976) by organic amendments of the soil with corn straw supplemented with nitrogen. Mehrotra and Claudius (1972) controlled the root rot and wilt of *Lens culinaris* by organic amendment of the soil, together with introduction of antagonists.

Breeding of Disease-Resistant Varieties

This forms the best control measure but has not been very successful so far. However, varieties of cabbage, tomatoes and peas that are resistant to Fusarial wilt have been successfully produced. Genetically engineering plants for disease resistance is the latest technique for bringing in genes from a wide range of organisms into crops, to make them disease-resistant. Many plants produce the enzyme chitinase as a

defence mechanism in response to fungal infection. Chitin is a component of the cell walls of all fungi except the Oomycetous ones. Two bacterial genes have been isolated and characterized for insertion into plants (Jones *et al*., 1986). Engineering these genes into crop plants will make them resistant to most of the fungal pathogens, including soil-borne ones (Defago *et al.*, 1990). Jones *et al*. (1995) reviewed the use of alien genes for the development of disease resistance in wheat.

An interesting discussion on the potential of biotechnology in solving untractable constraints to food security in Africa is provided by Gressel *et al*. (2004) and, in a more general approach, by Christou and Twyman (2004). Candidate genes include those that inhibit or destroy virulent attributes of the pathogen, such as destructive enzymes and toxins, and those that enhance defense. The latter include genes that increase the concentrations of saponins, antimicrobial peptides, and reactive oxygen species or modify the phytoalexin response, and those that switch on systemic acquired resistance. Paradoxically, transformation of plants with pathogen genes by means of coating protein and replicating genes of viruses confers resistance, and this phenomenon is known as pathogen-derived resistance. Finally, the ability to transform plants allows the pyramiding of resistance genes, including those that are normally found as alleles at the same locus (Strange, 2003).

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Questions

1. What is pre-emergence damping off and post-emergence damping off?
2. What are soil-borne diseases?
3. What is the essential difference between root diseases and diseases of aerial parts?
4. Define the following terms:
   (i) Rhizosphere
   (ii) Competitive saprophytic ability
5. What is biological control of plant pathogens? Give some suitable examples.
6. What is the difference between soil-inhabiting and root-inhabiting pathogens?
7. What are disease-suppressive soils?
8. Genetic engineering is the latest technique of bringing in genes for resistance from wide range of organisms. What are its pitfalls?

Seed-Borne Diseases

22

Botanical seeds and other propagating materials transport many plant pathogens, while viral diseases are mainly transmitted through vegetatively propagating materials. Seed-borne diseases are commonly observed in cereals and pulses. At the time of seed germination, the resting mycelium or spores lodged inside the seed or seed coat become active under suitable conditions, infecting the seedling. According to Wallen (1964), a large proportion of research on seed-borne diseases has been mycological in nature. As a result, hundreds of seed-borne fungi, both pathogenic as well as saprophytic, have been isolated and identified. Less work has been done on the bacterial and viral diseases. Nene and Agarwal (1978) have given information on some seed-borne diseases and their control. Suryanarayana (1978) gave a comprehensive account of the important seed-borne diseases. Some of the recent literature on seed pathology is that of Singh *et al.* (1991). Dalbir Singh (1994) reviewed "Seed Pathology in India: Present status and Importance." Some other relevant publications are those of Neergaand (1977) and Agarwal and Sinclair (1987-1989). Singh and Trivedi (1994) have brought out two edited volumes titled *Vistas in Seed Biology*, which provide information on various aspects of seed science, based on studies in India. Sharma and Sharma (1994) have reviewed the work on seed-borne nematodes, their distribution, biology, yield losses and management. Sharma and Sumbali (2007) have discussed pre and post harvest seed spoilage of cereals and prevalence of mycotoxin contamination.

Seed borne pathogens are of two types: (1) adherent to the outer covering of the seed and (2) borne inside the seed.

Seed-Borne Bacterial Diseases

Numerous bacterial diseases are seed-borne. *Xanthomonas campestris* pv. *oryzae* is seed-borne and the disease is transmitted to paddy seedlings when raised under high humid conditions. *Xanthomonas campestris* pv. *vesicatoria, Xanthomonas campestris* pv. *malvacearum,* and Xanthomonas *campestris* pv. *cassiae* are generally carried externally or internally by the seed, while *Clavibacter (Corynebacterium) michiganense* subsp. s*epidonicum* and *Ralstonia* (*Pseudomonas) solanacearum* in potato and *Xanthomonas campestris* pv. *vasculorum* in sugarcane are spread through vegetative plant parts used for propagation. The infection of seed by *Xanthomonas campestris* pv. *phaseoli* may take place through the vascular system of the plant. Infection can also take place through the dorsal suture followed by the invasion of the funiculus, the raphe, and the seed coat. Bacteria may enter the seed through the

micropyle after they have penetrated the pod cavity. Once in the seed coat, the bacteria invade the intercellular spaces and surround the cotyledons. Katznelson and Sutton (1951) first developed the phage method to detect and identify the seed-borne pathogenic bacteria. This technique has been used in other cases also. Recently, Gitaitis and Walcott (2007) have discussed the epidemiology and management of seed-borne bacterial diseases.

Seed-Borne Viral Diseases

In general, viruses are not transmitted through true seed or pollen, but at least 30 viruses including the potato spindle tuber viroid are transmitted in that way. In most virus-host combinations, the proportion of seedlings infected through the seed is very small (e.g., lettuce mosaic virus in lettuce). On the other hand, in tobacco ring spot virus in soybean, most of the seeds may be infected. Seed transmission provides a very effective means of introducing a virus into a crop at an early stage, giving randomized foci of infection throughout the planting. Viruses may persist in seeds for long periods and as a result, the commercial distribution of a seed-borne virus over long distances may be very effective. Fulton (1964) listed 36 known viruses that are transmitted internally through the seed. These include tobacco ring spot, lettuce mosaic, barley stripe mosaic, bean mosaic, southern bean mosaic, elm mosaic, and *Prunus* ring spot. Some interesting studies have been undertaken on the seed transmission of the virus responsible for false stripe diseases of barley. Particles of the virus have been found to be associated with the disease. These occur in large numbers on the leaves, embryos, endosperms, pollen and unfertilized pistils. The presence of the rods in seeds produced from healthy pistils pollinated by pollen from diseased-plants suggested that the disease is seed transmitted. Seeds from the pollen of diseased plants and healthy pistils produce a small percentage of diseased seedlings. There are examples of a virus being transmitted through the seed of one host species but not through the seed of another. Cucumber mosaic virus is transmitted through the seed of wild cucumber (*Micrampelis lobata)* but not through the seed of cultivated cucumber. Cowpea mosaic virus is transmitted through the seeds of asparagus bean (a variety of *Vigna unguiculata)* but not through the seeds of cowpea (*Vigna sinensis)*. Tobacco ring spot virus is carried through the seeds of *Petunia sp.* There are some other examples of transmissions of viruse through the seeds of other host plants. Certain viral diseases are seed transmitted with the help of nematode-borne viruses.

It is not known why fewer viral diseases are seed-transmitted. Viruses restricted to the phloem region cannot enter the seed because the phloem of the mother plant and the embryo are not connected directly. With other viruses, the possible explanation is that they cannot replicate and/or are degraded in meristematic tissues as is found in developing seeds. Weathers and Calavan (1959) showed that the seedlings grown from both true seed and nucellar seed of infected citrus are virus-free. In the case of southern bean mosaic, the virus is present in the embryos of immature seeds of *Phaseolus vulgaris.* The virus can come from either the pollen or ovule-parent but is deactivated as soon as the seed matures and is not transmitted. Seed-borne viruses can be detected by inoculating extracts of embryos or pollen into indicator plants. The particles of barley stripe mosaic virus can be found in extracts of individual seeds of barley by electron microscopy or microserological tests (Hamilton, 1965).

A large number of viruses are transmitted through vegetative parts. Thus, any vegetative part taken for propagation, such as tubers, bulbs, corms, runners and cuttings transmit the virus if the host plants from which these have been taken were infected.

Seed-Borne Fungal Diseases

A large number of fungal pathogens are transmitted through the seed and the vegetative propagating parts. Some of the fungal pathogens transmitted in this way are cereal smuts. Leaf blight of wheat caused by *Alternaria triticina,* leaf stripe and seedling blight of oats (*Helminthosporium avenae*), leaf stripe of barley (*H. gramineum*), anthracnose of French and summer beans (*Colletotrichum lidemuthianum*), red rot of sugarcane (*Colletotrichum falcatum*), seedling blight of flax (*Colletotrichum linicola*), die-back and ripe fruit-rot of chillies (*Colletotrichum capsici*), leaf, stem and pod spot of beans (*Ascochyta pisi*), frog eye leaf spot of tobacco (*Cercospora nicotianae*), blight of gram (*Ascochyta rabie*, stem gall of coriander (*Protomyces macrosporus*), the choke of grasses (*Epichloe typina*), ergot of rye (*Claviceps purpurea*), downy mildews of peas (*Peronospora pisi*) and late blight of potato (*Phytophthora infestans*). These are some important examples where the pathogens are transmitted on the seed surface, inside the seed coat or deeper in the embryo. A list of the important fungal, bacterial, viral, and nematodal pathogens prevalent in India, which are transmitted through the seed are given in Table 22.1. Seed infection by fungi, bacteria and viruses may arise in various ways, such as by floral infection, systemic invasion through the vascular system of the plant of the seed, direct penetration through pods or other fruits and invasion of the seed during storage and harvesting.

Table 22.1 Some Important Seed-borne Diseases and Their Control

Crop	Diseases	Pathogen	Nature	Control Measures
1	2	3	4	5
Paddy	Foot rot	*Fusarium moniliforme*	Externally seed-borne	Seeds are treated with organo-mercurial (1% active material at 1 : 400 parts by weight before sowing).
	Brown leaf spot	*Drechslera (Helminthosporium oryzae)*	–do–	–do–
	Blast	*Pyricularia grisea (oryzae)*	Seed as well as soil-borne	Seed treatment with organo-mercurial is recommended. Thirumulachar (1967) recommends 200 ppm of Aureofungin and 200 ppm of copper sulphate as seed treatment.
	Stack burn (Leaf spot Discoloration of grains)	*Trichoconis padwickii*	Soil as well as seed-borne.	Mancozeb, Thiophenate-methyl, Organomercurials as seed treatment (Shetty *et al.,* 1989).
	Udabatta	*Ephelis oryzae* (perfect stage *Balansia oryzae*)	Internally seed-borne.	Hot water treatment at 50°C for 10 minutes.

(*Contd.*)

(*Contd.*)

Crop	Diseases	Pathogen	Nature	Control Measures
1	2	3	4	5
	Bacterial blight	*Xanthomonas oryzae*	Internally seed-borne	Soaking of seeds in water at room temperature for 12 hours followed by hot water treatment at 53°C for 30 minutes (Sinha and Nene, 1967). According to Srivastava and Rao (1964), the bacterium can be eliminated by soaking in water containing 2.5 % Ceresan Wet and 15 % Agrimycin-100 before hot water treatment.
	White tip	Nematode *Aphelenchoides besseyi*	Infected seeds. The nematodes lie in a quiescent stage beneath the hulls of the seeds.	Seed treatment with hot water at 55°C for 10-15 minutes. Soaking of seeds in Isophenophos or Carbosulfan at 0.1 % for 12 hours.
	"Ufra"	Nematode *Ditylenchus angustus*	Externally attached to seeds	Delaying sowing or planting to revive the nematodes with first showers in the season and killing them by starvation, rotation with jute or sesamum. Application of nematicides such as Carbofuran, Mocap, Monocrotophos in between planting and flooding.
Wheat	Foot rot	*Helminthosporium sativum (Cochliobolus sativus).*	Externally and internally seed-borne	Treating the seeds with organomercurial compounds such as Agrosan GN eliminates external infection.
	Loose smut	*Ustilago segetum tritici*	Internally seed-borne	Hot water treatment, solar energy heat treatment, anaerobic seed treatment Hebert (1955), seed treatment with Vitavax or Benlate.
	Stinking smut or Hill bunt Flag smut	*Tilletia foetida* and *T. caries*, *Urocystis tritici*	On the surface and in the soil Soil and externally seed-borne	Treating the seeds with organomercurials (2-3 gm/27.2 kg). Seeds are treated with organomercurial compounds.

(*Contd.*)

(*Contd.*)

Crop	Diseases	Pathogen	Nature	Control Measures
1	2	3	4	5
	Leaf blight	*Alternaria triticina*	Internally as well externally seed-borne.	Normal mercurial seed dressing. Pre-soaking in water for 4 hours followed by a 10-minutes dip in hot water at 50°C (Prabhu and Prasada, 1966).
	Karnal bunt	*Tilletia (Neovossia) indica*	Soil and externally seed-borne	Thiram, Zineb, Benlate, Bavistin, Vitavax and Du-Ter (Fentin hydroxide).
	Ear cockle	*Anguina tritici*	Nematode galls along with seeds	Sanitation and use of clean seeds. Soaking of seeds in 18% brine solution in the floatation of galls.
Barley	Stripe disease	*Helminthosporium gramineum*	Internally seed-borne	Seed treatment with organomercurial dust.
	Covered smut	*Ustilago hordei*	Externally seed-borne	Treatment of seeds with organomercurial compounds.
	Loose smut	*Ustilago segetum* var. *nuda*	Internally seed-borne.	Treatment similar to that of loose smut of wheat.
	Foot rot and seedling blight	*Helminthosporium sativum.*	Seed and soil borne	Seed treatment similar to foot rot of wheat.
Oats	Covered smut	*Ustilago kolleri*	Externally seed-borne	Seed treatment with organomercurials such as Agrosan-GN.
	Loose smut	*U. segetum* var. *avenae*		
Bajra	Ergot	*Claviceps fusiformis*	Sclerotia get mixed up with the seed	Use of certified seeds, soaking of seeds in 1% solution of common salt (Nene and Singh, 1976).
	Green ear or downy mildew	*Sclerospora graminicola*	Oospores on the surface and mycelium inside the seeds.	Use of certified seeds crop rotation.
Jowar	Grain smut or Kernel smut	*Sphacelotheca sorghi*	Externally seed-borne	Treatment of seeds with organomercurials, sulphur (1:250 parts), Thiram or Captan treatment.
Cotton	Loose smut	*S. cruenta*	-do-	-do-
	Black arm or angular leaf spot	*Xanthomonas campestris* pv. *malvacearum*	Externally as well as internally seed-borne	Seeds are delinted with sulphuric acid and treated with organomercurial compounds like Vitavax or Plantavax, Streptomycin.
	Anthracnose	*Colletotrichum gossypii*	-do-	-do-

(*Contd.*)

(*Contd.*)

Crop	Diseases	Pathogen	Nature	Control Measures
1	2	3	4	5
Ground-nut	Pre-emergence blight	Various fungi, such as *Aspergillus niger*		Seeds are treated with Thiram and Captan or organomercurial.
Sugarcane	Red rot	*Colletotrichum falcatum (Glomerella tucumanensis)*	Internally seed(sett)-borne	Setts are treated with Agallol or Aretan. Sett treatment with Bavistin and Thiram (0.1%) each.
	Whip smut	*Ustilago scitaminea*	-do-	Ratooning to be discouraged. Selecting the setts form healthy areas. Setts are treated with an organomercurial at the time of sowing.
	Wilt of sugarcane	*Cephalosporium sacchari*	Sett-borne	Control as in the case of red rot.
	Grassy shoot and mosaic	Mycoplasmal, viral	Sett-borne	Disease-free seeds, control of aphids and hot air treatment (Singh, 1968).
Tomato	Bacterial canker	*Clavibacter (Corynebacterium) michiganensis* sub sp. *michiganensis*.	Seed-borne	Hot water treatment or by treating the seeds in organomercurial slurry or 50 ppm of streptomycin solution.
Potato	Potato spindle tuber viroid disease	Potato spindle tuber viroid	Transmitted by core grafts in the tubers, transmitted in both ovule and pollen, potato and tomato	Healthy seed tubers.
	Leaf roll	Potato-virus-1 or Solanum virus-14	Through infected seed tubers and aphids insect vector	Storage of seed tubers at 37.5°C in a humid atmosphere for 20-25 days.
	Rugose mosaic	Potato virus-X and Potato virus-Y	Though small sized tubers on diseased plants (*Myzus persicae* transmits virus-Y)	Certified seed sowing and control of aphids
	Mild mosaic	Potato latent virus or potato Mottle virus Potato virus X or Solanum virus-1	Sap inoculation, contact and through tubers	Certified seed sowing.
	Ring rot	*Corynebacterium sepedonicus*, now *Clavibacter michiganense* sub sp. *sepedonicus*	Seed-borne	Healthy seed tubers, disinfection of cutting knife.

(*Contd.*)

(*Contd.*)

Crop	Diseases	Pathogen	Nature	Control Measures
1	2	3	4	5
	Brown rot	*Ralstonia (Pseudomonas) solanacearum*	Soil and seed-borne	Selecting disease-free tubers, disinfecting the cutting knife with a suitable bactericide, pretreatment of seed tuber with 500 or 1000 ppm of Streptocycline.
	Late blight	*Phytophthora infestans*	Dormant mycelium in the seed and also through resting structures in the tubers or in the soil.	Use of disease-free seed tubers.
	Black scurf	*Rhizoctonia solani* and *R. bataticola*	Externally tuber-borne	Treatment of seed tubers with 0.1% mercuric chloride. PCNB treatment of seed potatoes.
	Potato rot nematode	*Ditylendues dipsaci*, and *D. destructor*	Infested potato tubers	"Seed" treatment with Carbofuran at 0.3 kg air /80 kg seed or soaking seeds in chloropyrifos at 0.1% for 12 hours.
Chillies	Die-back and fruit-rot	*Colletotrichum capsici*	Soil-borne and seed-borne	Seeds are treated with organomercurial compounds.
Tobacco	Frog eye leaf spot	*Cercospora nicotianae*	Seed-borne	Seeds are treated with $AgNO_3$ at 1 gm/L of water to treat 450 gm of seeds
Turmeric	Rhizome rot	*Pythium aphanidermatum*	Seed rhizome	Seed rhizomes are treated with a 1% mercuric chloride solution.
Ginger	Rhizome rot85	*Pythium myriotylum, Pythium aphanidermatum P. monospermum P. gracile*	– do –	– do –
Cabbage	Black rot	*Xanthomonas campestris*	Seed-borne	Seeds are treated with mercuric chloride (0.1% for 30 minutes) or with hot water at 49°C for 30 minutes.
Coriander	Steam gall	*Protomyces macrosporus*	Soil as well as seed-borne	Soil and seed treatment with Thiram.
Pea	Downy mildews	*Peronospora pisi*	Internally and internally seed-borne	Seed treatment at 50°C.
Gram	Blight	*Ascochyta rabiei*, now known as *Phoma rabiei*	Both externally and internally seed-borne	Hot water treatment, crop rotation of 3-4 years.
Bean	Bean mosaic	Phaseolus virus 1 or common bean mosaic virus	Seed transmission (internally seed-borne)	Use virus-free seeds.

In case of *Ustilago nuda* and *U. segetum tritici*, researches have been done on floral infection in the fields on the pathway of infection of the seed. Penetration usually takes place through the ovary wall and not via the stigma and style, as was previously assumed (Batts, 1955). The hyphae cross the cells of the pericarp (fruit coat), enter the testa (seed coat) and then move towards the ovary. After reaching the ovary, the hyphae turn sharply from the testa, enter the scutellum and pass into the growing point of the embryo. Mycelium is intracellular with in the pericarp and testa, but it becomes intercellular with the tissues of the embryo. There is apparently no effect on grain development and it is not possible to know if the seed is infected from external appearance. An excellent technique has resulted from the researches of Simmonds (1946) and Popp (1958). In Popp's technique, embryos are separated from the seed by boiling the seeds in a mixture of 3% (w/v) sodium hydroxide and 12% (w/v) ethanol, containing 15% (w/v) detergent. The embryos are then boiled in a 12% (w/v) ethanol, containing 15% (w/v) sodium hydroxide and heated in a 3: 1 mixture of ethanol and glacial acetic acid. This is done to clear the tissues and to remove oil from the embryo. The embryos are then heated in 45% (v/v) acetic acid containing 0.1% trypan blue to stain the fungus hyphae. The embryos are then mounted in 45% (v/v) lactic acid.

In the rye plant, when leaves are infected by the ascospores of *Claviceps purpurea,* the whole inflorescence is transformed into a typical horn-shaped sclerotium. When sown in the field, the sclerotia, in admixture with the healthy seeds, cause infection. In the case of linseed rust, pieces of straw containing resting teleutospores are commonly mixed with the seed and are the chief source of infection.

As already known, in addition to the plant pathogens, a large number of other microorganisms may be present outside or inside the seed. And some of the microorganisms on the seed surface are capable of colonizing the seedling roots. The importance of the microflora of the grain, from which roots arise, in providing an inoculum of fungi for root colonization in nature has not been fully appreciated. Further studies are needed to elucidate the contribution of the seed coat in providing the initial root colonists under natural conditions.

Seed-Borne Nematode Diseases

Nematodes have many adaptations for their survival and dispersal. One of the important adaptations is their ability to move passively with the seed, either as internally seed-borne or as seed-contamination along with infested plant debris, soil clods, and packaging material. Sharma and Sharma (1994) have reviewed seed-borne nematodes, their distribution, biology, yield losses and management. Some of the important seed-borne diseases due to nematodes are ear cockle of wheat, rye, oats etc., caused by *Anguina tritici,* bent grass nematode disease of bent grass (*Agrostis tenuis*) and ryegrass (*Lolium rigidum),* stem nematode disease of alfalfa (*Medicago sativa*), clover (*Trifolium pnatense*), white clover (*T. repens*), faba bean (*Vicia faba*), onion (*Allium cepa)* cause by *Ditylenchus dipsavi*; potato or nematode diseases of potato and groundnut caused by *Ditylenchus distructor*, white-tip nematode disease of rice due to *Aphelenchoides beneji,* testa nematode of groundnut caused by *Aphelenchoides besseyi*, and *Aphelenchoides arachidis.*

Use of clean planting, hot water treatment, and seed treatment with suitable nematicides, rotation of crops and soil fumigation are some of the prevalent methods for controlling these diseases.

Pathological Effects of Seed-Borne Diseases

Various types of fungi are known to colonize seeds, and many among them are plant pathogens. The fungi may affect the seeds in the form of seed abortion, shrunken seeds, reduced seed size, seed rot, sclerotisation or stromatisation of seed size, seed necrosis, seed discoloration, reduction or complete loss of germinability and some physiological changes.

Seed Abortion

Seed abortions due to fungi that occur during anthesis are: kernel smut and false smut of paddy, kernel smut of wheat, and ear cockle of wheat.

The diseased seeds of kernel smut of paddy, when examined, show minute black pustules or streaks bursting through the glumes. Normally, only a part of the seed is affected. The infected seeds can be detected by their dull color externally before the glumes burst open by the masses of spores. In false smut of rice (*Ustilaginoidea virens*), the fungus transforms the individual seed of the panicles into greenish spore balls with a velvety appearance. The moderately infected seeds look dark green while heavily infected grains are completely transformed into pseudomorphs.

Some pathogenic fungi imperfecti infect flowers and young seed structures. As the seeds mature, the degree of susceptibility decreases. When the pathogen infects the flowers, it causes deterioration of ovules and young seed primordia, thus reducing seed yield. According to Neergaard (1977), *Drechslera oryzae* in paddy, *Fusarium graminearum* in maize cause much damage to the seed development. *Peronosclerospora sorghi*, the downy mildew pathogen in sorghum and maize, and *Sclerospora graminicola* in pearl millet transform the floral primordia into vegetative leafy structure, causing 30-70% seed production loss.

Shrunken Seeds, and Reduced Seed Size

Fungal infection often substantially reduces the size and weight of seeds. Small seeds of wheat show a higher infection percentage of *Ustilago segetum tritici* than larger seeds. However, in Indian conditions, the seed size had no significant effect on smut percentage. Raut *et al.* (1983) reported that the wheat seeds collected from *Alternaria triticina*-infected plants were small and shriveled and based on 1000 grain weight, showed 46-75% reduction in weight over the control (uninfected) plants. Sharma and Bhowmik (1987) studied various aspects of groundnut seed deterioration due to *Macrophomina phaseolina* infection. Fungal infection caused both quantitative and qualitative damage to the seed. Infected pods were brown to black, smaller in size and numerous sclerotia could be seen on the surface of the shell. Low yields result primarily due to the formation of undersized pods and kernels, the latter being mostly shriveled.

Seed Rot

Many seed-borne fungi produce seed rot. In sorghum, *Fusarium moniliforme,* in paddy *Drechslera oryzae, Trichoconiella padwickii, Fusarium* spp. in maize, *Diplodia maydis, Botryodiplodia theobromae, and* in pearl millet, *Curvularia penniseti, Drechslera setariae, F. moniliforme, Phoma exigua* cause seed rot. Species of *Aspergillus* and *Penicillum* are the important storage fungi, which develop on seeds during storage and cause seed-rot in the sown crop.

Sclerotisation and Stromatisation

Here, floral organs or seeds are transformed into stromata or sclerotia as in the case of false smut of paddy and ergot of sorghum. Normally, the disease causes little loss in yield but under favorable conditions, heavy losses have been reported. Ergot severity on sorghum depends mostly upon weather conditions at flowering. If the days are cool, highly humid and cloudy during the anthesis period, the spread of the disease is rapid and damage is severe.

Seed Discoloration

Discoloration of seed is an important disorder, which often indicates the presence of pathogenic fungi in sowing seeds. Commercially, discolored seeds are considered to be of poor quality. The extent of discoloration depends on the seed microflora, environmental factors and host-cultivar physiology. Coating of fungal mycelium and sporulating structures of many saprophytic fungi often develops seed discoloration. In many cereals, this type of discoloration is common. The disorder is caused by profuse growth of pathogens such as *Drechslera sorokiniana* in different cereals (Neergaard, 1977). Contamination of *Alternaria tenuis* and *Cladosporium cladosporioides* is also common in many other kinds of seeds and often depreciates the market value. Pigments produced by different seed-borne fungi may indicate the presence of pathogens or saprophytes. The discolored seeds showed the presence of deoxynivalenol (Vomi-toxin), diacetoxyscirpenol, HT-2 and T-2 mycotoxins. The highest concentration of the mycotoxin was observed on the seed coat. All the symptom-free seeds germinated and developed into healthy seedlings, whereas the affected seeds failed to germinate.

Reduction or Complete Loss of Germinability

Fungal infestations in seed tissues reduce the germination, viability, longevity and vigor of seeds. According to Warham (1990), wheat infected with *Tilletia indica* had very little effect on seed viability, irrespective of the age of the seed, while germination of infected seeds appeared to depend upon the wheat cultivar and the age of the seed. Infected seeds had a lower survival rate in storage compared to healthy seeds of the same seed lot.

Physiological Effects in Seeds

Seed-borne and storage fungi produce a large number of metabolites that are toxic to seeds. Aflatoxins produced by *Aspergillus flavus* group are known to reduce seedling elongation, inhibit chlorophyll synthesis, inhibit various enzymes and degranulate the endoplasmic reticulum. Tentoxin (produced by the field fungus *Alternaria alternata* on cotton seed) is a cyclic tetra peptide that causes irreversible chlorosis in germinating seedlings of susceptible cotton, interferes with plastid development, binds especially to chloroplast coupling factor and inhibits photophosphorylation changes in protein content, amino acid and sugar content of seed due to fungal infection and reduced seed vigour, and this aspect has been reviewed by Bilgrami *et al.* (1979). Finally, it can be said that seed-borne fungal metabolites may affect seed metabolism at the cellular level. Aflatoxins and other mycotoxins affect the seed viability, germination and vigor, which ultimately affect the seed yield.

Quarantine Programmes for Seed Borne and Seed Stocks

Many pests and pathogens such as downy mildew of sunflower and onion, tomato spotted wilt virus, peanut mottle virus and peanut stripe virus in groundnut, bacterial blight of rice, golden nematode and wart disease of potato etc., have gained entry and widely established in our country due to inadequacy of quarantine laws, infrastructure and facilities for effectively enforcing the same. The importance of seed-borne pathogens in relations to quarantine and seed industry has been reviewed and the need for specific quarantine regulations for seed is greatly emphasized (Neergaard, 1970, 1980; Raychaudhuri, 1973; Raychaudhuri *et al.,* 1976; Reddy and Muthaiyan, 1994; and Trivedi, 1994).

In the past, a score of diseases got introduced and some of them got established wide across the country through indiscriminate import of propagative plant material. Some of the examples include apple scab (*Venturia inaequalis*), which was recorded for the first time in 1935 from Jammu and Kashmir. The disease was initially confined to Kashmir valley, appeared in epiphytotic form in Baramulla District in 1975 and later it has been reported in Himachal Pradesh State in 1977 (Rao *et al.,* 1984), Arunachal Pradesh and Sikkim (Malik *et al.,* 1984) and Tamil Nadu.

Potato wart was reported first from Darjeeling district of West Bengal in 1953. So far, wart is confined to Darjeeling. Similarly, golden nematode disease of potato caused by *Globodera rostochiensis,* now called as *Heterodera,* was recorded in 1961 in Ooty. Subsequently, it spread to Nilgiri Hills and also was also recorded in Kadaikanal. The potato cyst nematode has been introduced from Europe. Similarly, black shank of tobacco was reported in Andhra Pradesh in 1960 and now it is known to occur in Himachal Pradesh as well. Groundnut rust was recorded in 1969 in Punjab and in Tamil Nadu in 1972, but is now widely reported from states of West Bengal, Bihar, Assam, Maharashtra and Andhra Pradesh (Mayee *et al.,* 1977). Banana bunchy top was recorded in 1943 in Kottayam area of Travancore. Subsequently, the disease spread and was reported from Assam, Kerala, Orissa, Tamil Nadu, West Bengal, Bihar, Karnataka and Uttar Pradesh.

A score of pest/pathogens were intercepted in imported seed/propagative material through various plant quarantine stations and in germplasm introduction through National Bureau of Plant Genetic Resources and erstwhile Plant Introduction Division, I.A.R.I. A few cases of significant and historical interceptions are given below.

The golden nematode of potato and sugarbeet nematode were intercepted in bulk shipments of seed potatoes imported from Scotland, Northern Ireland and West Germany during 1965-68, through Madras Port and the entire shipment was dumped into sea and destroyed. Similarly, *Fusarium oxysporum* f. sp. *albedinis,* a pathogen of date palm, was recorded during post-entry quarantine inspection of suckers at Jodhpur, Bikaner and Hisar in 1978. The same was imported from USA through New Delhi Airport. About 75% of suckers of Medjod variety succumbed to the disease. All the affected plant material was destroyed by incineration and the pits were thoroughly drenched with suitable fungicide.

Ultimately, the Ministry of Agriculture (Department of Agriculture and Cooperation) introduced a new seed policy in 1988. It envisages providing the best planting material available in the world to the farmers to increase the productivity and thereby raise the agricultural economy of the country and at the same time take appropriate quarantine measures to prevent the introduction of destructive pests/pathogens affecting crops. The policy was formulated with special emphasis on import of high-quality seeds and hybrids, time-bound strengthening/modernizing plant quarantine facilities, effective observance of plant quarantine procedures including post-entry quarantine and to provide incentives to domestic seed industry. The imported seeds and propagative material are subjected to visual inspection, X-ray tests,

washing tests, incubation tests etc. to ascertain freedom from quarantine pests/pathogens and other pests or pathogens of major economic significance.

Seed Treatment

According to Leukel (1936), copper sulphate was first recommended as seed treatment for stinking smut of wheat (Schulthess, 1961). For many years, seeds have been treated with fungicidal dusts and liquids for the control of seed-borne pathogens and seedling diseases. During most of this period, mercurial fungicides were used. Many of them possessed a volatile component that effectively sterilized the seed coat by diffusion and untreated portion of seed by its vapor action (Lehman, 1943). Some of the organomercurials commonly used for seed treatment are Ceresan (ethyl mercury chloride), new improved Ceresan (ethyl mercury phosphate), Ceresan dry (phenyl mercury acetate), Aretan (Ethoxy ethyl mercury chloride), Agallol (Methoxy ethyl mercury chloride) and Agrosan-GN (Phenyl mercury acetate and ethyl mercury chloride). It may be mentioned that mercurial fungicides are no more recommended for disease control.

The number of fungicides available has increased considerably since 1960. The loss of methyl and ethyl mercury formulations led to the development of new organic fungicides, whose performance is equally good. The most extensively used seed treatment fungicides are Captan, Thiram, Zineb, and Maneb. These are contact fungicides and because they have a broad spectrum of fungicidal activity, their compatibility with insecticides is quite good. Carboxin, Benomyl and Carbendazim remove systemic and deep-seated infections. Carboxin is successfully utilized for the control of various seed-borne smut infections. The metalaxyl fungicides (Ridomil or Apron SD35) are good systemic fungicides to get rid of infections belonging to oomycetous pathogens such as *Pythium, Phytophthora, Sclerospora Peronosclerospora*, which are responsible for damping off and downy mildews. Dharamvir (1994) discussed advances in the chemical control of seed-borne diseases in India. Earlier, Kabana *et al.,* (1977) have discussed the control of soil and seed-borne diseases.

For internally seed-borne diseases, it was realized that chemical disinfectants will not work and disinfection would have to be accomplished by the use of physical agents. Jensen (1988) devised hot water treatment for the control of late blight of potato by treating the seed potatoes. Luthra and Sattar (1934) utilized solar heat energy treatment for the control of loose smut of wheat in India. There are various modifications of the hot water treatment and the solar energy treatment. In temperate countries, where solar heat cannot be used effectively, the alternative method (Tyner, 1957) of soaking the seed in 0.2% Spergon (having 98% tetrachloroparabenzoquinone) is used. Pre-soaking of seeds in cold water for some time also eliminates intercellular infection. Later a successful method adopted in the USA is the anaerobic seed treatment, developed by Hebert (1955). This method consists of soaking the seeds for 2-4 hours in water at a temperature of 16-17°C, keeping the moist seeds in air-tight containers for 65-70 hours and thereafter drying them. The efficacy of normal seed treatment is due to the production of quinones within the seed during anaerobic respiration.

Purdy (1967) discussed the equipment for application of seed treatment and materials. Taylor and Harman (1990) have reviewed the area of "concepts and technologies of selected seed treatments." The most common method of applying seed disinfectants is the dip method, in which, the seeds are soaked and thus coated with the disinfectant dust in rotating drums or in a closed earthen jar. In other countries, the slurry method is most commonly employed. Here, a thick water suspension of disinfectants is applied with the help of a special type of machine. The seeds are pelleted by heavily coating them with a

mixture of chemical disinfectant in a quick drying sticker (methyl cellulose + shellac). In most of the advanced countries of the world, especially the USA, fungicides are sprayed on seeds with the help of a special type of machine known as Mist-O-Matic. In this method, the liquid is atomized into a penetrating spray mist, which is retained in a mist chamber.

Seed Treatment with Fungicides

Seed treatments have been widely used for centuries on a commercial basis. During the last 15-20 years, seed treatment use has rapidly accelerated. Pressure to meet global demand for food and fuel has increased the motivation to maximize crop productivity. However, environmental pollution is a major disadvantage of using foliar and soil application of crop protection chemicals to meet this objective. Many new fungicidal seed treatments have been adopted on their own merits and along with the development of highly efficacious, new active ingredients. The adoption of triazole fungicides (Difenoconazole, Tubeconazole and Triticonazole), systemic seed treatment for cereals, the use of Fludioxonil as a low-rate, reduced-risk seed treatment, the introduction of Strobilurin fungicides as seed treatment products have direct benefits related to reduced microbial infection (Munkvold, 2009). Systemic fungicides such as Carboxin were already available for control of smut diseases in cereals but Carboxin did not have the broad spectrum as that of Difenoconazole, which provided good control against soil-borne pathogens. Systemic fungicides, when combined with Difenoconazole, provide protection against foliar pathogens of seedlings including leaf rust (*Puccinia recondita* and *Puccinia striiformis*) and Septoria leaf blotches (*Septoria tritici*). Difenoconazole has been used for improving yield from head blight-damaged wheat seed, contaminated with *Fusarium graminearum* (Schaafsma and Tamburic-Ilincic, 2005 and Wiersma and Kandel, 2004). Ipconazole, another triazole active ingredient, has been used as a seed treatment. Fludioxonil also showed improved efficacy against *Fusarium graminearum* (Munkvold and O'Mara, 2002). Growth in the use of seed treatments in soybean and maize also followed the introduction of Fludioxonil.

Azoxystrobin and Trifloxystrobin - strobilurin derivates, are widely used on a number of crops including maize and soybeans. Strobilurins are active against Ascomycota, Basidiomycota, Deuteromycota and Oomycota. The systemic activity of Azoxystrobin in seed treatment may also contribute to improve performance as a result of more complete root tissue protection and a longer duration of control compared to contact fungicides. Strobilurin fungicides have been shown to induce several physiological changes in plants (Bartlett *et al.,* 2002). They include increased level of abscisic acid, suppression of ethylene biosynthesis and enhanced antioxidative potential, resulting in delayed senescence (Grossmann *et al*., 1999; Reade *et al.,* 2003), increased tolerance to environmental stresses and increased water use efficiency as a result of reduced transpiration. Additional effect may include induction of plant-defense responses, resulting in better resistance to non-target pathogens. However, it is clear that Strobilurin seed treatments do provide consistent, economically beneficial crop performance gains through the combination of disease control and direct physiological effects.

Seed Treatment with Insecticides and Nematicides

The popularity of seed-applied insecticides began with the introduction of neonicotinoid active ingredient, beginning with Imidacloprid in 1991. It was used as a seed treatment for maize in 1995, followed by Thiamethoxam and Clothianidin. Seed treated insecticides are used for control of soil-borne insects. Their systemic properties enable the compounds to control above-ground, leaf and stem-feeding insects

(Munkvold, 2009). Several insects that are controlled by insecticide-treated seeds are vectors of plant pathogens and in some cases, seeds treated with insecticides have contributed to the reduction in diseases transmission. Seeds treated with neonicotinoid insecticides were shown in several studies to effectively prevent feeding by the corn flea beetle and significantly reduce transmission of *Pantoea stewartii*. Imidacloprid-treated seeds reduced the severity of bacterial wilt caused by *Erwinia tracheiphila* through control of its vector, the striped cucumber beetle (Fleischer *et al.,* 1998). Seeds treated with insecticides have also been effective in reducing aphid transmission of viruses in oats, sorghum, sugarbeet and wheat. Thiamethoxam-treated seeds can reduce soybean aphid damage, and have the potential to reduce spread of soybean mosaic virus by aphid vectors (McCornack and Ragsdale, 2006). There is potential for seed treatments to contribute to the management of additional pests and diseases that are currently beyond the reach of seed treatment and this approach will continue to be pushed.

Plant Extracts and Natural Compounds

Plants extracts contain natural antimicrobial compounds, and these can be used for seed disinfection as an alternative to fungicide treatments, or in combination with physical treatments. These plant extracts, including essential oils have been reported to be active against pathogens like *Ascochyta* spp., and *Alternaria* spp. (Riccioni *et al.,* 2013). Among the essential oils, thyme oil has most frequently shown the best effectivity in *in vitro* and field tests. Thyme oil contains thymol and other antifungal compounds, which provide general antimicrobial protection against seed-borne bacteria and fungi (Van der Wolf *et al.,* 2008). Onion-seed exudates include sugars, amino acids, organic acids and phenolic compounds, which can be released during seed imbibitions, and these have an inhibitory effect on pathogenic fungi (Nelson, 2004).

Chitosan is derived from crab-shell chitin, and it is a biopolymer with antifungal properties that have been shown to be effective against several fungi. Chitosan acts by chelating nutrients and minerals, which prevents pathogens from accessing them (El Hadrami *et al.,* 2010; Romanazzi *et al.,* 2013). When chitosan is applied to seeds, the most frequently produced effects are an increase in germination index, and greater shoot height, root length and root and shoot weights. The efficacy of chitosan as a seed protectant against several pathogens has been reported in numerous studies carried out on cereal crops, such as for wheat (Reddy *et al.,* 1999), maize (Lizarranga *et al.*, 2013), pearl millet and oil-bearing crops (Nandesh Kumar *et al.,* 2008) and also on several horticultural crops.

Biocontrol Agents

Biocontrol agent species belonging to the genus *Trichoderma*—which is a free-living fungus common in soil and root ecosystems—have been widely used as antagonistic agents against several diseases. Their biocontrol actions include mycoparasitism, spatial and nutrient competition, and antibiosis by enzymes and secondary metabolites and induction of plant defences. Experimental trials conducted with several crops have shown the effectiveness of *Trichoderma* spp. as seed treatments against soil and seed-borne pathogens such as *Pythium*, *Phytophthora*, *Rhizoctonia* and *Fusarium* spp. Among Plant Growth Promoting Rhizobacteria (PGPR), the Gram-negative bacteria *Pseudomonas* spp., are widely used because they have a number of positive features, such as the ability to grow rapidly *in vitro* and to be mass produced, rapidity in the use of seed and root exudates, colonization and multiplication in the rhizosphere and within the plant, production of a wide spectrum of bioactive metabolites (i.e. antibiotics, siderophores, volatiles and growth promoting substances), aggressive competition towards other

microorganisms and adaptability to environmental stress. Several species of *Pseudomonas* have been shown to be effective against plant diseases caused by soil-borne and seed-borne pathogens and the agents of canker. The major weakness of Pseudomonads as biocontrol agents is their inability to produce resting spores (as do many *Bacillus* spp.), which complicates the formulation of these bacteria for commercial use; indeed, they are formulated as frozen-cell pellets that must be kept on dry ice until application. Fluorescent Pseudomonads are most frequently applied as biocontrol agents for horticultural seed production.

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Questions

1. Give examples of externally seed-borne diseases.
2. Would you suggest ordinary contact fungicides for all types of seed-borne infections?
3. Suggest some suitable fungicides for treating seeds with internal infection.
4. Name some pathogens that have been introduced into our country because of lack of proper quarantine regulations or check.

Plant Diseases Caused by Mollicutes: Phytoplasmas and Spiroplasmas

23

Mycoplasmas, the free living or parasitic organisms, are not new to microbiologists and veterinary pathologists. *Mycoplasma mycoides* was cultured *in vitro* more than hundred years ago in Pasteur's laboratory (Nocard *et al.*, 1898), from animals suffering from bovine pleuropneumonia. Later on, similar types of organisms were isolated from a number of other animals and birds and these were named as pleuropneumonia like organisms (PPLO). Borrel *et al.* (1910) gave the first binomial to these organisms and called them *Asterococcus mycoides* and later, they were put under the genus *Mycoplasma* and *Borrelomyces.* Since *Asterococcus* was an algal genus, the Editorial Board of the International Bulletin of Bacterial Nomenclature and Taxonomy (1955) discarded the genus *Asterococcus* and recommended the use of either of the other two genera. Edward and Freundt (1956) proposed to retain only valid genus *Mycoplasma,* on grounds of priority. It is now an established and accepted genus of the order Mycoplasmatales under the class *Schizomycetes.* Although it resembles bacteria to a great extent, it cannot be classified with the so-called true bacteria.

Edward *et al.* (1967) proposed a new class, which they called *Mollicutes.*

The morphological features of mycoplasmas, which distinguish them from most other organisms, are:

1. They are pleomorphic. They range from small, spherical bodies of about 10 nm in diameter (elementary bodies) to large, irregular bodies with bigger diameters. There are also elongate forms and their forms vary with the cultural conditions.
2. They are both parasitic and saprophytic.
3. Cells are non-motile and usually form typical "poached egg" shaped minute colonies with a central nipple on agar media.
4. Cells are delimited by a unit lipoprotein membrane and lack rigid mucopeptide. The unit membrane is about 10 nm thick.
5. Within the cytoplasm are ribosomes, which are of similar size (14 nm diameter) as those of bacteria (cytoplasmic ribosomes of eukaryotes are about 18 nm in diameter). The mycoplasmal ribosomes resemble those of bacteria in the sedimentation characteristics of both the nucleoprotein and nucleic acid. The ribosomes are mostly 72S.
6. Also, within the cytoplasm, thin, stranded material (3 nm diameter) can be recognized which has been shown to be DNA. Extracted DNA is circular (a prokaryotic character) but can be distinguished from bacterial DNA by its low guanine and cytosine content.

7. Animal mycoplasmas also contain densely staining bodies with a diameter of about 20-60 nm. Lipid metabolism in believed to be connected to them.
8. Cells of mycoplasmas are usually resistant to the antibiotics, which act on cell-walls, for example, penicillin, cephaloridine, vancomycin, etc. Mycoplasmas are sensitive to tetracyclines.

Although mycoplasmas were earlier associated with animal diseases, in 1967, it became apparent that proliferation diseases, yellows type, big bud, witches' broom and certain stunting diseases are not of viral origin (Doi *et al.*, 1967; Nasu *et al.*, 1967). This inference was drawn on the basis of electron microscope observations and the recovery of the disease symptoms by tetracycline antibiotics. A group of Japanese scholars (Doi *et al.*, 1967 and Ishiie *et al.*, 1967) introduced for the first time an entirely new group of organisms to plant pathologists. There are several reviews and articles, which have covered the reports dealing with nearly 200 different diseases in the group (Maramorosch and Raychaudhuri, 1981; Bove, 1984; Da Garca, 1991; Hiruki, 1988; Paul Khurana *et al.,* 1988; Whitecomb and Tully, 1989). Verma and Ahlawat (1994) reviewed plant Mollicutes and bacteria-like organisms.

Since 1967, mycoplasma-like organisms have been associated with as many as 200 plant diseases, based on: (1) the constant presence of mycoplasma-like organism (MLO) in the phloem tissue of diseased plants and their complete absence from healthy plants; (2) the presence of similar microorganisms in insect vectors, which carry the infectious organisms and the absence of MLO from non-infective individuals; (3) the recovery of plants after the application of tetracyclines and the reappearance of MLO when the disease recurs; and (4) the disappearance of MLO from the plants and vectors which have been cured by heat therapy. Attempts were made in many laboratories to obtain cell-free cultures of the various MLOs. According to Maramorosch (1976), by the end of 1974, with the exception of MLO properly isolated from stubborn diseased citrus, none of the reports had been substantiated by the deposition of representative prototypes to a type culture collection.

Properties of True Mycoplasmas

Mycoplasmas are prokaryotic organisms that have no cell walls. They are members of the class Mollicutes, which has one order, Mycoplasmatales, which was further been divided into three families, each with one genus: Mycoplasmataceae, genus *Mycoplasma,* Acholeplasmataceae, genus *Acholeplasma,* and Spiroplasmataceae, genus *Spiroplasma*. Mycoplasmas lack true cell wall. They measure from 175 to 250 nm in diameter during reproduction but grow into various sizes and shapes. Mycoplasmas reproduce by budding and by binary transverse fission of cells. They have no flagella, produce no spores and are Gram negative. Nearly all Mycoplasmas are parasitic to humans and animals and all saprophytic ones can be grown on more or less synthetic media. Mycoplasmas have been isolated from healthy and diseased animals and humans suffering from diseases of respiratory and urogenital tracts. According to Agrios (2005), they have been associated with some arthritic and nervous disorders of animals and some have been found to exist as saprophytes.

Phytoplasmas

The organisms observed in plants and insect vectors, i.e. the Phytoplasmas, which do not include the Spiroplasmas, resemble Mycoplasmas of the genus *Mycoplasma* or *Acholeplasma.* According to Agrios (2005), genetically, Phytoplasmas are more related to *Acholeplasma* than to *Mycoplasma.* Phytoplasma lack cell wall, are bounded by a unit membrane and have cytoplasm, ribosomes and strands of nuclear material. The size of their chromosome varies from 350 Kilobases of DNA to 1130 Kilobases. The

shape of phytoplasmas is usually spheroidal to ovoid or irregular tubular to filamentous. Phytoplasmas and Spiroplasmas are generally present in the sap of a small number of phloem sieve tubes of the infected plants. Most plant mollicutes are transmitted from one plant to another by leaf-hoppers, but psyllids and plant-hoppers also transmit some. Plant mollicutes also grow in the alimentary canal, hemolymph, salivary glands and intracellular in various body organs of their insect vectors.

There is an incubation period for the multiplication and distribution of the mollicutes within the insect. Despite several attempts by several workers, no phytoplasmas could be cultured on the artificial media so far. Phytoplasmas have been extracted and their antisera, including monoclonal antibodies, have been prepared. Some of the modern techniques like specific antibodies, DNA probes, RFLP profiles and analysis of 16s rRNA genes using PCR amplification techniques have been utilized in the detection and identification of the phytoplasmas.

Spiroplasmas

Spiroplasmas are helical mollicutes. Spiroplasmas are known to cause the stubborn disease in citrus plants and the brittle root disease in horseradish (*Spiroplasma citri*), stunt disease in corn plants and a disease in periwinkle. They are helical in shape and hence called Spiroplasmas. *Spiroplasma citri* has also been found in several other dicots. Several other kinds of Spiroplasmas have been found to infect honeybees and several other insects, and several others live saprophytically on flowers and other plant surfaces.

Spiroplasma citri, the causal agent of citrus stubborn disease, was obtained in pure culture by Saglio *et al.*, in 1971 and by Fudr Allah *et al.*, in 1972. It took two to three years more before definite evidence was obtained that *S. citri* is the causal agent of the stubborn disease (Saglio *et al.*, 1973). Markham *et al.* (1974) confirmed Koch's postulates with regard to *Spiroplasma citri*.

As stated earlier, since 1967, wall-less micro organisms were seen with the electron microscope in the phloem of plants infected with one of several yellow-type diseases and in insect vectors of these diseases. These organisms were called as mycoplasma like organisms (MLO) because of their superficial resemblance to mycoplasmas. It was later shown that these are not mycoplasmas. Although all belong to the class Mollicutes, order Mycoplasmatales of prokaryotes. The true nature of mycoplasma like organisms (MLO), now called as phytoplasmas, is still not certain. Their genomes are only distantly related to true mycoplasmas (Agrios, 1996). Phytoplasmas have not been grown in artificial nutrient medium so far and in no case, Koch's proof has been obtained by directly inoculating with phytoplasmas on healthy plants.

However, Spiroplasmas, as already indicated (Koch's postulates have been proved), have been cultured in the laboratory and their pathogenicity had been proven. At the moment, phytoplasmas are considered to belong to the class Mollicutes, which include true mycoplasmas, but no family or genus name has been assigned to them. The citrus stubborn and the corn stunt organisms are placed in the genus *Spiroplasma* in the class Mollicutes.

Spiroplasmas are helical mollicutes. Spiroplasmas are cells that vary in shape from spherical or slightly ovoid, 100-240 nm or larger in diameter, to helical and branched non-helical filaments. Unlike mycoplasmas, phytoplasmas have not been cultured on nutrient media. They produce mostly helical form in liquid media and multiply by fission. They lack a true cell wall and are surrounded by a unit membrane. The helical filaments are motile, moving by a rapid rotatory or screw-like motion. There are no flagella. Spiroplasma colonies on agar media have a typical fried-egg appearance and are around 0.2 mm in diameter

Two distinct groups of Spiroplasmas have been reported to cause diseases in plants. One group, represented by corn stunt Spiroplasma has a narrow host-range limited only to species of Z*ea.* The other group is represented by *Spiroplasma citri* and has a much wider host range and has been found to cause stubborn diseases in citrus as well as in crucifers and other crop plants. Tully and Whitecomb (1983) have reviewed the work on Spiroplasmas.

More than 200 plant diseases, grafts or insects transmissible with characteristic symptoms such as abnormalities in floral and vegetative parts (virescence and phyllody), yellowing, proliferation of axillary buds, reduction in leaf lamina, general stunting, etc., have been reported to be due to mycoplasma-like bodies from various parts of the world (Agrios, 2005).

There are several important plant diseases, which are caused by mycoplasma-like organisms. Some of the most common, widespread and economically important diseases as well as the work done on them will be dealt with here.

Raychaudhuri (1991) has listed 33 plant diseases in India with MLO symptoms but etiology has been reported in 13 cases only.

Sandal Spike

McCarthy (Barber, 1903) first reported this disease from Coorg district of Karnataka in 1899. It has now spread to the entire southern part of Karnataka, as well as to the border areas of the neighboring states. This disease has become very serious and is threatening the sandal industry (Varadaraja-Iyengar, 1969). Earlier, graft transmissions (Coleman, 1917) and an insect-borne virus were believed to cause this. Sandal (*Santalum album*) tree is of great economic importance for its heartwood and oil, which are very fragrant. The tree is a semi-root parasite, grows in the forests of southern Karnataka and northern Tamil Nadu. A recurring annual loss of 194 million rupees has been reported earlier by Rajan (1977). Gosh *et al.* (1992) have reviewed the work on this disease.

Symptoms

Trees of all ages get infected. Sandal spike is of two types, rosette and pendular type. Rosette spike is more common and is commonly referred to as spike or spike disease. The disease is characterized by an extensive reduction in the leaf size and the internodes. The whole shoot looks like a spike bearing four rows of spiked bristles. Spiked plants do not bear flowers or fruits. Only occasionally, phylloid or abortive flowers are produced. Diseased trees display virescens. Disease also affects fine roots. Their ends(tips) die and causes their disconnection from the roots of the host plants causing death of the trees.

Pendulous spike is less common. Due to elongation of infected shoots that are disproportionate to their thickness, they droop. Rosette symptoms are absent in these branches. In this case, the root ends and haustoria do not die.

Causal Organism

In earlier years, spike disease was suspected to be root disease, a physiological disorder caused by unbalanced sap circulation brought about by adverse factors such as forest fires, a fungal disease and a disease caused by some ultramicroscopic bacteria. With the discovery of mycoplasma like organisms with yellows disease of aster, witches' broom of potato, paulonia and mulberry dwarf by Japanese Scientists in 1967 gave impetus to a reconsideration of the causative agents of plant diseases of unconfirmed

etiology. In 1969, the viral theory of sandal spike was disproved by three groups of workers who demonstrated mycoplasma like bodies in the phloem tissues of the diseased plants (Dijkstra and Ie, 1969; Hull *et al.*, 1969; and Varma *et al.*; 1969). Remission of symptoms confirmed mycoplasmal etiology. The MLOs are found in the phloem tissues of the leaves, petioles, stems, and roots of diseased plants. These bodies are mostly 40-750 nm in diameter, however some of the bodies are ovoid to ellipsoidal in shape. Typically, they are devoid of cell walls and the cytoplasm is bound by unit membranes 10-12 nm thick. The organism contains a fibrillar network of DNA and ribosomal bodies.

Physiology of the Diseased Plants

In diseased plants, there is huge accumulation of starch in branches and leaves (Coleman, 1917). Coleman further showed that spiked plants contain lower amounts of calcium and total ash content and higher nitrogen content in contrast to healthy plants. The ratio of Calcium to Nitrogen (C/N) can be used as a diagnostic character to see if the plants are infected or not. According to Iyengar (1960), deficiency of calcium in the vegetative parts is traced to the non-mobility of the same from the roots, where it is stored. Nitrate reductase activity is higher in diseased plants than the healthy ones. Spiked plants show lower iron content, resulting in chlorosis of the leaves (Parthasarthy and Rao, 1962). The ascorbic acid (AA) level in the leaves of spiked sandal is higher. Parthasarthy *et al.* (1963) stated that since AA is known to exert an inhibitory activity on the amylolytic activity, it might be a contributory factor for the abnormal accumulation of starch noticed in spiked plants. According to Parthasarthy *et al.* (1963), the higher respiratory rate in the diseased tissues cannot be ascribed to a higher AA content but due to other enzymatic respiratory systems. Ramaiah *et al.* (1964) demonstrated that the Krebs cycle is affected and there is disturbance of key amino acids. Parthasarthy *et al.* (1970) showed higher level of polyphenolase activity (PPA) due to which phenolics and tannins rise in the diseased plants.

Disease Cycle

The disease is transmitted through root contact and insects. More than one species of leaf-hoppers are involved in its transmissions, such as *Nephotettix virescens* and *Moonia albimaculata*. The MLO infect a large number of collateral hosts such as *Eucalyptus grandis, Vinca rosea, Zizyphus oenoplea* and *Dodonea viscosa* etc. The diseases can be transferred from *Vinca rosea* to Sandal and vice versa through dodder. Epidemiology of the disease is poorly understood. *Lantana camara* renders the trees susceptible to the disease and might be acting as a pathogen reservoir.

Disease Management

So far, no effective method of controlling the disease is known. In the past, attempts have been made to cure the trees by raising the temperatures above 38°C by building fires in trenches around the effected trees, but it was neither successful nor practicable. On the assumption that eliminating the source of infection might lessen the incidence of the disease and its spread, attempts were made in the past to remove or kill the spiked sandal trees by arsenical poisoning and also by removing the diseased branches. However, this resulted in the removal of large trees without significantly reducing the disease incidence.

Different host plants of sandal impart resistance or susceptibility to the disease in sandal. Kristen (1960) compiled a list of such host plants, based on field observations by earlier workers. Among the host plants, through which sandal acquires resistance are *Azadirachta indica, Bambusa arundinacea, Cassia siamea, Casuarina equisetifolia, Dalbergia sissoo, Dodonaea viscosa, Erythroxylum monogynum,*

Ficus benghalensis, Murraya koenigii , Ruta graveolens, Sarcostemma acidum, Semecarpus anacardium, Strychnos nux vomica and *Ziziyphus mauritiana*. Among the hosts that render sandal susceptible to spike are species of *Acacia, Caesalpinia coriaria, Cajanus cajan, Lantana camara, Ocimum sanctum* and *Pongamia* (*Milletia*) *pinnata.* It may therefore be possible to raise sandal in association with those trees, which impart relative resistance to the sandal trees.

In nature, sandal generally remains free from infection under a top canopy of host species such as *Casuarina, Eucalyptus, Cassia siamea* and *Azadirachta indica.*

Chemical Control

Sandal spike shows remission of symptoms after treatment with Tetracycline. The systemic fungicide, Benlate, has been reported to bring about temporary remission of symptoms. Application of the antibiotic by the girdling method has shown the recovery of these 25-30 days after treatment with antibiotic Terramycin, either alone or in combination with Benlate, and 30-35 days after treatment with another antibiotic Ledermycin alone, or in combination with Benlate (Raychaudhuri *et al.*, 1972). However, these treatments provide only temporary relief for 50-150 days and then the disease reappears (Singh, 2005). The production of spike-free plants via somatic embryogenesis and breeding for resistance will be useful for long-term management of the disease (Sita *et al.,* 1980).

Sesamum Phyllody

This disease is quite common in sesamum-cultivating regions. Though the disease has been known since a long time, Pal and Pushkarnath (1935) first reported it as a graft-transmitted viral disease. It is the most destructive disease of sesamum (*Sesamum indicum*) and causes huge losses in yield wherever it appears earlier in the crop season.

Symptoms

Vein-clearing of the leaves, phylloid flowers, stimulation of axillary buds, which results in profuse branching, reduction in leaf size and shortening of internodes are some of the characteristic symptoms of the disease. In affected plants, floral parts are transformed into green, leaf-like structures resulting in complete sterility.

Causal Organism

Cousin *et al.* (1970) reported pleomorphic Mycoplasma-like bodies (MLBs) in the phloem sieve tubes of phyllody-affected plants. MLBs have also been found associated by others in this disease.

Transmission

Disease is transmitted in nature by a leaf-hopper, *Orosius albicinctus* (Vasudeva and Sahambi, 1955) in a persistent manner. Sahambi (1970) also studied the ‘pathogen – vector’ relationship and the host range. The incubation period of the pathogen in leaf hoppers was found to be 15-63 days and 13-61 days in the host (Sesamum) depending upon the atmospheric conditions, especially the temperature. The causal MLO can infect 90 plant species belonging to 38 genera in 12 families.

Disease Management

Late-sowing of the crop results in reduction in the incidence of the disease. Leaf extract of *Vitex negundo* var. *purpurascens* and *Sesbania rostrata* reduced phyllody incidence by 68% (Srinivasulu and Narayanaswamy, 1993). An appropriate sowing time so as to circumvent insect transmission may be helpful for minimizing severity of the disease. A high degree of control can be achieved by spraying Metasystox (0.1%) along with soil treatment with Phorate or Thimet. Antibiotics like Tetracycline sprays of 500 ppm at flower-initiation stage have proved to be effective against phyllody but the recovery is temporary (Vyas *et al*., 1983).

Spraying manganese chloride has been indicated as a means of possible biochemical control. It appears that $MgCl_2$ oxidizes phenols and protects or inhibits the enzymes, bringing the auxin level to normal. Once hyper-Auxin is oxidized, the plant can regain its normal conditions. Soil treatment with Thimet 10 G at 10 kg/ha or Phorate 10G at 11 kg/ha or Temik 10 G at 25 kg/ha at the time of sowing are effective (Bhale *et al*., 1998).

A number of lines, i.e. S1289, S951, S2194, S2201, S2373, S2635 have been found to be resistant to phyllody.

Grassy Shoot Disease (GSD) of Sugarcane

This is one of the most serious diseases of sugarcane and is especially widespread in Maharashtra, from where it was first detected in 1949. The disease is also prevalent in Andhra Pradesh, Bihar, Uttar Pradesh, Madhya Pradesh and Tamil Nadu.

The disease was perhaps first observed by Barber (1919) and reported by Vasudeva (1955) from Belapuer (Maharashtra). The disease is now known to occur in India, Burma, Sri Lanka and Sudan and appears to be synonymous with the white-leaf disease of Taiwan and Thailand. In India, the disease has been reported from most of the sugarcane growing states, namely, Punjab, Uttar Pradesh, Haryana, Bihar, West Bengal, Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka, and Tamil Nadu. According to Agnihotri (1990), in the germplasm collection maintained at the Sugarcane Breeding Institute, Coimbatore, and the disease was seen in several sugarcane varieties imported from those countries where the disease is unknown so far.

Losses from grassy shoot are in the form of reduced germination of seeds from diseased canes, stunted growth of the canes and impairment of juice quality (Dhumal and Nimbalkar, 1983). Infection of up to 10% does not cause much reduction in yield and number of milleable canes (Pooniah, 1982).

Symptoms

GSD is characterized by the production of numerous thin, lanky tillers from the base of the affected plants. The tillers bear pale yellow, thin, narrow leaves resembling grasses. The internodes of these tillers are much reduced. If the crop is infected in the early stages of growth, the loss may be up to 100%. Premature and excessive tillering gives a crowded, grass-like appearance of the clump. Affected clumps are usually stunted and in many instances exhibit premature proliferation of axillary buds. Diseased plants exhibit varying degree of loss of chlorophyll, ranging from total green to white.

In a GSD infected clump, hardly one or two thin, weak and small milleable canes are produced in a clump having primary infection and these do not produce inflorescence or arrow.

The diseases display different types of symptoms at various stages of plant growth and because of this the disease has been referred as 'new chlorotic disease', 'albino disease', 'yellowing disease', and 'bunchy disease' or 'leaf tuft'. Sanosh *et al.* (1986), undertook anatomical studies on sugarcane affected with grassy shoot diseases and found them not much different from healthy canes. However, Dhumal and Nimbalkar (1982) made physiological studies on grassy shoot disease infected sugarcane cultivars and reported that the enzymes of sucrose synthesis were inhibited due to disturbed inorganic metabolism.

Causal Organism

Workers in the Sugarcane Research Institute, Lucknow, in collaboration with scientists from the USA, found the association of mycoplasma-like bodies (MLB) in the sieve cells of infected plants (Corbett *et al.*, 1972). Rishi *et al*. (1973) found two types of MLB spherical bodies, 300-400 nm in diameter and filamentous bodies, 30-53 nm in diameter associated with this disease.

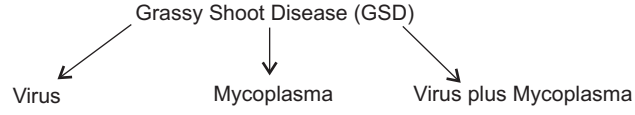
Concentration of MLO is high in canes growing at high temperatures (30°C or so). Such canes show severe symptoms. At lower temperatures, the symptoms are less severe. Staining of hand sections of diseased canes with Diene's stain gives a dark blue color to the infected phloem while the healthy phloem remains unstained (Shukla and Bhansali, 1985).

Transmission

The primary transmission of the disease is through diseased seed material, while the secondary transmission is through insect vectors (Chona *et al.*, 1960). GSD was reported to be sap and aphid-transmitted (Chona *et al.*, 1960). Singh and Shukla (1970) and Varma *et al.* (1970) reported the biology and the 'pathogen – vector' relationship respectively. This is probably the only known example of sap and aphid-transmitted yellows disease known so far. In addition to sugarcane, the disease also occurs on *Sorghum,* which serves as a collateral host (Singh and Shukla, 1966).

The grassy shoot MLO perennates through diseased canes used for seed and spreads through diseased cane setts and cane-cutting knives. However, Rishi *et al.* (1973) did not find proof of sap transmission. Ratooning of diseased crop is an important means of transmission of the disease. According to Shukla (1995), the pathogen is introduced into new fields through diseased cane setts. The vectors are *Rhopalosiphum maidis (Aphis maydis), Aphis sacchari*, and *Aphis idiosacchari.* Transmission by dodder (*Cuscuta campestris*) has also been reported (Jha *et al.*, 1973).

According to Agnihotri (1990), there is enough circumstantial evidence for the presence of three different types of grassy shoot disease in nature, perhaps caused by the following different organisms:



The successful transmission of the disease through sap and also by aphids, reported by Chona *et al.* (1960) and Varma *et al.* (1966), is a pointer that they were dealing with plants affected by virus alone. However, later work of Edison *et al.* (1976) has proved GSD to be of mycoplasma-origin and the role of aphids in transmitting GSD has to be viewed with caution. In 1973, Edison reported *Assamia moesta* transmitting GSD.

Disease Management

Healthy setts should be used for plantation purposes and should be selected from areas where the grassy shoot is not present at all. Rouging may help with consistent reduction in inoculum in the area. Both heat therapy and chemotherapy have been found effective against the GSD pathogen. In heat therapy, the setts should be kept in hot water at 50°C for 2 hours or in hot air at 54°C for 8 hours (Singh, 1968). According to Shukla and Singh (1990), moist heat at 54°C (90-95% humidity) from 1.5-2 hours is enough to deactivate the MLO in an air-tight chamber, especially designed for the treatment of canes.

Insect vectors can be controlled by weekly sprays of 0.16% Malathion. These treatments are possible for seed nurseries. Sattar and Ali (1976) successfully controlled the complete cure of the disease in CO 419 when its single-bud setts were treated with 500 or 1000 ppm of Ledermycin.

Little Leaf of Brinjal (*Solanum melongena*)

Little leaf of brinjal is a mycoplasmal (phytoplasmal) disease (Anjaneyulu *et al.,* 1968; Varma *et al.*, 1969). This graft-transmissible disease was first reported from Coimbatore (Thomas and Krishnaswami, 1939). It is very widespread in India and can cause losses of up to 90% in certain cases.

Symptoms

In the beginning, young leaves show chlorosis followed by axillary bud proliferation. Diseased plants show extreme reduction in the size of their leaves and nodes, giving a bushy appearance. In heavily infected plants, flower and fruit setting in negligible; virescent and phylloid flowers are very common (Anjaneyulu and Ramakrishnan, 1972a) and these remain green and there is a total loss in yield.

The plant has a bushy appearance because the internodes of the plant are shortened and a large number of axillary buds are stimulated to grow into short branches with small leaves.

Accumulation of metabolites occurs in the diseased leaves, presumably due to obstruction in their translocation to the roots (Mitra and Bhattacharya 1904). The diseased plants have reduced growth (Datar, 1985).

Causal Organism

Prior to 1968, the causal agent of little leaf of brinjal was considered to be a virus. Varma *et al.* (1969) showed MLB, spherical to ovoid (40-300 nm) structure in the phloem cells of diseased plants. The disease is caused by Mycoplasma-like organisms. Kothari *et al.* (1982) studied the ultra structural changes in the diseased plants.

Transmission

The disease is transmitted in nature by insects (leaf hopper) such as *Hishimonus phycitis,* which is common on brinjal crop. MLOs have also been reported in the hemolymph and fat bodies of the vector (Shanta and Lakshmanan, 1984). The same MLO occurs on *Datura fastuosa* and *Vinca rosea*. Bitter gourd, ground gourd, watermelon, menthi, Sunnhemp, carrot and *Solanum xanthocarpum* are good hosts of the vectors while beet, castor, soybean, *Chenopodium amaranticolor* are suitable hosts for oviposition. Dhawan and Sethi (1977) have pointed a possible relationship between root knot nematode infection and little leaf.

Disease Management

No effective method of controlling the disease is known. Anjaneyulu and Ramakrishnan (1959, 1972), Raychaudhuri *et al.* (1970) and Varma *et al.* (1973) have obtained temporary masking of symptoms by using Terramycin, Achromycin, Aureomycin and Ledermycin.

The severity of the disease can be reduced by destruction of affected plants and spraying of insecticides reduced the severity of the disease. New crop should be planted only when diseased plants in the field and its neighborhood have been removed. Methyl Demeton 25 EC (2 ml/L), Dimethoate 30EC (2 ml/L), and Malathion 50EC (2 ml/L) have been recommended for vector control. Although it is reported that mycoplasmas are suppressed by Tetracyclines, field application of this method has not been recommended.

Cultural practices like eradication of weed hosts, destruction of diseased plants and control of insects which are vectors of these diseases by suitable insecticides will go a long way in controlling this disease. Cultivars BB-7, BWR-12, Pant Rituraj, Pusa Purple cluster, Akra sheel, Aushy, Manjari gota, Banaras giant and H-8 are reported to have shown some resistance to little leaf (Singh, 1998).

Coconut (Root) Wilt

According to Koshy (1999), "root (wilt) disease of coconut" is of national importance as the disease causes an annual loss of 968 million coconuts in Kerala. According to Jayasanker (1992), the disease is of international importance. Menon (1961), Lal (1964, 1969), Shanta and Radha (1975), Jayasanker (1981), Nayar and Jayasanker (1981) and Koshy (1999) have earlier reviewed coconut root-wilt disease. According to Jayasanker (1992), the disease was first recorded around (1874) from Erattupetta in the erstwhile state of Travancore (now Kerala), India. Butler (1908) initiated systematic investigations. Since then, more than 350 publications by 154 scientists have appeared on this disease during the last 100 years or so. A comprehensive survey was undertaken by the Central Plantation Crops Research Institute and the Department of Agriculture, Kerala and other agencies during 1984-1985 to estimate the area of spread, intensity of the disease and production loss due to the disease. This survey showed that the disease was prevalent more or less contiguously in 4,10,000 ha in eight southern districts of Kerala, the intensity of the disease was the highest in Kottayam district. Loss of husk per unit nut was 25.8% and loss of oil copra and oil was 9.08 and 11.3% respectively. According to Koshy (1999), 60% of the leaves were found damaged due to the disease.

Symptoms

Butler referred it as root disease, probably due to the rotting of the roots of the affected palm trees. McRae (1916) called it the leaf rot disease, Menon (1935) as the coconut leaf disease, and Menon and Nair (1948) as the leaf-rot disease of coconut. Nagaraj and Menon (1955) felt that the name root wilt is more appropriate taking the foliar symtoms into consideration. From then onwards, the disease was named as root-wilt disease. However, Koshy (1999) believes that the name root wilt disease seems to be a misnomer. Dr. M.S. Swaminathan in 1976 called it as coconut decline (quoted in Koshy, 1999).

Wilting and drooping of the leaves, flaccidity, paling and yellowing and necrosis of the leaves were considered to be the typical symptoms of the disease. Characteristic bending and ribbing of the leaflets of the leaves in the central and outer whorls are the earliest symptoms. Foliar yellowing and marginal

necrosis, the other two symptoms, set in with the advancement of the age of the palm trees. Flaccidity is considered to be the most consistent symptom in palms of all ages.

Based on the relative contribution of flaccidity, yellowing and necrosis, George and Radha (1973) developed a method for quantifying the disease intensity. The method has been further simplified by taking the rating of the leaves of the five spirals into consideration (Nambiar and Pillai, 1985).

Inflorescence necrosis, lack of ability to produce female flowers and pollen sterility render the plants unproductive. Shedding of immature nuts, even before the appearance of other visual symptoms or after is another important character. In seedlings below 5 years of age, rotting of spear leaf (spindle) is the first symptom to appear. Many experts considered rotting of roots as one of the most important symptoms. Joseph and Jayasanker (1981) did not consider root rot as a characteristic symptom of the disease as they could not observe more than 10.6% root decay in diseased palms as compared to the healthy ones. Anatomy of the roots of the diseased palms revealed degenerated pith, disorganized tracheal elements and tyloses in metaxylem (Indira and Ramadasn, 1968; Govindankutty and Vellaichary, 1983).

Diagnosis of the Disease Before the Onset of Visual Symptoms

A number of tests were developed to forecast or detect infection sufficiently before the visual manifestation of symptoms. Joseph and Shanta (1963) developed a color test based on the succinic dehydrogenase activity, but this was neither consistent nor conclusive. Pillai and Shanta (1965) observed an accumulation of amino acids, particularly arginine, in paper chromatograms, but its elution and estimation of intensity were not successful enough to employ it as a tool for diagnostic purposes. Infrared aerial photography was also tried but was not successful. Dwivedi *et al.* (1977) developed a rapid biochemical test on the basis of colorimetric estimation of the constituents extractable in ethylenediaminetetraacetic acid (EDTA), but the results were inconsistent. However, Solomon *et al.* (1983) developed an agar gel double diffusion test, which could be used to detect the disease in palms irrespective of their age group. Enzyme linked Immunosorbent Assay (ELISA) have been standardized for the quick detection of root (wilt) disease (Anon, 1996). A diagnostic test based on the changes in stomatal opening (regulation) has been successfully formulated (Rajgopal *et al.*, 1986).

Physiology of the Diseased Plants

A number of physiological derangements were noticed in the root functioning, water relations, mineral nutrition, respiration, and photosynthesis and phenol metabolism of the diseased plants. The uptake and transport of water through the trunks in diseased plants was reported to be 35% less than the healthy plants (Ramadasan, 1964). According to Mathew (1981), root (wilt) diseased palms were seen to have higher stomatal frequency than that of healthy palms. Rajgopal *et al.* (1986) found abnormal stomatal opening in the infected palms with impaired regulation leading to excessive water loss, irrespective of the time of the day, season, or growing conditions. The root-infected plants had consistently lower leaf-water potential than the healthy palms at any given time (Rajgopal *et al.*, 1987). Michael (1978) reported higher rates of respiration in root (wilt) diseased palms as compared to healthy ones. Percentage increase of the chlorophyll content in the first fully opened and middle leaves of healthy palms was 16 and 70 respectively, over that in diseased palms (Dwivedi *et al.*, 1978). In spite of higher total reducing and non-reducing sugars in the leaves of the diseased palms, depletion of these sugars occurred in the roots of the diseased palms, which is a clear indication in the derangement in translocation (Mathew, 1977). Low protein values were reported in the leaves of the diseased palms, compared to the healthy palms

(Padmaja *et al.*, 1981). Varkey *et al.* (1969) reported reduced C/N ratio in the roots and the leaves of the diseased plants. According to Pillai and Shanta (1965), there is greater accumulation of free amino acids, especially arginine, in the leaves of diseased palms. Pectin lyase activity was found to be six times higher in the roots of the diseased palms compared to the healthy palms. Joseph and Jaysanker (1973) reported higher concentration of polyphenols in the roots of diseased palms. Dwivedi *et al.* (1979) reported reduced utilization of absorbed phosphorus constituted organic substances in diseased palms. Joseph (1983) reported clear-cut differences in isozyme pattern of polyphenol oxidase between healthy, apparently healthy and diseased palms. The sap collected from the inflorescences of the diseased palms showed higher content of arginine and aspartic acid among amino acids; malonic and lactic acids among organic acids; and glucose and galactose among sugars (Chempakam *et al.*, 1991). Rajgopal *et al.* (1998) had reviewed the work on the physiology and biochemistry of the disease.

Causal Organism

The sporadic occurrence and spreading nature of the disease suggested the involvement of a pathogen in the disease. A number of biological agents such as fungi, bacteria and nematodes were associated with the disease. However, pathogenicity studies revealed that none of them are involved in the causation of the disease, either singly or in combination. Researches reported the role of a sub-microscopic agent, possibly a virus but no virus could be isolated or consistently associated with the diseased plant tissues. Polyacrylamide gel electrophoretic studies of isolated nucleic acid also did not corroborate the associations of the viroid type of pathogen with the disease. Investigations on the soil and nutritional aspects indicated no direct involvement of any major or minor nutrient in the incidence of the disease (Cecil and Amma, 1991).

The presence of phytoplasma was identified in the sieve tubes of roots, tender stem, petiole and developing leaf bases of root (wilt) diseased plants (Solomon *et al.*, 1983, 1998). Tissues of healthy palms were free of such organisms. Constant association of phytoplasma with the disease has been established with the detection of the organism in tissues of all the 75 diseased palms. The palms investigated were of various age groups, intensities of disease and from different locations. Abnormal bluish discoloration in sieve tubes of diseased palms, following Diene's staining is indicative of accumulation of DNA in extra nuclear sites and thus the presence of phytoplasma. Uneven distribution of phytoplasma was observed. However, a higher number of phytoplasma was observed in the juvenile tissues.

Pleomorphic forms ranging from circular to oval and occasionally beaded or filamentous were observed. The coccoid forms were in the size range of 250-400 nm. Intracellular presence of the organisms and their absence from healthy palms and the cellular changes noticed further strengthened the etiological role of phytoplasma with the disease. As already indicated, phytoplasmal infection could be seen under optical microscope employing Diene's staining or fluorochromes such as DAPI stain (4', 6-diamidino-2-phenylindole is a fluorescent *stain* that binds strongly to A-T rich regions in DNA) of free hand sections of fixed tissues.

Disease Cycle

It is now believed that the lace bug, S*tephanitis typicus,* has been shown to transmit this phytoplasma from diseased to healthy palms. Phytoplasma was observed in brain and salivary glands of lace bug, given an acquisition plus incubation period (A+ I) ranging from 18-23 days; whereas, phytoplasma was not observed in lace bugs collected from healthy areas as well as in bugs, offered an acquisition and

incubation period of less than 18 days (Mathen *et al.*, 1987). Phytoplasma has also been observed in the salivary glands of the plant-hopper, *Proutista moesta,* given an acquisition and feeding period of more than 30 days (Anon., 1991). Transmission of phytoplasma to periwinkle (*Catharanthus roseus*) was also achieved through *Cassytha filiformis* (Sasikala *et al.*, 1980). Phytoplasma from coconut could not be cultured though various methods were tried.

Disease Management

Field trial was initiated towards controlling the disease by means of Oxytetracycline (Pillai *et al.*, 1981) with Neomycin, Penicillin and distilled water control. Fifteen palms, each in the early stages of infection, were given different treatments at quarterly intervals. Fifty-three palms, treated with 3 and 6 g of OTC showed remission of symptoms. In contrast, palms treated with Penicillin and distilled water deteriorated significantly.

Spraying the leaves with Monocrotophos (0.05 ml/L) or Endosulfan (1 ml/L) is effective in killing the insects. Application of 50 kg of farmyard manure/palm/year is also effective in controlling the disease. The disease can be controlled by spraying 1% Bordeaux mixture or 0.3% Copper oxychloride or Mancozeb. Proper drainage of water during rainy season is also quite effective.

One redeeming feature of this disease is that it is not lethal but a declining malady, which responds to ideal management. Based on the experimental evidences, twin strategies - one for the highly diseased contiguous areas and another for mildly-affected areas have been formulated. In the former, the yield of palms can be sustained or even improved through adoption of the integrated disease management practices such as removal of diseased, advanced, and juvenile palms, application of balanced fertilizer, addition of organic matter, raising green manure crops in the basin and their incorporation into soil, irrigation during summer months, adopting inter and mixed cropping and practising plant protection methods (Murlidharan *et al.,* 1986, 1991; Rajagopal *et al.*, 1987).

Strategy in the mildly affected areas is to contain the disease from spreading and this involves the eradication of all diseased palms.

Since there are no curative or prophylactic measures available to control the disease, the long-term option is to breed for disease resistance. However, not much progress has been made so far in this direction.

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Questions

1. What are mycoplasmas?
2. Who discovered mycoplasmas like organisms as plant pathogens?
3. How would you control diseases caused by phytoplasmas?
4. List some important phytoplasmal diseases of your area.
5. Name any mycoplasmal disease in which Koch's postulates have been proved.
6. Why diseases caused by phytoplasmas or spiroplasmas are not controlled by cell-wall synthesis inhibiting antibiotics like Penicillin?
7. Do spiroplasmas move by means of flagella?
8. Differentiate between mycoplasmas, L forms of bacteria, true bacteria and viruses.
9. How would you control the following diseases:
   (i) Sandal spike
   (ii) Grassy shoot diseases (GSD) of Sugarcane
   (iii) Little leaf of Brinjal
   (iv) Coconut (root) wilt

Bacteria and Bacterial Diseases

24

Structure, Reproduction and Classification of Bacteria

In 1675, Anton von Leeuwenhoek, for the first time, made notable observations on bacteria, using crude microscopes that could hardly magnify 150 times. The importance of these observations was, however, realized only after Pasteur (1876) had demonstrated their role in fermentation and decay, and Koch (1876) had proved that bacteria could cause diseases such as anthrax, tuberculosis and Asiatic cholera.

It is now well recognized that of all the organisms, bacteria are the most closely related to man's life. T.J. Burrill of the University of Illinois (1878) was the first to prove the association of a bacterium with a plant disease. There are about 1600 species of bacteria, of which 50% are true bacteria. Bacteria are present almost everywhere except the pits of volcanoes and deep strata of rocks. They include the photosynthetic and chemosynthetic autotrophs, saprophytes, capable of decomposing all imaginable organic materials, parasites, pathogens, symbionts of plants and animals and other protista, and even predators. Very few bacteria are autotrophic. The average size of bacteria is about 1.25 μm in diameter. The smallest is rod-shaped, measuring 0.15 μm, almost touching the last limit of visibility. The largest is *Spirillum volutans*, measuring up to 15 μm in length and 1.5 μm in diameter. Some interesting calculations have been made to drive home the minute size of bacteria. A single drop of water may contain 50 thousand million bacteria.

Forms of Bacteria

Bacteria are of various shapes. They may be spherical (*Coccus*), rod-shaped (*Bacillus*), curved (*Vibrio*) or spiral-shaped (*Spirillum*).

Flagella and Locomotion

Hair-like projections called flagella help bacteria to move. On the basis of the number and arrangement of flagella (Fig. 24.1), bacteria may be classified as follows:

Monotrichous A single flagellum at one end of the cell.

Lophotrichous Two or more flagella at one or both ends of the cells.

Amphitrichous One flagellum at each end.

Peritrichous Large number of flagella surrounding the cell.

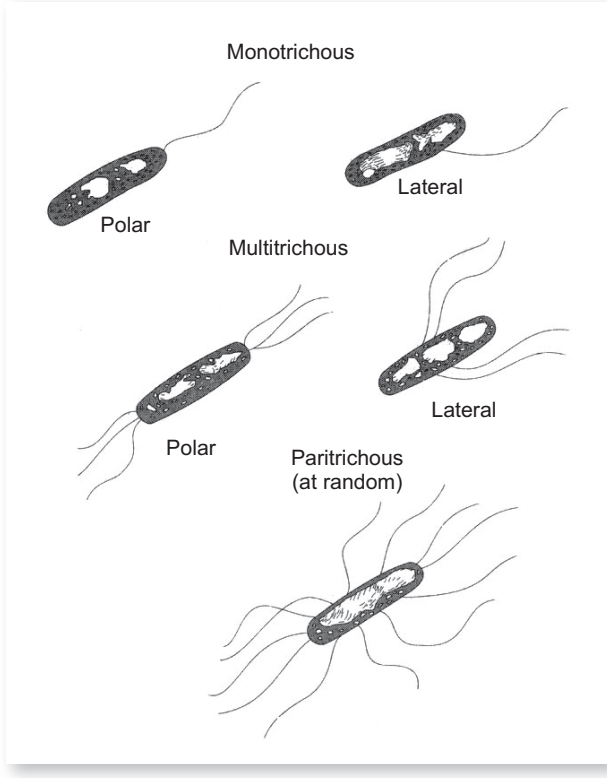


Figure 24.1 Various types of flagellation in bacteria

Bacterial flagella differ structurally from those of the eucaryotes. They range in thickness from 15 nm to more than 24 nm, and consist of single or entwined structures that resemble microtubules but are composed of a single protein called flagellin. It is interesting to note that similar proteins occur in animal muscle. The flagella of some bacteria are anchored in a specialized peripheral region of the cytoplasm.

Pili

A structure somewhat analogous to the flagellum but which is not involved in motility is the pilus (plural, pili). Pili are considerably shorter than flagella and are more in number on the cell. They may be chemically similar to flagella. Not all organisms have the pili. The ability to produce pili is an inherited character. Certain pili are involved in the transfer of genetic material from one bacterial cell to another; they are the sex pili. The functions of other types of pili are not known. In some cases, they may enable the organism to stick to inert surfaces or to form pellicles on the surface of liquids.

Capsules, Slime Layers

Some bacteria secrete slimy or gummy materials on their surface, which can sometimes be seen by negative staining. When the material is around the cell surface in a compact manner, it is called a capsule, but when the material is diffused, it is called a slime layer. Capsules and slime layers are usually composed of polysaccharides, polypeptides or polysaccharide protein complexes.

Bacteria are prokaryotic in their organization, that is, they do not have membrane-bound organelles or a well-organized nucleus, and their cell-wall composition is entirely different. The difference between the prokaryotic and eukaryotic organization is given below:

Comparison between a Procaryotic and a Eucaryotic cell

	Procaryotes	Eucaryotes
Nuclear body	No nuclear membrane, no mitosis	True nucleus, nuclear membrane, mitosis
DNA arrangement	Single molecule, not complexed with histones	In several or many chromosomes, usually complexed with histones
Composition of membranes	Lack sterols	Contains sterols
Respiratory system	Respiratory system part of plasma membrane or of mesosome, mitochondria absent	Present in membrane bounded organelles, the mitochondria
Photosynthetic apparatus	Photosynthetic apparatus in organized internal membranes, chloroplasts absent	Present in membrane bounded organelles, the chloroplasts
Ribosome size	70S (50S + 30S)	80S (60S + 40S), except for ribosomes of mitochondria and chloroplasts, which are 70S.
Cytoplasmic movement	Cytoplasmic streaming rare or absent	Cytoplasmic streaming often occurs
Cell wall	Chemically complex cell wall structure (peptidoglycan)	When present, composed of simple organic or inorganic materials
Flagella	Submicroscopic in size, each flagellum composed of one fibril of molecular dimension	Microscopic size, each flagellum composed of 20 fibrils in a distinct pattern $2 \times 9 + 2$
Sexual reproduction	Elementary fragmentary process, no meiosis, usually only portion of genetic complement reassorted	Regular process, meiosis, reassortment of whole chromosome complement
Vacuoles	Rarely present	Often present

Outer Covering of the Cell

The outer covering consists of three layers. The inner layer is the cytoplasmic membrane and consists of lipids and lipoproteins. The middle layer of cell wall is the rigid part of the outer covering of the cell. The purified cell wall contains lipids, carbohydrates, proteins, phosphorus, and other inorganic substances. Here, the rigidity is due to a unique polymer, the mucopeptide, which is based on a backbone of alternating molecules of N-acetyl glucosamine and N-acetyl-muramic acid. Attached to the muramic acid are short chain molecules of amino acids, which are cross-linked to other muramic acid molecules in adjacent chains. The cross-linking enables the complex to form a rigid network. The third layer is known as the slime layer. If the slime layer surrounding the bacterial cell is thick, gummy, and mucilaginous, it is called a capsule. It protects the cell against adverse conditions. Capsules and slime layers

of certain bacteria are made up of polysaccharides, a material composed of dextrans, levans, and so on, and appear like plant gums. Capsules or slime of certain other bacteria are composed of polypeptides and peptides.

The Cytoplasm

The cytoplasm is granular, viscous and lies between the nuclear material and plasma membrane. Brock (1970) has called the cytoplasm "Cell pool". The protoplasm of bacterial cells is similar to that of other living organisms. The moisture content is 70-85% and is colloidal in nature. The cytoplasm is densely packed with ribosomes of 70S.

Mesosomes

These are invaginations of the plasma membrane. Their function is a matter of conjecture. They have a role in wall-synthesis and secretion of extracellular products. It has also been shown that all the cells, via the mesosomes, take up the process of transforming DNA. The nuclear body gets attached to the mesosome at the time of replication. Recent view is that they appear to be artifacts of specimen preparation observed in electron microscopy.

Nuclear Region

The difficulty of demonstrating the nuclear material in bacterial cells is due to the extreme basophily of the cells, because of the presence of RNA in the cytoplasm. It is now believed that the nucleus in bacteria is present in the cell as a discrete body. Feulgen tests applied in the case of bacteria have revealed the presence of purple-stained bodies in a large number of cases. Procaryotic organisms do not possess a true nucleus as eucaryotes do. In electron micrographs of thin sections, the nuclear region is seen as a weakly contrasting area that contains thin fibrillar material but which lacks a definite membrane. The fibrillar material is DNA, the genetic material of the cell. When a bacterial protoplast is lysed gently, this DNA pours out of the cell in a long, single fibre; probably a single molecule, whose length when stretched out would be many times the length of the cell itself. For instance, the length of an *E. coli* cell is about 2 μm whereas the length of *E. coli* DNA is 110-1400 μm, so the DNA must be folded multiple times. With the help of radio autographic techniques, Cairns (1963) has demonstrated the circular DNA of *E. coli*.

The Gram Reaction

One of the most important cytological features of bacteria is their reaction to a simple procedure called, after its discoverer, the Gram stain. The procedure involves staining the cells with the stain crystal violet and all bacteria are stained blue. The bacteria are then treated with an iodine solution and decolorized with alcohol. Gram-positive bacteria retain the stain crystal violet, whereas the Gram-negative bacteria are decolorized. Thus, there is a fundamental difference between Gram-positive and Gram-negative bacteria. The structures of the cell walls of Gram-positive and Gram-negative bacteria are quite different (Fig. 24.2). The differences between the two types of bacteria are given below:

Gram-positive bacteria	Gram-negative bacteria
1. Cell wall thicker, contains only traces of lipids	Cell wall thinner, may contain upto 20% lipids
2. Striking simplicity of amino acids glutamic acid occurs in large amounts	All amino acids are present.
3. Diaminopimelic acid in some	Diaminopimelic acid in some
4. More muramic acid	Less muramic acid
5. Teichoic acid present	Teichoic acid absent

Acid Fast Stain

A covering of fatty or waxy substances surround some bacteria. These organisms are not readily stained, but when stained, are able to retain the color even after treatment with drastic decolorizing agents. They are called acid fast because the stained bacteria are resistant to decolorization with acid alcohol. Two well-known members of the group are *Mycobacterium tuberclosis* and *M. leprae*. In Ziehl Neelsen's method, the primary stain is carbol fuchsin.

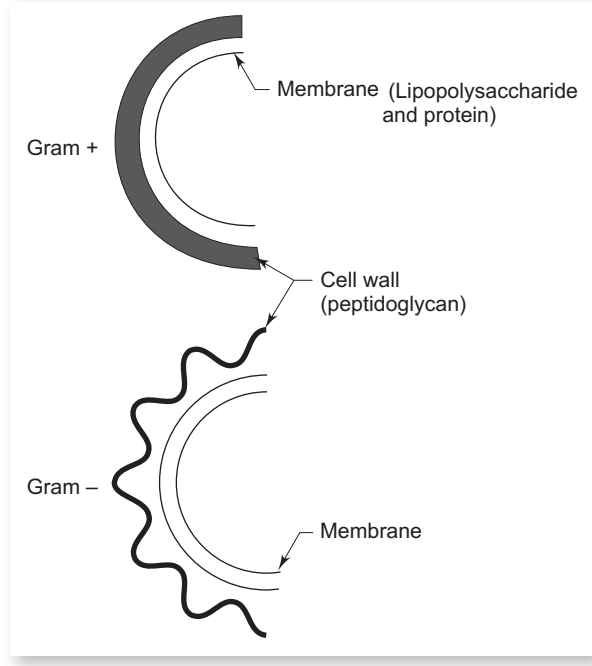


Figure 24.2 Cell walls of Gram positive and Gram negative bacteria (schematic representation)

Nutrition

Bacteria can be divided into two groups, on the basis of the way in which they derive their nutrition. Those, which can utilize carbon dioxide as the sole source of carbon, are called autotrophs. Very few bacteria fall into this category, the limited number being separated into phototrophs and chemotrophs. Phototrophs (e.g. *Clorobium limicola*) can use the energy of sunlight, and chemotrophs (e.g. *Thiobacillus thioxidans*) can use the chemical energy of inorganic compounds, during the synthesis of their own protoplasm. The photosynthetic process in bacteria differs from the photosynthesis of higher green plants. The photosynthetic bacteria possess chlorophylls (bacteriochlorophylls) different from those of plants in terms of structure and light-absorbing properties. Bacteriochlorophylls absorb light in the near-infrared region (660-870 nm). They are not contained in chloroplasts but are found in extensive membrane systems. Oxygen is not liberated during the photosynthetic process and the hydrogen source is not water but usually a sulphur compound (e.g. hydrogen sulphide). Most of the bacteria derive their energy from the organic compounds contained in or produced by other organisms. These are the heterotrophs. Heterotrophic bacteria may be further subdivided into saprophytes, organisms that live on organic matter, and parasites, organisms that grow upon living materials. Most heterotrophs are saprophytes and the laboratory-media upon which they grow reflect the environments in which they are found in nature to some extent. A commonly used medium is a nutrient broth (when agar is added it is called "nutrient agar") or sometimes "beef extract-peptone" broth (or agar). There are various other media for growing various types of bacteria.

Growth and Reproduction In Bacteria

Growth of Cells

If conditions are favourable, cell division is normally followed by a period of increased growth. Through absorption of water and food manufactured by the protoplasm, the cell enlarges to its original size. Under favourable conditions of moisture, nutrition, pH, and temperature, some kinds of bacteria, for example, *E. coli*, may double in mass about every 20 minutes since their generation. If this rate of increase were to continue for even a few days, enormous numbers of bacteria would be produced. However, there are several factors that prevent this from happening. In some cases, there may be an exhaustion of the food supply or an accumulation of waste products.

Different bacterial species show various shapes of growth curves, depending on the generation time and the maximum population attainable under the prevailing conditions. During a period of one to several hours, there may be a lag phase in which there is little or no increase in cell number. During the first part of the lag phase (which may be called the initial stationary phase), the cells adapt themselves to the new environment.

When the growing cells begin to divide, they usually continue to do so over a regular interval until the maximum growth that can be supported by their environment is approached. This period of rapid cell-division is known as the logarithmic phase of growth. The stationary phase occurs when rapid growth is halted by the depletion of nutrients, accumulation of waste products or other factors. Unless the cells are transferred to a new environment capable of supporting continued growth, they will eventually die.

Cell Division

Cell division requires the doubling of all cell constituents and their orderly partitioning into two daughter cells. The first step in cell division is the doubling of the DNA complement of the nuclear body, which occurs by the separation of two strands and the replication along each strand of a new complementary strand. Due to the enormous length of the DNA, the cell faces a problem in partitioning the doubled DNA between the two daughter cells. It is thought that the DNA molecule is attached at some point to the cell membrane and after replication, each one of the double strands, while still attached, swings towards one of the cell halves. Only after this step has been accomplished can the new cross wall form. At the time of the formation of the new cross wall, a mesosome of the membrane system can be seen attached to the growing cross wall, and it is likely that this mesosome plays some role in the synthesis of the cross wall. The DNA is probably attached to the other end of the same mesosome.

The highly coordinated process—DNA doubling /DNA partitioning/cross wall formation—is equivalent of the mitotic process in eucaryotic organisms.

Bacterial Endospores

Though no plant pathogenic bacteria produce endospores, they are produced in some other bacteria. Endospores are highly-resistant structures and are an important means of perennation and tiding over unfavourable conditions. Such spores are characteristically produced by two genera of bacteria—*Bacillus* and *Clostridium*—both of which are widely distributed in soil.

Sexual Reproduction

In all fundamental aspects, the genetics of bacteria is the same as that of other organisms. Conjugation is most closely related to sexual reproduction. Conjugation occurs in several different bacteria but is best known in *Escherichia coli*.

Bacterial Conjugation

This method entails directly transferring genetic material from a donor to a recipient bacterium. Lederberg and Tatum first discovered it in 1946, in *Escherichia coli*. It has also been observed to occur in strains of other bacteria such as *Salmonella*, *Pseudomonas*, *Shigella*, and *Vibrio*. After cell contact, actual chromosome transfer occurs; the process is unique to bacterial conjugation and is vastly different from chromosome transfer in higher organisms. Sexual conjugation in bacteria involves the union of cells designated as male and female (Fig. 24.3 and 24.4). Electron micrographs of paired cells show them connected by a short tube, 50 nm wide and it has been assumed that this pairing represents conjugation. It has also been suggested that the fimbriae attached to the surface of male cells are responsible for the transfer of DNA from the male to the female cells. It has been suggested that a sex factor is attached to the plasmalemma at the point where a fimbria forms.

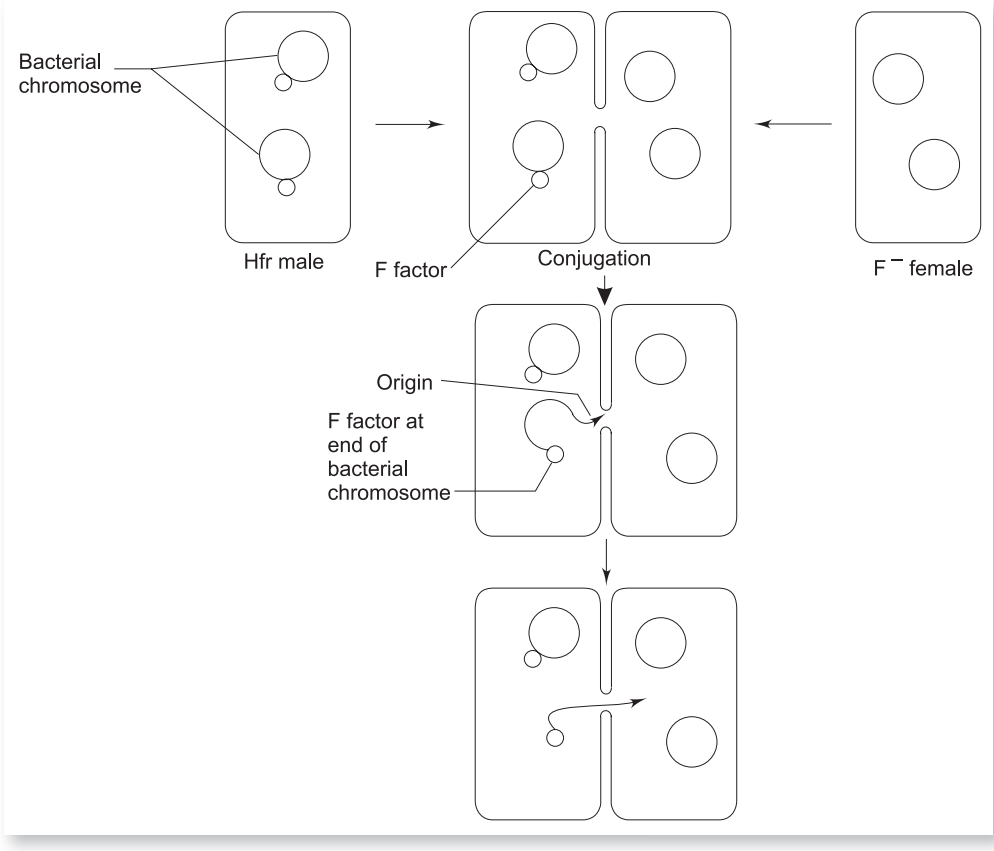


Figure 24.3 Conjugation in bacteria; chromosome from an Hfr male is transferred to F⁻ female

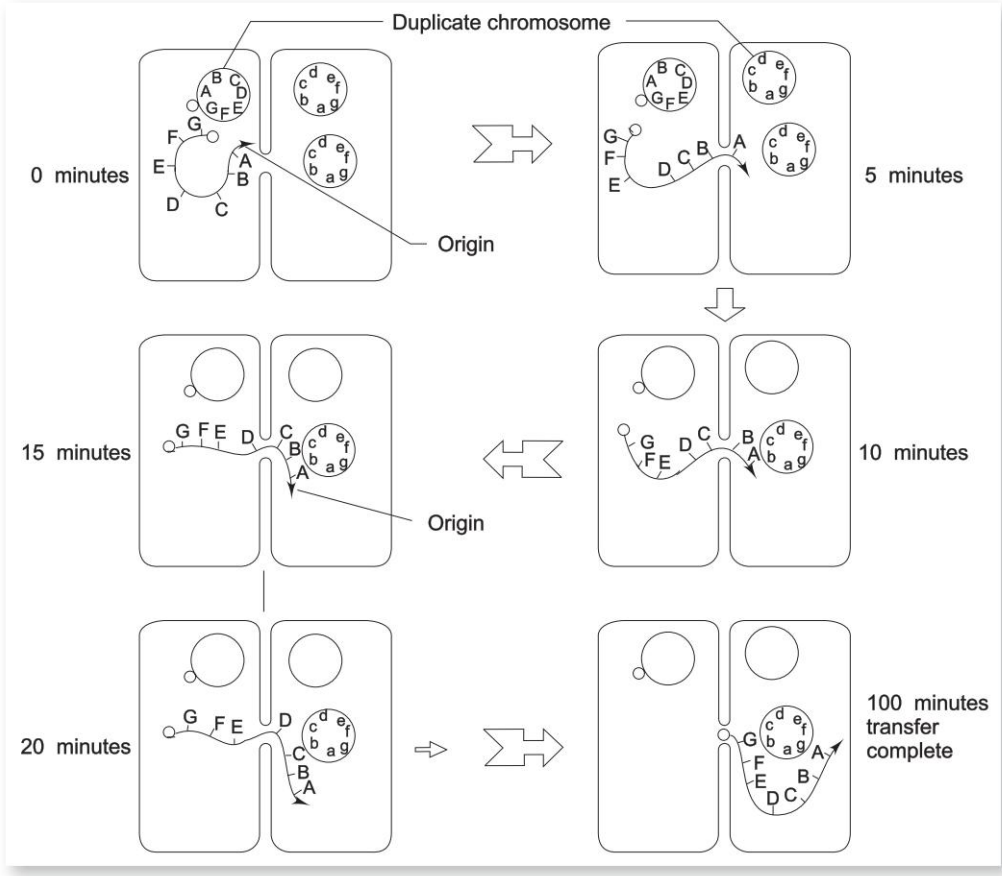


Figure 24.4 Results of interrupted mating experiments, which show how the chromosome is transferred during conjugation.

Recombination frequencies from $F^+ \times F^-$ crosses were never particularly high. In the early 1950s, some *Escherichia coli* strains were isolated, in which the frequency of recombination was up to 10,000 times greater than what was observed in F^+ strains. These were called Hfr strains. The discovery of Hfr strains made the actual physical act of conjugation feasible and a number of fine electron micrographs showing a conjugation bridge between bacterial cells have been obtained.

The discovery of Hfr strains also enabled Jacob and Wollman (1962) to determine the mechanism of transmission of the bacterial chromosome. When a female cell receives a sex factor either by pairing or by fimbrial conjugation, it changes to a male cell and subsequently can conjugate with another uninfected female cell. During the conjugation process, the male cell slowly injects a genophore into the female cell so that the genes enter the female cell in a linear way. The episome becomes attached to the female genophore and is the last-part to enter the receptor cell (Figs. 24.6 and 24.7). It takes about 100 minutes for a complete transfer of the genophore to occur. The transfer is usually not complete and the resulting zygote is usually a partial diploid. Crossing over between bacterial chromosome and exogenous DNA will then occur, thus resulting in recombination (Fig. 24.5).

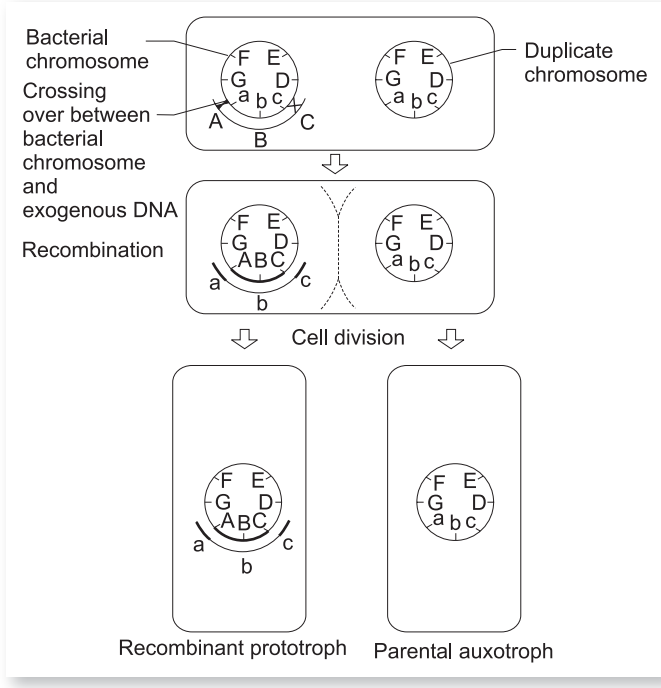


Figure 24.5 Recombinantion in *E. coli*

Bacterial Transformation

In 1928, Griffith injected mice with non-capsulated and heat-killed capsulated pneumococci; neither of these proved fatal when infected separately. When both these bacteria were injected together, some of the mice died and living capsulated bacteria were recovered from their blood. The transformation (Fig 24.8) could be brought about by the cell-free extract of the capsulated bacteria. Griffith at first thought that the transforming principle was the bacterial polysaccharide. But Avery, MacLeod and Mc Carty (1944) demonstrated that the transforming principle was DNA.

Bacterial Transduction

Zinder and Lederberg's (1952) experiement in a U-tube on *Salmonella typhimurium* indicated that bacterial viruses or phages are responsible for the transfer of genetic material from one bacterium to the other (Fig. 24.9). Zinder, a student of Lederberg, was studying various strains and mutants to see if recombination would occur. An experiment was performed in such a manner that direct cell-contact was not possible. This was done by physically separating the bacteria using a sintered glass filter impermeable to bacteria. High-frequency recombinants were still obtained. It became clear that a filterable agent passed from one cell to another, transferring genes between them. This phenomenon is called transduction. Transduction has been demonstrated in many bacteria.

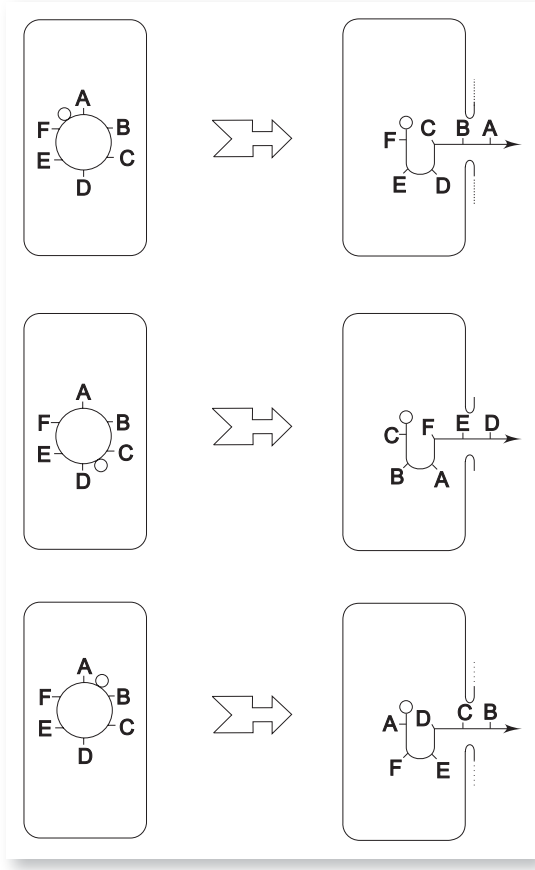


Figure 24.6 Conjugation in *E. coli*. Chromosome opens in regions adjacent to the site of attachment of F factor

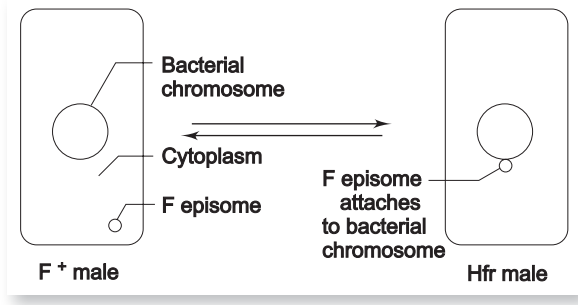


Figure 24.7 Relationship between F^+ and Hfr strains. Attachment of F (episome) to bacterial chromosome converts F^+ male to Hfr male

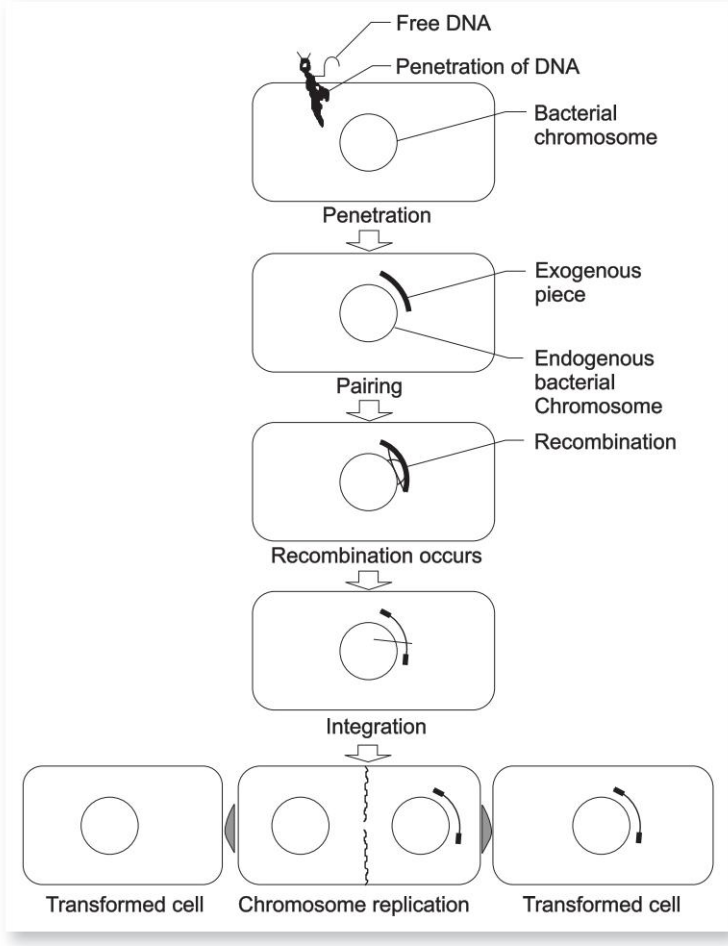


Figure 24.8 Transformation in bacteria

Classification of Bacteria (Procaryotes)

The procaryotes—eubacteria and archaebacteria—evolved along different lines. Modern classification systems for the Procaryotes reflect a phylogenic relationship, placing some procaryotes in the kingdom *Eubacteria* and the other in the kingdom *Archaea*. Some of the important criteria for classifying bacteria are cellular characteristics (cell shape, cell size, arrangement of cells, arrangement of flagella, capsule, endospore), staining reaction (Grams stain, acid fast stain), growth and nutritional characteristics (colonial morphology, pigmentation, energy sources, carbon sources, nitrogen sources, fermentation products, modes of metabolism), biochemical characteristics (cell-wall constituents, pigment biochemicals, storage inclusions, antigens, RNA molecules), physiological and ecological characteristics (temperature range and optimum oxygen reationships, pH tolerance range, osmotic tolerance, salt requirements and tolerance and antibiotic sensitivity), and genetic characteristics (DNA molecular, % G+C, DNA hybridization).

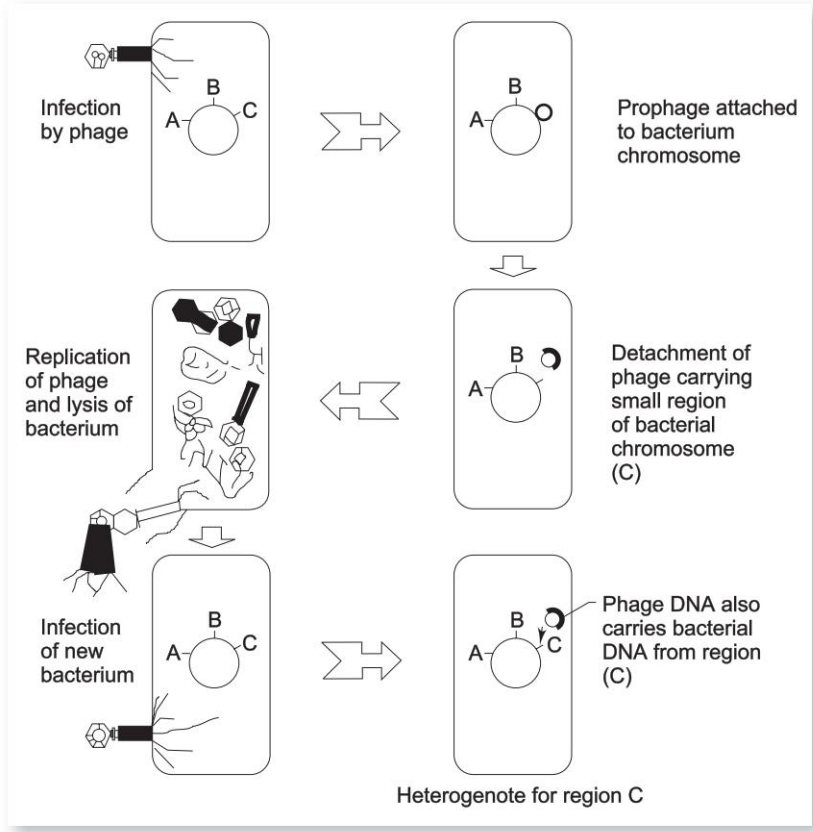


Figure 24.9 Transduction in bacteria

Bergey's Manual of Determinative Bacteriology was published in 1923. From 1923 to the turn of the century, a lot of information has been published on various groups of bacteria. Bergey's manual was revised a number of times. After the publication of the eighth edition of the manual in 1974, a new series, under the title *Bergey's Manual of Systematic Bacteriology* has been started and has been published in four volumes.

The eighth edition of *Bergey's Manual of Determinative Bacteriology* had all procaryotic organisms in the kingdom *Procaryotae.* Prokaryotae is distinguishable into two divisions, the Cyanobacteria (includes all the blue green algae) and the bacteria. The division, bacteria, was further subdivided into 19 parts. The modern system of classification for the prokaryotes reflects phylogenetic relationships, placing some prokaryotes in the kingdom *Eubacteria* and the others in the kingdom *Archaea*. The systematics of prokaryotes is always in a state of flux, with descriptions of new bacterial and archaebacterial genera, and species, and revisions of older taxonomic classifications frequently appearing in the International Journal of Systematic Bacteriology. Periodically, the status of bacterial and archaebacterial taxonomy is summarized in a comprehensive volume, *Bergey's Manual of Determinative Bacteriology*. The ninth edition of Bergey's Manual, published in 1994, is the most comprehensive volume. In addition to *Bergey's Manual of Determinative Bacteriology, Bergey's Manual of Systematic Bacteriology* gives the description of the Eubacteria and Archaebacteria. The latter manual is a multivolume treatise: Volume 1 covers the Gram-negative bacteria of general, medical, plant pathogenic (*Pseudomonas*, *Xanthomonas*,

Erwinia, *Agrobacterium* and the Mollicutes i.e. MLOs and *Spiroplasma*) or industrial importance, Volume 2 covers the Gram-positive bacteria other than Actinomycetes, Volume 3 covers the Archaebacteria, Cyanobacteria and remaining Gram-negative bacteria, Volume 4 covers the Actinomycetes.

At present, the bacteria (Eubacteria) are classified into groups or parts. Twenty-two groups or parts are recognized of Eubacteria while Archaebacteria are classified into five groups.

A. Eubacteria

Group or Part-I	Phototrophic Bacteria
Group or Part-II	The Gliding Bacteria
Group or Part-III	The Sheathed Bacteria
Group or Part-IV	Budding and Appendaged Bacteria
Group or Part-V	The Spirochaetes
Group or Part-VI	Spiral and Curved Bacteria
Group or Part-VII	Gram-negative Aerobic Rods and Cocci
Group or Part-VIII	Gram-negative Facultatively Anaerobic Rods
Group or Part-IX	Gram-negative Anaerobic Bacteria
Group or Part-X	Dissimilatory Sulphate or Sulphur-reducing Bacteria
Group or Part-XI	Anaerobic Gram-negative Cocci
Group or Part-XII	Gram-negative Chemolithotrophic Bacteria
Group or Part-XIII	Gram-positive Cocci
Group or Part-XIV	Gram-positive Endospore Forming Rods and Cocci
Group or Part-XV	Gram-positive Asporogenous Rods of Regular Shape
Group or Part-XVI	Gram-positive Rods of Irregular Shape
Group or Part-XVII	Mycobacteria
Group or Part-XVIII	Rickettsias and Chlamydias
Group or Part-XIX	Actnomycetes
Group or Part-XX	Mycoplasmas
Group or Part-XXI	Anaerobic Non-filamentous or Filamentous rods
Group or Part-XXII	Endosymbionts

B. Archaebacteria

Group or Part – 1	Methanogens
Group or Part – II	Archae Sulphate-reducers
Group or Part – III	Extremely Halophilic Aerobic Archaebacteria Halobacteria
Group or Part – IV	Cell-wall less Archaebacteria
Group or Part – V	Extremely Thermophilic and Hyperthermophilic Sulphur Metabolizers

Part I – The Phototrophic Bacteria The phototrophic bacteria are different from others in their ability to use light energy to drive the synthesis of ATP. They are capable of using CO_2 as the source of carbon. There are two major groups, anoxygenic phototrophic and oxygenic phototrophic bacteria. The anoxygenic phototrophic bacteria are photosynthetic but do not produce oxygen while oxygenic phototrophic bacteria split water to form oxygen as part of their photosynthetic metabolism. The group contains a single order, Rhodospirillales. The order contains three families Rhodospirillaceae, Chromatiaceae, and Chlorobiaceae. Examples are *Rhodospirillium, Rhodomicrobium, Chromatium, Chlorobium*, etc.

Part II – The Gliding Bacteria The gliding bacteria represent a phylogeñetically heterogeneous group. The bacteria are so known because of their gliding movement that is brought about by flagella. The cells are flexuous, Gram-negative and are rod-shaped, either occurring singly or forming filaments. Some are chemolithotrophs and others are chemoorganotrophs. There are two orders, Myxobacteriales (produce large fruit bodies), and Cytophagales (do not produce fruit bodies). Myxobacteriales are divided into different families, namely Myxococcaceae, Archangiaceae, and Cytobacteriaceae. Cytophagales are also divided into four families. Examples of these two orders are *Myxococcus, Cytophaga*. None of them are plant pathogenic.

Part III – The Sheathed Bacteria The sheathed bacteria comprise bacteria whose cells occur within a filamentous structure called a sheath. This sheath helps the bacteria to attach to solid surfaces and helps in absorbing nutrients from water, and also gives protection against predators and parasites.

Part IV – Budding and Appendaged Bacteria It is a heterogenous group on the basis a particular morphological feature that is the formation of extensions or protrusions from the cells. In some bacteria, the cellular extensions have a reproductive function and others have a physiological function. Some bacteria reproduce by budding while others by binary fission. Nutritionally, the group comprises both chemoorganotrophic and chemolithotrophic members. External deposits of iron or manganese characterize few genera. Important genera are *Rhodomicrobium, Hyphomicrobium, Hyphomonas, Caulobacter* and *Seliberia*.

Part V – The Spirochaetes Spirochaetes are characterized by a spiral-shaped body, from a single order *Spiroachaetales* and a single family Spirochaetaceae. The cells measure 3-500 μm in length and are flexuous and motile. These are found in fresh, sea and polluted water. Some are free living, many are parasitic and several are pathogenic. *Treponema pallidum* subsp. *pallidum* causes syphilis in humans. Some members of the genera *Leptospira, Cristipira* are animal and human pathogens.

Part VI – Spiral and Curved Bacteria The members of this group are included in a single family Spirillaceae. The cells are curved or spiral, but are rigid (unlike spirochaetes). Locomotion is brought about by polar flagella. Some are found in fresh or salt water and some are parasitic. Species of *Bdellovibrio* are parasitic on a variety of bacteria. Typical examples are *Spirillum* and *Bdellovibrio*.

Part VII – Gram-Negative Aerobic Rods and Cocci This group encompasses a number of taxonomic units. Several major families and genera are given below:

Family	Flagella	N_2 Fixation	Carbon Source	Important Genera
Pseudomonadaceae	Polar	No	Numerous	*Pseudomonas, Xanthomonas, Acidovorax, Rhizobacter, Rhizomonas, Xylophilus, Xylella Ralstonia*
Rhizobiaceae	Peritrichous or polar	Yes	Numerous	*Rhizobium, Bradyrhizobium, Agrobacterium*

(*Contd.*)

(*Contd.*)

Family	Flagella	N_2 Fixation	Carbon Source	Important Genera
Azotobacteraceae	Peritrichous or polar	Yes	Numerous	*Azotobacter, Beijerinckia*
Methylococcaceae	Polar or none	Yes	C1 Compounds only, methane oxidized	*Methylobacterium, Methylococcus*
Legionellaceae	Polar or lateral	No	Various	*Legionella*
Neisseriaceae	None	No	Various	*Neisseria, Moraxella*
Acetobacteraceae	Peritrichous or polar	No	Various	*Acetobacter*

Part VIII – Gram-Negative Facultatively Anaerobic Rods

1. Enterobacteriaceae: Members are motile by means of peritrichous flagella. Important genera are: *Escherichia*, *Edwardsiella, Salmonella, Shigella, Klebsiella, Enterobacter, Serratia, Proteus, Erwinia* and *Yersinia.*
2. Vibrionaceae: Members are motile by means of polar flagella. Important genera are: *Vibrio, Aeromonas, Plesiomonas* and *Photobacterium.*

   Several genera of uncertain affiliation are placed in this group. These include the genera *Chromobacterium*, *Flavobacterium, Zymomonas,* and *Streptobacillus.*

   The genus *Erwinia* is the only genus, which has plant pathogenic species. It belongs to the family Enterobacteriaceae.

Part IX – Gram-Negative Anaerobic Bacteria There is only one family Bacteriodaceae. The straight, bent or pleomorphic rods and even cocco bacilli of this group are strictly anaerobic. The members are known for their fermentative production of a large number of acids of industrial importance. *Desulphovibrio* of unknown affiliation is characterized by the reduction of sulphate or other sulphur compounds to H_2S. The genera *Bacteriodes* produce succinic, acetic, formic acid while *Fusobacterium* produces butyric acid, and *Leptotricha* produces lactic acid. Some members are human pathogens. There is no plant pathogen in this group.

Part X – Dissimilatory Sulphate or Sulphur-reducing Bacteria Several genera of bacteria are characterized by their ability to reduce sulphate, other oxidised sulphur containing compounds or elemental sulphur. Some of the important members are *Desulfovibrio, Desulfuromonas, Desulfomonas Desulfococcus, Desulfobacter, Desulfobulbus, Desulfosabarcina, Desulfotomaculum* and *Desulfonema*.

Part XI – Anaerobic Gram-Negative Cocci There are three genera included in the family Veillonellaceae. Species of *Veillonella* are parasitic in the mouth, respiratory tract and intestine of man.

Part XII – Gram-Negative Chemolithotrophic Bacteria The metabolic activities of this group are important in the bio-geo-chemical cycle. These bacteria oxidize inorganic compounds to generate ATP; these are given in a tabular form below:

Family: Nitrobacteriaceae	Oxidize ammonia to nitrite
Genera: *Nitrobacter, Nitrospina*	or nitrite to nitrate
Nitrococcus, Nitrosomonas	

(*Contd.*)

(*Contd.*)

Nitrospira, Nitrosococcus	
Family: Siderocapsaceae	Oxidize iron or manganese
Genera: *Siderocapsa, Naumanniella,*	
Ochrobium, Siderococcus	
Chemolithotrophic Sulphur Oxidizers	Oxidize Sulphur and Sulphur
Genera:*Thiobacillus, Thiobacterium*	Compounds
Macromonas, Thiovalum, Thiospira	

Part XIII – Gram-Positive Cocci The Gram-positive cocci have three families.

1. Family: Micrococcaceae (Occur in irregular clusters or tetrads)
   Genera: *Micrococcus, Staphylococcus, Planococcus*
2. Family: Streptococcaceae (Occurs in pairs, chains or tetrads)
   Genera: *Streptococcus, Leuconostoc, Aerococcus, Gemella*
3. Family: Peptococcaceae (irregular, Chains, tetrads or octads)
   Genera: *Peptococcus, Peptostreptococcus, Ruminococcus, Sarcina*

Part XIV – Gram-Positive Endospore-Forming Rods and Cocci This is an important group because of the heat resistance of the endospores. It has two families.

1. Family: Bacilliaceae (Rods or cocci)
   Genera: *Bacillus, Sporolactobacillus, Clostridium, Desulfotomaculum, Sporosarcina.*
2. Family: Micromonosporaceae (Filamentous thermophile)
   Genera: *Thermoactinomyces, Micromonospora*

Part XV – Gram-Positive Asporogenous Rods of Regular Shape This group includes only one family, Lactobacillaceae. Lactobacillus is extremely important in the dairy industry. There are several genera of uncertain affiliation; these include *Listeria, Erysipelothrix*, and *Caryophanon*.

Part XVI – Gram-Positive Rods or Irregular Shape This group includes the genera *Corynebacterium, Arthrobacter, Brevibacterium, Cellulomonas*, and *Kurthia*. Many species of *Corynebacterium* are plant or animal pathogens. Since 1981, all the plant pathogenic species have been transferred to genera *Rhodococcus, Arthrobacter, Curtobacterium* and *Clavibacter*. Thus, now *Corynebacterium sensu stricto* has no plant pathogenic species. *C. diphtheriae* is the causative agent of diphtheria.

Part XVII – Mycobacteria The known genus *Mycobacterium* are slow-growing, aerobic, rod-shaped, Gram-negative, and acid fast. *M. tuberculosis* is the causative agent of tuberculosis and *M. leprae* causes leprosy.

Part XVIII – Rickettsias and Chlamydias It has two orders, Rickettsiales and Chlamydiales. Rickettsias are Gram-negative parasitic bacteria. The natural hosts for the coccoid or rod-shaped members of the order Rickettsiales are arthropods, through which they are transmitted to human beings.

Chlamydias are small and rigid-walled elementary bodies that can survive outside the host extracellularly. During multiplication inside a cell, they are converted into large, thin-walled, non-infectious bodies. *Rickettsia* and *Chlamydia* are the representative genera of the two orders.

Part XIX – Actinomycetes Actinomycetes are characterized by the formation of branching filaments that superficially resemble the mycelia of filamentous fungi. The order Actinomycetales includes five families.

Order-Actinomycetales

1. Family: Frankiaceae (Symbionts in plant nodules)
   Genus: *Frankia*
2. Family: Actinoplanaceae
   Genera: *Actinoplanes, Spirillospora, Amorphosporangium, Pilimelia*
3. Family: Dermatophiliaceae
   Genera: *Dermatophilus, Geodermatophilus*
4. Family: Streptomycetaceae
   Genera: *Streptomyces, Streptoverticillium*
5. Family: Micromonosporaceae
   Genera: *Micromonospora, Microbispora, Micropolyspora*

Part XX – Mycoplasmas (For details see chapter 23) The mycoplasmas are classified as mollicutes because they lack a cell wall but are bounded by a single triple-layered membrane. Important genera are *Mycoplasma* and *Spiroplasma*.

Part XXI – Anaerobic Non-Filamentous or Filamentous Rods There is a single family, Propionibacteriaceae, which contains Gram-positive rods that produce propionic acid, acetic acid or a mixture of organic acids by fermentation. Important genera are *Propionibacterium* and *Eubacterium*.

Part XXII – Endosymbionts Here, bacteria are obligate endosymbionts of invertebrates without adversely affecting the host. For example, the protozoan *Paramecium aurelia* can harbour various endosymbiotic bacteria such as *Caedibacter, Tectibacter, Pseudocaedibacter, Lyticum* and *Hotospora.*

Archaebacteria

Members of the archaebacteria are phylogenetically related to each other on the basis of analysis of their 16S rRNA molecules. They are placed in a separate kingdom, *Archaea*. There are several morphological and physiological features that make them distinct from other prokaryotic microorganisms. These bacteria lack murein in their call walls and the other unusual linkage in their phospholipid molecules. These archaebacteria are divided into five groups as follows:

Part I – Methanogens (Methane-Producing Bacteria) It is a highly specialized physiological group. There are four families in this group with three orders.

1. **Order**: Methanobacteriales (strict anaerobes, reduce CO_2 to methane)
   Family: Methanobacteriaceae
   Genus: *Methanobacterium*
2. **Order**: Methanomicrobiales (strict anaerobes, reduce CO_2 to methane)
   Family: Methanomicrobiaceae
   Genera: *Methanomicrobium, Methanogenium*, *Methanospirillum*
   Genus: *Methanosarcina*
3. **Order**: Methanococcales
   Family: Methanococcaceae
   Genus: *Methanococcus*

Part II – Archae-Sulphate Reducers The genus *Archaeoglobus* contains archaebacterial sulphate reducers. It grows at 65-90°C, 1-4% salt, and during growth, sulphate, sulphite or thiosuiphate are reduced to hydrogen sulphide.

Part III – Extremely Halophilic, Aerobic Archae Bacteria (Halobacteriaceae) All members of the family Halobacteriaceae are obligate halophiles (15% NaCl). Important genera are *Halobacterium* and *Halococcus.*

Part IV – Cell Wall-Less Archaebacteria The genus *Thermoplasma* is acidophilic (pH-2), thermophilic (59°C) and lacks a cell wall. It grows both aerobically and anaerobically.

Part V – Extremely Thermophilic and Hyper Thermophilic Sulphur Metabolizers It contains microorganisms that metabolize sulphur and grow at very high temperatures. It has three orders.

1. Order: Thermoproteales (extreme thermophiles: 85-105°C, Gram-negative) Genera: *Thermoproteus*
2. Order: Sulfolobales (extreme acidophiles: pH 2-3)
   Genera: *Sulfolobus, Acidianus*
3. Order: Thermococcales (Thermophiles)
   Genera: *Thermococcus, Pyrococcus*

Plant Pathogenic Bacteria

The following is the list of important plant pathogenic bacterial genera—*Pseudomonas, Xanthomonas, Xylella, Xylophilus, Acidovorax, Agrobacteriurn, Erwinia, Clavibacter, Curtobacterium* and *Streptomyces.*

Plant Pathogenic Bacterial Genera

Gram-negative	Gram-positive
1. *Pseudomonas*	1. *Clavibacter*
2. *Xanthomonas*	2. *Curtobacterium*
3. *Xylella*	3. *Streptomyces*
4. *Xylophilus*	
5. *Acidovorax*	
6. *Agrobacterium*	
7. *Erwinia*	
8. *Ralstonia*	

Pseudomonas Pseudomonads are straight to curved rods, 0.5-1 × 1.5–4 µm. They are Gram-negative and are motile by one or more polar flagella. Some plant pathogenic species produce a fluorescent pigment on a medium with low iron content, strict aerobes, and chemoorganotrophs. Its metabolism is respiratory and never fermentative.

Xanthomonas Cells are predominantly single rods, usually with the size range of 0.4-0.7 × 0.7-1.8 µm, Gram-negative, and motile by means of a single polar flagellum, do not produce sheath, no resting spores known, copius extracellular slime is characteristic of the genus, metabolism respiratory, never fermentative, oxidase reaction negative or weak, catalase positive, strict aerobes.

Xylella Cells are mostly single, straight rods, 0.3 × 1-4 μm, produce long filamentous strands under certain conditions, colonies are small, with smooth or finely undulated margins. Nutritionally fastidious, require special media for growth, habit is xylem tissues of the plant, Gram-negative, non-motile, aflagellate, strictly aerobic and non-pigmented.

Xylophilus Formerly, this was not a separate genus from *Xanthomonas* or *Pseudomonas*. De Vos and De Ley (1983), while reporting intra and integeneric similarities of *Pseudomonas* and *Xanthomonas* ribosomal RNA cistrons, suggested that *Xanthomonas ampelina* (responsible for bacterial gummosis of grapevine) is at a great distance from *Xanthomonas* and *Pseudomonas*. Some of the characters of this genus are a slow growth, maximum growth at a temperature of 30°C, strong urease production, utilization of tartrate, no utilization of glucose, mannose, fructose, sucrose or propionic acid and no hydrolysis of gelatin or esculin. Willems *et al*. (1987) transferred this species of *Xanthomonas ampelina* to *Xylophilus.*

Acidovorax Based on the percentage of certain fatty acids, *Pseudomonas acidovorans* was transferred to a new genus *Acidovorax.*

Agrobacterium The bacteria are rod-shaped, 0.8 × 1.5-3 μm. They are motile by one to four peritrichous flagella. When only one flagellum is present, it is more often lateral in position than polar. Gram-negative; when growing on carbohydrate-containing medium, bacteria produce copious polysaccharide (slime). The colonies are non-pigmented and usually smooth. These bacteria are rhizophere and soil-inhabitants and produce galls in plants.

Erwinia Cells are predominantly single, straight rods, measuring 0.5-1.0 × 1.0-3.0 μm, motile by peritrichous flagella and are Gram-negative. *Erwinia* are the only plant pathogenic bacteria that are facultatively anaerobic. *Erwinia* of the amylovora group do not produce pectolytic enzymnes, while carotovora group has strong pectolytic activity and causes soft rots of tissues.

Ralstonia Until very recently classified under *Pseudomonas,* these resemble the latter (*Pseudomonas*) in most respects with the important difference that its cells do no produce fluorescent pigment.

Clavibacter Cells are shaped straight to slightly curved rods, 0.5-0.9 × 1.5-4 μm, Gram-positive, non-acid fast, pleomorphic rods, no coccoid cells are seen, non-endospore forming, non-motile, strict aerobes. The genus was proposed by Davis *et al*. (1984) for those plant pathogenic species that contain diaminobutyric acid as the major constituent of the cell wall and which were formerly included in the *Corynebactrium michiganesis* group.

Curtobacterium Before 1980, the plant pathogenic *Corynebacteria* (which were Gram-positive, pleomorphic, non-branching and rod-shaped) were assigned to the genus *Corynebacterium,* although they did not exactly satisfy the description of a typical *Corynebacterium*. After 1980, these plant pathogenic species were transferred to other genera such as *Curtobacterium, Calvibacter, Rhodococcus*, and *Arthrobacter* on the basis of the chemistry of the cell wall, G+C contents of the DNA and genetic homologies. The genus *Curtobacterium* is characterized by small rod-shaped cells, loose Gram-positiveness, motile by lateral flagella, cell division by bending type of cell division, major cell-wall amino acid is ornithine, G+C content of the DNA ranges from 66-71 mole percent.

Streptomyces This is the best-recognized genus of the family Streptomycetaceae and is widely distributed in soils. Slender coenocytic filaments of 0.5-2.0 μm in diameter produce aerial mycelium, form chains of three to many spores. They are slow-growing and Gram-positive. *Steptomyces* species are producers of antibiotics and a variety of color pigments. *S. scabies* is pathogenic.

Symptoms of Bacterial Plant Diseases

In contrast to fungal pathogens, bacteria are incapable of mechanically penetrating the cutinized plant tissues, cuticle, periderm, and so on. The only ways they can gain entry into the plants are through: (1) non-cutinized areas such as root hairs, stigmas; (2) natural openings such as stomata; and (3) incidental wound or those initiated by insects, nematodes, and other microbes. On entry into a suitable host, the bacteria may establish themselves intercellularly, intracellularly, intravascularly or in more than one combination. The host tissues react differently to the invasion and exhibit different types of disease symptoms. The various types of disease symptoms caused by phytopathogenic bacteria are leaf spot, canker, fasciation, dwarfing of plants, distortion of plant parts, and discoloration of tissues, rotting, gall formation, wilting and others. Chlorosis and halo induced by phytopathogenic bacteria retard the photosynthetic activity of the host plant. The following are the major types of plant disease symptoms commonly observed due to bacterial pathogens.

Local Lesions

Bacterial lesions or localized spots occur on the leaf blade and sometimes in the petiole, stem, and other parts. Such spots may also occur on fruits, resulting in the discoloration of fruits. The leaf spots start as minute water-soaked specks, spreading rapidly to form circular, irregular or angular spots, bound by veins and veinlets. The pathogen, which enters the host tissues through stomatal openings, establishes in the substomatal space and remains so in the parenchymatous tissues, causing necrotic lesions. This necrosis may spread rapidly in certain cases, such as in fire blight of apples and pears.

Soft Rots

The soft rot symptoms are found mainly on fleshy parts. The major effect is the softening of tissues due to disintegration of cells and dissolution of middle lamella as a result of the action of enzymes and very often, dirty liquid oozes out of the affected parts. The enzymes diffuse in advance to dissolve the middle lamella of the cells. Plasmolysis and death of cells follow and the bacteria grow upon the dead plant tissues rather than upon the living cells.

Vascular Disease

In some of the bacterial leaf spots, the organism moves into the vascular tissues and becomes systemic. In others, the invasion is concentrated in the vascular tissues, causing typical wilt of the affected plants. Due to vascular infection, there may be hypoplasia, leading to the stunting of the entire plant. Common examples are cucurbit bacterial wilt, brown rot of potato, ring rot of potato, bacterial wilt of tomato, etc. Certain polysaccharides produced by phytopathogenic bacteria *in vitro* possess wilt-inducing properties. In other studies, it has been found that certain pectinolytic and celluloytic enzymes possess wilt-inducing properties. Pathogenic infection causes the movement of water in the vessels to decline due to an increase in the viscosity of vessel fluid. This is because of the formation of a slimy or gummy substance surrounding the bacterial mass. Tylose formation and vessel collapse may also contribute to wilting.

Tumours and Galls

In many bacterial diseases, the effect of invasion by the pathogen is the hyperplasia and hypertrophy of invaded tissnes. As a result, tumors develop on the affected organs. The formation of root nodules in leguminous roots is response to bacterial action also has a similar effect. Crown gall (*Agrobacterium tumefaciens*) is the best-known example of this. The pathogen seems to trigger a chain of reactions to produce some chemicals in the vicinity, which cause hyperplasia and hypertrophy of the host cells.

Scabs and Cankers

Scabs and cankers are corky outgrowths, which are formed on leaves, twigs and all other plant parts above the ground. These outgrowths are the result of the reactions of the host tissues to the pathogen. Such reactions are mostly localized and often confined to the parenchymatous tissues of the host plant. The incubation period normally required for such infections is about ten days after infection. Scab is formed by epidermal infection and is not deep-seated. The powdery scab of potatoes is a familiar example. Cankers, on the other hand, are deep-seated and involve the cambium layer. They entail the destruction of woody tissues and are common infections on stems of citrus, mango and many fruit and forest trees.

Bacterial Plant Diseases

Bacterial Blight of Paddy

Bacterial leaf blight is widespread in Asia. It has been reported from Sri Lanka, China, Cambodia, India, Japan, Indonesia, Taiwan, Vietnam, the Philippines and Thailand. It has not been reported from any rice-cultivating countries outside Asia.

In Japan, it is known as 'white withering disease'. Ishiyama established the bacterial nature of the disease in 1922. In India, the bacterial blight was first recorded in 1951 in the Khopoli area in the erst-while Bombay state, but at that time, the pathogen was not identified (Bhapkar *et al*., 1996). Srinivasan *et al.* (1959) reported the bacterial blight to be caused by a strain of *Xanthomonas oryzae*. Studies have shown the disease to be present in most of the rice-cultivating states of India (Mizukami, 1964; Srivastava and Rao, 1964; Srivastava, 1967). Since the introduction and cultivation of new, high yielding but susceptible rice varieties over a large acreage, the disease has become one of the most serious problems of rice cultivation in India (Srivastava *et al*., 1967). Srivastava (1972) had given a detailed account of the disease in his presidential address to the members of the Indian Phytopathological Society. Devadath (1992) has reviewed the work done on this disease up to that period. According to Mew *et al*. (1993), bacterial leaf blight is one of the most destructive diseases of rice in Asia. In India, the losses had been estimated to vary from 6-60% (Srivastava and Rao, 1966). Rao and Kaufman (1971) estimated the losses to reach up to 50%. Ahmad and Singh (1975) estimated the losses under controlled conditions in different varieties of rice, which ranged from 7.1% in IR-20 to 74.2% in the variety Bala. According to Devadath (1992), losses in the range of 6-60% have been reported in India.

Symptoms

In the temperate regions, the disease usually becomes noticeable in the field at the heading stage. In severe cases, it may appear earlier but it is rare in the seed-bed. The disease is typical vascular wilt; leaf blight is only the mild phase of the disease, which results from secondary infection (Singh, 1998). There are three phases of diseases (1) the leaf blight phase (2) the most destructive form of the disease is "Kresek" or wilt phase and (3) the pale yellow leaf phase in the tropics.

In the seed-bed, it first appears as tiny, water-soaked spots at the margin of fully developed lower leaves. As the spots enlarge, the leaves turn yellow, dry rapidly and wither. On leaf blades, lesions usually begin at the margin; a few are formed at the tip as water-soaked stripes. The lesions enlarge both in length and width, have a wavy margin and turn yellow within a few days (Fig. 24.10a). The region adjoining the healthy parts shows water soaking. Lesions may start at one or both edges of the leaves. As the disease advances, the lesions cover the entire blade, turn white and later become greyish due to the growth of saprophytic fungi. In susceptible varieties, the lesions extend to the leaf sheath where they may reach the lower end. The infected blades in susceptible varieties wilt and roll as the diseased portions enlarge while the leaves are still green. The entire blade may soon become involved and gets dried up. On more resistant varieties, a yellow stripe appears just inside the margin of the leaf blade with no formation of necrotic lesions. On the surface of young lesions, milky or opaque dew-drops may be observed in the early morning. These dry up in the form of yellowish spherical beads (Fig 24.10b) which fall down in the field. In severely diseased fields, the infection may reach the grains. The glumes get discolored and water-soaked spots develop on them.

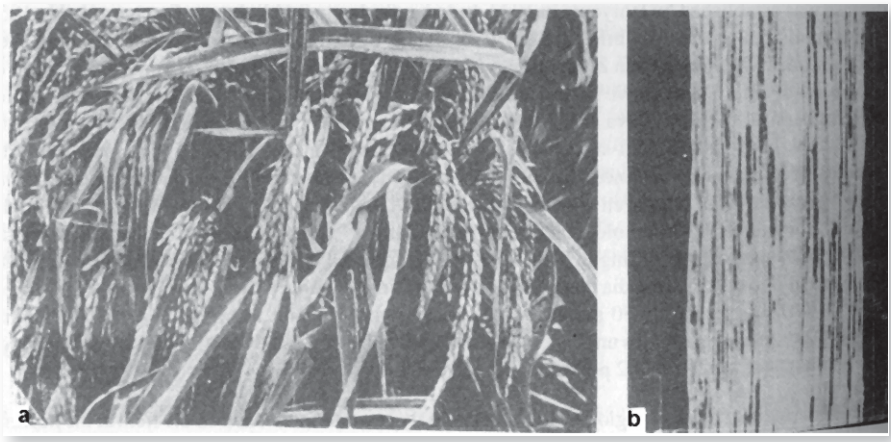


Figure 24.10 Bacterial blight of rice caused by *Xanthomonas oryzae pv. oryzae:* (a) symptoms (b) portion of the blighted leaf magnified to show bacterial exudates.

In Indonesia, the disease has been known for a long time by the name, 'Kresek'. It affects young plants, the outer leaves of which wither, become greyish green or light brown and often float on the surface of water. Some of the younger leaves roll up and wither. The bacterium spreads through the xylem vessels to the growing point of the young plant and infects the base of other leaves. As a result, the entire plant dries. Bacterial exudate can be seen from the leaf blades or sheaths (Goto, 1964).

In India, symptoms become visible one week after infection. The disease is characterized by the drying of the leaf tip and inward rolling and twisting of the leaf blade. Linear, yellowish stripes may

develop along the midrib or on the blade; marginal necrosis may also occur. The disease extends to the leaf sheaths and cuims, killing single tillers or the whole plant. Srivastava and Rao (1966) have described the symptoms in detail.

In Japan, the disease becomes noticeable only late in the season—at the tillering stage. The first symptoms appear along the leaf margins. In Sri Lanka, where the disease was first reported in 1958, symptoms are best seen on the flag leaf, where the typical undulate marginal necrosis occurs (Seneviratne, 1962).

Causal Organism

The disease is caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al*. (1990). It is a rod-shaped bacterium with rounded ends, 1-2 × 0.8-1 µm and monotrichous flagellum of 6-8 µm. It is Gram-negative and non-spore forming (Ishiyama, 1922). Bacterial cells are surrounded by mucous capsules and joined to form an aggregated mass, which is relatively stable in water. Colonies of the bacterium are yellowish on artificial media and the yellow pigments are insoluble in water.

The strains of bacterial leaf blight organism vary in their virulence. Most of the tropical strains are virulent forms, capable of causing considerable damage and 'Kresek' symptoms. Strains also differ in their susceptibility to bacteriophages. Six types of phages have been found in the Philippines and five in Japan. There is no correlation, however, between virulence and phage reaction. In India, a particularly virulent strain, differing in pathogenicity, morphology and biochemical characteristics from the normal strains has been distinguished as the Rajasthan strain (Chakravarty and Rangarajan, 1967). Some minor differences were noticed in the physiologic and biochemical characters by different workers.

Disease Cycle and Predisposing Factors

The bacterial leaf blight is principally a vascular disease. It enters the leaf through wounds, for example, by the chafing and striking of leaves against one another in the wind, through the cut ends of seedling leaves, through broken roots at transplantation or through the hydathodes. Once inside the leaf, the bacterium multiplies and moves in both directions. Lesions may be seen about ten days after artificial inoculation. Bacterial exudates are produced on the surface of leaves and during rain storms, these bacteria disperse and come in contact with other leaves or fall into the water. Bacteria are also carried by irrigation water from one field to another; this bas been demonstrated by using the bacteriophage technique of estimating their numbers.

The bacterium appears to be soil-borne in Japan. The organisms may live in soil for 1-3 months depending upon soil moisture and acidity. However, the soil is not considered to be an important source of infection as the short period of survival is not sufficient to carry over the disease to the next year's crop.

In Japan, the bacterium can also overwinter in wild grasses (such as species of *Leersia* and *Zoysia*) growing along irrigation ditches and in swampy areas. The grass, *Leptochloa chinensis,* has been found to be a potential source in the Philippines. Several Japanese workers have reported the perpetuation of the pathogen in the infected straw left over in the field or the barn and also in the stubble. Under the conditions prevalent in northern India, these sources have not been found to be effective means of primary inoculum (Srivastava and Rao, 1968; Rao, 1970).

Several workers have demonstrated seed infection and the survival of the bacterium in the seeds for varying lengths of time, up to the next growing season and the disease from such seeds. Detailed studies conducted by Kauffman and Reddy (1975) on the seed transmission of pathogen in rice indicate that the

bacterium remains viable in infected seeds for only two months. In India, the pathogen is propogated from season to season through infected seeds (Devadath, 1992).

The question of weeds being sources of primary inoculum has evoked considerable interest. Several researchers from Japan (Goto *et al*., 1953a; Wakimoto, 1956; Watanabe and Kurita, 1958; Mizukami, 1961; Tagami *et al*., 1963) and a few others (Chattopadhyaya and Mukherjee, 1968; Rao and Kauffman, l971) have been successful in infecting several weed hosts but the ones considered to function as active natural hosts are *Leersia oryzoides* and *Leersia oryzoides* var *jáponica* (Goto, 1953a; Yoshimura, 1959; Yoshimura *et al.,* 1959). However, these species of *Leersia* are not known to occur in endemic rice areas in India. Chattopadhyaya and Mukherjee (1968) regarded *Cyperus rotundus* as a host but other researchers have not confirmed this. A report (Roa and Kauffman, 1971) of *Leersia hexandra* being a possible collateral host is again open to doubt. Pandey (1970) tested 32 Indian common weeds. Although the pathogen could be successfully established around the site of inoculation in six such weeds, none of them could be considered a possible active source. Pandey (1970) failed to demonstrate the bacterium in the rhizosphere of several non-hosts. According to Devadath (1992), chaffs dumped near threshing areas seem to play an important role in primary infection.

According to Devadath (1992), the hibernated bacterial cells, on coming in contact with the rice seedlings, are activated and multiply and invade through wounds caused by root development at the basal part of the stem or through hydathodes on the leaf. The pathogen can also enter the seedlings through stomata and then multiply in the intercellular spaces of the parenchyma in the coleoptile and leaf sheath. No external symptoms are visible at this stage. Devadath (1992) has discussed the possibility of perpetuation of the pathogen during off-season. Several possibilities have been discussed, such as the survival of the pathogen in dry and growth form, in soil and in field water, in stubbles/ratoons, in infected straw and self-sown plants, in/on seed, on weeds and other crops including epiphytic survival.

In spite of the extensive work done in Japan and elsewhere on the perpetuation of the bacterium from one season to the next, there is as yet no complete information on the sources that contribute to the primary inoculum. According to Devadath (1992), disease forecasting is very essential to use need-based chemical application. Several methods are available, but the validity of some of these methods is questionable. These are (1) by inspecting the natural infection in the seedlings towards the end of the nursery stage (2) by climatic conditions and (3) by estimating the bacteriophage population.

In connection with the forecasting of bacterial blight, climatic factors have been subject to analysis. The incidence of the disease has been found to be correlated with total precipitation, the occurrence of heavy rain, flooding, the presence of deep irrigation water and severe wind. Relatively high temperatures during the growth of the rice crop increase the incidence of disease but severe heat and drought suppress it. In India, the disease develops readily above 25°C. The rice plant is susceptible to infection at all stages of its growth, but with age, the susceptibility to vascular infection decreases.

There are several reports that an excess of nitrogen or unsuitable combinations of NPK favour disease development. High doses of silicate, magnesium and shale have been reported to increase disease severity, whereas potassium has been shown to decrease the disease. On the other hand, Kim and Cho (1970) have reported that potassium and phosphorus applications at twice the normal level tend to stimulate lesion development.

There is considerable variation in the susceptibility of rice varieties to bacterial blight. During 1968, a total of 4828 varieties from the genetic stock of the Central Rice Research Institute, Cuttack, were screened under the mass-screening technique developed at the Institute and 33 varieties were found to exhibit resistant reactions to the disease (Padmanabhan, 1969; Nayak and Padmanabhan, 1970;

Chakravarty and Devadath, 1971). Padmanabhan *et al.* (1971) studied the inheritance of resistance to bacterial blight of rice.

The ratios between the total sugar and total nitrogen content of plants were found to be larger in rice varieties resistant to bacterial blight than those susceptible to blight. There was however, no correlation between resistance to bacterial blight and the total nitrogen content of the leaf sheaths; rice varieties resistant to bacterial blight were reported to have a low reducing sugar/total nitrogen ratio, while the susceptible varieties had a higher ratio (Srivastava, 1972). In a comparative study of certain post- infectional changes in resistant and susceptible varieties, Mohan (1971) showed that the resistance to blight was due to a higher peroxidase activity and low ascorbic acid content, leading to an active oxidation of phenols to quinones, which are known to be antimicrobial.

Kuo *et al.* (1970) purified the toxin of *X. oryzae* and reported that it contained mannose and glucose and possessed four fractions with molecular weights of 200,000, 153,000, 147,000, and 29,200. Rai (1978) investigated the toxin production by virulent and avirulent strains of *X. oryzae*. The virulent strains produced more toxin than the avirulent ones.

Disease Management

As *X. oryzae* pv. *oryzae* is suspected to be seed-borne in India, seed treatment is advocated. Soaking the seeds for 12 hours in Ceresan plus Agrimycin 100 (75 g) followed by hot-water treatment for 30 minutes at 52-54°C has been recommended. Later, it was found that the preliminary soaking of seeds could be done in plain water (Sinha and Nene, 1967). Jain *et al.* (1966) suggested an alternative method, which did away with hot-water treatment. This involves an eight-hour soaking in 0.1% Ceresan wet and Streptocycline at 0.3 g/10 L.

Spraying in the field is also advocated, particularly in Japan. A combination of 60-120 ppm of antibiotic Chloramphenicol with an organomercurial such as phenyl mercury acetate at 10-20 ppm metallic mercury has been found effective. Another mixture used is 100 ppm each of Dithianone and Cellucidin. Plants should be sprayed as soon as the first signs of the disease appear. A disease forecasting service is necessary. A method has been devised in which the bacteriophages of the leaf blight organism give an indication of the intensity of the disease. Padmanabhan and Jain (1966) suggested the chlorination of irrigation water, which could help in reducing infection.

Sometime back, the antibiotic preparation called Streptocycline was used on a large scale in India (Thirumalachar, 1968). Trials were conducted at several locations after the performance of this antibiotic was reported to be unsuccessful. The results of these experiments showed that the antibiotic is not at all effective against this disease (Srivastava, 1972). Singh (1975, 1976) had recommended 5 sprayings of Agrimycin plus copper oxychloride at 12-day intervals. Fungicides TF-130 and RH-893 were also quite efficacious in controlling the disease.

In Japan, the seedbeds and fields are kept from being too deeply flooded, but the value of this method in the tropics is doubtful. The burning of crop remains, to destroy the bacteria present in infected straw and stubble, is recommended in India (Sulaiman *et al.*, 1965). Eliminating volunteer plants and ratoon rice is recommended in Phillipines. Srivastava (1969) and Kauffman (1971) have suggested that the sowing of nursery beds in disease-free, isolated areas to prevent the inflow of primary inoculum into the fields might reduce the disease and arrest the build-up of an epidemic. Injury of the host plays an important role in infection, as injury is a prerequisite for the entry of the bacterium. Pruning of top portions of all varieties, which is a common practice in many parts of India, should be avoided in order to reduce disease occurrence, especially in places where the disease has established itself.

It is well established that nitrogenous fertilization is an important predisposing factor for the occurrence of bacterial blight. The method of application of nitrogen also influences disease. Nitrogen application at the tillering stage increases the incidence of the disease but a similar application at the time of transplanting, or a later application at the time of flower initiation, results in lower incidence of the disease (Have and Kauffman, 1972). Reducing N doses by means of better application methods results in a better growth of the plants and increased disease intensity (Have and Kauffman, 1972).

In Japan, no varieties of rice have been identified to be highly resistant to the most virulent strains of *X. oryzae* pv. *oryzae*. More than 6,500 varieties were screened for resistance at the International Rice Research Institute between 1964 and 1966. About a dozen or more varieties have a broad resistance to the organism and some of them are being used in the Institute's breeding program.

In India, two varieties (W529 and W348), out of the 16 tested in a trial of early maturing varieties, were highly resistant in the field (Padmanabhan, 1965). Srivastava *et al*. (1967), using five virulent isolates of the pathogen, showed that 13 out of the 128 varieties tested showed resistance. The resistant varieties included Tainan 3 and Chainung-292.

Concentrated efforts have been made in India to develop durable resistant cultivars in India and a number of fixed lines are being evaluated in multilocations to identify high-yielding, broad-spectrum resistant lines. But the so-called resistant cultivars released in India falied to show durable resistance. Singh (2004), using molecular markers, studied diversities present in *Xanthomonas oryzae* pv. *oryzae* and based on these, pyramided three bacterial blight-resistant genes: Xa5, Xa13 and Xa21, in the background of rice varities PR106 and Pusa44. Some of the resistant cultivars available in India are IR64, Sona Mahsuri, Swarna, Janaki, Sujata, Asha, Udya, etc., (Reddy, 2004).

Bacterial Leaf Streak

Although less damaging than leaf blight, bacterial leaf streak is of some importance. It was first definitely identified as a distinct disease in 1957, when the pathogen was described in China (Fang *et al.* 1957). It was earlier confused with leaf blight. The disease occurs in India and was first reported by Srivastava *et al*. (1967). Subsequently, Shekhawat and Srivastaa (1971, 1972 a), Chattopadhyay and Mukherji (1968), reported it from other states. Singh *et al.* (1980) have investigated the effect of bacterial leaf streak on the quantitative and qualitative characters of rice.

Symptoms

Bacterial leaf streak appears as fine, interveinal, long or short lines on the leaf blade which are water-soaked and greyish. Yellowish beads of dried bacterial exudate are seen on closer scrutiny (Fig. 24.11). The lesions enlarge and coalesce to form larger patches and become yellow.

Causal Organisms

The disease is caused by the bacterium *Xanthomonas oryzae* pv. *oryzicola* (Fang *et al*.) Swings *et al*. (1990a). Fang *et al.* (1957) described the morphology and physiology of the bacterium. The bacterium is rod-shaped, 1.2×0.3-0.5 µm, single, occasionally in pairs but not in chains; no spores and no capsules; motile by a single polar flagellum. The bacterium is Gram-negative, aerobic, grows favourably at 28°C. Colonies on nutrient agar pale yellow, circular, smooth, margin entire, convex or viscid.



Figure 24.11 Bacterial leaf streak of rice caused by *Xanthomonas oryzae* pv. *oryzicola*: (a) symptoms (b) portion of the infected leaf magnified to show the bacterial beads.

Disease Cycle

Shekhawat *et al.* (1969), Shekhawat and Srivastava (1972 a, b) and Devadath (1971) reported that seed from an infected crop harbours the bacterium and is a potential source of the disease and its dissemination to distant places. The bacterium is found to be present both inter and intracellularly. It gains entry through the injured epidermal cells or directly through the stoma of the leaf, colonizing the substomatal cavity and mesophyll tissues. Slight discoloration of the invaded cells is noticed in the early stages of infection but in advanced stages of the disease, the leaves turn completely yellow and degeneration of the protplasts in the mesophyll occurs. The vascular bundles are not invaded by the bacterium. Shekhawat and Srivastava (1972 c) worked out most of infection in bacterial leaf streak of rice and histology of the diseased leaf.

The bacterium seems to survive the off-season under the glumes and when the seed is sown, it contaminates the plumule during germination. Infection of coleoptile, leaf sheaths and first leaf occurs through the stomata. The bacterium is carried to the foliage by the first leaf. Seed infection in the panicles is caused by the bacterial exudate from the flag leaf causes. The epidemiology of the disease has been studied by Shekhawat and Srivastava (1971, 1972 a, b).

The bacterium survives from one season to another in the infected seed but not in the infected debris or soil. Young leaves are more susceptible to the disease than the older ones. Continuous high humidity for 2-3 days or during early morning hours favours infection. Lesion development is high at moderate temperatures (20.3-26°C). Spread of the disease can be prevented by heavy doses of nitrogen, shade, and close planting. According to Seshagiri Rao and Devadath (1978b), the disease severity increases with higher doses of nitrogen. Hairiness of the leaves has ben correlated with higher disease intensity (Seshagiri Rao and Devadath, 1978 a).

Disease Management

In China, seed treatment has been found to give partial control using a soak treatment in 0.5% Thiram or Ceresan for three hours or 0.2% Thiram for two hours. Shekhawat and Srivastava (1971) reported that seeds soaked in 0.025% Streptocyclin and subsequently transferred to hot water at 52°C for 30 minutes

are effective in eradicating the seed-borne infection. Vitavax at 0.15-0.3% as a spray prevents leaf infection and spread. Banerjee *et al.* (1984) have recommended Streptocylin or Agrimycin 100 at 10-day intervals in controlling the disease. The use of resistant varieties will hopefully also give good control. In India, 132 out of 372 varieties tested at The Central Rice Research Institute, Cuttack, were very resistant (Padmanabhan, 1965) and in the Philippines, most Japonica varieties have been found to be highly resistant, although most of the indica varieties tested were highly susceptible (Goto, 1965). According to Singh (1998), IR-20, Krishna and Jaganath have shown good tolerance to the disease.

Citrus Canker

In India, citrus canker was first reported from Punjab (Luthra and Sattar, 1942). Its occurrence was further recorded in Tamil Nadu, Andhra Pradesh, Karnataka, Rajsthan, Assam and Uttar Pradesh. Further, the disease appears as a serious problem wherever acid lime (*C. aurantifolia*) is grown on a large and commercial scale and has become a permanat major problem to citrus cultivation in India (Das, 2003). Fawcett and Jenkins (1933) found lesions of citrus on Kew specimens of *Citrus medica* collected in Dehradun, in 1827 and 1831. Canker lesions have been found on *C. aurantifolia* collected in Java between 1842 and 1844. This suggests that the disease might have originated in India or Java and then spread to other countries. The disease was first recorded in Japan. Now, it is known to occur in practically every citrus-cultivating region of the world. Citrus canker is regarded as a disease of international importance. Goto (1992) has reviewed the work done on this decease. It was recognized as a new disease in Florida in 1913. A campaign to eradicate citrus canker has begun in 1915 in the southern states of America, including Florida, Alabama, Georgia, Lousiana, Mississipi, South Carolina, and Texas. It was declared that Citrus canker was eradicated from these areas by 1947. This achievement has been considered as a rare instance of successful eradication of a plant disease that is so firmly established in certain ecosystems (Goto, 1992). However, it has re-appeared in Florida since 1984 and again the same drastic measures have been reinitiated. The disease is of worldwide importance and millions of dollars have been spent on eradication campaigns, in addition to direct disease losses.

Symptoms

Citrus canker may affect any part of the tree above the ground, although the leaves, twigs, young branches and fruits are the most susceptible parts. On the leaves, canker first appears as small, watery, translucent spots, usually of a darker green color than the surrounding tissue and with a raised convex surface. The spots first become evident on the lower surface, and as the disease advances, the surface of the spots become white or greyish and finally rupture, exposing a light brown, spongy, central mass developed in a crater-like formation. These spots usually become surrounded by a halo, which persists in very old lesions. Old lesions become corky and brown and may appear pinkish. The development of the pink color may also be due to scab disease. In susceptible varieties, lesions on the leaves but acquire a cankerous appearance and the surface membrane completely disappears. The canker lesions on the fruit appear similar to those on the leaves, except that the yellow halo is usually absent and the crater-like appearance is more noticeable. The spongy rough eruptions may be scattered over the surface, or several may coalesce, forming an irregular, scurfy, and scabby mass (Fig. 24.12).

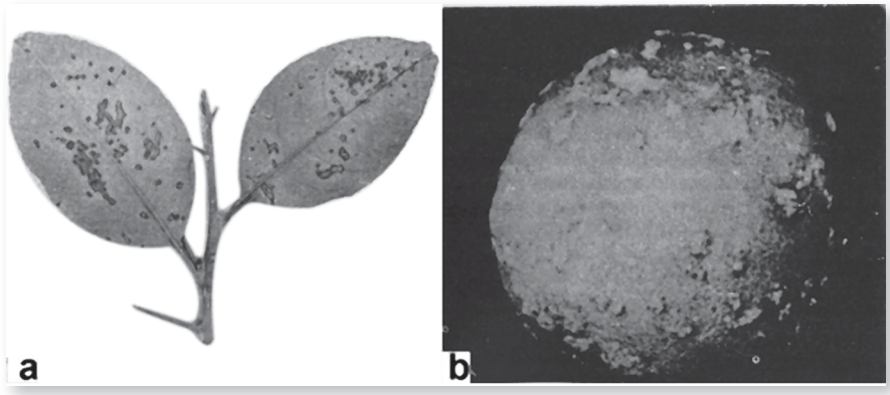


Figure 24.12 Citrus canker caused by *Xanthomonas axonopodis* pv. *citri:* (a) symptoms on the leaves (b) cankers on fruit.

Causal Organism

The pathogen responsible for citrus canker is *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin [Syn. *X. citri* (Hasse) Dowson and *X. campestris* pv. *citri* (Hasse) Dye]. The bacterium *Xanthomonas* is rod-shaped, measuring 1.5-2.0 × 0.5-0.75 µm, Gram-negative and has a single polar flagellum. Hasse (1915) was the first to establish the bacterial nature of the disease and to describe the organism as *Pseudomonas citri*. Breed *et al*. (1957), who subsequently studied the organism, included it under *Xanthomonas citri* (Hasse) Dowson. Following the most recent classification scheme of the bacterium, the pathogen should be called *X. axonopodis* pv. *citri* (Hartung *et al*., 1996). It may be mentioned that Gabriel *et al.* (1989) have proposed the restoration of the original name *X. citri*. However, Schaad *et al.* (2000) very recently proposed that pv. *citri* should be retained as pathovars of *X. campestris*. It is a short, rod-shaped bacterium, motile by means of a single polar flagellum. It is 1.5 - 3 µm long and 0.5 - 1.5 µm broad and strictly aerobic. On 2% sucrose peptone agar, there is a yellow, copiously mucoid growth. Gelatin and starch are hydrolysed. Nitrite is not formed from nitrate and aesculin is hydrolysed.

Disease Cycle

Diseased twigs and leaves provide the inoculum. The pathogen enters the host through various types of wounds, natural openings, and so on. On penetration into the host, it multiplies in the intercellular spaces, dissolves the middle lamella and establishes itself in the cortex. Cankerous outgrowths now develop, within which bacteria multiply and are released with exudations. Wind, rain, and insects chiefly disseminate the disease.

Citrus leaf miners are very important for the dissemination of the disease. However, man, who seems to be the chief agent of dissemination of the pathogen, transports the disease through infected nursery trees.

It has been found that an atmospheric temperature of 20-35°C, with an optimum of about 30°C and free moisture for 20 minutes or more on the surface of rapidly growing tissues, are essential for the infection of leaves. Infection is related to the density and maturity of the stomata, being heaviest during their development. The organism has been found to survive in infected leaves for more than six months, in sterilized soil for 52 days and in unsterilized soil from 9-17 days (Vasudeva, 1958; Rao and Hingorani, 1953).

Physiology of the Diseased Plant

Padmanabhan, D. *et al*. (1974) studied the physiology of canker-infected citrus leaves and found certain differences in the physiological activities of healthy leaves and canker and the halo regions in the infected leaves. The respiratory rate was found to be higher in the halo region than in the canker tissue and the healthy tissue. A reduction in total chlorophyll was observed in the canker and halo regions, resulting in a reduced photosynthesis rate in these tissues. A reduction in the total major sugar content and total nitrogen was observed in the canker and halo regions as compared to the healthy tissue. The total phenolic content in the halo region was three times that in the healthy ones.

Disease Management

Citrus canker incidence can be reduced considerably by taking integrated management approach consisting of (1) using canker-free nursery stock; (2) pruning all the infected twigs before monsoon and burning them; (3) periodical spraying of suitable copper-based bactericides along with an insecticide; (4) taking some precaution to reduce the risk of spread of disease in orchards and nurseries; and (5) by evolving canker-resistant varieties suited to local environmental conditions (Das and Singh, 2001). Exclusion by means of rigid quarantines is the best method of control where the disease does not exist or it has been eradicated. Control measures include exclusion by quarantine, eradiction by the destruction of infected trees, use of canker-free nursery stock, sanitary precautions and the growth of resistant varieties. Because wind greatly facilitates wound infection, the establishment of windbreaks around citrus orchards is effective in preventing infection. Spraying the trees before planting in new orchards with a copper fungicide and in old orchards, pruning of the affected twigs and spraying with copper fungicides at periodic intervals, especially during the rainy reason is recommended. Spraying of neem cake at the rate of about 80 kg per acre has been reported to be highly effective in checking the citrus canker as well as leaf miners.

Rangaswami *et al.* (1959 a, b) have shown that the disease can be controlled under field conditions with a spray of antibiotics, such as streptomycin sulphate at 500-1000 ppm or crude agricultural preparation containing an equivalent amount of the antibiotic at intervals of 15 days. Other effective antibiotics were Agrimycin and Streptocycline, in combination with Bordeaux mixture. Application of neem cake solution on the foliage reduced the canker in nurseries. Integrated application, pruning of infected twigs, copper oxychloride (0.3%), Streptocycline (100 ppm) and neem cake suspension were found very effective in controlling the disease (Das, 2003). Grapefruit, sweet oranges, limes and some lemon varieties are very susceptile. Varieties of *Citrus reticulata*, including tangerines, king, mandarin and satsuma oranges are resistant to canker.

Studies on the biological control of citrus canker are still in a preliminary stage. Some strains of bacteria viz., *Pseudomonas syringae*, *Erwinia herbicola*, *Bacillus subtilis* and *P. fluorescens* were reported to be antagonistic *in vitro* to the canker pathogen.

Bacterial Blight, Angular Leaf Spot and Black Arm of Cotton

The disease occurs in all cotton-growing regions of the world, including the USA, South America, Egypt, Sudan, and other African countries, Russia, Pakistan, Sri Lanka, China, Australia, and others. In India, the disease was first observed in Tamil Nadu in 1918. Since then, it has been found to occur in various degrees in all the major cotton-growing areas of the country where the exotic American cotton

is grown. The disease symptoms and intensity depend largely on the climatic conditions prevailing in each tract. In Egypt, Sudan and Uganda, the black arm infection is most serious; in the USA, seedling blight is more common; and in India, angular leaf spot and boll rot symptoms are severe. Earlier the disease remained unimportant because of cultivation of the indigenous cotton, *Gossypium arboreum* and *Gossypium herbaceum,* which are comparatively tolerant to the disease. Several epiphytotics of the disease occurred, when a campaign was started to replace the rain-fed desi cottons with high-yielding good quality tetraploid cottons (*G. hirsutum* and *G. barbadense*), which were highly susceptible to the disease (Chopra, 1998). Verma (1995) discussed advances in bacterial blight of cotton. Verma and Sheo Raj (1992) have reviewed the work on this disease.

Symptoms

The bacterium is capable of infecting all the portions of the plant, which are above the ground. There are four major types of symptoms: (1) seedling infection; (2) angular leaf spot of cotton; (3) black arm of cotton; and (4) boll rot of cotton.

Seedling Infection The earliest symptom of the disease is noticed on the cotyledons of the germinating seed. Water-soaked, circular lesions appear first on the lower surface of the cotyledons. The infection soon spreads and forms angular patches. The diseased tissues turn brown, distort the shape of the cotyledons and finally cause the drying and withering of the seedling. The whole seedling may collapse and wilt in severe infections.

Leaf Spots On the leaves, the spots are usually angular. These first appear on the under surface and then on the upper surface. The disease advances along the main veinlets and veins to cause finger-like lesions on the leaf blade (Fig. 24.13) and then the disease is identified as 'vein blight'. The leaves affected by such vein blights become crinkled and twist inwards and dry. The infection may also spread through the leaf blade to the petiole, causing severe defoliation. On the surface of the affected tissues, there may be profuse exudation of the bacterial slime, which may dry and form a thin film.



Figure 24.13 Angular leaf spot of cotton caused by *Xanthomonas campestris* pv. *malvacearum:* symptoms on leaves (Courtesy of Professor R.K. Grover)

Black Arm The pathogen causes dark brown or black lesions on the stem and fruiting branches. The lesions are linear and sunken from the surface, after covering the whole circumference of the stem or branch. Under favourable climatic conditions, most of the branches and the main stem of the plant are affected by the disease. As the disease advances, the branches dry and soon the plant withers.

Boll Lesions The bacterium affects the boll or fruit also. The first symptom appears as water-soaked lesion on the surface. Such lesions turn dark brown and finally black. They are invariably sunken on the surface. When young bolls are attacked with several such lesions they may drop off from the plant. In several cases, the bacterial infection spreads inside the boll or the fruit and causes severe reduction in size, quality and quantity of the lint formed inside. As the disease advances, the seeds invaded by the bacterium suffer a reduction in their size and loss of viability.

Causal Organism

The pathogen is currently named as *Xanthomonas citri* pv. *malvacearum*. Intially, the responsible bacterium was *Pseudomonas malvacearum*, then *Bacterium malvacearum*, *Xanthomonas campestris* pv. *malvacearum* and *Xanthomonas axonopodis* pv. *malvacearum*. It is an aerobic, Gram-negative, rod-shaped, 1-1.2 μm × 0.7-0.9 μm. The bacterium is motile by a single polar flagellum. On a 2% sucrose peptone agar, a yellow, copiously mucoid, wet, shining growth takes place. Gelatin, casein and soluble starch are strongly hydrolysed by it. Nitrite is not produced from nitrate by the bacterium. Aesculin is hydrolysed by it. Singh *et al.* (1977) identified 14 races of *X. malvacearum* in Delhi. Verma and Singh (1975) worked out the cellulases, pectinases and proteases of India isolates of *X. malvacearum*. According to Verma (1995), as many as 26 races of the possible 32 races have been described from India, upto that period. The exopolysaccharides (EPS) produced by the pathogen have a role in pathogenicity. Virulence has also been correlated with the production of protease, endo PG and endoglucanase (Verma, 1995).

Epidemiology

Under North Indian conditions, trash, self-sown seeds and soil were not as important as the seeds in providing the primary source of inoculum (i.e. carry over the infection from one season to another), specially because cotton is a not a continuous crop, and generally, a cotton-wheat cropping pattern is followed. Intensity of the disease (disease grades) at 6-weeks crop age was considered important in forecasting disease development which can be helpful in planning for need-based chemical sprays. *X. campestris* pv. *malvacearum* was present (10^2- 10^6 cfu/ g fresh weight of leaves) in the latent phase in apparently healthy leaves (this population was considered the tolerance level of the host to *X. campestris* pv. *malvacearum* population), and symptoms were produced only when the population of the pathogen reached 1-5 × 10^{10} cfu/g fresh weight of leaves (Verma, 1995).

Disease Cycle

The pathogen is both externally as well as internally seed-borne (Massey, 1931; Hunter and Brinkerhoff, 1963; Verma and Singh, 1974; Verma and Sheo Raj, 1992; Verma, 1995).

The primary infection starts mainly in the seed while the bacterium may be present as a slimy mass on the fuzz or inside the seed. Infected cotton bolls, leaves and twigs, which fall on the soil surface, also form an important source for the carryover of the disease. The secondary spread of the pathogen occurs mainly through the agency of wind-driven rain.

Verma *et al*. (1977a) studied the survival of *X. malvacearum* under North Indian conditions. They found that the pathogen remained in a viable state for six and three months in the trash applied on the

surface of the soil and buried 15 cm deep respectively. In the cases of flooding or sowing of wheat following trash application, the pathogen survived for less than one month. Self-sown seeds germinate in March-April upto July to produce diseased seedlings, which served as a focus of disease spread. Standing diseased plants remained ineffective till the next season. Detailed work has been done on the specific bacteriophages against *X. campestris* pv. *malvacearum*, where two groups of phages have been isolated (Singh, 1972). These phages have been used to detect the presence of bacterium in seeds, trash and soil, and have provided a useful tool for epiphytological studies (Nayak, 1972). Two insect pests, *Earias* spp. and *Dysdercus koenigii*, transmit the disease mechanically. A large number of collateral hosts are also known but their role in disease cycle is not well defined.

Disease Management

Seed treatment for the control of bacterial blight is an effective means of reducing crop loss. Methods vary widely between different countries; for example, delinting with sulphuric acid followed by dusting with mercury or copper compounds, the former being more effective but more harmful to seed germination than the latter. Delinting by flaming is claimed to be both effective and non-dangerous to seed.

Rangaswamy (1957) observed that when seeds are treated with organomercurials, external infection is checked but internal infection persists and when such seeds are sown, the bacterium multiplies to cause infection. Treating the seeds with antibiotic substances such as Streptomycin sulphate, results in complete eradication of both internal as well as external infection of the seed.

Certain antibiotic mixtures (Streptomycin + Tetracyclin) could control bacterial blight disease effectively; particularly, when combined with copper fungicides. However, the use of antibiotics in human use is hazardous and their use should be minimized to seed treatment. However, certain fungicides (e.g. Carboxin, Oxycarboxin, Busan-72) used are good anti-microbial or even better under certain conditions. These have an additional advantage because the danger of selection of antibiotic fungicide-resistant mutants is eliminated.

For the control of secondary spread, spray of copper-oxychloride/ Vitavax/ Plantavax alone, or in combination with Streptomycin sulphate is very effective. Spraying is usually started when the crop is 6-8 weeks old and subsequent sprays are made at 20-day intervals. Management of insect pests by spraying of insecticide also reduces the disease incidence upto some extent (Chopra, 1998). Verma *et al.* (1977b) claimed that hot water treatment of seeds at 56°C for ten minutes eliminates both external and internal infection of *X. campestris* pv. *malvacearum,* without affecting seed germination.

Breeding varieties resistant to the disease has attracted the attention of several scientists all over the world. Knight (1946) had done considerable work on the genetics of black arm resistance in Sudan. *Gossypium herbaceum* and *G. arboreum* are mostly immune to the disease, whereas *G. barbadense* and *G. herbaceum* var. *typicum* and *G. hirsutum* are susceptible. Five main genes governing the resistance of *Gossypium* spp. have been isolated and identified: B 1, B21 B3, B4 and B5.

Several resistant sources to bacterial blight are available. These are: 101-102 B, BJA-592, P- 14-T128, Raba B-50, HG-9, Tamcot-CAMDE, Tx Bonham etc., which have been used in breeding programs for development of resistant varieties.

In India, a variety BJR (bacterial blight jassid resistant) has been developed. It is serving as an excellent source for combined resistance to bacterial blight pathogen and jassid attack in the breeding program. At Punjab Agriculture University, Ludhiana, a *hirsutum* variety LH 900 has good resistance against bacterial blight and is also a good yielder. Neem-based ecofriendly botanical pesticides were found to be quite effective. These are Agricare and Plantotype.

Biological Control

A few phylloplane bacteria of cotton (namely species of *Aeromonas*, *Flavobacterium*, and *Pseudomonas*), on preinoculation, protected the leaves from infection by a challenge dose of the pathogen. Field trials using these phylloplane bacteria have been quite successful. These seem to produce elicitor-like substances to cause a phytoalexin-type of response (Verma *et al.,* 1983b). A protective effect of preinoculation of avirulent strain, heat-killed races of the pathogen and phylloplane bacteria has been tried by Verma *et al.* (1974-1978) at IARI. Field trials conducted at Ludhiana, Hisar and IARI in recent years indicated that a spray of these bacteria could be included in the disease management program.

Black Rot of Crucifers

It is very widely distributed and has been reported in over 90 countries of the world. It is found on a number of cultivated cruciferous vegetables, including cabbage, cauliflower, turnip, Brussels's sprout, broccoli, kohlrabi, radish, mustard, and Indian mustard. Patwardhan (1928) was the first to report its occurrence on cabbage in India. Patel *et al.* (1949) reported black rot of cabbage caused by *Xanthomonas campestris*, now called *X. campestris* pv. *campestris*, from Bombay. Chattopadhyay and Mukherjee (1955) reported a severe incidence of black vein disease of cabbage in West Bengal, caused by *X. campestris*. Rao and Srivastava (1964) reported widespread occurrence of black rot of cabbage in Katrian, Kulu, and Jammu and Kashmir. The disease is regarded as of international importance. Onsando (1992) has reviewed the work on this disease.

Symptoms

The first sign of the disease appears near the leaf margin, characterized by chlorosis, which progresses toward the centre of the leaf blade (Fig. 24.14a.). In the affected portions, the vein and veinlets turn brown and finally black (Fig. 24.14b). In cases of severe infection, the leaves wither. The vascular blackening may extend to the main stem, causing systemic spread of the infection. From the affected plant parts, bacterial ooze may also be seen. In cauliflower and cabbage, if the heads are already formed, soft rot of the head may set in.

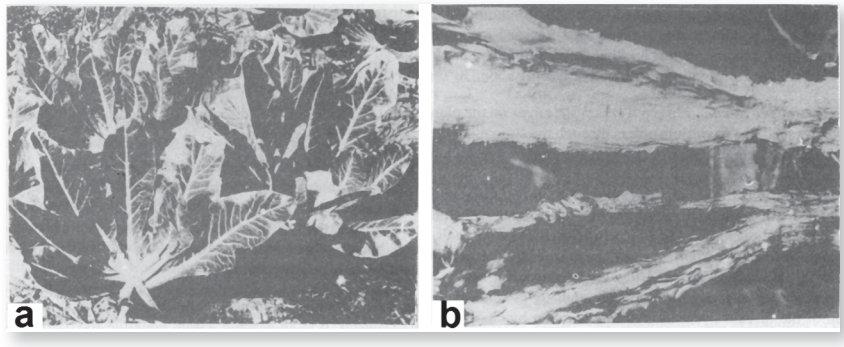


Figure 24.14 Black rot of cabbage caused by *Xamthomonas campestris* pv. *campestris* (a) water soaked spreading lesions, rotting of tissues and severe discoloration are charateristics symptoms (b) blackening of the vascular tissue.

Causal Organism

The pathogen responsible for black rot of crucifers is *X. campestris* pv. *campestris* (Pammel) Dawson. It is an aerobic, Gram-negative rod, 0.7-3.0 × 0.4-0.5 µm occurring singly or in pairs, motile with a single polar flagellum, on solid medium containing a utilizable carbohydrate. The colonies are yellow, convex, shining and of a mucoid consistency. It produces a yellow extracellular polysaccharide (EPS) called xanthan on media containing glucose. *X. campestris* pv. *campestris* hydrolyses starch, a characteristic that is used for easy recognition of the bacterium on Schaad's selective medium. It prefers neutral or alkaline medium. The *in vitro* survival temperature range is very wide (5-38°C) with the optimum temperature being about 30°C and lethal at about 50°C.

Disease Cycle

The pathogen *X. campestris* pv. *campestris* lives on the plant residue in the soil or in the seed. In a germinating seed, the bacterium enters through the stomata of the cotyledons and spreads first intercellularly in the substomatal region and then systemically into the vascular bundles. Bhide (1949 a, b) studied the mode of entrance of the bacterium in cabbage and reported that the organism invades the host normally through the hydathodes situated in the leaf margins. The secondary spread of the disease in the field is mainly through irrigation water, wind, implements, tools, and so on.

The organism is found pathogenic on the following hosts: *Beta vulgaris*, *Brassica oleracea* var. *botrytis*, *Cucumis sativus*, *Daucus carota*, *Hibiscus esculentus*, *Ipomoea batatas*, *Lycopersicon esculentum*, *Mangifera indica*, *Musa paradisiaca*, *Phaseolus vulgaris*, *Pisum sativum*, *Raphanus sativus*, *Solanum melongena*, *S. tuberosum*, and *Vigna catjung*.

Cook, Walker and Larson (1952) gave a general account of the disease cycle in crucifers. Onsando (1992) has given the latest information on this disease and discussed the disease cycle.

Disease Management

Seed treatment with 0.1% mercuric chloride for half an hour is effective in eradicating seed-borne infection. Hot water treatment of the seed at 50°C for 30 minutes is also effective. Drenching the seedbed with 5% commercial formalin may help in checking the disease (Patel *et al*. 1949). In a small-scale field trial, Agrimycin 100 gave good control of the disease. Streptocycline was found to be effective in checking the *in vitro* growth of *X. campestris* (Chakravarty *et al*., 1969).

The crop should be rotated. A three-year rotation is desirable. Strict sanitary precautions should be observed to minimize the incidence of black rot.

Resistant varieties of cabbage have been found to possess a higher proportion of mechanical to parenchymatous tissue than susceptible varieties. Seedling resistance may be affected by balance of nutrients, as in potassium deficiency, where mechanical tissue is reduced, resulting in increased susceptibility.

Biological control of the disease is still at the experimental stage. Protozoa have been associated in the decline of the population in soil.

Wilt and Brown Rot of Potato

Bacterial wilt/Brown rot of potato is widespread in the tropical, subtropical and warm temperate parts of Asia, Africa, Australia, Europe, North America, Central America, and West Indies. The disease is

widespread in India and has become a serious problem. It may damage the crop in two ways, i.e., premature wilting of standing crop and rotting of tubers both in the field and storage.

In India, Cappel recorded the disease on potato in 1892 and was the first bacterial disease recorded from this country. According to Shekhawat and Gadewar (1998), this disease of potato is still referred to as Rassa (moisture disease), bangle blight or Bangdi, Parrya, Ghera, and Utka. In other countries, it is known as brown rot (USA), slime disease (Java and Sumatra), mattey eye, grey eye, sore eye, murcha (Brazil), green wilt of die young (Vietnam) and so on. Internationally, it is known as bacterial wilt or brown rot of potato.

According to Shekhawat and Gadewar (1998), the endemic nature of the pathogen, many a times, took epiphytotic form and caused large-scale damage to potato crops in the world. In some areas, the economic depredations were so high that the farmers had to change the cropping patterns of the region. For example, in Ranchi district of Bihar (India), cultivation of rain-fed potato was abandoned due this disease. Similar instances were recorded from Indonesia and Peru. Latest surveys have indicated the prevalence of this disease in 16 states of India and extensive damage in a few pockets only. In India, losses reported are up to 55% in Kumaon hills, 0.33 -40% in Maharashtra and 20-25% at Hydrabad and over 75% in some localities of Karnataka.

According to Hayward (1991), the host range of this pathogen is very extensive and includes several hundred species, representing 44 families of plants. Apart from solanaceous crops like potato, tomato, chillies, the pathogen is known to attack groundnut, caster, papaya, cabbage, radish, banana and a host of other species.

Symptoms

The disease affects both the above and underground parts of the crop. The infected plants show sudden wilting, sometimes show temporary recovery followed by wilting in some branching or complete collapse of plants (Fig. 24.15b). According to Shekhawat and Gadewar (1998), yellowing of lower leaves and stunting of plants are observed in many countries but are not in India. Potato tubers from diseased plants may or may not show external symptoms. In some tubers, brown discoloration may be seen through the skin, indicating a well-advanced stage of infection. A bacterial mass oozes out of the tubers' eyes. Examination of the cross-section of the tubers indicates brown discoloration of the vascular bundles and if such tubers are gently squeezed, cream-like drops of sticky liquid containing bacteria are exuded from the several vascular bundles (Fig. 24.15a). The disease may be distinguished from ring rot

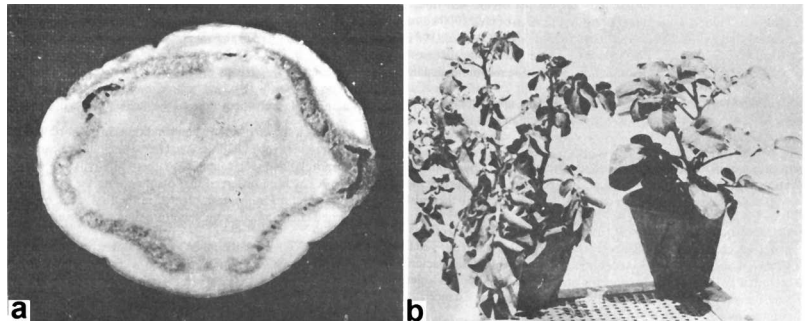


Figure 24.15 Brown rot and wilt of potato caused by *Ralstonia solanacearum* (*Pseudomonas solanacearum*): (a) vascular discoloration of the cut tuber and glistening bacterial ooze at the cut edge (b) wilt symptoms (Courtesy of Dr. B.B. Nagaich)

of potato tuber caused by *Corynebacterium sepedonicum* (now *Clavibacter michiganense* subsp. *sepedonicum*) by the more variable nature of the ring of infected vascular bundles and surrounding tissues which may not always be accompanied by brown discoloruation and the bacterial ooze which may be like putty rather than viscous or slimy.

If the stem of a diseased plant is cut across, white bacterial ooze may appear and the xylem vessels are discoloured to a dark brown color. The bacterium is mainly confined to the vascular region, but in advanced cases, it may invade the cortex and pith regions and cause dicoloration of the tissues. In wet soils, the tuber may start rotting and the bacterial mass may exude from the eyes of the tuber, to which soil adheres.

Causal Organism

The brown rot of potato and wilt of tomato is caused by the bacterium *Pseudomonas solanacearum* (Smith) Smith [now known as *Ralstonia solanacearum* (Smith) Yabnuchi *et al*.]. It is an aerobic, Gram-negative rod (0.5 × 1.5 μm), motile with one or several polar flagella. All strains except those from banana and other musaceous hosts produce a dark brown diffusible pigment on a variety of agar media containing tyrosine. The isolated cultures of the bacterium easily lose their virulence when grown on laboratory media. Shekhawat *et al*. (1992) and Chakravarty *et al.* (1995) have reported spontaneous reversion of afluidal mutants to wild type in some strains of the bacterium. There are 5 races of the species on the basis of host specificity and 5 biovars on the basis of ability to utilize and/or oxidize several hexose alcohols and disaccharides. Only races 1 and 3 Biovars II, II and IV are present in India.

Disease Cycle

The pathogen lives in the soil on diseased plant residues and initiates infection in the field. Often, cutting knives used for preparing the seed material spread the infection from diseased to healthy tubers and then to the field. Infection takes place through the wounds caused by cultural operations and by insects. The organism may also spread through irrigation and rainwater. Potato tubers are infected by way of stolons. Infection in tomatoes takes place via injured roots but the pathogen can also enter the host through wounds on the stem and through stomata. According to Pathak and Nath (1993), root knot nematodes aid in the penetration if they attack the host before it comes in contact with the bacterium. Potato tubers carry the infection in three ways: through vascular tissues, on the tuber surface, and in the lenticels. The latent infection is the most important and significant method of the transmission of the disease through the tubers.

According to Hingorani *et al.* (1956), the disease on potato is favoured by relatively high temperatures and high soil moisture levels; the infection develops rapidly at 21-38°C and 50-100% water-holding capacity of the soil. There is a decrease in disease intensity with an increase in the age of the plant. The organism can remain in the soil for up to 16 months and on diseased plants up to nine months.

Winstead and Walker (1954) found that *P. solanacearum* (now called as *Ralstonia solanacearum)* produced a toxin (polysaccharide), which is partly responsible for causing the wilt. Strobel and Hess (1968) presented evidence that the compounds produced by bacteria bring about wilting by interfering with the semipermeable membrane of the host cell and that plugging does not seem to be a factor.

Disease Management

Use of Disease-free Seeds In India, trials were conducted in the infested soils in Shillong (eastern hills), Ootacamund (southern hills), and Deccan plateau to study the role of infected tubers. The incidence of wilt was higher (5-25 times) in crops raised from the seeds (potato tubers) from wilt-endemic areas than the seeds from wilt-free areas. Thus, seeds for planting potatoes should invariably be taken from wilt-free areas.

Soil Disinfection Under Indian condition, Shekhawat *et al.* (1988) observed that application of bleaching powder at 12 kg/ha, mixed with fertilizer drill or soil drenching, before or after first earthing up reduced the bacterial wilt by 80% and also increased potato yield.

Soil Solarization The pathogen is susceptible to high temperatures. Raising the soil temperature by covering the soil with a polythene sheet has been found effective in Japan for race-1 but not for race-3. In the eastern hills of India, jhumming is the practice among the tribals where the forest vegetation is cut, spread on the soil and burnt. According to Shekhawat and Gadewar (1998), wilt incidence is negligible in such areas.

Proper Crop Rotation Crop rotation as a means to reduce the wilt incidence has been successful in both, hilly regions and the plains. The incidence of wilt has been reduced to the extent of 94% in case of crop rotation with wheat, maize, oat, barley, sunhemp, finger millet, and vegetables like cabbage, onion, garlic and others.

Proper Date of Planting It has been shown that in Indian conditions, in the northwestern and northeastern hills, early planting of the summer crop in February and harvesting in June can avoid disease. However, in Shillong (northeastern hills) and Ootacamund (southern hills), disease is always more in the autumn crop (Aug-Dec/Jan). It has been observed that planting after the last week of July reduced bacterial wilt and was negligible to nil in the crops planted in the first week of September. Produce obtained in these periods carried latent infection and therefore should not be used for seed purposes.

Seed Treatment The knife used for cutting the tuber seeds into parts should be properly disinfected with a suitable bactericide and tubers obtained for seed purposes should be obtained from disease-free fields for planting and should be treated with 0.02% Streptocycline for 30 minutes after giving a 4-5 mm deep cut.

Soil Amendments Shekhawat *et al.* (1978) found that the pH of the soil does not govern the persistance of *R. solanacearum* under Indian conditions. Application of urea at 1000 kg/ha has been found to be effective in reducing the wilt incidence (Kelman, 1953; Shekhawat *et al.,* 1981, 1982). Kumar (1970) found that town compost should not be used as it increases the disease. Application of Chloropicrin or sulphur is helpful but due to their high cost, cánnot be recommended in India.

Foliar Application Under experimental conditions, foilar application of Agrimycin 100, Chloramphenicol or Streptocyclin sulphate, one day prior to inoculation is effective (Sitaramaiah and Sinha, 1983) in case of brinjal.

Cultural Operations Rain or irrigation water should not be allowed to flow from infested to healthy fields. Infected plant materials should always be removed and burnt. After harvest, the field should be ploughed to expose soil to the summer heat of May and June in the plains to kill the soil-borne inoculum.

Genetic Engineering Techniques and Biocontrol According to Singh (1998), efforts are being made to incorporate the lysozyme, cecropin and other potent antibacterial proteins derived from insects into potato plants as a means of augmenting resistance to bacterial wilt and other bacterial diseases. Attempts to control bacterial wilt of potato by antagonistic rhizobacteria and avirulent strains of *P. solanacearum* have been made. The mechanism involved may be induced resistance and colonization of the rhizosphere by the antagonistic bacterium.

Yellow Ear Rot or Tundu Disease of Wheat

Hutchinson (1917) was first to describe the disease from Punjab in India. Milne (1919) observed that the disease incidence dependent on mechanical injury to the plant part by eelworms and that the bacterium could grow only on the excretions of the eelworms. The disease has subsequently been reported from Egypt, China, Australia, Cyprus, Asiatic USSR, Israel, Syria, Pakistan, New Zealand, Brazil, USA, Canada, and several European countries. Experiments have shown that the nematode, *Anguina tritici*, is responsible for transforming the developing grains in wheat ears to galls (popularly called 'gegle' and 'mamni') or ear cockle disease is associated with the bacterium in causing the rot. Choudhary (1935) reported that he could produce the disease with pure cultures of the bacterium alone, but detailed studies carried out at IARI, New Delhi, by Vasudeva and Hingorani (1952 a, b) and Sabet (1954 a, b, c) have shown conclusively that the infection can take place only when nematode galls are mixed with the bacterial inoculum.

Symptoms

Twisting of the stem, distortion of the earhead and rotting of the spikelets with a profuse oozing of yellow liquid from the affected tissues characterize the 'tundu' disease. Hence, the name of the disease is yellow ear rot (Fig. 24.16). The ooze contains masses of bacterial cells. The nematode alone causes wrinkling, twisting and various other distortions of the leaves and stems, and also forms small round galls on the leaves. The infected plants are shorter and thicker than the healthy plants. In the distorted earheads, dark galls are found in place of kernels (Singh *et al.,* 1959; Gupta, 1966; Midha, 1969). When the bacterium is associated with the nematode, the disease symptoms are intensified at the flowering stage and the yellow ear rot sets in. Due to the combined action of the nematode and bacterium, the earhead becomes chaffy and the dark nematode galls, which are also contaminated with the bacterium, replace the kernels. The disease is severe during the rainy season.

The infected plants produce more tillers than the healthy ones do. Another interesting feature is the

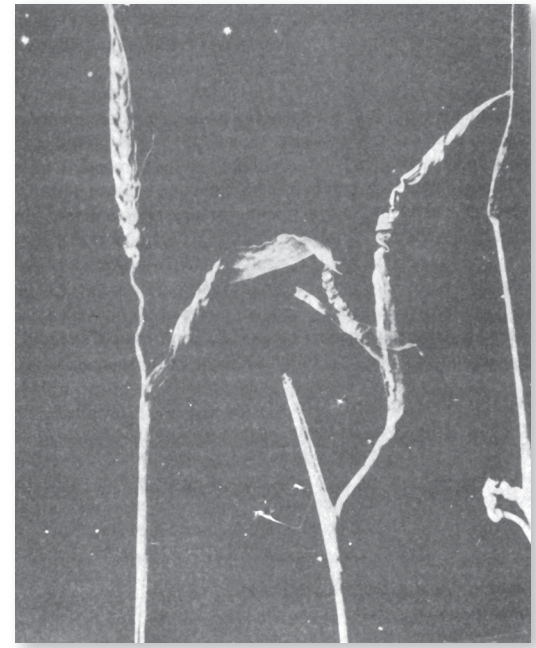


Figure 24.16 Yellow ear disease of wheat caused by *Clavibacter tritici* earlier named as *Corynebacterium tritici*: symptoms

early emergence of ears in the nematode-infected plants, which is about 30-40 days earlier than the healthy ones. Suryanarayana and Mukhopadhyaya (1971) observed that the kernels are modified into galls and bear a superficial resemblance to normal kernels. The number of nematode larvae per gall may range between 80-32,000.

Causal Organism

Rathayibacter tritici (ex Hutchinson) Zgurskaya*, et al.* 1993 is the causal organism and earlier it was called as *Clavibacter tritici* (Carlon and Vidaver) Davis *et al.* The pathogen was earlier named as *Corynebacterium tritici*. The bacterium is rod-shaped, 2.3 × 1 μm in size. It is motile by a single polar flagellum. It is Gram-positive. Colonies on agar media are bright yellow, becoming orange, convex, round, glistening, moist, opaque in the centre and opalescent at the edges. The margin of the colonies is smooth. The substrate changes to brown. The broth is clouded with a thin pellicle, the gelatin is not liquified and nitrates are reduced. The cell-wall amino acid is diamino butyric acid. It may be mentioned that the disease occurs due to the combined action of the wheat nematode *Angunia tritici* and the bacterium *Clavibacter tritici*. Vasudeva and Hingorani (1952) suggested an obligate etiological relationship between the two. Swarup and Singh (1962) confirmed this.

Disease Cycle

The disease starts from seeds contaminated with the nematode galls. When such contaminated seeds are sown in the field, they absorb moisture from the soil. The larvae escape from the galls and shortly climb up the young wheat plants. The nematodes have a tendency to seek the tender growing points of the plants, where they remain as ectoparasites. After the plants flower, the nematodes enter the floral parts and form galls in the ovaries. While within the galls, sexual differentiation occurs, copulation takes place and the female lays eggs, up to 2000 in number. The eggs hatch into larvae under favourable conditions and become active parasites. Once the nematode is inside the tissues of the ovary, the bacterium becomes active and causes rotting. The yellow ooze coming out of the rotting earhead provides the inoculum for the secondary spread of the disease, which is favoured by wind and rain. The nematode probably functions as a vector, transporting the bacterium to the otherwise inaccessible meristematic regions of the host. The nematode secretes some substance, in the presence of which the bacterium can cause the disease. Chand (1967) observed that the bacterium *C. tritici* survived in soil debris under laboratory conditions for about seven months and concluded that the diseased debris probably does not take any part in primary infection because of the limited survival of bacterium in the soil.

Mathur and Ahmad (1964) reported that bacteria remained viable for at least five years in the galls of *A. tritici*. The nematode galls are reported to remain in the soil for 20 years or more and perhaps the bacterium can survive inside the gall for a fairly long time. Midha (1971) has shown that the galls serve as nutrient sources to the bacterium. Pathak and Swarup (1984) have shown that the bacterium does not survive in the free state in the soil, nor do the nematodes separately carry the bacterium on their bodies. Nematode galls show the presence of bacterium in up to 40-50% of cases and it is believed that the main source of survival of the pathogen is the nematode galls.

Disease Management

The sowing of gall-free seeds in non-infested soil will help to reduce the incidence of disease. The seeds can be freed from the galls by being floated in brine, at 160 g of sodium chloride per litre of water. Wheat, barley, oat or other susceptible crops should not be sown in infested soil. Solar heat during May

and June in the northern wheat-belt of India can be utilized for destroying the galls and nematodes. Infected plants should be carefully taken out and burnt. Seeds for sowing purposes should be taken from disease-free areas only. Soil treatment with Aldicarb or Carbofuran destroys the nematode in the soil and prevents infection.

Bacterial Soft Rot and Blackleg of Potato

Bacterial soft rot and blackleg of Potato is caused by *Pectobacterium carotovorum* var. *carotovora* (earlier known as *Erwinia carotovora* subsp. *carotovora*) and *Pectobacterium carotovorum* var. *atrosepticum (*earlier known as *Erwinia carotovora* subsp. *atroseptica*) occurs in most parts of Europe, the USA, and Canada. In India, Hingorani and Addy (1952, 1953) investigated the disease in detail and the characteristics of the causal organisms have been established. According to Arora (2012), the pathogen affects potato tubers under poor storage conditions, especially in warm and wet climate. Losses under bad handling of the produce, poorly ventilated storage or transit may go upto 100%.

Symptoms

The disease appears sporadically in the field. The plants turn pale green or yellow and soon wilt and die. The affected haulms are jet black in color at the soil level; later, the discoloration extends into the old-seed tuber also. The cortical tissue of the basal stem becomes black and shrivels and gives the appearance of a blackleg. Sometime, the young seedlings, which emerge from the diseased tuber, may be destroyed soon after emergence or they may not emerge at all. During the wet season, the disease progresses rapidly and the entire stool may rot. When the infection takes place late in the crop season, the organism is harboured on the tubers and the damage continues in storage godowns. In soft rot of tubers, which may occur in the field if the soil is moist and temperature high, or during transit and storage, the tubers are transformed partly or wholly in a soft decayed pulpy mass covered by the corky epidermis, which is not attacked.

In storage, bacteria cause severe rot of the tubers, sometimes resulting in almost 100% damage. When tubers are kept in storage under damp conditions, decay sets in and the tubers become soft with a watery mass of bacterial ooze accompanied by a sulphurous odour.

Causal Organisms

Bacterial soft rot and blackleg of Potato is caused by *Pectobacterium carotovorum* var. *carotovora* and *Pectobacterium carotovorum* var. *atrosepticum*.

Disease Cycle

The organism lives saprophytically in decaying plant debris and enters the tubers through bruises, sunscald, insect wounds, etc. The seed corn maggot, *Hylema platura* is a common pest of potato and it helps in the infection of seed potato by the bacterium. Infection also takes place through enlarged lenticles in tubers. When the tuber is infected, the organism enters the vascular bundle and by means of enzyme action, brings about the soft rot. In the growing plant, the organism becomes systemic, causing the browning of vascular bundles along the shoot, down to the stem and newly formed tubers. The organism may enter the tuber through bruises caused during harvesting, transit and storage or transmitted from

the basal portion of the stem. The organisms are capable of growing under a wide range of storage temperatures moisture, and even in water logged areas.

Hingorani and Addy (1953), who studied three species of *Erwinia (*now known as *Pectobacterium*), viz, *E. carotovora* (*E. carotovora* subsp. *carotovora*), *E. atroseptica* (now *E. carotovora* subsp. *atroseptica* and *E. aroideae),* found that temperature and aeration were important predisposing factors for the soft rot. Also, immature potatoes were found to be more susceptible to the rot than the fully matured ones. *E. carotovora* and *E. atroseptica* were more destructive at low temperatures and *E. aroideae,* during transit when temperatures tended to be high.

Five isolates of *E. carotovora* obtained from soft rot of radish, potato, onion, turnip, and ladyfinger fruits, when tested for their pathogenic ability, were comparatively more virulent on their respective fruits. When the five isolates were compared for their capacity to reduce pectin in a liquid medium, except for slight initial variations, all of them were found to be almost equally efficient in reducing 77.80% of pectin in the medium within eight days. For breaking down the pectin, all the five isolates produced protopectinase and polygalacturonase and not pectin methyl esterase (Sanne Gowda and Rangaswami, 1963). Fox (1971) dealt with the etiology and spread of the storage rot of potato caused by *E. carotovora* var. *atroseptica*).

Chakravarti and Hegde (1971) reported that *X. campestris* and *E. atroseptica* were often the causes of soft rot of cabbage in Rajasthan.

According to Somani *et al.* (1988 b), there is a progressive increase in the incidence of the disease in tubers harvested from January to March and kept in country stores. The level and source of nitrogenous fertilizer also influences the soft rot incidence (Somani *et al.* 1988a). Heavy nitrogenous manure increases the incidence of the disease, while higher calcium decreases the disease due to better structural integrity of the cell walls of the tubers.

Disease Management

Only healthy tubers should be sown when cut tubers are sown. The cut tubers used for seed purposes should be stored at temperatures of 12-15°C in a dark room for 4 days. This will allow cork formation at cut surfaces and will prevent entry of bacteria through cut surfaces. The cut tubers used for seed purposes should be dipped in a fungicidal solution.

The wounding and bruising of tubers should be minimized at the time of harvest and storage. Chlorinated water should be used for washing the tubers.

Crop rotation, following green manure–potato–wheat reduces soft rot. Calibration of equipment to minimize bruises during harvest, avoiding direct exposure of tubers to sunlight, proper aeration in transport and storage are some measures that help in the reduction of soft rot. Planting of whole seed potato well suberized seed pieces, in well-drained soil with temperature around 10-13° C at low depth reduces incidence of blackleg (Arora, 2012).

Control of soft rot through biopesticides, such as essential oils and extracts of hemp flower and common wheats or microbial antagonists such as strains of *Pseudomonas putida*, *P. fluorescens*, *Bacillus subtilis* strain BS107 and *Erwinia herbicola* Eh 252, has been found effective. Certain endophytic bacteria such as *Pseudomonas* species promote plant growth and induce resistance of potato plants towards infection by *Pectobacterium atrosepticum* (Pavlo *et al.,* 2011).

Fire Blight of Apples and Pears

The fire blight of apple (*Pyrus malus*) and pear (*Pyrus communis*) is one of the most serious known diseases of orchard plants. It was noticed in the Hudson valley as early as 1780 and is believed to have spread from there to other parts of the USA. Widespread epiphytotics were reported, which wiped out entire pear orchards and nurseries and led to the abandonment of this crop in New York and other states. In 1878, Burrill established the bacteriological nature of the disease in USA—one of the early discoveries of bacteria as plant pathogens. Singh (1943) reported the possible occurrence of the disease on apple in the Kumaon Hills, Uttar Pradesh, but the disease is as yet not very serious in India. According to Byrde and Hutcheon (1985/1986), the disease is absent in North India. Thompson (1992) reviewed all aspects of the disease comprehensively.

Symptoms

The first symptoms of fire blight appear usually on flowers, which become water soaked, then shrivel rapidly, turn brownish to black in color and sometimes fall off. Soon, the symptoms spread to the leaves on the same spur or on nearby twigs, starting as brown-black blotches along the midrib and main veins or along the margins and between the veins. As the blackening progresses, the leaves curl and shrivel, hang downwards and usually cling to the curled, blighted, twigs.

Terminal twigs and water sprouts (suckers) are usually infected directly and wilt from the tip downwards. Their bark turns brownish-black and is soft at first but later shrinks and hardens. The tip of the twig is hooked and the leaves turn black and cling to the twig. From fruit spurs and twigs, the symptoms progress down to branches where cankers are formed. Toxins have been suggested as the pysiological basis for symptom expression by *E. amylovora*. Goodman *et al*. (1974) reported the production of a host-specific phytotoxic polysaccharide (EPS) from bacterial ooze. Isolates without EPS are often avirulent.

Infection usually takes place through the pedicel, but direct infection is not uncommon. The fruit becomes water-soaked, turns brown, shrivels, mummifies and finally turns black. Dead fruits may also cling to the tree for several months after infection.

Under humid conditions, droplets of milky-colored, sticky ooze may appear on the surface of any recently infected part. The ooze usually turns brown soon after exposure to the air. The droplets may coalesce to form large drops, which may run off and form a layer on parts of the plant surface.

Causal Organism

The fire blight-causing organism, *Erwinia amylovora* (Burrill) Winslow *et al.,* is a Gram-negative or Gram-variable rod, the living cells are about 0.8 × 10-3.0 μm, occurring singly, in pairs or in short chains, usually capsulated and motile by 1-8 peritrichous flagella. The bacteria are facultative anaerobes and require nicotinic acid as a growth factor. It is identified from the symptoms it causes and serological tests.

Disease Cycle

The bacteria overwinter at the margins of cankers formed during the previous season on pear trees and other susceptible hosts and possibly a bud and apparently healthy wood tissue. In spring, the bacteria in these ‘holdover’ cankers become active again, multiply and spread into the adjoining healthy bark.

Bacterial ooze on cankers is carried into open blossoms by rainfall and more commonly by flies, ants, and honeybees. Aphids and leaf-hoppers also transmit the pathogen to growing terminals and water sprouts. Multiplication of the bacteria in the floral nectaries provides secondary inoculum, which may be spread by rain splash and insects. Apple buds infected during the previous growing season may provide a source of inoculum in orchards, where there are no overwintering cankers and also in young trees, where blossom infection cannot occur. Wounds in the bark or branches caused by pruning, hail damage or wind may serve as points of entry.

The spread of the pathogen is favoured by warm, humid weather, which encourages the rapid growth of young shoots that are especially susceptible to infection on apple. The development of the disease requires maximum daily temperatures of over 18.5°C during flowering with precipitation or very high humidity.

Disease Management

Several measures need to be adopted successfully to control fire blight. Control measures include excision and destruction of overwintering cankers or twig infections before the blossoms open. The application of 0.1% mercuric chloride solution was found to be effective in checking primary infections. One or two sprays of 1% Bordeaux mixture have been moderately successful. Application of Streptomycin at 100 ppm, three times during the pre-blossom and blossom periods effectively controlled the disease in the USA. A special insect trap has been developed by which the bees emerging from the hives pass through the Streptomycin dust, which covers them and thus gets transmitted to the flowers. In the flower, the antibiotic gets distributed and inhibits the growth of the bacterium.

Crown Gall of Stone Fruits

Distribution

The disease has been known in the USA since 1870. Cavara from Italy isolated the bacterium from stem galls on grapes and proved its pathogenicity. Smith and Townsend (1907) correctly identified the bacterium. The disease has been reported from USA, South Africa, New Zealand Australia, Asia, Canada, and Europe. In India, Singh (1943) reported the occurrence of crown gall. Durgapal (1971) in Shimla, Himachal Pradesh, isolated the organism and established its pathogenicity. In India, the disease has been reported from cherry (*Prunus avium*), plum (*P. domestica*), almond (*P. dulcis*), apricot (*P. armenica*), peach (*P. persica*), apple (*Malus domestica*), and pear (*Pyrus communis*) (Durgapal, 1971; Jindal, 1990). Crown gall bacterium is a natural genetic engineer and has been utilized in many biotechnological transformations (genetic engineering). Plant biologists are using it as the main tool (vehicle) for transferring all kinds of genes between related and unrelated plants and even other organisms, e.g. insects or viruses, plants and animals.

Symptoms

The galls are usually 7-100 mm in diameter, spherical to irregular with a hard rough surface, generally present on the stem, more often at the graft union of fruit tree and also on roots or just below the soil line. Tumors can appear on any portion of the plants (roots, stem, branches, petiole and leaf veins).

Secondary tumors can appear far away from the primary tumor. The secondary tumors do not contain bacteria and may not be connected with the primary one. In early stages, the tumors are more or less spherical in shape, white in color and soft in texture. Later, the outer tissues become dark brown or black. The exterior of the hypertrophied growth is corky. The attacked plants may remain stunted and in advanced stages may be killed. Unlike other galls, the crown gall (Fig. 24.17) is an example of a non-self-limiting tumor.



Figure 24.17 Crown gall caused by *Agrobacterium tumefaciens:* symptoms

Causal Organism

Crown gall is caused by the bacterium *Agrobacterium tumefaciens*. Another species, *A. radiobacier,* is saprophytic and does not produce galls. *A. tumefaciens* and *A. radiobacter* are identical except for the plant pathogenic nature of the former. The bacteria contain one to several plasmids (small chromosome-like bodies composed of circular double-stranded DNA). One of these plasmids carries the genes for tumor induction and is called Ti (tumor inducing) plasmid. The Ti plasmid also carries the genes that determine the host range of the pathogenic bacterium and the kinds of symptoms that will be produced. The most characteristic property of this bacterium is its ability to introduce part of the Ti plasmid (T-DNA) into plant cells and transform normal plant cells to tumour-producing cells. The Ti plasmid contains *vir*-genes, which code for proteins required for the transfer of T-DNA (transforming DNA). The *vir*-genes are expressed after induction by plant-specified phenolic compounds such as p-hydroxybenzoic acid and vanillin. Damaged plant tissues produce these phenolic-inducer molecules. Once inside the plant cells, the T region-containing DNA is transported into the nucleus and integrates into plant chromosomes at a number of sites. The T-DNA contains oncogenes that code for either octopine or nopaline (two opines that serve as a carbon sources for the infecting bacteria). The bacterium can grow on relatively simple medium but its isolation from the soil requires selective media (Patel, 1926).

After the inducing bacteria have been in direct contact with the plant cells at the wound site for several days, viable bacteria are no longer necessary for tumor formation. The plant cells are transformed and escape from normal control mechanisms. The arrangement of the transformed cells becomes highly irregular. The gross morphology of the crown gall is dependent upon the inducing bacterium strain of *A. tumefaciens*. Some are highly irregular and leaf-like, which are only partially transformed, while unorganized tumors are completely transformed.

Disease Cycle

The pathogen overwinters or oversummers in the soil. There are several hosts. All the root stocks used for the propogation of the temperate fruit plants except *Cotoneaster bacillaris*, *C. microphylla*, and *Malus baccata* were found to be highly susceptible to crown gall (Durgapal, 1977).

Disease Management

For controlling the disease destruction of the infected plant material, adoption of rigorous sanitation in the nursery, rotation of nursery site, use of well pitted and properly wrapped (with antiseptic tape) grafts, avoidance of highly susceptible rootstocks, and preference of budding over grafting are recommended. However, the most effective and cheap method of controlling the disease is by dipping the seeds, cutting and roots of young plants before transplanting, in a suspension of *A. radiobacter* strain K84. This strain is antagonistic to most virulent strains of *A. tumefaciens*. It is postulated that the antagonistic strain (*A. radiobacter* K84) produces the bacteriocin agrosin 84, which is inhibitory to most virulent strains of *A. tumefaciens*. Unfortunately, some strains of *A. tumefaciens* inherited resistance to agrosin 84, from strain K84. Therefore, a new strain K-1026 is now recommended for control of the disease. The strain K-1026 lacks the ability to transfer its resistance to pathogenic *Agrobacterium* strains. There is an immediate need to test these strains under Indian conditions (Jindal, 1990).

Other antagonists reported for biological control of crown gall in temeperate fruits are *Bacillus subtilis* and *Streptomyces* spp. Gupta and Khosla (2007) observed antagonistic activity of *Bacillus subtilis* against *A. tumefaciens*. Al-momani *et al.* (1999) have reported inhibitory activity of *Streptomyces* spp., isolated from the temperate fruit orchards soil against *A. tumefaciens* infected almond trees.

According to Pathak *et.al* (1996), painting with the chemical Elgetol (Sodium dinitro-o-cresylate) helps considerably in protecting the plants from infection. Avoiding wounding the crown and control of root-chewing insects is also recommended.

Stalk Rot of Maize

Rosen first reported this disease in 1922 in the USA, and named it *Pseudomonas dissolvens*. Since then, it has been known to occur in South Rhodesia, Israel, Greece, and South Africa. In India, Prasad (1930) reported the occurrence of the disease in Pusa, Bihar. It is considered to be a major disease and is prevalent in Himachal Pradesh, Punjab, Delhi, Uttar Pradesh, Bihar, West Bengal, Rajasthan, and Andhra Pradesh. Hingorani *et al.* (1959) identified the pathogen as *Erwinia carotovora* f. sp. *zeae* Sabet. Thind and Payak (1985) have reviewed the work on this disease.

Symptoms

First, the basal portion of the leaves rots. The basal internodes become soft and discolored (Fig. 24.18). The stalk becomes soft and pliable and the affected plants topple down rapidly. Ear shoots and cobs occasionally get infected, which then droop down and hang limply (Fig. 24.18).

Causal Organism

The disease is caused by *Erwinia chrysanthemi* pv. *zeae* (Sabet). Victoria *et al.* Earlier, the pathogen was known as *Erwinia carotovora* f. sp. *zeae*. *E. dissolvens,* and *Pseudomonas syringae* pv. *lapsa* are also reported to be associated with stalk rot of maize. According to Sabet (1954) and Sabet *et al.* (1964), the maize stalk rot organism is a typical soft rot bacterium. However, only maize isolates can attack maize and onion, *E. carotovora* isolates do not infect maize. For this reason, the variety *zeae* has been retained to indicate its specificity. Phages specific to this pathogen occur in nature (Thind, 1970).

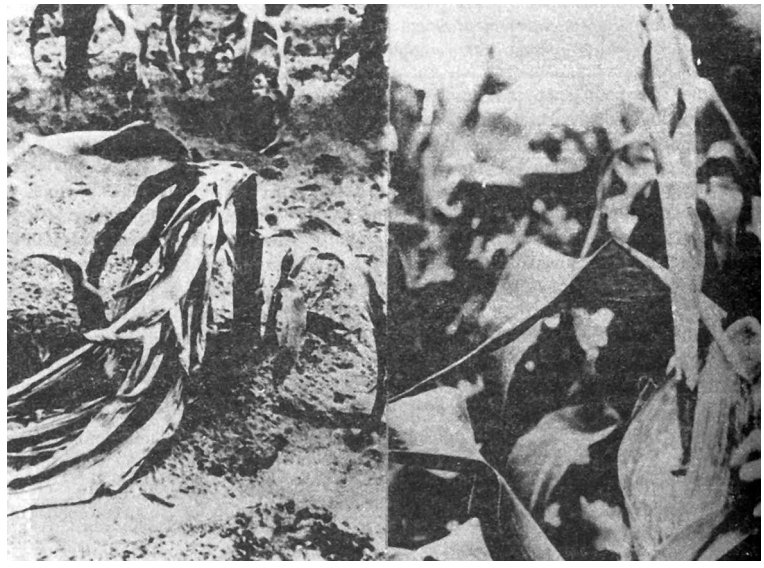


Figure 24.18 Stalk rot of maize caused by *Erwinia chrysanthemi* pv. *zeae* (*Erwinia carotovora* pv. *zeae*)

Disease Cycle

The organism is soil-borne. Injuries and wounds serve as avenues to enter the host. The isolates of stalk-rot pathogen considerably affect the germination of seeds (Rangarajan and Chakravarti, 1970). All the varieties tested were found to be susceptible to stalk-rot pathogen.

Disease Management

Agricultural antibiotic combinations such as Streptocycline and Agrimycin 100, as well as bleaching powder were found to be effective in controlling the disease (Chakravarti and Rangarajan, 1966; Thind, 1970). Lal *et al.* (1970) reported reduced disease incidence with chlorinated water (100 ppm). Water-logging in the field and dense planting should be avoided.

Common Scab of Potato

Distribution

This disease occurs throughout the world. It is most prevalent and important in neutral or slightly alkaline soil and light sandy soils. In India, since 1976, the disease has become widespread in the plains of Uttar Pradesh, Haryana, and Punjab (Nagaich, 1983). In India, the disease has been reported from U.P., Assam, Meghalaya, West Bengal, Lahaul Valley of H.P. and the plains of Bihar, Punjab, and Maharashtra. The losses due to this disease can be upto 40%. The greatest loss from the disease is the reduction in market value of tubers due to rough and blemished skin, and deep scabs increase the waste in peeling. The disease was earlier considered to be of minor importance.

Symptoms

The symptoms of common scab of potato are seen mostly on tubers. There are two types of lesions, the shallow and the deep scab formed on the tubers. In shallow scab, the affected tubers show superficial roughened areas, sometimes raised above and often slightly below the plane of the healthy skin. The lesions consist of corky tissue, which arises from the tuber periderm. The lesions vary widely in size and shape, sometimes darker than the healthy skin. In deep scab, the lesions measure 1-3 mm or more in depth and are darker than the shallow scab.

Causal Organism

Streptomyces scabies causes common scab of potato. The vegetative form consists of slender, branched, aerial, grey-colored mycelium with few or no cross walls. The conidiophores are branched, septate, spirally coiled with terminal branches. Segmentation of these hyphae forms spores (conidia) in chains, which break loose and are disseminated. Spores are smooth-walled, cylindrical or barrel-shaped, about 0.6×1.5 mm in size. These spores germinate by means of one or two germ tubes, which develop into the myceloid form.

Disease Cycle

The pathogen is spread through soil water, wind-blown soil, and on infected potato seed tubers. However, infected tubers play the main role of its distribution over larger areas. The bacterium can survive through the digestive tract of animals and can be disseminated by manure. The pathogen penetrates tissue through lenticels, wounds, stomata, and in young tubers directly. After penetration, the pathogen grows between or through a few layers of cells, the cells die, and the pathogen then lives off as a saprophyte. In the meantime, the pathogen secretes a substance that stimulates the living cells surrounding the lesion to divide rapidly and to produce several layers of cork cells that isolate the pathogen and several plant cells. And the cells that are cut off by the cork layer die, the pathogen subsists on them. Usually, several such groups of cork cells are produced and as they are pushed outward and sloughed off, the pathogen grows and multiplies in the additional dead cells and thereby large scab lesions develop.

Various soil factors influence the severity of the disease. *S. scabies* is most active in dry soil. Disease is significantly decreased when soil pH is brought down to below 5.2; within the pH range of 5.2 to 8.0, the severity of the disease increases with pH. Disease can develop in a temperature range of 11-30.5°C.

Disease Management

1. Control of the common scab of potato is through the use of certified scab-free seed potatoes.
2. Tubers with deep lesions should not be used for seed.
3. Dipping the tubers for five minutes in a 0.25% suspension for five minutes can disinfect them.
4. Using certain crop rotation methods and bringing the soil pH to about 5.3 with sulphur might help in obtaining a fair degree of disease control. Rotational crops such as maize, cotton, sorghum, wheat, cabbage and onion have been observed to reduce incidence of common scab.
5. Lapwood and Hering (1970) controlled scab in field plots by maintaining soil moisture near field capacity during tuber formation, until all the internodes of the tubers are formed. Thus, maintaining the soil moisture at field capacity from the fifth week, after planting, upto the ninth week, by frequent light irrigation is recommended. In India, Singh and Singh (1982) have recommended

maintaining the available soil moisture at 75% and above from tuber initiation to maturity, to reduce tuber infection.

6. Green manuring and cover crops of soybean promote activity of antagonistic bacteria. Therefore, in infested fields, green manuring before planting or cultivation of potato may be useful. It may be mentioned that Millard and Taylor (1927) reported control of scab of potatoes grown in sterilized soil and inoculated with *S. scabies* through simultaneous inoculation with *S. praecox*, a saprophytic species that is antagonistic to pathogenic *S. scabies.* The disease was reduced by incorporation of rapeseed meal into the soil and by adding a mixture of *T. viride* isolates in furrow at planting (Hilton *et al*., 2006).
7. Soil solarization with transparent polyethylene mulching during hot summer season was found effective for control of russet scab. The disease severity by soil solarization reduced to almost one-third compared to the unsolarized plots (Arora, 2005).
8. Non-pathogenic, antibiotic producing *Streptomyces* spp. have been reported to reduce severity of potato scab when added to disease-conducive soil (Hiltunen *et al*., 2009). *Bacillus subtilis*, non-pathogenic *Streptomyces* spp. and biopesticides such as *Geranium pretense* have been reported to control common scab. *Pseudomonas* strain R1, when applied with vermicompost, has been reported to show reduction of common scab (Singhai *et al.,* 2011).
9. Resistance has been discovered in *S. andigena* × *S. tuberosum* hybrids and in red tuber varieties like Kufri Sindhuri (Nagaich, 1983). Thus, using resistant or tolerant potato varieties can control the disease.

Ratoon Stunting Disease (RSD) of Sugarcane

In 1978, Hughes stated that ratoon-stunting disease is one of the most important diseases of the sugarcane industry. It is found in almost all the sugarcane-cultivating regions of the world. For many years, a virus was believed to be the cause of this disease. But now, it is established that it is caused by a bacterium. In India, Professor S.J.P. Chilton first observed this disease in COS 510 at Golakokarannath (Uttar Pradesh), in 1956 (Chona, 1956). Later on, Rafay (1957) suspected it in variety Co 738.

Ratoon-stunting does not have well-described external symptoms. Stunting, though present in many cultivars, is absent in others. In mature diseased canes of many cultivars, there is a reddish discoloration of the vascular bundles at the level of nodes. Typical symptoms are seen only when the canes are split open longitudinally. The disease is present in both plant and ratoon crops, although the effect has been more pronounced in ratoon crops. Affected crops look stunted with less vegetative growth in spite of all the inputs and proper agronomical practices. The disease, although present in the stalks, is not expressed under good-growing conditions in many instances. Such suspected canes, if split open longitudinally, show pinhead-like pink colored spots at the nodes, and most towards the upper nodes.

Causal Organism

For many years, a virus was believed to be the cause of ratoon-stunting, as the disease had many characteristics of viral diseases. Ratoon-stunting disease caused by the xylem-inhabiting, slow-growing, recently named *Leifsonia xyli* subsp. *xyli* is one of the most important sugarcane diseases in world. Work by Gillaspie *et al*. (1973), Maramorosch *et al*. (1973) and Teakie *et al.* (1973) indicated that a bacterium of the coryneform group might be the causal agent. Subsequently, the causal organism was cultivated

on artificial media by Davis *et al*. (1980) and Lio and Chen (1981). Gillaspie *et al.* (1989) have given a comprehensive account of the disease. In culture, the bacterium is rod-shaped, 0.25-0.35 × 2-5 µm, Gram-positive, non-motile, lacking endospores, aerobic and of club-like or V-form morphology. Davis *et al.* (1980) have named the pathogen *Clavibacter xyli* subsp. *xyli*. The bacterium is filamentous and the length of the filaments may be 10 microns or even more. The cells may be straight, bent, curved and/or swollen at the tip or in the middle.

The bacterium can be observed in nodes, internodes, leaves, young tillers, and also in tissues just below the growing point. This gives an indication that infection is systemic. Currently, serological detection of the pathogen is a common and reliable method by which the disease can be diagnosed easily.

Disease Cycle

The pathogen is found in all parts of the sugarcane plant and is easily transmitted by mechanical means. Juice from diseased plants at dilutions of 1/25,000 is infectious. Cutting knives used for preparing cuttings or setts (seeds) and cutter-planting machines transmit the bacterium readily. Man is the primary agent in spreading the bacterium. Rats have been shown to transmit the bacterium by chewing. The pathogen does not survive in the soil; there is no evidence of transmission of the pathogen through true seeds. No insect vector is known to transmit the bacterium. A number of grass hosts have been found infected by experimental inoculation. Elephant grass (*Pennisetum purpureum*) is a very useful indicator plant. The role of alternate/collateral hosts in the epidemiology of the disease is not clear.

Disease Management

The control measures involve healthy planting material, preventing disease spread in the field and the use of resistant cutivars. Canes meant for planting should be treated either by hot water (50°C for 2 hours), aerated steam (53°C for 4 hours) or hot air (54°C for 8 hours). Afterwards, these clean canes are planted and maintained disease-free. Seed setts should be taken from robust plants only and ratooning of weak or stunted crop should be completely avoided.

Relatively little work has been done on breeding for resistance to ratoon-stunting disease.

Citrus Greening Disease (CGD)

The disease is one of the most important, widespread diseases in India (Fraser *et al.*, 1966; Nariani *et al.*, 1973). The disease has been reported from Jammu and Kashmir, Punjab, Haryana, Rajasthan, Uttar Pradesh, Madhya Pradesh, and Karnataka. The disease has completely wiped out the citrus trees from the Coorg region. According to Kapur (1992), tristeza and greening are wide spread. Greening is more serious in Punjab, followed by Assam, where the incidence of the disease is 64.8 and 41.1% respectively. According to Chohan and Knorr (1970), the greening disease may be more devastating than tristeza in India due to its widespread occurrence. When tristeza and greening occur together, the two bring about citrus die-back and quick decline in trees (Martinez *et al.*, 1970).

Symptoms

Citrus greening is characterized by yellow type of symptoms, which are highly variable. There is yellowing in the mid rib and lateral veins of mature leaves. Young leaves of infected plants are mottled and chlorotic as though they have been affected by zinc deficiency, the yellow tissues of the leaf lamina

show scattered green island. Since chlorosis can be caused by mineral deficiency of zinc, mironutrient spray temporarily halts or masks the symptoms of greening. A characteristic symptom is that the yellow areas are delimited by the midrib and expand towards the leaf margins. Soon after a tree becomes infected with citrus greening, tree growth slows down, trees become stunted, and twigs and branches die-back. But the trees do not die and in some cases trees may appear to recover. Leaf size is reduced; leaves become thicker and usually remain more erect. The internodes and branches are shortened and the branch usually gives a bushy appearance. Fruits of the affected trees are smaller. The diseased trees flower earlier. The root number is considerably reduced.

Causal Organism

Citrus greening disease was first reported by Reinking in 1991 by the name Huanglongbing, meaning 'yellow shoot'. The pathogen is a phloem-limited, uncultivable, Gram-negative bacterium. Initial reports suggested it was a virus but later on, it was considered a mycoplasma organism. This pathogen was defined as a Gram-negative bacterium in 1984 by Garnier *et al.* (1984) and was further characterized by Jagoueix *et al.* (1996). The genus is *Liberobacter* from Asia and Africa and two geographically distinct *candidatus* species, *L. candidatus asiaticum* and *L. candidatus africanum* are widely distributed (Jagoueix *et al.,* 1996). The size of the bacterium ranges from 350-550 × 600-1500 nm and its thickness is 20-25 nm. They are generally rigid, rod-like and are pleomorphic bodies during growth.

Earlier, a virus was considered to be the cause of the disease. Later, a mycoplasma-like organism was suspected to be involoved. It was in 1970 that Lefleche and Bove reported that the disease is caused by a phloem-inhabiting fastidious bacterium with a double-membraned cell wall, distinct from MLO's cells. Since the causal organism has not been cultured in the laboratory so far, its characterization has not been possible. The cells of the phloem-restricted fasidious bacteria are Gram-negative, non-motile, non-pleomorphic, rigid, and rod-shaped measuring 1.10-2.0 µm. The bacterial cells occur in mature sieve elements and are irregularly distributed among vascular bundles.

The presence of the bacterium can be confirmed by indexing either in the immunofluorescence tests using greening specific antibodies or Southern blotting using cDNA probes or by ultrathin sectioning using an electron microscope. The causal agent of Huanglongbing (HLB) or citrus greening disease was deducted by the total DNA isolated from the midrib or the leaves of citrus trees by PCR amplification of ribosomal protein gene by Baranwal *et al*. (2004).

Disease Cycle

The cirtus greening organism is transmitted through vegetative propagation and by two species of citrus psylla, *Diaphorina citri* and *Trioza erytreae*. In South Africa, electron microscopic studies have confirmed the role of *T. erytreae* in transmission of the organism (Moll and Martin, 1973). In India, these two vectors are more common in North and Central India than in other regions of the country. The vector once infected remains viruliferous throughout its life. Gamier and Bove (1983) have demonstrated the transmission of the bacterium from sweet orange to *Vinca rosea* (periwinkle), *Cuscuta* or dodder plant.

Disease Management

The disease can be controlled by a combination of the following measures:

1. Removal of diseased plants from the orchards.
2. New plant should be raised from indexed stock or nuceller seedlings.

3. Spray Diazinon (0.02%), Eldrin (0.02%) or Parathion (0.02%) to control the insect vectors.
4. Spray mixture of Bavistin and Ledermycin (500 ppm each) six times at 10-day intervals (Kapoor and Cheema, 1983; Cheema *et al.,* 1985). Tetracycline hydrochloride showed good results in reducing leaf symptoms.
5. Thermotherapy is another approach found to be very effective in the control of citrus greening. Graftwood is heated to 48-50°C for several minutes for complete elimination of the pathogen.
6. Attempts have been made to control the vectors through different approaches, for example, by means of chemical, cultural or biological measures. Systemic insecticides like Dimethoate and Monocrotophos to eliminate *Diaphorina citri*. Methomyl or Melathion sprays on citrus trees at 10-12 days intervals were not effective against citrus greening.
7. Biological control has been tried in India. *Tetrastichus radiata* is reported to be drastically reducing the psylla population (Singh, R.S., 2007)

Angular Leaf Spot and Wildfire of Tobacco

Distribution

The disease is quite prevalent in all parts of the world where tobacco is grown. This disease comes year after year in some areas and is very destructive, whereas in others, it appears sporadically. The pathogen attacks other hosts such as bean and soybean. Angular leaf spot disease occurs in Karnataka in India, and is one of the worst diseases of tobacco in the United States of America.

Symptoms

Characteristic symptoms of the leaf spots may occur at any stage of plant growth, including nursery seedlings. In the nursery beds and in young seedlings, two to three dark spots with a yellow halo may be found. In the field, the spots are small, angular and black or dark brown in color but frequently develop into larger lesions. In wet weather, the disease spreads so wildly and rapidly that this phase of the disease is called wildfire of tobacco.

Causal Organisms

Angular leafspot of cotton was earlier belived to be caused by *Pseudomonas angulata* and wildfire disease by *P. tabaci,* which has been renamed *P. syringae* pv. *tabaci*. Strains of *P. syringae* pv. *tabaci* produce mutants that have lost the ability to produce the toxin (Tox^-). Toxin producing strains produce a pathotoxin tabtoxinine, which is the active moiety. The toxin inactivates glutamine synthetase activity, which leads to depleted glutamine levels and as a consequence, accumulation of toxic concentrations of ammonia. The toxin destroys the thylakoid membranes of the chloroplasts, thereby causing chlorosis and ultimately necrosis. Thus, it is increasingly clear that both angular leaf spot of tobacco and wildfire disease are caused by the same bacterium, *P. syringae* pv. *tabaci*. The one producing the toxin causes wildfire symptoms, and the one lacking the capacity to produce the toxin causes the angular leaf spot symptoms.

Disease Cycle

The pathogen spreads in the field through air-borne infections. The bacteria responsible for primary infection are most likely harboured in the plant debris. The method of perpetuation of the disease is not

properly understood. Wet weather accompanied by strong winds cause heavy splashing of water, due to which bacteria are disseminated. Bacteria are usually carried to the field on the roots of seedlings at the time of transplanting and cause the disease later on.

Disease Management

The site of nursery should be changed every year and the refuse of tobacco of the previous years should be removed. Seedlings showing infection on the leaves should not be used for transplanting. As the causal organism is likely to be carried through the seed, seed treatment with Silver nitrate (1: 1000) would give some protection. The seed-bed soil should be sterilized before sowing. After seedlings emerge, seedbeds should be sprayed with Copper fungicide and Streptomycin.

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Questions

1. What are procaryotes and eucaryotes?
2. Name some important genera of phytopathogenic bacteria. How will you differentiate them at generic level?

3. What is Gram stain? Give examples of Gram-positive and Gram-negative bacteria causing plant diseases.
4. How will you differentiate plant pathogenic bacteria?
5. How will you control the following diseases?
   (i) Bacterial blight of paddy
   (ii) Citrus canker
   (iii) Bacterial Blight and Angular leaf spot of cotton
   (iv) Black rot of crucifers
   (v) Brown rot of potato
   (vi) Yellow ear rot or tundu disease of wheat
   (vii) Blackleg and soft rot of potato
   (viii) Crown gall of stone fruits
   (ix) Stalk rot of maize
6. What are the different antibiotics used for controlling plant bacterial diseases?

Viruses, Viroids and Their Diseases

25

Plant Virsues and Plant Viral Diseases

Viral diseases of plants, although not recognized as such, were known long before the discovery of bacteria. Breaking of flower colour of tulips was mentioned as early as 1576. Transmission of leaf variegation from the scion to the stock of woody plants was recorded as early as 1700. By 1870, potatoes were known to degenerate in some parts of Europe. Anderson (1778) showed it to be transmitted with the seed tuber and likened the disease to small pox in nature in its infectiveness. Peach yellows were recognized as early as 1791 in the eastern USA. Swieten identified tobacco mosaic in Holland in 1857. German-born Adolph Mayer (1886), a Dutch investigator in Holland, was the first to point out that tobacco mosaic is readily transmissible and infectious. He showed that the juice of the infected plants, if applied to healthy plants, could cause the disease. He could not see or isolate any bacteria in the clarified sap. But he still believed that bacteria are the cause of the disease.

Ancient Romans used the word *virus*, to mean poison, venom or secretion – all unpleasant meanings. When the field of bacteriology began to flourish, the medical use of the term *virus* implied any microscopic etiologic agent of disease. The field of virology really began to develop after the invention of bacterial filters by Charles Chamberland in the year 1884.

Dimitri Ivanowski (1982), a Russian botanist made a most remarkable discovery. He was also involved in the task of investigating mosaic disease of tobacco. He was specifically trying to find out the nature of the agent causing the disease. He carried out most of Mayer's experiments and confirmed some of the results. For example, he also found that the sap obtained from the leaves of infected plants was capable of causing fresh infection in healthy plants. He also demonstrated that the power to infect was lost if the sap was previously heated. He, however, strongly refuted Mayer's observation that the capacity to infect was lost when the sap was passed through a pad of filter papers. He reported that infectiousness was retained even when the sap was passed through more sophisticated filters (bacteriological filters) small enough to retain bacteria. He called these agents filterable, which indirectly meant that they were of a different nature. However, Ivanowski could not think of a different type of organism altogether. He simply hinted at the possible presence of bacteria smaller than they were supposed to be. It was M.W. Beijerinck, a distinguished Dutch microbiologist, Professor of Microbiology at the Technical University of Delft, who got interested in these filterable agents causing the mosaic disease of tobacco. He demonstrated in 1896 that the tobacco mosaic infectious agent could diffuse through an agar membrane and

concluded that the tobacco mosaic was caused by a non-corpuscular 'contagium vivum fluidum', which he called virus.

Despite all these developments, however, the controversy regarding these agents persisted and branched into a new direction. In the year, Beijerinck proposed the new concept, two German Scientists, Loeffler and Frosch, suggested that these filterable agents were particles, perhaps of a new type. They were searching for the causal agent of foot and mouth disease of cattle during these years. Beijerinck was opposed to their idea. He observed that he "cannot agree with Mr. Loeffler as regards the corpuscular nature of the virus of foot and mouth disease". This sort of uncertainty regarding the nature of the filterable agents was to continue for more than thirty years. Gibbs and Harrison (1976) likened the search for viruses in plant extracts even in the early 1930s to trying to find a black cat in a dark cellar without being certain of its presence. This was true in spite of the fact that many more diseases of plants, animals and human beings could be traced to such agents. Baur (1904) showed that the infectious variegation of *Abutilon* could be transmitted by grafting but not by mechanical inoculation. Beijerinck and Baur used the term 'viruses in describing the causative agents of these diseases to distinguish them from bacteria. Earlier, workers used the term 'virus', almost synonymously with bacteria. As more and more diseases of this sort were discovered, the unknown causative agents came to be called 'filterable viruses'.

The discovery of the relationship between viruses and insects was not made in a day, and a number of years elapsed between the time when insects were first suspected of transmitting plant viruses and the actual demonstration of this method of transmission. In 1894, a Japanese farmer, Hashimoto, was the first to prove the relationship between an insect and a virus. He showed that the leafhopper, *Nephotettix apicalis* var. *cincticeps* is connected with the dwarf disease of rice. However, this was not known to the western world. Around 1907, three workers in America – Ball, Adams and Shaw – suggested that there was some connection between curly top and the leafhopper, *Eutettix tenella.* In 1926, the first enzyme was isolated, crystallized and identified as a protein. The isolation of others soon followed. In the early 1930s, workers in various countries began attempting to isolate and purify plant viruses, using methods similar to those that had been used for enzyme assays. Following detailed chemical studies suggesting the infectious agent of TMV might be a protein, Stanley (1935) announced the isolation of this virus in a crystalline state. At first, Stanley (1935, 1936), working in the USA, considered that the virus was a globulin containing no phosphorus. For this discovery, he was later awarded the Nobel Prize. But Stanley overlooked the essential component of TMV. Bawden and Pirie (1936), working in Britain, described the isolation from TMV-infected plants of a liquid crystalline nucleoprotein containing nucleic acid of the pentose type. They showed that the particles were rod-shaped, thus confirming the earlier suggestion of Takahashi and Rawlins (1932), based on the observation that a solution containing TMV showed anisotropy of flow. Bernal and Fankuchen (1937) applied the technique of X-ray analysis to the purified preparations. They obtained accurate estimates of the width of the rods and showed that the needle-shaped bodies produced by precipitating the virus with salt were regularly assayed on only two dimensions and, therefore, were better described as paracystals than as true crystals. The isolation of other rod-shaped viruses and spherical viruses, which formed these crystals so on followed. All were shown to consist of protein and pentose acid.

Electron microscopy and X-ray crystallography have been the major techniques used to explore the structure of virus particles. Early electron micrographs confirmed that TMV was rod-shaped and provided approximate dimensions, but the micrographs were not particularly revealing because of lack of contrast between the virus particles and the supporting membrane. The development of shadow casting

with heavy metals greatly increased the usefulness of the method for determining the overall size and shape of the virus particles. High-resolution microscopy and negative staining in the 1950s became important tools for studying viruses.

Until about 1948, attention was focused on the protein part of the viruses. There are probably several reasons for this. Quantitatively, protein constitutes the large part of the virus particle. Markham and Smith (1949) isolated TYMV and showed that purified preparations contained two classes of particles – an infectious nucleoprotein with about 35% of RNA, and an identical protein particle which contained no RNA and which was not infectious. The results clearly indicated that the RNA of the virus was important for biological activity.

Experiments conducted by Hershey and Chase (1952) showed that when *Escherichia coli* was infected by a bacterial virus, the viral DNA entered the host cell, while most of the protein remained outside, which emphasized the importance of nucleic acids in viral replication.

However, the classic experiments of Gierer and Schramm (1956), Fraenkel-Conrat and Williams (1955), and Fraenkel-Conrat (1956) demonstrated the infectivity of naked TMV–RNA and the protective role of the protein coat. Subsequent work has shown that for viruses such as TMV, the RNA is present as one strand and a single cleavage in this strand leads to inactivation of the virus particle.

Until a few decades ago, all the plant viruses that had been examined contained single-stranded RNA as their genetic material. It is now known that wound tumour and rice dwarf viruses contain double-stranded RNA, while cauliflower mosaic virus contains double-stranded DNA. Viruses containing single-stranded DNA have also been found (Gemini- viruses and banana bunchy top virus).

During the last few decades, much more information on the relationship of plant viruses with their vectors has been accumulated. It is now clear that in addition to the insects that transmit plant viruses, other organisms such as mites, nematode worms and fungal spores have all been considered vectors. Other advances in the study of plant viruses include increased knowledge of incomplete viruses, the discovery of satellite viruses (Kassanis, 1966) and viroids (Diener and Raymer, 1967).

There are some recent reviews on the role of satellite RNA in the expression of symptoms caused by plant viruses (Collmer and Howell, 1992), and plant viral RNA synthesis in all free system (Graaff and Jaspars, 1994). Matthews (1987) comprehensively discussed the changing scenes in plant virology. Brakke (1988) reviewed perspectives on progress in plant virology. Raychaudhuri (1996), and Varma and Ramachandran (1994) have reviewed the development of plant virology in India

There has been an enormous progress in the field of virology, especially in the 90's, in the molecular aspects/virus taxonomy. The frontier areas, where great breakthroughs were made, include virus movement/spread, viral genome and its expression, advances in detection, identification of viral genes and genes for resistance and their utilization for the development of transgenics, gene silencing, use of viruses as vectors for genes and so on.

Kassanis (1962) was the first to describe the satellite virus (SV), which has been found only in association with tobacco necrosis virus (TNV), the latter being the activator. TNV is often formed alone and multiplies indefinitely in plants without the production of SV, which has to be introduced as such. SV is a very small virus measuring only 17 nm in diameter, it has an RNA content of 20% by weight and its maximum molecular weight is 340,000 Dalton. Both viruses occur in the roots of normal plants and both are transmitted by the zoospores of *Olpidium brassicae*. SV cannot multiply by itself. TNV alone can multiply indefinitely without causing the production of SV and the two viruses are anti-genetically unrelated and the activator does not influence their antigenicity. The antigenicity depends on the strain of SV used in the inoculation, which shows that its own RNA codes the protein of SV (Kassanis, 1964,

1966). Since SV codes for its own protein, the help that is needed by SV from the activator is not known at present. Dodds (1998) had reviewed the work done on satellite tobacco mosaic virus.

Zaitlin and Palukaitis (2000), have divided plant virus research into "Classical Discovery Period" from 1883-1951, "Early Molecular Era" from 1952-1983, and "Recent Period" from 1983 to the present era, when techniques have been developed to modify plant virus genomes to detect non-structural gene products, to determine the functions of viral gene products and to transform plants to elicit novel forms of resistance to viral diseases. In this period, plant virology has played a significant role in formulating and understanding the mechanisms of gene silencing and recombination, plasmodesmatal function, systemic acquired resistance and in developing methods for pathogen detection. The term 'viroid' had been introduced to denote a newly recognized class of sub-viral plant pathogens (Diener, 1971). The viroids currently known consist solely of a short strand of RNA with a molecular weight of about 75,000 – 12,50,000 Dalton. The introduction of this RNA of low molecular weight into susceptible hosts leads to replication of RNA, and in some hosts to disease. The first viroids came to light during efforts to purify and characterize the agent of potato spindle tuber disease (PSTV), a disease, which, for many years, had been assumed to be of viral etiology (Diener and Raymer, 1971). Diener and Raymer (1967) reported that the infective agent of this disease is a free RNA and that virus particles apparently are not present in the infected tissues. Later sedimentation and gel electrophoretic analyses conclusively demonstrated that the infectious RNA has a low molecular weight (Diener, 1971) and that the agent, therefore, differs basically from conventional viruses. Low molecular weight RNA that is viroids, are also known to cause citrus exocortis (Semancik and Weathers, 1968) and cucumber pale fruit (Van Dorst and Peters, 1974). The recognition of viroids as a newly identified class of pathogens raises several questions that have potential important implications for microbiology, molecular biology and plant pathology as well as for veterinary and human medicine. Cadang-cadang (dying) disease of coconut and other palms had been found to be of viroidal origin.

To conclude this introductory section, some definitions of a virus are appended. According to Lwoff and Tournier (1966), the following characteristics separate viruses from other agents:

1. Only one type of nucleic acid – either DNA or RNA – is present in virions, other agents possess both types of nucleic acids.
2. Virions are reproduced from their sole nucleic acid – either DNA or RNA - whereas other agents reproduce from the integrated sum of their constituents.
3. Virions are incapable of growing or undergoing binary fission.
4. The genetic information for the synthesis of Lipman System (the system responsible for the production of energy with high potential) is absent in viruses.
5. Viruses make use of the ribosomes of their host cells. Bawden (1964) defined viruses as submicroscopic, infective entities that multiply only intracellularly and are potentially pathogenic. On the other hand, Hahon (1964) defined viruses as bits of infectious heredity in search of a chromosome.

Symptoms of Viral Infections in Plants

Viral diseases of plants exhibit a variety of symptoms. The most common is the mosaic symptom. Interspersion of various degrees of chlorosis with the normal green colour of the leaf results in a mosaic with yellow and green. This results in a mosaic pattern. This is the most common symptom encountered

in viral infections and the viral diseases with this type of common symptom are popularly called mosaic diseases. On the contrary, when there is a uniform reduction of chlorophyll, the symptom is referred to as the yellows type. It should, however, be emphasized that these are now believed to be caused by mycoplasmas rather than viruses. Some of the other symptoms due to viral diseases are as follows.

Vein Clearing and Vein Banding

These commonly appear in systemically infected leaves before mottle or mosaic clearing or chlorosis of the tissue in or immediately adjacent to the veins. This pattern is referred to as vein clearing. Vein banding consists of broader bands of green tissue in that portion, set off by chlorosis or necrosis in the interveinal parenchyma. Vein clearing or vein banding may be transient and precede mottle or it may remain as the major symptom of the disease concerned, for example, potato vein banding disease on tobacco.

Ring Spots

On inoculated or systemically infected leaves, the symptoms appear in localized spots. These spots consist of various types of chlorosis and necrosis. They may be circular chlorotic areas, in which case they are called chlorotic ring spots. In other cases, necrosis may appear in rings alternating with normal green. Such spots are called necrotic ring spots. The centers of either type of spot may eventually also become necrotic. Ring spots are formed due to tobacco ring spot virus on *Nicotiana rustica.*

Necrosis

In diseases of both the yellow and mosaic groups, necrosis appears in various forms. The most drastic effect of viruses on their host plants, though not the commonest one, is to kill cells, tissues or the whole plant. Necrosis is not confined to chlorophyll-containing tissues. Chlorophyll-less leaves, hypocotyls or even roots can develop necrotic lesions similar to those in green leaves.

Wilt

In certain virus-infected plants, the outer whorls of leaves droop and become yellow and the inner whorls also become pale yellow in colour. The leaflets soon turn brown in color and start drying up from their tips.

The leaves start shedding in quick succession after a plant is infected. The leaves become smaller and stunted in size. There is also considerable reduction in root growth.

Stunting

It may affect all parts of a plant, involving a reduction in the size of the leaves, flowers and fruits, and a shortening of the petioles and internodes; for example, bean yellow mosaic virus on bean. The extent of stunting varies according to the time during which the infection occurs in the developmental stage of the plant. Sometimes, some parts of plants may be more stunted than those same parts in other plants.

Changes in Leaf Form

In mosaic diseases, there is often uneven growth of leaf lamina. Leaves become curled, brittle (crinkling) and show prominences and depressions (puckering). In some cases, there may be upward and

inward rolling of the leaf, such as in potato leaf roll. In certain other cases, the lamina gets extremely reduced, giving a 'fern leaf' effect (filiform) or shoe-string symptoms, such as TMV or CMV on tomato. In cherry leaves, the lamina develops perforations, giving a 'tatter leaf' appearance.

Production of Outgrowths

Various types of abnormal growths and malformations are common in many viral infections. Foliar outgrowths and vein swellings are generally formed on the underside of the leaves of infected plants, for example, tobacco leaf curl disease, Fiji disease of sugarcane, etc.

Tumours or swellings are formed on stems and roots in the case of sweet clover (*Melilotus* spp.) infected with wound tumor virus, cocoa infected with swollen shoot virus and woody gall of citrus. Less commonly, the stem is affected, for example, grape vine fan leaf virus is responsible for fasciation in grape vine stems.

Flower Breaking

Variegation in flower color due to viral infections is very common. This is called breaking of flower colour, for example, tulip breaking. Similar symptoms are produced by turnip mosaic virus in wall flower (*Cheiranthus cheiri)*.

Phyllody, Sterility and Witches' Broom

These symptoms are characteristic of yellows type of diseases, most of which are now related to mycoplasmal infections. The most characteristic symptom is the virescence or greening of floral parts. The axillary buds are stimulated to grow and these give a bushy appearance to the plants, as in Sesamum phyllody. Flowering and seed production may be decreased or even stopped. In the case of broad bean (*Vicia faba*), infected with bean leaf roll virus, all the flowers abscess, no more are produced and the ovules already fertilized produce small seeds.

Fruit Abnormalities

A variety of symptoms may develop on the fruits in certain viral infections; for example mottling on cucumber and papaya due to mosaic type of viruses and deformed fruits in apples and plums due to plum pox virus.

Internal Symptoms

The detailed structure of virus-infected plants has been much studied, initially using the light microscope and then more recently, by phase contrast and electron microscope. There are several review articles on this subject. Some important reviews are by Bald (1966a): Some cell reactions during plants virus synthesis seen under the light microscope; Bald (1966a): Cytology of plant virus infections; Esau (1967): Anatomy of plant virus infections; Diener (1963): Physiology of virus infected plants; Matsui and Yamaguchi (1966): Some aspects of plant viruses *in situ*; and De Zoeten (1976): Cytology of virus infection and virus transport. Hull (1989) reviewed movement of viruses in plants.

Several cytological and histological abnormalities can be observed within the virus-affected plants. The histology deviations, although especially seen in tissues, are actually of purely cytological origin.

The effects of hyperplasia, such as tumours or enations, and necrotic reactions are of shown in the histology of the plant, but other changes can also be seen. Many viruses affect the vascular tissues. The xylem elements of grape vine, infected with grape vine fan leaf virus, contain characteristic lignified strands, which are known as endocellular cordons (these are uncommon in virus-free plants). Tyloses are formed in the xylem elements in the barley yellow dwarf and other leaf yellowing viruses. The phloem cells degenerate or die and callose deposition occurs on the phloem sieve plates.

According to Esau (1967), the phloem tissue degenerates followed by hyperplasia of phloem and xylem, and supernumerary sieve tubes are produced in sugar beet, tobacco and tomato plants infected with curly top virus. In the cacao swollen shoot virus disease, the swellings are the result of over-production of xylem elements, whereas in the wound tumours caused by wound tumour virus, the tumour is the result of excessive and abnormal meristematic activity of phloem parenchyma cells.

When the fine structure of the infected cell is studied, many alterations are seen. Some viruses affect particular organelles. Tymovirus induces the formation of marginal vesicles in the chloroplasts. Some isolates of tobacco rattle virus greatly modify mitochondria and aggregate them to form the so-called inclusion bodies. The most characteristics change is the presence of virus particles. These are usually confined to the cytoplasm but they may also be found in other parts of the cell. The particles of TMV are only found in the cytoplasm whereas those of a strain, U5, can be found in the chloroplasts or nuclei. Particles of some rhabdoviruses are found in the space between the outer and inner nuclear membranes, nucleocapsids of the virus are found in the nucleus and it appears that they acquire their outer membranes when they bud through the inner nuclear membrane into the perinuclear space.

The virus particles may be scattered in the cytoplasm or they may be aggregated in various ways. Particles of tomato spotted wilt virus are found in membranous bags, those of beet yellows virus occur in bundles, while those of tobacco rattle virus are found packed around mitochondria.

A major cytological effect of virus infection is the development of inclusion bodies, visible by light microscopy in infected cells. Matz (1919), Kunkel (1921) and Smith (1929) were among the first researchers to observe these inclusions and for a time, a controversy raged their nature. One school of thought considered them to be organisms while others thought them to be the side-effects of disordered metabolism. There seems to be little doubt that some of these inclusions are aggregates of virus particles large enough to be seen in the light microscope.

There are two main types of inclusions, crystalline and amorphous; the latter are known to occur in the cytoplasm of infected cells and have bizarre structures in the shape of ‘pin wheels’ and ‘cat-o-nine-tails’. These bodies occur in infections by flexuous rod-shaped viruses of the potato virus Y group. The exact nature of these bodies is not known. They have a very close connection with the endoplasmic reticulum and it is unlikely that they are virus particles.

Generally, the inclusion bodies are most common in the epidermal cells of leaves and stems, but they also occur in roots and flowers and in most tissues, except the phloem sieve elements. McWhorter (1965) has tabulated the various types of inclusions.

The behavior and appearance of infected living cells has been studied under a light microscope. The cytoplasmic streaming has been found to increase after infection by viruses. Chloroplasts in such cells may clump and coalesce, and in the cells around a developing lesion, the nucleoli are often much swollen.

Transmission of Plant Viruses

One of the cardinal characteristics of a virus is the fact that it is infectious. Viral diseases differ from other infections and diseases in one important respect – the causal entity is not visible under the light microscope. Plant viruses are more dependent on the organism for their transfer from infected to healthy plants than any other pathogen. Five important methods are known for the transmission of plant viruses. These are grafting, mechanical transmission, seed transmission, pollen transmission, and vectors.

Graft Transmission

Theoretically, grafting can transmit any virus. The only limiting factor is the tissue compatibility of the stock and scion. Grafting can transmit viral diseases, which are systemic. Graft transmission is generally practiced in experimental virological work. There are several such procedures, such as cleft or wedge grafting, bud grafting approach or in arch grafting, patch or bark grafting and core grafting.

Mechanical Transmission

Transmission by this means comprises of the removal of the plant sap from a diseased plant by artificial or natural means and its transfer to a healthy plant by rubbing or injecting. Most of the basic knowledge of plant viruses has resulted from mechanical inoculation. Earlier, workers used a variety of methods for the mechanical transmission of viruses. Mayer (1886) sucked the juice of diseased plant and injected it into the midribs of healthy plants. Beijerinck (1898) injected juice with a syringe and inserted pieces of dry leaves into the wounds in the stem. Schultz and Folsom (1923) inoculated potatoes by brushing leaves with their fingers and then applying the juice of the virus-affected potato on healthy leaves. The efficiency of the inoculum is greatly improved by the addition an abrasive, such as carborundum powder (silicon carbide), 300-500 mesh or celite, sand or animal charcoal. The abrasive causes slight injury to the cells and thus facilitates the penetrations of the juice. It removes the cuticle and hair and makes the leaf highly susceptible to infection. Yarwood (1955) considers that a brief washing of inoculated leaves immediately after inoculation increases infection, but reduces it if prolonged for more than 20 seconds. There are many modifications and special methods with unstable viruses or those, which are difficult to transmit mechanically. Some plants, which are difficult to infect by these methods, are easily infected when the inoculum containing a fine abrasive is sprayed onto the plant by a pressurized airbrush. This is a relatively quick way of inoculating a large number of plants. The success of mechanical inoculation depends on a number of host factors such as the host plants, inoculum, inhibitors of infections and virus deactivators present in the host plants.

The ease with which a virus is transmitted mechanically usually has a bearing upon the degree of natural spread. Insects, for instance, seldom spread tobacco mosaic virus, but it is readily transmitted by the hands of workmen in the process of removing ‘suckers’ in tobacco and in greenhouse-grown tomatoes. Pickers spread cucumber mosaic virus when they remove green fruit from the plants if their hands or implements are contaminated. Spindle tuber viroid of potato is spread by the knife blades used for cutting seed tubers. Viruses of the mosaic group are, in general, more easily transmitted mechanically. Potato virus X spreads in potato crops by contact, when the leaves of a virus-infected plant brush against those of virus-free plants.

Seed Transmission

It is believed that more than 100 viruses are transmitted through seeds. Seed transmission can occur in two ways: (1) virus particles are attached to the surface of the seeds and during germination the seedlings are infected through the little wounds caused by soil particles; this is called surface seed transmission; (2) virus particles enter the reproductive organs in some phase of the generative process; this is known as internal seed transmission.

Two things are needed if a virus has to be externally transmitted. These are: (1) the high stability of the virus, and (2) peculiar structure of the fruit. Tobacco mosaic virus infecting tomato is carried externally in the seeds of tomato while the same virus is not carried in the seeds of the tobacco. The reason for this is that in the former case, the seeds are embedded in a gelatinous material in the fruits. The virus-containing substance, in a dried state, adheres to the seeds and infects them during germination. In the case of tobacco plants, the seeds are not enveloped in any gelatinous substance, and hence tobacco mosaic virus is not carried in the seeds of tobacco. With red pepper, a mechanism similar to that existing with tomatoes is responsible for the transmission of tobacco mosaic virus by red pepper seeds. Milicic and Udjvinac (1960) have shown that the tissues of red pepper contained a large amount of TMV crystals.

The transmission of viruses through seeds is dependent on the host–virus combination. Tobacco ring spot virus is transmitted by bean seeds but not by tobacco seeds. Bean plants transmit bean mosaic virus through seeds but the same host plant infected by bean yellow mosaic virus does not transmit the virus through the seeds. Many factors affect seed transmission; seed-borne viral infections are common in the family, Leguminosae (Fabaceae). Viruses belonging to nepoviruses are typically seed-borne. If seed transmission has to take place, the plants must be infected before the ovules are fertilized. Certain viruses are transmitted through the pollens. Blakeslee (1921) pointed out that the mosaic of *Datura stramonium* was transmitted through the pollen to the seed of 79% of the offspring. Reddic (1931) offered evidence of the fact that the bean mosaic virus is transmitted by this method. Das and Milbrath (1961) infected squash plants with stone fruit ring spot virus through pollen. Johansen *et al.* (1994) reviewed the current perspectives of seed transmissions of viruses. Mink (1993) reviewed pollen and seed-transmitted viruses and viroids.

Vectors

Walker (1969) claimed that no other group of diseases is so generally dependent upon insects for dissemination of the infectious causal entity as are viruses. Most viruses are transmitted by insects, which belong to the Hemiptera. From an epidemiological point of view, insects play the most important role in the transmission of plant viruses. Vector relationships and the development of epidemics have been discussed by Thresh (1974). Virus-transmitting insects are called vectors. For an insect to be a vector, it is necessary that its mouth is formed in such a way that they can pick up and eject viruses. The most suitable for this purpose are those of Hemiptera.

No other type of insect was implicated in virus spread until Doolittle (1916) showed that the aphid, *Myzus persicae* could transmit potato leaf roll virus. About 400 species of insects are now known to transmit over 200 different viruses. These include the biting and sucking insects, mites, beetles, nematodes, and fungi belonging to chytridiales. Harris and Maramorosch (1980), in an edited book, have brought in complete information on this aspect till that period.

Aphids

The biology and habits of aphids facilitate the spread of viruses between plants and they are pre-eminent as virus vectors. They include more vector species and transmit a far greater number of viruses than any other group. One species alone, *Myzus persicae,* is the vector of 60 different viruses. Aphid-transmitted viruses can be divided into three groups, namely, non-persistent, semi-persistent and persistent, based on the length of the period. The infective or viruliferus aphids remain capable of transmitting the virus. Kennedy *et al.* (1962) have introduced terms which describe the relationship between the insect and virus and which give some indication of the location of the route followed by the virus in the insect. They gave the term 'stylet-borne' viruses for non-persistent and 'circulative' and 'propagative' for persistent aphid transmitted viruses.

Barley yellow dwarf (Nagaich and Vashisth, 1963), cardamom dwarf or foorkey disease (Varma and Capoor, 1964) and groundnut chlorosis (Sharma, 1966) are persistently transmitted viruses in India. Cardamom 'Katte disease' is one of the semi-persistent transmitted viruses, while banana mosaic, barley mosaic, bean mosaic, cardamom 'Chirke' disease, chili mosaic and pea mosaic are some of the non-persistently transmitted viruses. Harris and Maramorosch (1977) have complied information on aphids as vectors of plant viruses.

Leafhoppers and Plant Hoppers

Next to aphids, leafhoppers are the most important vectors of viral diseases. Almost all the leafhopper-borne viruses are transmitted in a persistent manner except rice tungro virus, which is transmitted in a non-persistent manner by *Nephotettix implicticeps* (Raychaudhuri *et al.*, 1966: John, 1968). Some other examples of leafhopper-transmitted viruses are maize streak by *Cicadulina mbila,* rice dwarf by *Nephotettix nigropictus* and beet curly top by *Circulifer tenellus.* Leafhoppers are known to be the vectors for the disease of the yellows type, which are now attributed to mycoplasmas.

Transmission by Whiteflies

Whiteflies (Aleyrodidae), particularly *Bemisia tabaci*, are the vectors of several important virus-like agents. Some of the viruses that are transmitted by whiteflies are leaf curl of tobacco, yellow mosaic of *Acalypha indica* and yellow mosaic of *Phaseolus aureus.*

Mealy Bugs

Mealy bugs are much more sedentary than other vectors of viruses. Swollen shoot caused by cacao rarely spreads between cacao trees unless the latter are in contact. Crawling nymphs of mealy bug probably spread this disease, as they are more mobile than adults. Mealy bugs are particularly important as vectors of swollen shoot virus in West Africa.

Thrips

Tomato spotted wilt virus is transmitted by a thrip known as *Frankliniella insularis.* Other species of thrips have also been found to be vectors.

Mites

Some Eriophyid mites transmit several important viruses. All vectors species are four-legged with tubular, worm-like bodies and piercing/sucking mouths. Free-living species feed and reproduce on leaves; others infest buds, sometime causing galls. Eriophyid mites in India transmit fig mosaic and pigeon pea sterility (PSV).

Beetles

Viruses such as squash mosaic, turnip yellow mosaic (Markham and Smith, 1949), turnip crinkle, turnip rosette, and potato virus X (Nagaich *et al.*, 1972) are transmitted by beetles. Fulton *et al*. (1987) reviewed beetle transmission of plant viruses.

Grasshoppers

Walters (1952) showed that *Melanoplus differentialis* could carry the virus – tobacco mosaic. The turnip yellow mosaic virus is transmitted by two species of grasshoppers and an ear-wig. Turnip crinkle virus is transmitted by *Locusta migratoria.*

Nematodes

Two groups of viruses are transmitted by soil-inhabiting plant parasitic nematodes. These are the Nepoviruses and the Tobraviruses. The nematode vectors are in two corresponding groups, of the order Dorylaimida. None of the plant parasitic nematodes belonging to the group Tylenchida transmit viruses. Nepoviruses are transmitted by members of the family Dorylaimidae (*Xiphinema and Longidorus* spp.), while Tobraviruses are transmitted by members of the family Trichoderidae (e.g., *Trichodorus* spp.). *Xiphinema index* transmits fan leaf virus of grapes and grapevine yellow mosaic virus. Arabis mosaic is transmitted by *X. diversicaudatum,* while tomato black ring is transmitted by *Longidorus elongatus* and *L. attenuatus*. According to Sol and Seinhorst (1961), tobacco rattle is transmitted by *Trichodorus pachydermus.* Nematode vectors are unable to travel far through the soil and their slow progress towards roots seems to be of lesser importance in spreading virus than the growth of roots towards the nematodes. The movement of virus through the root system of infected plants is also important for increasing the availability of virus to nematodes. Brown *et al*. (1995) reviewed the transmission of viruses by plant nematodes.

Chytrid Fungi

Some of the root-infecting fungi act as vectors of some soil-borne viruses. Species of *Olpidium* transmit tobacco necrosis, satellite and cucumber necrosis viruses and also the agents causing tobacco stunt and lettuce big-vein diseases. *Polymyxa graminis,* a member belonging to the *Plasmodiophorales,* transmits potato mop-top virus. Teakle (1962) carried out a number of experiments to prove definitely that *Olpidium* does infect transmit TMV. Teakle and Gold (1963) have demonstrated that virus transmission is not just a question of surface contamination; the *Olpidium* spore acquires and hardours TMV and so is a genuine vector. Fry and Campbell (1966) have given details of relationships between the fungus, virus and roots of the host. Temmink (1971) has made an ultra-structural study of *Olpidium brassicae* and its transmission of tobacco necrosis virus. Campbell (1996) reviewed the fungal transmission of plant viruses.

Dodder Transmission

Dodder (*Cuscuta* spp.) are parasitic plants with slender straggling stems which, produce root-like haustoria that connect with the vascular bundles of the host, when they touch the stems of the host plant. Bennett (1940) observed that dodder could transmit viruses from plant to plant, such as sugar beet curly top virus (BCTV) with *Cuscuta subinclusa* and CMV with *C. californica.* Johanson (1941) transmitted aster yellow, CMV, DCTV, tomato bushy stunt virus (TBSV) and TMV with *C. campestris.*

Control of Viral Diseases

The control of viral diseases, which is a complicated program, can be classified under the following headings:

1. Eliminating the sources of virus infection
2. Avoiding the vectors.
3. Directly attacking the vectors.
4. Breeding resistant varieties.
5. Curing virus infected plants.
6. Adopting special methods of propagation.

Eliminating the Sources of Virus Infection

It is obvious that there will be no virus problem if the crop is free of virus when planted and if there is no source of infection in the field or none near enough to allow its spread into the crop. There can be several sources of infection: (1) perennial weed hosts in which the virus is seed transmitted or annual weed hosts which have several overlapping generations throughout the year, (2) perennial ornamental plants which often harbour infection in a mild form, (3) unrelated crops, (4) volunteer plants of the same species remaining from a previous crop, (5) biennial seed crops of sugar beet and many vegetables may approach maturity about the time the annual crop emerges.

Elimination of the sources of infection will reduce viral infections but in practice such elimination is not possible.

Two discoveries in 1960s have emphasized the importance of weeds as sources of virus infection. One is the implication of nematode worms as vectors of several viruses affecting raspberry and strawberry plants; the other is the fact that these viruses are transmitted through the seeds of many common weeds, which carry the viruses without symptoms. This is doubly important due to the fact the over wintering nematodes lose their infectivity but regain it in the spring from germinating infective weed seed. The survival of the virus through the winter is thus ensured.

Cooper and Harrison (1973) studied the importance of weed hosts and the distribution and activity of vector nematodes in the ecology of tobacco rattle virus. *Viola arvensis* and *Stellaria media* acted as over wintering hosts of the virus. The virus is retained for long periods in the vector *Trichodorus pachydermus.*

Plants remains in the soil may harbour mechanically transmitted viruses and act as a source of infection for the next season crop. In the case of TMV, general cleanliness is very important for control.

Sometimes it may be necessary to remove infected plants from a crop. The removal of all diseased plants as soon as they are spotted has proved advantageous in crops like potato and sugarcane.

Obtaining Virus-free Planting Material

Virus-free planting material that has been inspected carefully at regular intervals and from which all infected plants have been removed (rogued) is recommended. In the case of vegetatively propagated crops, the main source of virus is chronic infection in the plant itself. One of the most successful forms of control with such crops lies in the development of virus-free clones.

Where a vegetatively propagated cultivar is not totally virus-infected, tests can be done to find virus-free plants to be used as mother plants; but where the cultivar is totally infected, special techniques have to be applied to obtain virus-free plants. These include heat therapy (35-54ºC), meristem tip cultures, cold treatment and chemotherapy. Raychaudhuri and Varma (1977) reviewed the control of viral diseases by means of heat, radiation and meristem culture.

Kunkel (1941, 1943) made a noteworthy contribution by applying heat therapy for the first time for curing peach potted trees infected with peach yellow, little peach, red suture and rosette. Raychaudhuri (1953) reported the cure of bayberry yellows in periwinkle by heat therapy. Hollings and Kassanis (1957) freed chrysanthemum plants from infection by aspermy stunt and ring pattern viruses by keeping them at a temperature of 30ºC for 3-4 weeks.

A number of fruit trees, such as apple, cherry peach, strawberry, raspberry, etc. have also been cured by heat therapy. Grant (1957, 1958) obtained virus-free bud wood by treating key lime with T_3 strain of tristeza virus at 36.6 – 40ºC for 86-100 days.

Considerable interest has been shown by growers in other countries in the heat therapy of certain crops, such as strawberry, raspberry, chrysanthemum, carnation, sugarcane, citrus, pome, stone fruits, etc. Thirumalachar (1954) showed that potato tubers stored in thatched huts in Bihar for six months at an average temperature of 29-36ºC were freed from leaf roll, whereas others placed in cold storage were all found to be infected. Now, MLOs have been found to be associated with this disease.

Apical Meristem and Tip Culture

The apical meristems of systemically infected plants may contain little or no virus. Morel and Martin (1952, 1955) reported that virus-free dahlia and potato plants could be grown from apical meristems excised from infected plants. The meristem tips are dissected from plants in sterile conditions and grown into small plants on nutrient agar or on filter paper wicks dipped into a nutrient solution.

Apical meristem or tip culture is now widely used to produce virus-stocks of vegetatively propagated plants and is often combined with heat treatment or chemotherapy. Kassanis (1957b) freed the King Edward variety of potato from potato paracrinkle virus with which all the stocks of this cultivar were infected.

A very interesting technique is used to obtain virus-free plants of various Citrus species. Citrus seedlings are of two types: one is sexually produced and the other is produced from the nucellus and thus derived from the female parent. Nucellar seedlings are rarely infected with the viruses that occur in the parent plant. After suitable selections, virus-free mother plants can be obtained.

Avoidance of the Vectors

This can be achieved in various ways – by isolation, by breaking the cycle of the vector, virus and host plant, and by creating artificial barriers to exclude the vector.

The growing of seed potatoes in particular areas of Scotland and Ireland is a practical illustration, on a large scale, of the control of potato virus diseases by avoiding the vector. Sometimes, it is possible

to avoid a bud infestation of an aphid vector by early sowing. Rotation of crops may reduce soil-borne viral diseases. Whitefly transmitted virus of tobacco, known as leaf curl, was successfully controlled by breaking the crop cycle, though whitefly had bred on the suckers of the previous year's crop. A fallow period of three weeks after the destruction of the entire crop controlled the disease. Screens or cages may be used to ward off flying insects, such as aphids and leafhoppers, or a living screen or 'barrier crop' is sometimes quite effective.

Control of the Insect Vectors

Various pesticides may be used to kill or inhibit the activity of plant virus vectors, but for various reasons, pesticides have been successfully used to control only a few viruses. Pesticides seem to be useful in controlling viruses that are acquired and transmitted by their vectors only during long feeds.

There have been many reports of the successful use of insecticides for controlling viral diseases. The results are not very convincing. Broadbent *et al.* (1956) tried several insecticides such as D.D.T., Endrin (organochloride insecticide), Sohradan, Mipufax, Malathion and Systox, and successfully prevented the spread of potato leaf roll virus from infected plants within the crop. In the case of non-persistent viruses, for example, potato virus Y, these insecticides could neither protect the entry of the virus into the host, nor stop its spread even though the incidence had decreased slightly (Patkar *et al.*, 1969; Nirula and Kumar, 1967).

Antiviral Agents

Antiviral agents have also been tried for the control of viral diseases. Antiviral agents may be divided into two categories: (1) inhibitors against virus infection, and (2) inhibitors against virus multiplication. Hiral (1977) reviewed the action of antiviral agents. Some of the inhibitors against virus infection are from plant extracts, microorganisms and insects, oxidized phenolic compounds, Ribonuclease, photosensitizing dyes and milk. Inhibitors against virus multiplication are the antimetabolites such as purine and pyrimidine analogues. A number of antibiotics have also been tested for their inhibitory effects on plant virus multiplication. In Japan, antibiotic Blasticidin S, isolated form *Streptomyces griseochromogenes,* has been found most inhibitory against TMV multiplication in tobacco leaves. There are several other antibiotics with inhibitory effects in viral multiplication, such as Trichothecin against potato virus Y (PVY) infections of tobacco, Gentamycin against TMV multiplication, and Actinomycin D (AMD) against cowpea yellow mosaic virus. Ribavirin, applied as a spray or injected into plants, reduces symptoms drastically and may eliminate the virus from the treated host (Agrios, 1996).

Structure of Plant Viruses

Viruses are the smallest biological structures that have the ability to replicate only inside living cells. Essentially, they consist of a shell of protein which encloses a core of nucleic acid- either ribonucleic acid (RNA) or deoxyribonucleic and (DNA).

Most viruses fall in the size range of 10-200 millimicrons; in other words, between the fortieth of a wavelength and half a wavelength of violet light. Since objects smaller than the wavelength of light cannot be seen in an ordinary microscope, viruses can be observed only with the aid of an electron microscope. These instruments employ a beam of electrons whose wavelength is much smaller than the

dimensions of a virus. Viruses can also be studied indirectly by placing crystals of a pure virus preparation in an X-ray beam and recording the diffraction patterns produced when the X-rays are reflected from the planes of atoms in the crystal. An analysis of such X-ray diffraction patterns suggested that the protein sub-units forming the virus shell are arranged symmetrically. The tobacco mosaic virus, for example, showed up in early electron micrographs as a slender rod without visible sub-units. When the virus was examined by X-ray diffraction, however, one could see patterns suggesting that the sub-units are arranged in a helix.

Morphology and Biochemistry of Plant Viruses

All virions, even if they possess other constituents, are constructed around a nucleocapsid core (indeed, some viruses consist of only of a nucleocapsid). The nucleocapsid is composed of a nucleic acid, either DNA or RNA, held within a protein coat called capsid, which protects viral nucleic acid and aids in the transfer of the nuclear material between host cells.

There are four general morphological types of capsids and virions structures:

1. Some capsids are icosahedral in shape. An icosahedran is a regular polyhedron with 20 equilateral triangular faces and 12 vertices (such as potato leaf roll virus or barley yellow dwarf virus).
2. Other capsids are helical and shaped like hollow protein cylinders, which may be either rigid or flexible (such as tobacco mosaic virus).
3. Many viruses have an envelope, an outer membranous layer surrounding the nucleocapsid. Enveloped viruses have a roughly spherical but somewhat variable shape even through their nucleocapsid can be either icosahedral or helical (such as tomato spotted wilt virus and potato yellow dwarf virus).
4. Complex viruses have a capsid symmetry that is neither purely helical nor icosahedral. They may possess tails and other structures (e.g. many bacteriophages) or complex multilayered walls surrounding the nucleic acid (e.g., pox viruses such as Vaccinia).

Nucleic acids of viruses – all cellular organisms contain two types of nucleic acids, the DNA, which is found in the chromosomes and the RNA, which is present in the cytoplasm. Viruses are neither cellular organisms nor they have nucleus or cytoplasm, but possess only one type of nucleic acid—either DNA or RNA (not both in the infective particle). Either DNA or RNA serves as the genetic material in case of viruses.

Before 1968, all plant viruses that were studied contained only RNA but in 1968, cauliflower mosaic virus was found to contain DNA and not RNA

Now, we know that plant viruses can be arranged into 7 groups. These are: (1) Single-stranded positive RNA (+) ssRNA; (2) Single-stranded negative RNA (-) ssRNA; (3) Double-stranded RNA (dsRNA); (4) double-stranded DNA (dsDNA); and (5) Single-stranded DNA (ssDNA) (Fig. 25.1).

Classification of Plant Viruses

The International Committee of Nomenclature of Viruses (ICNV) established a subcommittee of plant viruses in 1966 and recommended that plant viruses should be classified into 16 subgroups, including monotypic groups (Harrison *et al.,* 1971).

1. Tobravirus group
2. Tobamovirus group

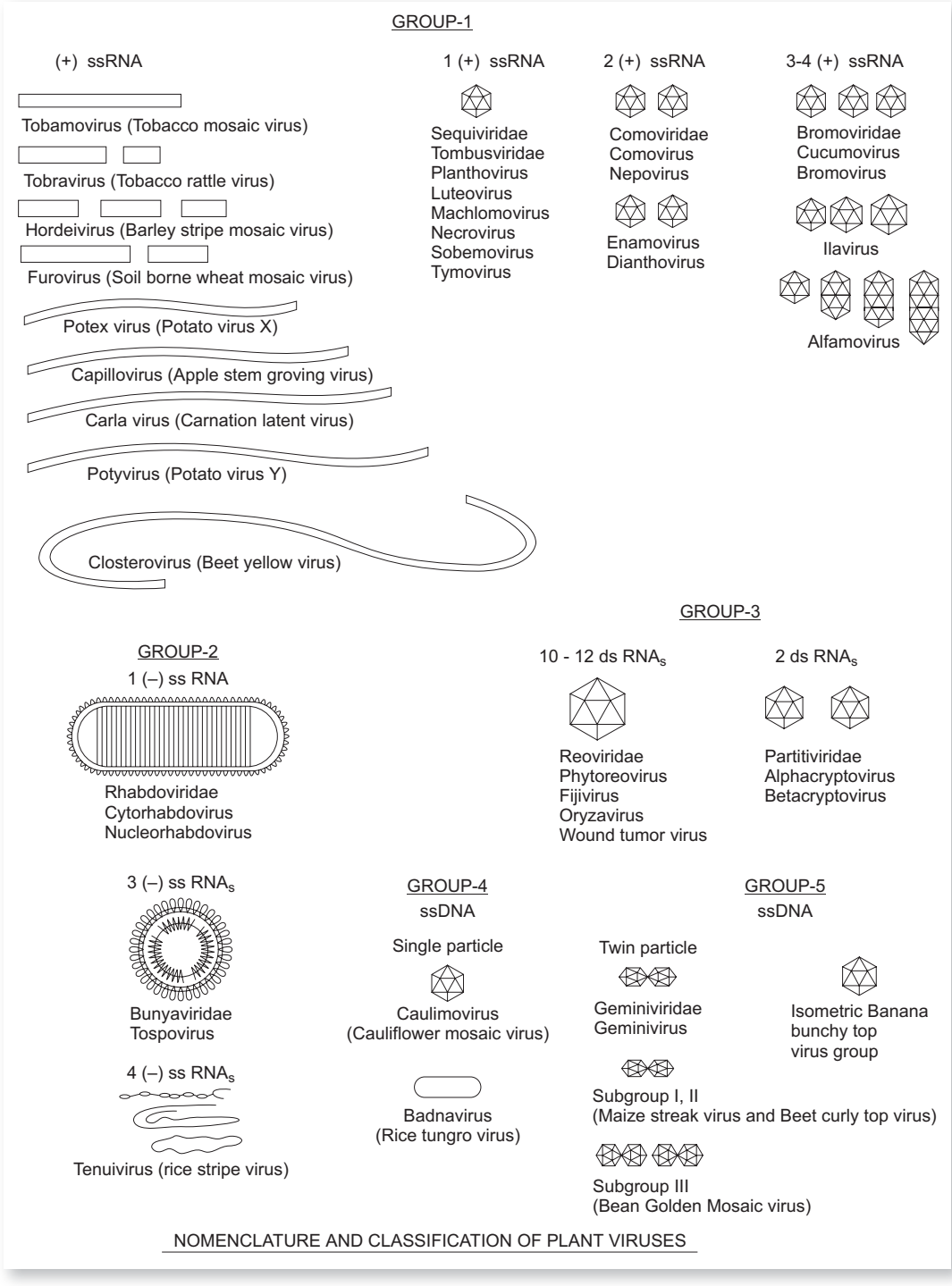
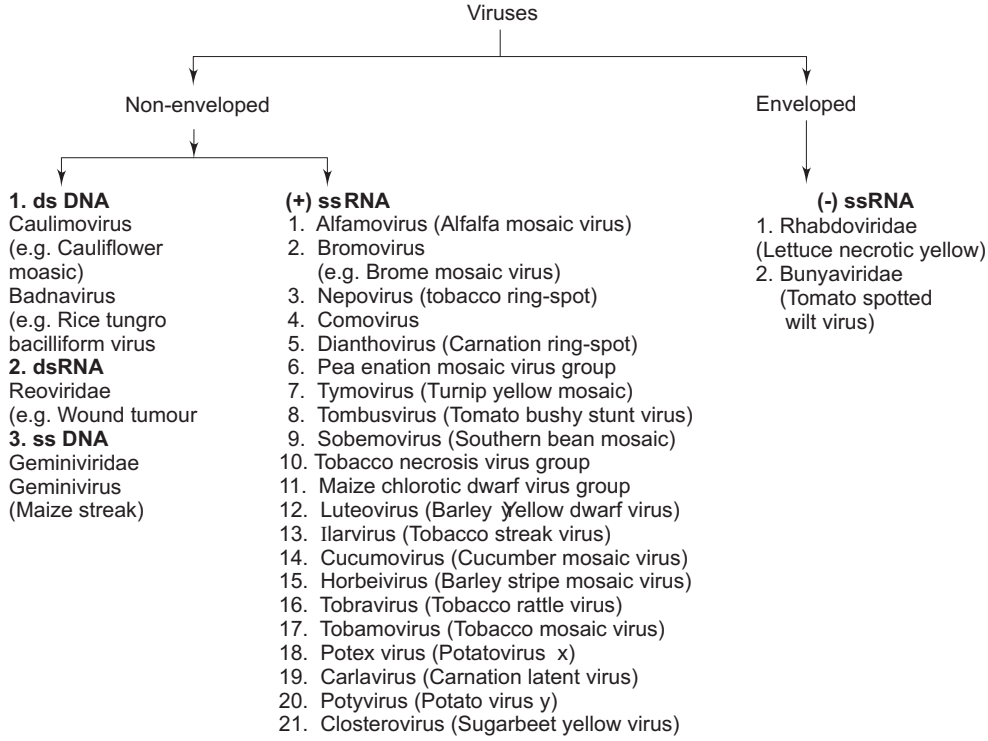


Figure 25.1 Schematic diagram of families and genera of viruses that infect plants [Modified from Murphy *et al.* (1995) and Agrios (1996)]

3. Potexvirus group
4. Carlavirus group
5. Potyvirus group
6. Cucumovirus group
7. Tymovirus group
8. Comovirus group
9. Nepovirus group
10. Bromovirus group
11. Tombusvirus group
12. Coulimovirus group
13. Alfalfa mosaic group
14. Pea enation mosaic virus group
15. Tobacco necrosis virus group
16. Tomato spotted wilt virus group.

Plant Virus Taxonomy

Like other type of viruses, plant viruses are classified according to properties such as nucleic acid, capsid symmetry, size and the presence of an envelope. The following is an outline classification of plant viruses:



Virus Purification

Virologists must be able to purify viruses and accurately determine their concentration in order to study the structure and reproduction of the virus. There are four methods used for purification of viruses:

1. Differential and density gradient centrifugation
2. Precipitation of viruses
3. Denaturation of contaminants
4. Enzymatic digestion of cell constituents

Gradient Centrifugation

Here, viruses can be purified based on their size and density by use of gradient centrifugation.

Differential Gradient Centrifugation

1. Old host cells that contain mature virions are used as the source of material.
2. Infected cells are first disrupted in a buffer to produce an aqueous suspension or homogenate containing cell components and viruses.
3. Viruses can be then isolated by differential centrifugation, the centrifugation of a suspension at various speeds to separate particles of different sizes.
4. The homogenate is first centrifuged at high speed to sediment viruses and other larger cellular particles and the supernatant, which contain the homogenate's soluble molecule, is discarded.
5. The pellet is next re-suspended and centrifuged at a low speed centrifugation that sediment the viruses. This process may be repeated to purify the virus particles further.

Density Gradient Centrifugation

1. A sucrose solution is poured in a centrifuge tube so that its concentration smoothly and linearly increases between the top and the bottom of the tube.
2. The virus preparation, often after purification by different centrifugation, is layered on the top for the gradient and centrifuged.
3. The particles settle under centrifugal force until they come to rest at the level where the gradient's density equals theirs (isopycnic gradient centrifugation).
4. Viruses can be separated from other particles only slightly differential in density. Gradients also can separate viruses based on differences in their sedimentation rate (rate zonal gradient centrifugation).
5. When this is done, particles are separated on the basis of both size and density; usually the largest virus will move most rapidly down the gradients.
6. Viruses differ from one another and cell components with respect to either density (gram per milliliter) or sedimentation co-efficient(s). Thus, these two types of gradient centrifugation are very effective in virus purification.

Precipitation of Viruses

1. Viruses like many proteins can be purified through precipitation with concentrated ammonium sulphate.
2. Initially, sufficient ammonium sulphate is added to raise its concentration to level just below that, which will precipitate the virus.

3. After precipitation, more ammonium sulphate is added and the precipitated viruses are collected by centrifugation.
4. Viruses sensitive to ammonium sulphate are purified by precipitation with polyethylene glycol (PEG).

Denaturation of Viruses

Viruses are less easily denatured than many normal cell constituents. Contaminants may be denatured and precipitated with heat or a change in pH to purify viruses. Because some viruses also tolerate treatment with organic solvents like butanol and chloroform, solvent treatment can be used to both denature protein contaminants and extract any lipids in the preparation. The solvent is thoroughly mixed with the virus preparation, and then allowed to stand and separate into organic and aqueous layers. The unaltered virus remains suspended in the aqueous phase while lipids dissolve in the organic phase. Substances denatured by organic solvents collect at the interface between the aqueous and organic phases.

Removal of Cellular Proteins and Nucleic Acids

Cellular proteins and nucleic acid can be removed from any virus preparations through enzymatic degradation, because viruses are usually more resistant to attack by nucleases and proteases than free nucleic acids and proteins. For example, ribonuclease and trypsin often degrade cellular ribonucleic acids and proteins while leaving virions unaltered.

Viroids

Viroids are shown to cause at least 40 plant diseases (Agrios, 2005). The term ‘viroid’ has been introduced to denote a comparatively recently recognized class of subviral plant pathogens (Diener, 1971). The first viroid came to light during efforts to purify and characterize the agent of the potato spindle tuber disease (PSTV), a disease, which for many years, had been assumed to be of viral etiology (Diener and Raymer, 1971). Diener and Raymer (1967) reported that the infectious agent of this disease is a free RNA and that virus particles are apparently not present in infected tissue. Later, sedimentation and gel electrophoretic analyses conclusively demonstrated that the infectious RNA has a very low molecular weight (Diener, 1971b) and therefore the agent differs basically from conventional viruses.

Several plant diseases – citrus exocortis, chrysanthemum stunt, chrysanthemum chlorotic mottle, cucumber pale fruit and cadang-cadang disease of coconut are caused by low molecular weight RNAs that are by viroids. As already indicated, viroids are low molecular weight ribonucleic acids (RNA) that can infect plant cells and replicate themselves and cause diseases. Viroids differ from viruses in at least two aspects: (i) the virus RNA is enclosed in a protein coat while the viroids lack any protein coat and exist as free RNA (ii) the size of the RNA, which consists of 250 to 370 nucleotides (bases) in viroids in comparison to 4 to 20 kb for viruses. Viroids are circular, single-stranded RNA molecules with extensive base paring in plants of the RNA strand. As a result of base pairing, some sort of hair-pin structure is formed. This results in single-stranded and double-stranded regions of the same viroid. This gives stability to the viroidal RNA.

How viroids replicate is still not known and how viroids cause disease is also not known so far.

Transmission of viroids from diseased to healthy plants is primarily by mechanical means, mainly through sap carried on tools or hands or other implements. Pollens and seeds are believed to transmit potato spindle tuber, cadang-cadang, and tomato bunchy top, but the rates of such transmission are very low.

In nature, viroids survive in dead plant matter for varying periods of time. Perennial hosts are a very good means for the perpetuation and over seasoning of the viroids.

Viroids are quite resistant to high temperatures and cannot be inactivated in infected plants by heat treatment. Use of viroid free planting material, rouging of infected plants, clean cultural operations can help in controlling the viroid.

Physical and Chemical Properties of Plant Viruses

Allard (1914, 1915, 1916, 1917, 1918) was the first to use certain physical and chemical agents to study the nature of the tobacco mosaic virus. Johnson (1927) believed that a safer means of distinction among viruses was that based on differences among the viruses rather than the differences among the diseases that they incite. He applied certain physical and chemical agents used by Allard, such as reaction to heat, to dilution, to ageing *in vitro* and to formaldehyde, alcohol and other chemicals. The tests have been widely used since then, and in the modern characterization of mechanically transmitted viruses, several of them are commonly applied. Those most extensively used are the thermal inactivation point (i.e., the constant temperature at which a virus is completely inactivated when exposed for 10 min.) and the ageing *in vitro* dilution inactivation point. These are referred to as physical properties and are helpful in placing unknown viruses in different groups.

Host Range

Host range is useful in determining whether or not two viruses are distinct. Cucumber mosaic virus and tobacco mosaic virus incite symptoms on tobacco, which are similar; but the cucumber virus affects cucumber systemically, while the tobacco virus ordinarily does not. However, much importance cannot be given to finding out the differences in the host range for distinguishing viruses from one other, as some viruses with similar properties have markedly different host ranges. Viruses are inoculated into indicator plants, which develop typical symptoms, often local lesions, when infected by specific viruses. It may be necessary to use several different species of indicator plants since most of the latter react to several viruses, but some are used to detect specific viruses and in virus assay (Steere, 1995). Hollings (1966) and Ross (1964) have discussed indicator plants.

Cross Protection Tests

Wingard (1928) reported that tobacco plants infected with tobacco ring spot virus recovered from the apparent symptoms and continued to remain healthy in appearance. The virus was however, recovered from such plants. Successive vegetative generations remained symptom less but the virus was continuously present. This type of recovery has been found in various other instances among plant viruses. There are several such examples now of the ability of one virus to inhibit the entrance of another strain of the same virus into the same plant. It is generally assumed that one virus, preceding another in the tissue, prevents or markedly impairs the increase of the second virus; this behavior indicates the

relationship of the virus previously inoculated. The cross protection test is only an indicative one which must be used in conjunction with other characteristics.

Serological Tests

When a macromolecular substance that is antigenic in nature (antigen) is introduced parenterally (not via the digestive tract) into animal tissues, antibodies are produced. Antibodies are proteins in nature and occur in the γ-globulin fraction of the serum of the animal, which has been immunized. A serum containing the antibodies is known as antiserum. Antibodies have the property of combining *in vitro* with the antigen that has induced their production. This *in vitro* test is called serological reaction. The most important features of serological reactions are their great specificity and high sensitivity. The former property of serological reactions is used in plant virology for the identification of plant viruses and the latter property for the estimation of virus concentration. If strains of viruses differing in disease symptom patterns are tested against the same serum, they react similarly. When a group of viruses gives similar precipitation reaction to the given antiserum, it is usually regarded as good evidence that it is distinct from the virus used to produce the antiserum. The serological test is a useful tool in determining virus similarities and dissimilarities.

Each antigen, such as a virus, has many different antigenic determinants (distinct groups of 6-10 amino acids) at its surface, and since each of them prescribes the production of a different kind of antibody, the antiserum (serum containing antibodies) of the animal contains a mixture of antibodies. Such mixtures of antibodies are called as polyclonal antibodies.

Infections, Multiplication and Movement of Viruses Within Plants

In their mode of replication, viruses differ greatly from cellular microbes, which multiply by the division of mature cells, often in a binary fashion. Hence, bacterial cells, for example, are intact at all stages of division and viable and recognizable as bacteria. By contrast, soon after their entry into host cells, viruses partially or wholly disassemble themselves into constituent macromolecules. The virus genome or messenger RNA transcribed from it is translated by the host's translation apparatus into new virus proteins, the nucleic acid genome is replicated and the progeny nucleic acid together with the molecules of one or more of the new virus proteins assemble to form new virus particles. Thus, intact virus particles are not present during part of the replication cycle of a virus. The virus genome is incorporated into the host's protoplasm and is totally dependent upon it.

Hamilton (1974) reviewed the replication of plant viruses. De Zoeten (1976) discussed the cytology of virus infection and virus transport and the temporal patterns of virus spread had been reviewed by Thresh (1974).

The first stage of infection is the entry of viruses into the cells, and this happens in nature through breaks in the cell walls made by vectors or by abrasion. There is no unequivocal proof that viruses enter through wounds made during mechanical inoculation, although viruses have been found in wounds caused by abrasions (Kontaxis and Schlegel, 1962). Ectodesmata have been implicated as possible routes of infection for plant viruses (Brants, 1964; Thomas and Fulton, 1968). When intact virus particles enter a cell they disassemble ("uncoat") and uncoating may, in part, control host range. Atabekov (1975) discussed host specificity of plant viruses. The question as to what factors determine the host specificity of viruses is not simple to answer. Atabekov (1975) has discussed the problem of

host range specificity of the plant viruses in terms of the possibility of expression of the plant virus genetic information in different types of cells. It has been assumed that different functions coded by the viral genome, if not performed in certain plant cells, may be the factors controlling the host range of the virus.

When infective brome mosaic virus RNA was coated with TMV protein, the particles were not infective for barley leaves, which were susceptible to infection by intact particles and by RNA of brome mosaic virus (Atabekov, 1971). According to Bancroft *et al.* (1971), the ability to uncoat depends upon the structure of the virus coat protein and can be greatly changed by a single amino-acid replacement. Evidence that uncoating takes place is found by comparing the sequence and timing of events after inoculating French bean leaves with either intact tobacco necrosis virus particles or with the infective RNA extracted from them. When inoculated with RNA, the virus appears earlier and the symptoms also appear earlier than when inoculated with intact virus particles.

It is also possible to stop infection by inactivating the leaves with ultraviolet light immediately after inoculation, if inoculation has been done with the nucleic acid. On the other hand, resistance to ultraviolet inactivation starts to increase almost at once in leaves inoculated with nucleic acid, whereas no such increase occurs in leaves inoculated with intact virus particles until three hours later (Kassanis, 1960). Mandels (1967), in his studies of polio virus (a simple RNA containing animal virus), came to the conclusion that uncoating might be initiated at the cell surface but completed intracellularly by means of pinocytotic movement. Cocking (1970) calls the movement of macromolecules into the cellular environment after attachment to the cell membrane (plasmalemma), rhopheocytosis, while Dales (1965) calls it viropexis. The situation in the case of plant virus infection is very unsettled and if uncoating takes place on the cuticle, the viral RNA has to get into the cell either by direct (active) transport through the plasmalemma or through viropexis. Viropexis has been inferred to exist in plant protoplasts. Cocking (1970) and Otuski *et al.* (1972) found this to be true from TMV moving into tomato fruit protoplasts and for TMV, PVX and the cucumber mosaic virus (CMV) moving into tobacco protoplasts. Motoyoshi *et al.* (1973), have reported successful tobacco protoplast infection, probably by the viropexis of cowpea chlorotic mottle virus (CCMV) and its RNA.

Tobacco and Tomato Mosaic

Tobacco mosaic, the first recognized viral disease of plants, is worldwide in distribution. It is most common on tobacco crops in India, appearing in every tobacco tract of the country. The same virus occurs on many other plants and over 20 different virus diseases occur on tobacco. The mosaic disease is most important.

Symptoms

The first symptoms are light discoloration along the veins of the youngest leaves. Soon, the leaves develop a characteristic light and dark green pattern; the dark green areas usually being associated with the veins. This typical mosaic symptom is sometimes accompanied by the blistered appearance of leaves due to the more rapid growth of the dark green tissue. At times leaf spots may also appear. Plants that become infected early in the season are usually very much stunted, while those infected late in the season show little reduction in size. In severe infections, the leaves are narrow, puckered and thin, in general, malformed beyond recognition. While it is easy to recognize a diseased plant in the main field, it is almost impossible to differentiate the diseased seedlings in the nursery. The disease causes extensive

damage by way of reduction in yield and quality of the product. Frequently, yellow patches develop on the leaves due to the presence of more than one strain produced by mutation. The disease causes partial sterility of the pollens.

Causal Virus

Nicotiana virus 1 (Smith) is the causal organism of tobacco virus. Several workers have made chemical studies on the properties of tobacco mosaic virus and voluminous literature is available. The virus is rod-shaped, measuring about 280 × 15-18 nm with a central hollow tube of about 20 Å in diameter. It is made up of centrally-placed ribonucleic acid molecules covered with a protein coat (Fig. 25.2).

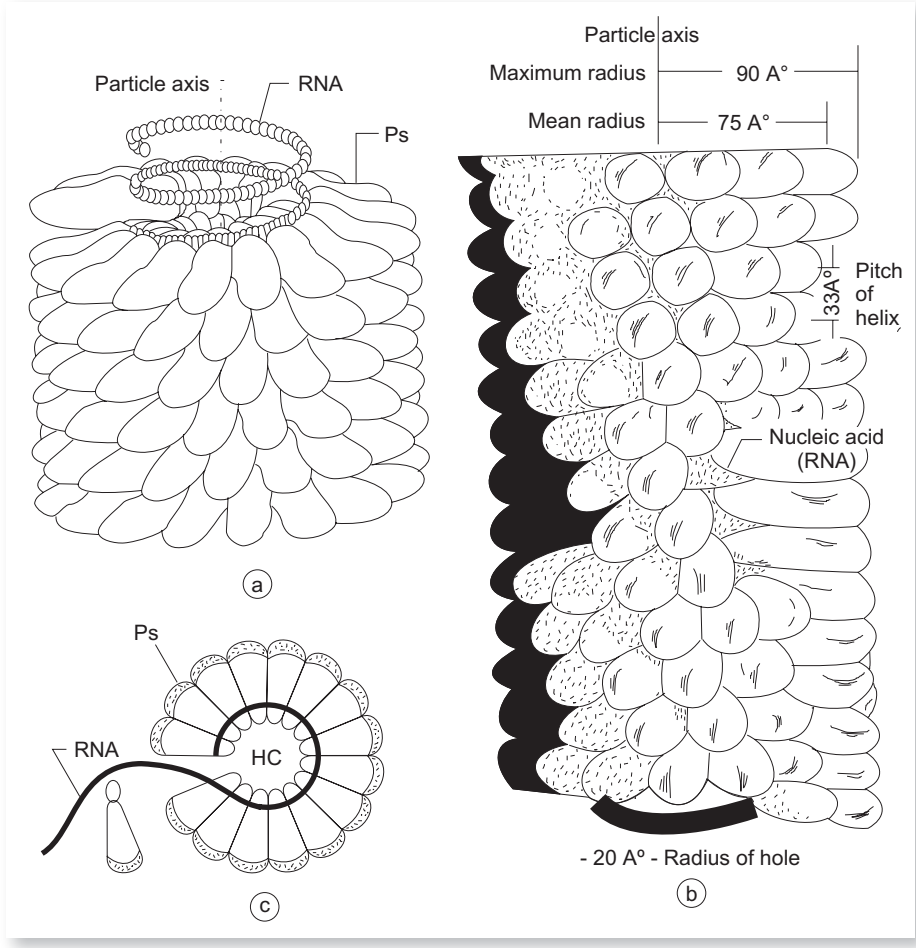


Figure 25.2 Structure of TMV: (a) drawing to show the relationship of DNA and protein sub-units. The RNA shown free of protein could not maintain this configuration in the absence of protein sub-units. There are three nucleotides per protein sub-units or 49 per turn of this major helix spaced about 5Å apart (b) photograph of a model of TMV with major dimensions indicated. The structure repeats after 69 Å in the axial direction. The repeat containing 49 sub-units distributed over three turns of the helix (c) cross-section of (b). Ps- Protien subunit; HC- Central hollow.

This virus particle has a molecular weight of 39 million Dalton. It is cylindrical, with dimensions of 160×3000 Å.

The protein coat of this virus contains 2130 identical protein sub-units; each with a molecular weight of 18,000. The sub-unit is composed of a single chain of 158 amino acid residues of known sequence. The folding of the chain produces an ellipsoid with an axial ratio of 3: 1. The RNA of the virus is single-stranded and the molecular weight is 2.4 million Dalton. It consists of 6400 nucleotides. The RNA strand forms a helix with a radius of 40 Å that can be seen under an electron microscope (Fig. 25.2), with a special staining technique when phosphotungstic acid is used as a negative stain. The RNA has four open reading frames (ORF) and is translated into four proteins, one of which is (17.6 kDa) is the coat protein, two (126 and 183 kDa) are components of the RNA polymerase enzyme and the fourth (30 kDa) is associated with cell to cell movement of the virus (Agrios, 2005).

Each plant virus consists of at least a nucleic acid and a protein. It appears that first new RNA produced is not the viral RNA but a complementary strand that is a mirror image of the virus and which, as it is formed, is temporarily connected to the viral strand. Thus, they form a double-stranded RNA, which soon separates to produce the original virus RNA and complementary (–) strand, the latter serving as a template for more virus (+) strand RNA synthesis.

Virus Protein Synthesis

The viral RNA itself (or the part of the viral coding for the viral protein) plays the role of messenger RNA. The virus utilizes the amino acids, ribosomes and transfer RNAs of the host, but it becomes its own blueprint (messenger RNA) and the protein formed is for the exclusive use of the virus as a coat. The RNA is translated into four proteins, one of which is the coat protein, two are components of the RNA polymerase enzyme and the fourth is associated with the cell-to-cell movement of the virus.

During virus synthesis, it appears that part of its nucleic acid also becomes involved with the synthesis of protein, other than the viral coat protein. Each protein sub-unit of TMV consists of 158 amino acids with a notch at 40 Å. Amino acids of each TMV protein sub-unit add up to a total of 17,531.

Since each TMV virus particle weighs approximately 39,000,000 molecular weight units, of which 95% or 37,000,000 units are protein, it is easily calculated that the protein of each virus particle consists of approximately 2100 protein sub-units.

Disease Cycle

The virus is sap-transmissible and enters the host through wounds. From the point of entrance (wounds), the virus moves from cell to cell through plasmodesmata, multiplies in and infects each cell, and when it reaches phloem, travels systemically through it and infects the whole plant. No insect vector is known. It is most resistant to adverse environmental and climatic conditions and hence, when it reaches the field, it may become effective any time the crop is grown. Besides, it has a wide host range, affecting nearly 50 plant species belonging to nine different families. The virus is not seed-transmitted in tobacco except as a contaminant on the surface, but it is reported to be seed-transmitted in tomato. The virus produces different types of symptoms on different host species and their varieties. In some cases, the host is a symptom-less carrier of the virus, making it difficult to implement control measures with such hosts. The intensity and type of symptoms produced by the virus on a given host also vary with the physiological condition of the plant and the environmental factors, such as temperature.

The virus is easily transmitted in the field by mechanical means and by wind and water. Various cultural operations cause wounds on the plant, which become portals of entry of the virus. Such operations as topping or clipping the shoots spread the virus form diseased to healthy plants. Even the workers in the field may transmit the virus to the standing crop. Thus, there is unlimited scope for the virus to set in and cause damage to the crop. The disease cycle of TMV is depicted in Figure 25.3.

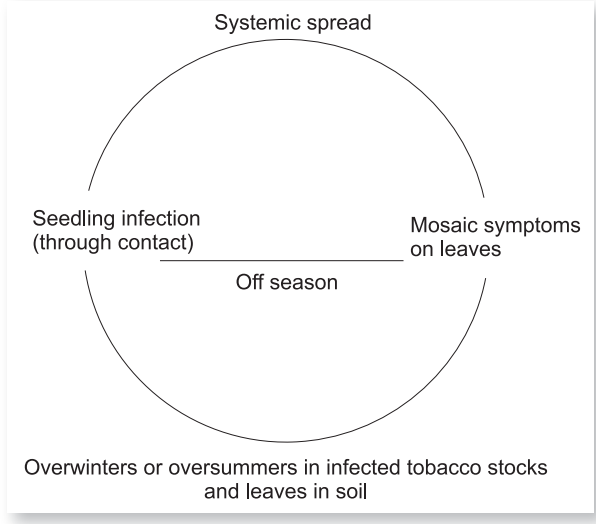


Figure 25.3 Disease cycle of TMV

Disease Management

The various physical and biological properties, which impart highly resistant qualities to the virus and the varying modes of spread of the virus, make disease control almost impossible. The only method seems to be to grow resistant or at least tolerant tobacco varieties and practice field sanitation. The field should be weeded thoroughly. After weeding, the workers should wash their hands thoroughly with soap and running water. Regular rouging of diseased plants and weeds in the field should be carried out. Utmost care should be taken while handling the diseased plants.

Sanitation and the use of resistant varieties are the two main means of controlling TMV. It has been shown that infection by TMV is inhibited by whole milk (Hare and Lucas, 1959). Some states in the USA are now recommending spraying of plants with milk before transplanting or otherwise handling them, or dipping the hands in milk during transplanting and handling, since this greatly reduces the spread of tobacco mosaic virus.

In the case of tobacco mosaic virus, cross-protection had been reported if the plants are inoculated with milder strain first. Genetic engineering has made it possible to produce tobacco plants with the gene coding of the TMV coat protein. The N gene is the most widely used gene conferring resistance to TMV. The N gene was transferred into *Nicotiana tabacum* from *N. glutinosa*. Once a plant containing the N gene is inoculated with TMV, a hypersensitive reaction occurs at the viral points of entry. The interaction of the N gene product with the TMV virus causes the plant to kill its own cells at any point where virus entered. Subsequently, lesions form on any inoculated leaf, restricting the movement of the virus to other plant parts and more importantly, to other plants.

Viral Diseases of Potato

Leaf Roll of Potato

Leaf roll is of great economic importance, being one of the main causes of potato degeneration.

Causal Virus Potato leaf roll virus (PLRV) (Syn.= phloem necrosis virus), also known as potato virus 1 or *Solanum* virus 14, is the virus responsible for this disease. The virus is a member of Luteovirus group.

The name 'leaf roll' is partly descriptive of the disease. In many varieties, the margins or the tips of the leaves sometimes become yellow. The leaves of an infected plant roll up along the margins, leaving the midrib at the bottom of the trough. In secondary infection (in plants with diseased seed tubers) in plant with diseased seed tubers, this rolling of leaves starts in the lower leaflets and progresses upward throughout the entire plant (Fig. 25.4). In some varieties, individual leaflets have a tendency to be more erect, giving the diseased plant an upright appearance. The infected leaves turn leathery brittle, pink or brown pigmented. Infected plants remains stunted chlorotic and make rattling noise when shaken. As a result of infection, necrosis occurs in the phloem and there is excessive accumulation of starch. Often non-viral or pseudo "leaf roll" symptoms are seen in the field, induced either by reasons like purple top roll (phytoplasmic), cutworm canker, black scurf (fungi) or calcium deficiency or water logging (Khurana, 2000).



Figure 25.4 Leaf roll of potato (Potato virus I or *Solanum* virus 14): symptoms (Courtesy of Dr. B.B. Nagaich)

Damage The number of tubers produced per plant and their size in a diseased crop are greatly reduced.

Transmission The virus is not transmitted through the sap. Transmission of the virus in nature occurs through infected seed tubers and through the agency of insects. The main insect vector is an aphid- *Myzus persicae.* The virus persists in the body of *Myzus persicae* for many days even if the vector has fed on many immune hosts after feeding on the diseased plants.

Host Range *Datura stramonium, D. tatula, Physalis angulata, Physalis floridana* and *Lycopersicon esculentum* comprise the host range.

Disease Management Potato seed certification and rouging are recommended for disease control. According to Kassanis (1950), if tubers are stored at 37.5°C in a humid atmosphere for 20-25 days, they become free of the leaf roll virus. In India, tubers can be free from PLRV infections by storing in ordinary stores in summer months at temperature 29-35°C. Hot water treatment of (50-55°C for 15-20 minutes) has also found effective in eradicating the virus. Resistance to PLRV is rather difficult to incorporate yet it has been identified in *S. brevidens* and *S. acaule OCH* 13823. Buildup of incoming aphid vectors be controlled by application of suitable contact/systemic insecticides to prevent aphid build up and virus spread in storage.

Mild Mosaic of Potato

This is another cause of degeneration in the crop. Several types of mosaic virus affect potatoes, but in India, latent or mild mosaic, rugose mosaic and crinkle are most important.

Mild Mosaic Potato latent virus, potato mottle virus, potato virus X or Solanum virus 1 cause mild mosaic. The virus contains a large number of strains.

The disease, although occurring widely in most of the seed stocks, causes very slight or negligible symptoms of interveinal mottling or mosaic. Under favourable growth conditions of the host, the symptoms are masked, thus making recognition of the disease difficult. The mosaic symptoms are almost completely masked at high temperatures (above 21ºC), and in many cells, plants carry the virus without any visible symptoms. There is only slight dwarfing of the plant or deformation of the foliage (Fig 25.5). The virus produces top necrosis in the varieties Key Edward, Arran, Crest and Epicure.

The damage is not much compared to leaf roll or other viruses infecting potatoes in India.

Potato virus X is resistant to ageing at room temperature for several weeks to a year and can tolerate dilutions up to 1: 100,000 to 1000, 000. It is deactivated at 74ºC. The particles of the virus are long, flexuous rods (515 nm long) and ssRNA. The same virus affects tomato, *Datura, Solanum nigrum* and tobacco. Raychaudhuri reported a ring spot strain in 1962.

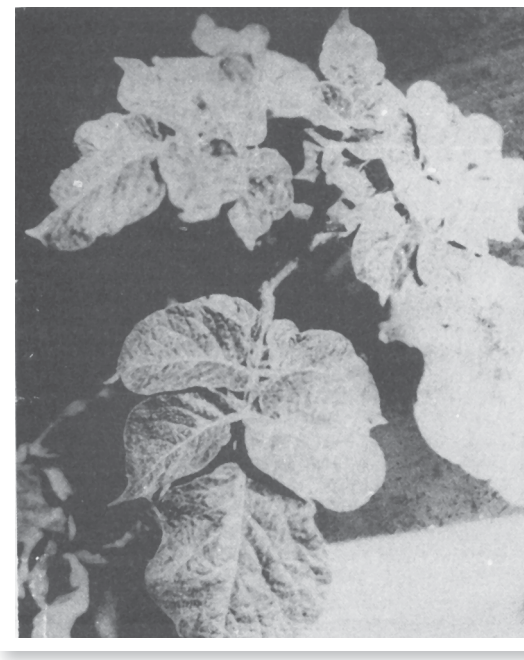


Figure 25.5 Mild mosaic of potato (Potato virus X or *Solanum* virus 1): symptoms (Courtesy of Dr. B.B. Nagaich)

Histopathology X bodies are present in the mottled areas of potato plants infected with the virus.

Transmission The virus is easily transmitted by sap inoculation and is one of the few viruses that spread in the field by contact between healthy and diseased plants. Virus can be transmitted by core-grafting and by dodder, *Cuscuta campestris*. No insect vector is known. However, the source of perennation and spread in the field under natural conditions is mostly the diseased seed stock.

Rugose Mosaic of Potato

The disease causes very severe damage to individual plants. The foliage is not only mottled but is also severely wrinkled, puckered and markedly reduced in size. The leaflet margins are rolled downwards and the entire plant is dwarfed. The lower leaves generally have black necrotic veins. While the symptoms of mottle may be masked by high temperature, roughness or rugosity and abnormal hairiness of leaves and dwarfing of the entire plant persist. In severe cases, plants may even die before producing any tuber.

A combination of two viruses – potato virus X (mild or latent mosaic) and potato virus Y, causes this disease. The latter causes blightening of the leaves in severe attacks and is also known as vein banding virus or *Solanum* virus 2.

Potato virus X (PVX) consists of a single, rather flexuous rod that is from 470-580 nm long and 11-12 nm in diameter. The genome is a positive, single-stranded RNA and the virus particle consists of a single species of protein sub-unit. Potato Virus Y (PVY) consists of a single flexuous rod-shaped particle, 680-900 nm long and 12 nm in diameter. The virus consists of a single positive RNA species.

Under field conditions, the transmission of the virus is through small-sized tubers. On diseased plants, *Myzus persicae* is capable of efficiently transmitting virus Y, which is one of the components of the complex.

Crinkle of Potato

The disease resembles rugose mosaic very closely but the yellowish patches on the foliage are bigger, and hence, more prominent. As the plants begin to die this color becomes more pronounced and is accompanied by rust spots, beginning near the tip of the leaves. The foliage is brittle and is easily injured (Fig. 25.6).

Potato virus X and potato virus A (*Solanum* virus3) cause super mild mosaic. This virus complex is also tuber borne. Sap inoculation transmits only potato virus X. Potato virus A particles are 730 × 13 nm flexuous rods transmitted by aphids.



Figure 25.6 Potato crinkle mosaic (Potato virus X and A): symptoms

Disease Management of Viral Diseases of Potato

1. Systemic rouging of diseased plants as soon as they are noticed and their destruction by burning is recommended.
2. Indirect rouging of diseased seed tubers is recommended at planting time by using large-sized potatoes (about 3.75 cm in diameter).
3. Raising a mosaic-free seed crop by planning large-sized seed tubers during the coldest season helps to check the disease considerably.

Recently, the Central Potato Research Institute, Shimla, has suggested the following way in which disease-free crops for seed tubers can be raised:

1. Obtain certified seeds from a reliable source and plant them early in the season.
2. Rogue out the sick-looking plants as soon as located.
3. Detop (removal of foliage) the plants in the third or fourth week of December when aphid infection starts, and leave the tubers in the soil to mature. The yield may be less but the tubers will be free of virus.
4. Use of true (Botanical) potato seed (TPS) also holds promise for areas where quality seed is not available. TPS offers various advantages, viz., reduced cost, early storage and transportation of seed material, and low incidence of diseases, but it requires more labour, and lacks uniformity etc.
5. In the late crop, spraying systemic insecticides is recommended to control the insect vectors (such as 0.1% Metasystox). In recent studies, it has been found that applying systemic insecticides to the soil in the furrows delays the progress of the buildup of aphid population at the time of planting.

Leaf Curl of Tomato

The disease has been reported from many parts of the world. It is fairly common in the winter season in India.

Symptoms and Causal Virus

Dwarfing, puckering, twisting and curling towards the dorsal side of leaves, mottle, vein clearing, excessive branching, shortening of the entire plant, and partial or complete sterility are the characteristic symptoms (Fig. 25.7). The curl of tomato is caused by the tobacco leaf curl virus, which belongs to the Gemini virus group. The geminate particles of the virus measure 25-40 × 15-20 nm. In Gemini viruses, the single-stranded DNA (ssDNA) molecule is circular.

Transmission

It is a seed-borne disease. The principal insect vector is *Bemisia tabaci.*

Disease Management

There is no definite control measure. *Lycopersicon peruvianum* shows a high degree of resistance. Spraying with Ekatox (0.2%) and Rogor (0.05%) at intervals of 10 days helps to reduce the incidence of the disease. There are indications that growth regulators like Gibberellic acid and 2,4-D reduce disease incidence and increase the yield of fruits.



Figure 25.7 Tomato leaf curl: symptoms

Mosaic Diseases of Tomato

Several viruses initiate mosaic diseases in tomato:

1. Tobacco mosaic virus (Nicotiana virus)
2. Cucumber mosaic virus
3. Potato mild or latent mosaic virus
4. Potato vein banding virus

Tomato aucuba mosaic is caused by a strain of *Nicotiana virus* 1. The predominant symptom is downward curling of the whole leaf.

The virus causing the common mosaic of tomato is known as Tomato mosaic Virus (Tobamovirus group). Lycopersicon virus 1 is a synonym of this virus. There are many strains of this virus producing different symptoms, which are often described as different diseases. The virus is very similar to the one causing tobacco mosaic virus disease. Particles of the virus are straight tubules with a helical construction and measure 300 × 18 nm. The single-stranded RNA (ssRNA) constitutes about 5% of particle weight. The virus occurs in all the tissues, including the pollen and seed but not in the embryo.

The virus can remain infective in laboratory condition even after 24 years. The virus is sap-transmissible and is principally transmitted by man through contact. Seed transmission also occurs, mainly externally. Diseased crop residue is a source of primary infection of the crop.

Disease Management

Use of virus-free seedling is the most important step towards controlling the disease. Seedbeds should be those where no susceptible solanaceous crop has been grown for six month or so. Soil sterilization by heat should be done. Seeds should be treated with hot water at 52°C for 25 minutes or the seeds should be treated with 20% trisodium phosphate solution. Field workers should avoid using tobacco products while working in the field.

Viral Diseases of Papaya

Leaf Curl of Papaya

The disease is common in kitchen gardens and commercial orchards around Delhi. The disease has also been reported from Bihar. Crinkling and curling of the leaves, accompanied by vein clearing and reduction in the size of the leaves are the marked symptoms. Leaves become leathery and brittle and the interveinal areas are raised on the upper surface due to hypertrophy, which produces rugosity. The most prominent symptom is the downward and inward rolling of leaves and the thickening and dark green color of the veins (Fig. 25.8). Sometimes, all the leaves at the top of the plant show these symptoms. The petioles are twisted in a zigzag manner. The severely affected plants fail to flower or bear fruit. In the advanced stages of the disease, defoliation takes place and plant growth is stunted.



Figure 25.8 Papaya leaf curl (Tobacco virus 16 or Nicotiana virus 10): symptoms

Causal Virus Tobacco virus 16 or Nicotiana virus 10 is responsible for leaf curl of papaya. It is a geminate (twin) virus containing single-stranded DNA (ssDNA).

Transmission The virus of papaya leaf curl is not mechanically transmitted (Nariani, 1956). It can be readily transmitted by grafting or juice inoculation. In nature, the important agent of its transmission is the whitefly, *Bemisia tabaci* (Nariani, 1956).

Host Range The host range of the virus includes papaya, tomato, tobacco, *Zinnia and* hollyhock *(Althaea).*

Disease Management No suitable control method is known. Rouging is helpful in reducing the incidence of the disease. No other control measure of the disease can be recommended except that insect vectors should be kept under control by spraying of suitable insecticides.

Mosaic of Papaya

Capoor and Verma (1958) reported this disease from Bombay. The disease is widespread in India in states such as Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh and Maharashtra. Papaya ring spot virus (Potyvirus group) causes the mosaic. It is a flexuous filamentous virus 11-16 nm in diameter.

Symptoms The disease is characterized by mosaic symptoms on the leaves with blister-like patches of green tissue distributed all over the yellowish green lamina. The thermal inactivation point of the virus is 53°C. The dilution end point lies below or between 10^{-4} and 10^{-3}. The virus survives ageing *in vitro* for 26 but not 28 hours.

Transmission The papaya mosaic virus is not seed-borne. Mechanical sap inoculation and wedge and bud grafting can transmit the virus. Aphids such as *Aphis malvae, Aphis gossypi* and *Aphis medicaginis* are known vectors. Under natural conditions, it is only transmitted through insect vectors. According to Capoor and Verma (1958), the disease is not soil-borne.

Host Range Cultivated curcurbits, *Trichosanthes anguina, Cucurbita maxima, Cucurbita pepo*, *Citrullus vulgaris, Cucumis sativus*, *Luffa acutangula* and *Lagenaria siceraria* could be artificially infected with virus.

Disease Management Direct control by the use of insecticides is not possible. According to Capoor and Verma (1961), *Carica cauliflora,* a wild papaya, has been found to be immune to the virus. It can be utilized in the breeding program. The resistance is gene-controlled. According to Bhargava and Paul Khurana (1969), weekly sprays with 1% groundnut oil help to prevent infection.

Yellow Vein Mosaic of Bhindi (*Abelmoschus esculentus*)

Yellow vein mosaic disease of bhindi is a serious problem wherever this crop is grown in India and Sri Lanka. When the infection sets in early, the entire crop is destroyed.

Symptoms

The main symptom of the disease in bhindi is vein clearing followed by veinal chlorosis of the leaves. The yellow network of veins is very conspicuous and the veins and veinlets become thick. In severe cases, the chlorosis may extend to interveinal areas and may result in the complete yellowing of leaves. Fruits are dwarfed, malformed and are yellowish green in color (Fig. 25.9).



Figure 25.9 Yellow vein mosaic of bhindi symptoms

Causal Virus

The virus responsible for the disease is *Hibiscus virus* 1 or yellow vein mosaic virus. Not much is known of the nature and properties of this virus.

Transmission

Capoor and Verma (1950) had shown that the virus is not sap-transmissible but under artificial conditions, it can be transmitted by grafting, which is not the natural means of propagation of this crop. They reported that the virus is transmitted in nature by whiteflies (*Bemisia tabaci*) and the okra leafhopper, *Empoasca devastans.* The virus persists in female whiteflies throughout their life if the insects are allowed to feed on diseased plants for 4-6 hours (Varma, 1955).

There are several hosts of the virus. *Croton sparsiflorus, Malvastrum tricuspidatum* and *Ageratum* sp. are common roadside plants harbouring the virus in Bihar (Jaha and Misra, 1955). The virus can infect several species of *Hibiscus* and *Abelmoschus.*

Disease Management

At present, no definite control measures are known. Pusa Sawani variety is reported to be tolerant (Joshi *et al.*, 1960). Ekatox (0.02%) or Rogor (0.05%) sprays have been reported to reduce disease incidence (Sastry and Singh, 1973). Spraying should be started soon after the emergence of the plants. Soil application of Thimet or Di-syston granules is also recommended. Use of trap crops and crop-free season can reduce vector population. Destruction of weed hosts should be done.

Viral Diseases of Sugarcane

Edgerton (1958), in his book, *Sugarcane and Its Diseases*, has discussed the following viral diseases of sugarcane.

1. Fiji disease
2. Sereh disease
3. Chlorotic streak
4. Mosaic disease
5. Australian dwarf
6. Ratoon Stunting diseases of sugarcane (This is now believed to be caused by xylem inhabiting fastidious bacteria.)

In addition, two more diseases have received attention in India. They are: (1) Grassy shoot (Chona *et al.,* 1960) and Stripe (Sharma and Jha, 1957). Agnihotri (1990) has discussed only two important viral diseases i.e. mosaic and spike of sugarcane.

Mosaic Diseases of Sugarcane

It was first noted in India in 1921. It is now widespread in all cane-growing areas of India. Chona (1944) observed that even 100% infection of a crop with mosaic does not cause more than 10% loss in yield and that the juice quality remains unimpaired. Later, Chona (1958) reported that mosaic could become a potential threat through the development of a more pathogenic strain. Kumar *et al.* (1963) have listed mosaic as one of the most serious diseases of sugarcane. Bhargava (1975) has discussed this disease in detail.

Symptoms Six weeks after planting, pale patches or blotches in the green tissue of the leaf appear. Diseased leaves show a characteristic mottling of chlorotic or light colored, elongated, more or less irregular stripes or streaks (Chona and Rafay, 1950).

The cells of affected leaves show, in one part of the cytoplasm, an area of proteolysis. This can be seen under a microscope as being more heavily stained than the rest and consisting of a vacuolated mass representing the X body.

Causal Virus The virus responsible for sugarcane mosaic is called Sugarcane Mosaic Virus (SCMV) of *Saccharum* Virus 1 or Sugarcane Virus 1. The particles of the virus are flexuous filaments measuring 620-750 × 13-15 nm. Maximum concentration of the virus is found in young leaves and minimum in roots of old infected plants. There are several strains of the virus (A, B, C, D, E, F, G, H, J, K). Rishi and Rishi (1985) had worked on the purification, electron microscopy and serology of strains.

Host Range Maize, sugarcane, sorghum, *Saccharum spontaneum* and many other wild grasses are the hosts for this virus.

Transmission The virus is transmitted from an infected sugarcane stalk to another by a large number of species of aphids. Transmission is in the non-persistent manner.

Disease Management The use of selected healthy setts for seed, systemic roguing out of the infected canes if infection is not very severe, elimination of weed hosts wherever possible, and use of resistant varieties are recommended for controlling the disease. However, due to continuous evolution of strains of the virus, resistant varieties are not a permanent solution of the mosaic problem in sugarcane (Rishi *et al.,* 1973). Heat therapy is effective against certain strains and can be used for raising disease-free nurseries.

Bunchy Disease of Banana

The disease was first reported from Australia. It also occurs in Fiji, Egypt and Sri Lanka. In India, infected suckers brought from Sri Lanka into the state of Kerala are believed to have introduced the disease. It has now spread to almost all the banana-growing areas in India.

Symptoms

In the infected plants, the leaves become bunched together at the apex of the pseudostem to form a rosette or bunchy top. The margins of the leaves become waxy and in the later stage roll upwards. As a result of the reduction in the size of the petioles, the whole plant gets stunted. Due to the failure of the leaf stalks to elongate, the leaves invariably show dark green streaks.

Causal Virus

Banana virus 1 or *Musa* virus 1 causes bunchy top of banana.

Transmission

The virus is not transmitted by mechanical inoculation. The chief insect vector is *Pentalonia nigronervosa*. The aphid acquires the virus in a feeding period of 24 hours and transmits the virus to healthy plants in an infection period of one and a half hours. The aphid retains the virus for a period of 13 days and the incubation period for the virus ranges from a few hours to two days.

Perpetuation

The virus perpetuates through the infected suckers.

Host Range

Musa cavendishii, M. sapientum, M. fehi and *Musa textilis* are the host of this virus.

Disease Management

Diseased plants should be thoroughly and carefully destroyed. They should be carefully dug out and destroyed by burning. An alternate procedure could be to kill the plants along with the aphids feeding on them by pouring kerosene over them. Hormone weed killer such as MCPA (2-methyl-4 chloro-phenoxy acetic acid) can also kill the plant.

Control of banana bunchy top depends primarily on adopting control measures that help to avoid or minimize viral infections. Such measures will include quarantine to keep the virus out from an area where the disease does not occur. The use of virus-free propagating material will go a long way in controlling the disease. Only certified virus-free suckers should be used for planting.

Bean Common Mosaic

Infected plants are stunted and appear bushy. The leaves of infected plants develop mottle, blisters and lamina curls downward. Infection causes the shedding of flowers and the setting of the pods is delayed. The yield is considerably reduced (Yeraguntaiah and Nariani, 1961).

Causal Virus

Phaseolus Virus 1 (Smith) is the common bean mosaic virus (BCMC), which is a Potyvirus. The virus is inactivated after being exposed for ten minutes at 62°C but not at 60°C. The virus remains infective up to a dilution of 1: 50, 000. It remains infective up to 24-30 hours at room temperature.

Transmission

The virus is sap-transmissible. The insect vectors are *Aphis craccivora* and *A. gossypii*, *A. evonymi* and *Myzus persicae*. The virus is also carried through the seed. According to Raychaudhuri and Nariani (1977), heating the seeds for 30 minutes at 50°C and treating them with thiouracil does not deactivate the virus.

Host Range

Phaseolus mungo, *P. aureus, P. Iunatus* and *P. calcaratus, Vicia faba, Cicer arietinum*, *Cyamopsis tetragonoloba, Trigonella foenum-graceum* and *Crotalaria sericea* are symptomless carriers of the virus.

Disease Management

The use of virus-free seed is recommended for the control of this disease. The resistant varieties—Kentucky Wonder, Contender and Pusa Parvati may prove to be a good means of controlling this disease.

Tristeza of Citrus

Distribution

This disease occurs in all citrus-cultivating states of India. The virus has been reported from almost all the citrus-cultivating regions of the world (Vasudeva and Capoor, 1958; Nariani and Raychaudhuri,

1968), except in certain parts in Mediterranean, some Central American countries and some isolated islands.

Tristeza affects all types of citrus plants but mainly orange, lime and grapefruit. Losses from tristeza vary because it depends upon the virulence of tristeza virus and the abundance of the vectors. In California, almost 4 lakh trees were destroyed by this pathogen in the first 15 years after the discovery of the disease. In Brazil, about 75% of the orange trees were destroyed by tristeza. Lee and Rocha-Pena (1992) have reviewed the work and have regarded it as a disease of international importance. Citrus tristeza virus exists in the form of many strains, having different biological activities. These biological activities belong to one or more of the following five major categories (i) mild (ii) seedling yellows (SY) (iii) decline on sour orange (iv) stem-pitting on grapefruit and (v) stem-pitting on sweet orange.

Symptoms

The typical symptoms appear as a quick or chronic decline of trees budded on susceptible rootstocks, but seedlings or budded trees may also develop stem pitting. In older trees, the symptoms include suppression of new growth and change of color of leaves from bronze to yellow. With the passage of time, the older infected leaves begin to fall off and later twigs begin to die back from the tip. Twig growth becomes weaker every season until the tree dies. The infected plants show root symptom consisting of a marked depletion of starch, death and decay of the feeder rootlets. Seedlings and budded trees of many plants develop stem pitting as a result of infection. Stem pitting consists of longitudinal grooves in the stem. Stem pitting decreases vigor of the trees, poor bushy growth and small, distorted fruits. Seedlings, when attacked with tristeza, develop the so-called seedling yellows symptoms. As a result of seedling infection, the first leaves are small and yellow.

Causal Virus

The causal organism is Citrus Tristeza virus (CTV). It is a phloem-limited Closterovirus (thread like virus) and is one of the largest plant viruses, appearing as a threadlike particle (2000 nm long and 11 nm in diameter). The virus contains a single-stranded RNA (ssRNA). The virus is transmitted by grafting, as it is only present in the phloem cells. The most important vectors are all aphids particularly citrus aphid, *Toxoptera citricida,* which is a stylet-borne vector. The vector becomes viruliferous after feeding for a few seconds and transmits the virus after equally short feedings.

Disease Cycle

The spread of the pathogen is through the use of tristeza-infected propagated material and through several aphid species in a semi-persistent manner. The virus is also transmitted by dodder. After infection of citrus plants with the tristeza virus, cells adjacent to a phloem sieve tube begin to degenerate and become necrotic. In plants that develop stem pitting, the degeneration spreads first into the cambium and inhibits the formation of normal xylem and phloem cells. In some feeder roots, the cambium is affected and therefore no normal xylem or phloem is produced after the infection. In plants that develop seedling yellows, the degeneration occurs in cells adjacent to phloem sieve tubes, which in leaves and stems are only mildly affected, but most of these in feeder roots become extensively necrotic. In plants, tristeza symptoms appear 7-8 months after inoculation with the virus. During this period, the root utilizes and finally exhausts the reserve starch. When the reserve starch is depleted, the root rot and decline or collapse follows.

Disease Management

1. It is more satisfactory and economical to remove susceptible trees after they become infected and to replant with a resistant plant.
2. In recent years, more satisfactory control of tristeza virus has been obtained by inoculating young citrus trees with selected mild strains of the virus in cross-protection or immunization.
3. According to Agrios (2005), considerable efforts are being made to express CTV genes such as the coat protein-gene that might make the trees resistant to tristeza.

Citrus Exocortis (Viroid Disease)

Distribution

Exocortis is worldwide in distribution. The pathogen (viroids) affects oranges, sweet lime, some lemons, grapefruit and other citrus trees grafted on exocortis-sensitive rootstocks. The pathogen causes great reduction in growth and yields are reduced up to 40%.

Symptoms

The infected plants show narrow vertical splits on the outer bark that give the bark a cracked and scaly appearance. Infected plants may also show yellow blotches of twigs on young infected stems and some plants show leaf and stem epinasty and cracking and darkening of leaf veins and petioles. All infected plants show stunted growth and have lower yields. Under field conditions, the symptoms of cracking and scaling are all evident on the bark, below the union of various *Citrus* species such as grape fruit, mandarins, and sweet oranges budded on above rootstocks.

Causal Viroid

The disease is caused by Citrus exocortis viroid (CEV). Viroids are small, low molecular-weight ribonucleic acid (RNA) without protein coat that can infect plant cell, replicate themselves, and cause disease. Citrus exocortis viroid consists of 371 nucleotides arranged in a circular or linear manner. CEV is transmitted from diseased to healthy plants by budding knives, pruning, by hand, by animals and by dodder (*Cuscuta* sp.). On contaminated tools, the pathogen retains its infectivity up to 8 days and can remain active at room temperature for several months. The thermal inactivation point of extracted sap is about 80°C for 10 minutes. According to Agrios (1996), in the past 15 years, CEV identification has been made by electrophoresis of infectious sap and by using radioisotope-labeled DNA probes complementary to CEV.

Diseases Cycle

The pathogen (CEV), survives in citrus plants and many herbaceous hosts and spreads to healthy plants by budding or wedge grafting and by contaminated cutting tools. No insect vector in known. The viroid enters the phloem elements and spreads in the plant. The viroid is associated with the nuclei and internal membranes of host cells and results in aberrations of the plasma membranes. The pathogen also brings about several metabolic changes in diseased plants. These changes include an increase in oxygen uptake in respiration, sugars and certain enzymes.

Disease Management

1. Propagating exocortis-free nursery trees can control the disease.
2. Dipping tools in a 10-20% solution of sodium hypochlorite for disinfection.
3. Removal and destruction of viroid-infected plants.

Cadang-Cadang Disease of Coconut (Viroid Disease)

Cadang-cadang disease of coconuts is found only in the Philippines. The name 'cadang-cadang' means dying in Bikol, a Philippine language. The disease was first reported from the Philippines in the year 1937; it has killed an estimated 30 million trees since then in that limited area. According to Agrios (1996), the disease is of great economic importance to the Philippines because of the great dependence of the local people on coconut plantations for food and lumber, and as a major cash crop through the export of coconuts and copra (the dried coconut meat from which oil is extracted).

Symptoms

The symptoms of the disease develop extremely slowly, over a period of 8-15 years and the tree may not die from the disease for 5-10 years after the first symptoms appear. Coconut palm trees usually become infected after they start flowering. The first symptoms appear on the coconuts, which develop scarification on their outer surface and the leaves start to show bright yellow spots. Three to four years later, the inflorescences are killed, which results in an abrupt stop in production of any further fruits on the palms. A few new leaves may develop, on which more and larger spots develop, leading to the chlorotic appearance of the disease from distance. Five to seven years from the beginning of the symptoms, the constantly increasing number of leaf spots gives the whole crown of the leaf a bronze colour and the number and the size of the leaves progressively reduced. Ultimately, the growing bud dies, falls off and leaves the trunk of the trees like telephone poles.

Causal Viroid

The disease is caused by a viroid (Hanold and Randles, 1991) known as cadang-cadang viroid (CCCVd). The viroid is known to consist of 246 nucleotides and in fact, is the smallest viroid known so far. The viroid is accompanied by a 247-nucleotide form of the identical viroid, along with an additional cytosine nucleotide. In later stages of infection, two viroids of longer forms appear and replace the smaller viroids in the fronds. These longer forms of viroids are of 296 or 297 nucleotides. These longer forms are the result of duplication of part of the right-hand end of the viroid molecule of the short forms. Thus, there is a changing pattern of the viroids of different nucleotides in the cadang-cadang disease of coconut. It is also interesting to note that this is the only viroid known to be infecting monocots and which kills its host plants.

Disease Cycle

The viroid is believed to survive in most palm tissues, including the husk and embryo of coconut. It is also transmitted to a small extent through seeds of coconut. Pollens of affected palms carry the viroid. However, it is not very definite or clear as to how the viroid spreads from one tree to another in nature.

The viroid may be carried on mouth parts of several chewing insects, mechanically on the machetes used to cut steps at the base of the palm, to dislodge the nuts, and to make cuts to the inflorescence for tapping the sap, and also possibly through the infected pollens.

Disease Management

No effective method of control of the disease is available and no resistant cultivars are available against the disease so far. Tissue culture can help in the production of viroid-free seedlings. Recent techniques of electrophoresis and nuclear techniques that help in the rapid detection of the viroid in parental material will go a long way in solving the problem of production of healthy seedlings free of any viroids, through the use of tissue-culture techniques or through healthy seeds (Agrios, 2005).

Potato Spindle Tuber (Viroid Disease)

Distribution

The disease potato spindle tuber is common in the United States, Canada, Russia and South Africa, where it causes quite severe losses. It has been reported from India as well (Owens *et al.*, 1992). It attacks all varieties of potatoes and is one of the most destructive diseases of potatoes due to its ability to spread rapidly.

Symptoms

The infected potato plants appear erect, spindly and dwarfed. The infected leaves are darker green and sometimes show rolling and twisting. The infected tubers are elongated with tapering ends. Yields are reduced to 25%.

Causal Viroid

The causal entity is Potato Spindle Tuber Viroid (PSTV). This is the first recognized viroid. PSTV is an infectious RNA of low molecular weight (100000 Dalton). The RNA is a single-stranded molecule of 359 nucleotides and about 50 nm in length. The pathogen is mechanically transmitted and is spread mainly by knives and tools used to cut healthy and infected potato 'seed' tubers and during handling and planting of the crop. Several insects, including aphids, grasshoppers, and beetles transmit PSTV. According to Singh (1970), true seed transmits the viroid in potatoes and tomatoes. It is also transmitted through pollen (Singh *et al.*, 1992).

Disease Cycle

After inoculation of a tuber with PSTV by means of a contaminated knife, or of growing plants with a sap from an infected plant, the viroid replicates itself and spreads systemically throughout the plant.

Disease Management

1. The disease can be controlled effectively by planting only disease (PSTV)-free potato tubers in fields.

2. Washing of hands and sterilizing tools after handling viroid-infected plants before moving on to healthy plants is recommended.

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Questions

1. What are viruses? How do they differ from other plant pathogens?
2. Viruses do not generally disseminate in regions of high altitude. Explain why this is so and how this feature has been utilized in agriculture.
3. List some important viral diseases and their vectors in a tabular form.
4. Discuss the nature of the development of the concept of viruses.
5. How will you find out whether a disease is due to virus or due to nutritional deficiency?
6. What are viroids? Who discovered viroids?
7. Name some important diseases of viroids.
8. Give one example each of ssRNA (positive RNA), ssRNA (negative RNA), ssDNA and dsDNA viruses.
9. Name a few diseases of international importance, caused by viruses and viroids.
10. How can tissue culture techniques help in controlling some of the diseases caused by viroids?

Diseases Caused by Nematodes and Flagellates 26

Diseases Caused by Plant Parasitic Nematodes

Nematodes are the most highly developed of the Pseudocoelomates. They belong to the phylum Nemata, previously named Phylum Aschelminthes. They are widely present in the soil, fresh water or marine water. Nematodes are the only plant parasites belonging to the animal kingdom, which are studied in plant pathology. Nematodes, sometimes called eelworms, are worm-like in appearance but quite distinct taxonomically from the true worms. Numerous species of nematodes attack and parasitize man and animals and cause various diseases. Several hundred species are known to feed on living plants as parasites and cause a variety of plant diseases. Crofton (1966) gave a general account of nematodes. Wallace published one of the most interesting works on the biology of plant parasitic nematodes in 1963. Swarup and Seshadri (1987) reviewed nematology in India. Khan *et al.* (2014) have discussed the methods of controlling nematodes. Dropkin (1977) discussed nematodes as parasites of plants, their ecology and the process of infection.

Literature on nematodes as plant pathogens and their biology, ecology, pathology control include those of Sitaramaiah (1984), "Plant Parasitic Nematodes of India"; Swarup (1990), "Nematode-Microorganism Interactions"; Khan and Khan (1993), "Nematode Problem of Vegetables"; Sitaramaiah and Singh (1993), *Hand Book of Plant Nematology*; Singh and Sitaramaiah (1994), "Plant Pathogen-The Nematodes", and "The Four Major Species of Root Knot Nematodes – Current Status and Management" by Khan (1997).

There are some other useful reviews and books on plant parasitic nematodes. These are of Luc *et al.* (1990), Duncan (1991), Sterling (1991), Brown *et al.* (1995) and Barker (2003).

All plant parasitic nematodes belong to the phylum Nemata (Nematoda). Most of the important genera belong to the subclass Secernentea, and order Tylenchida.

The characteristic features of the subclass Secernentea are: Excretory canals (protoplasmic) present; terminal excretory duct cuticularized; caudal glands absent; plasmids usually present; amphids usually minute, pore-like and cephalic in position; sensory organs papilloid, seldom setose; hypodermal glands absent; male with or without caudal alae.

Phylum: Nematoda
Order: Tylenchida
Sub order: Tylenchina

Super family: Tylenchoidea

The characteristic features are: Dorsal gland orifice in procorpus usually near base of stylet.

Family: Anguinidae

Genus: *Anguina,* wheat or seed gall nematode

Genus: *Ditylenchus,* stem or bulb nematode

Family: Belonolaimidae

Genus: *Belonolaimus*, sting nematode of cereals, cucurbits and legumes etc.

Genus: *Tylenchorhynchus,* stunt nematode of cotton, tobacco etc

Family: Pratylenchidae

Genus: *Pratylenchus,* lesion or meadow nematode of crop plants and trees

Genus: *Radopholus,* burrowing nematode of coffee, sugarcane, citrus etc.

Genus: *Nacobbus*, false root-knot nematode

Family: Haplolaimidae (oesophageal glands overlapping intestine, not enclosed in oesophageal wall)

Genus: *Hoplolaimus,* lance nematode of corn, cotton etc.

Genus: *Rotylenchus,* spiral nematode of many plants

Genus: *Helicotylenchus,* spiral nematode of various plants

Genus: *Rotylenchulus,* reniform nematode of tomato, tea, papaya etc.

Genus: *Scutellonema*, dry rot nematode of cassava and yam

Family: Heteroderidae (Female pear-shaped to lemon-shaped, males without highly specialized caudal alae)

Genus: *Globodera*, round cyst nematode of potato

Genus: *Heterodera*, cyst nematode of cereals, soybean, sugar beets and tobacco

Genus: *Meloidogyne,* root knot nematode of almost all crop plants

Superfamily: Criconematoidea

Family: Criconematidae (Anterior part of stylet usually greatly enlarged, cuticular striations moderate to coarse, even spinate)

Genus: *Criconemella,* ring nematode of woody plants

Genus: *Hemicycliophora,* sheath nematode of various plants

Family: Paratylenchidae

Genus: *Paratylenchus*, pin nematode of many plants

Family: Tylenchulidae (Excretory pore posterior to middle of body)

Genus: *Tylenchulus*, citrus nematode, olive, grapes etc

Suborder: Aphelenchina

Family: Aphelenchoididae (Female tails elongate, conoid to filiform. Male tails ventrally arcuate, Intestine joined directly to median bulb)

Genus: *Aphelenchoides,* bud and leaf nematode of strawberry, rice etc.

Genus: *Bursaphelenchus*, pine wilt and coconut palm or red ring nematode

Order: Dorylaimida

Family: Longidoridae

Genus: *Longidorus,* needle nematode

Genus: *Xiphinema,* dagger, nematode of trees

Family: Trichodoridae (spear and spear extension an elongate dorsal, Anterior part of stoma tubular with cuticularized walls, tail short)

Genus: *Trichodorus,* stubby root nematode sugar beet, potato etc.

Genus: *Paratrichodorus*, stubby root nematode of cereals and vegetables

The morphology and relative size of various plant parasitic nematodes are given in Figure 26.1

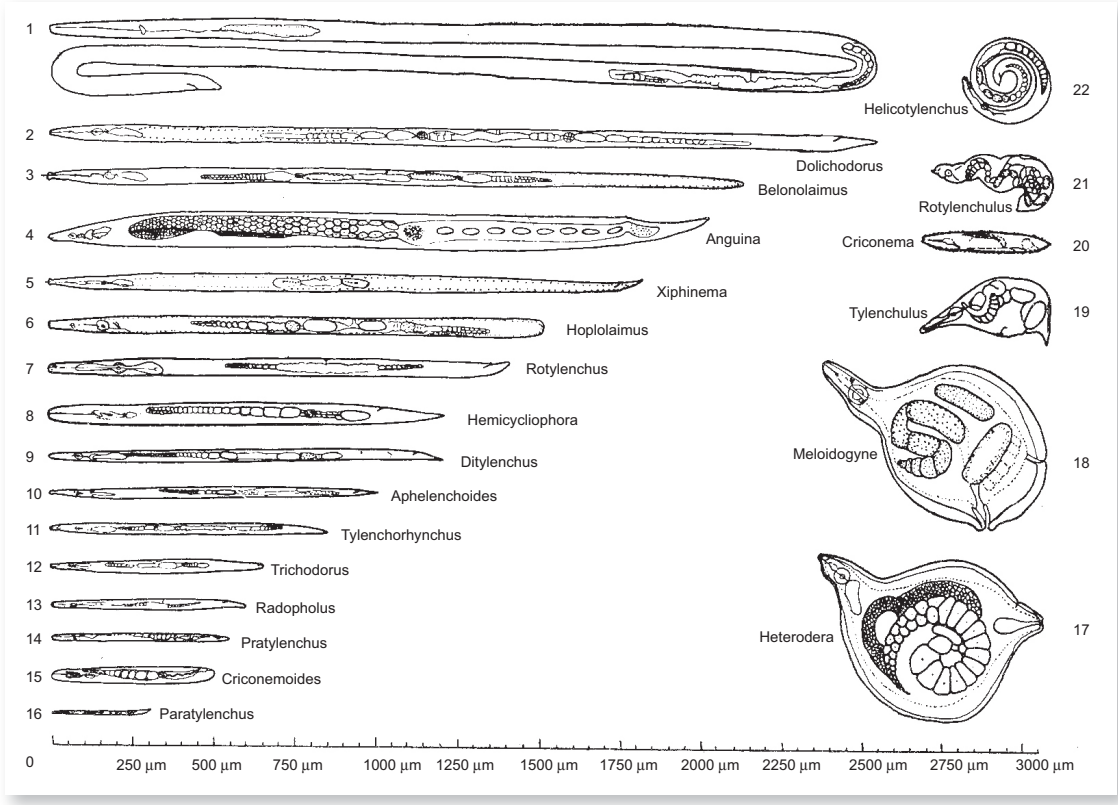


Figure 26.1 Morphology and relative size of the most important plant parasitic nematodes (Redrawn from G.N. Agrios (1969), Plant Pathology, Academic Press, London, p. 502

Isolation of Nematodes

Plant-parasitic nematodes are isolated from the roots or from the soil surrounding the roots, on which they feed. A few nematodes attack above-ground plant parts and these nematodes are isolated from the plant parts they infect.

A. Isolation of Nematodes from Soil

There are three methods of isolation of nematodes from soil viz. (i) Baermann funnel method, (ii) Sieving method, and (iii) Centrifuge or sugar flotation method (Fig. 26.2).

Baermann Funnel Method A Baermann funnel consists of a 12-15 cm diameter glass funnel to which a small piece of rubber tube is attached. The funnel is placed on a stand and filled with water. The soil sample is placed in the funnel on porous, wet-strength paper. Sometimes supported by a 5 to 6 cm circu-

lar piece of screen, or in a beaker over which a piece of cloth is fastened with a rubber stand. The beaker is then inverted in the funnel with the cloth and all the soil below the surface of the water and allowed to stand overnight or for several hours. The live nematodes move actively and migrate through the cloth or porous paper into the water and sink to the bottom of the rubber tubing just above the pinchcock. About 90% of the live nematodes are recovered by this method.

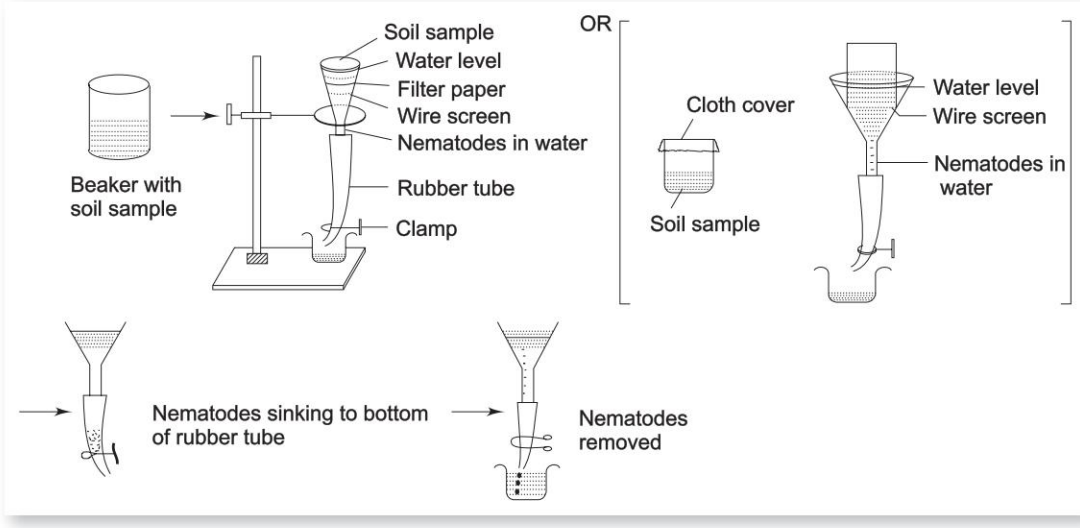


Figure 26.2 Isolation of nematodes from soil by Baermann funnel method

Sieving Method The second method (sieving method) is based on the fact that when a soil sample is mixed in water, the nematodes float in the water and can be collected on sieves with pores of certain sizes. First, the soil-water mixture is stirred and then allowed to stand for 30 seconds or so. The sieves are placed in the following order: 20-mesh sieve, followed by 60-mesh sieve and 200-mesh sieve. The supernatant is first poured through a 20-mesh sieve, which holds large debris but allows the nematodes to pass into 60 mesh sieve, which holds the larger nematodes and some plant debris and then passed through a 200-mesh sieve, which holds the small nematodes. The nematodes are then washed from 60 and 200 mesh sieves into petri dishes for examination and further isolation and identification (Fig. 26.3).

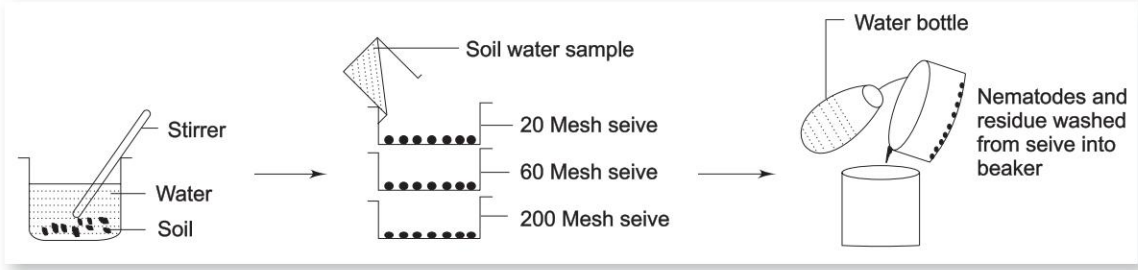


Figure 26.3 Isolation of nematodes from soil by sieving method

Centrifuge or Sugar Flotation Method The third method is Centrifuge or Sugar flotation method. Here, after following steps of sieving method, place beaker contents into centrifuge tubes and spin at 3000 rpm for 4 minutes. Now, discard the supernatant and retain the residue i.e. nematodes and debris. Fill tube with sugar solution and close it with stopper and shake until residue (nematodes and debris) is suspended. Again, fill tube with sugar solution and centrifuge at 3000 rpm for half to two minutes. Again discard the supernatant and retain the residue. Again, fill tube with water and shake until nematodes are suspended. Decant the supernatant into fine sieve. From the sieve, the nematodes can be flushed into petri dish with the help of water bottle for counting, observation and identification.

B. Isolation of Nematodes from Plant Material

Regardless of the type of plant material containing nematodes, the material is cut into very small pieces and is then placed in the Baermann funnel, as described above. The nematodes leave the tissue and move into the water in the tubing, from which they are collected in a petri dish for observation and identification.

Symptoms Caused by Nematodes

Nematodes infect the roots as well as the parts of the plant, which are above the ground. Root symptoms may appear as hypertrophy, necrosis or abnormal growth and include the following:

Root Knots or Root Galls

These are enlargements of the roots caused by the feeding of the nematodes, which may not necessarily be enclosed within them. The swelling may vary in size from 1 mm to more than 2 cm. The feeding of nematodes induces the formation of 'giant cells' in the host tissue and cell division is stimulated. This leads to the formation of galls of various sorts. Bird (1974) has discussed the response of plants to root knot nematodes under two major headings: First, the responses of entire plants; and second, the responses of their cells. Whole plants respond to infection by reducing their photosynthetic rate, growth and yield. It is suggested that the nematode influences the physiology of the plant by interfering with the synthesis and translocation of growth hormones produced in the roots. Cells of susceptible plants are changed from normal, undifferentiated cells to highly specialized syncytia, also known as giant cells or multinucleate transfer cells. This process involves a chain of events, starting with nuclear and nucleolar enlargement, followed by cell-wall breakdown, synchronous mitoses and incorporation of adjacent cells. This highly specialized syncytium is induced and maintained by, and is completely dependent on a continuous stimulus from the nematodes.

Root Lesions

There are discolored and often-collapsed portions of the root, consisting of cells on which nematodes have fed. They vary in size, from being as minute as to be almost invisible to the naked eye to lesions girdling the whole root. Toxic salivary secretions injected during the feeding of nematodes like *Radopholus* probably cause necrotic lesions.

Excessive Root Branching

This is caused by the formation of numerous short laterals in the vicinity of nematode injury.

Root Rots

Root rots occur when plant-pathogenic or saprophytic bacteria and fungi accompany nematode infections.

Injured Root Tips

This happens when the nematodes feed on or near root tips and cause them to stop growing, enlarging or disintegrating. Nematodes, feeding ectoparasitically at the root tips, suppress cell division in the apical meristem and result in short roots, as in *Trichodorus,* the stubby root nematode.

Nematode root infections are usually accompanied by non-characteristic symptoms in the above-ground parts of plants, appearing primarily as reduced growth, nutrient deficiencies such as yellowing of foliage, excessive wilting in hot or dry weather, reduced yields and poor quality of products.

Nematodes that attack the aerial parts of the plant may cause discoloration, necrosis, blotches, spots, distortion and galls on the leaf, stem and seed. Buds, growing points, or flower primordia are attacked by some nematodes, resulting in the abnormal growth of the affected plant. These symptoms are thought to result from substances secreted by the eelworm or perhaps by the invaded plant tissue. Hussey (1989) has discussed the disease inducing secretions of plant parasitic nematodes.

Mechanism of Nematode Injury to Plants

The mechanical injury directly inflicted upon plants by the nematodes during feeding is slight. Most of the damage seems to be caused by a secretion injected into plants while the nematodes are feeding. This secretion, called saliva, is produced in three glands from which it flows forward into the oesophagus and is ejected through the stylet. Some nematodes are such rapid feeders that in a matter of seconds they pierce a cell, inject saliva, withdraw the cell contents, and move on. Others feed more leisurely and may remain at the same juncture for hours or days.

The sedentary forms (*Heterodera* and *Meloidogyne*) remain attached to one point in the tissue throughout their lives. The males of the species may not penetrate the roots but the females invariably get established in or on the roots in a fixed position. In such species, it is the female that is responsible for the destruction of the host.

Nematode saliva has various functions, depending on the habit of the organism. The saliva of the plant nematodes seems to aid the parasite to penetrate the cell walls and possibly to liquefy the cell contents, making them easier to ingest and assimilate. The saliva, being toxic, proves disastrous to the plant tissues and its effects may reach up to the leaves even if the nematode is present only in the roots. As a result of the action of this saliva, any one of the following host responses may appear as symptoms of the disease: (i) cellular hypertrophy and hyperplasia, (ii) suppression of mitosis, (iii) cell necrosis, and (iv) stimulation of growth.

Dropkin (1969) and Sijmons *et al.* (1994) have reviewed the work on parasitic strategies of root nematodes and associated host cell responses and have discussed the various cellular responses to nematode infection.

The feeding of some nematodes produces only slight trauma in host cells. When the nematode *Tylenchorhynchus dubius* feeds upon the root hairs of *Lolium perenne,* a spherical mass is formed at the tip of the stylet within the host cells. For about 30 seconds, the nematode remains quiet, after which its bulb begins to pulsate and the spherical mass starts diminishing in size and finally disappears after one minute (Fig. 26.4a). In some associations, the stylet acts simply as the organ of penetration and suction tube. The nematode *Paraphlenchus acontiodes,* penetrates the host cells of *Pyrenochaeta terrestris* and withdraws their contents within 2 to 3 seconds (Fig. 26.4b).

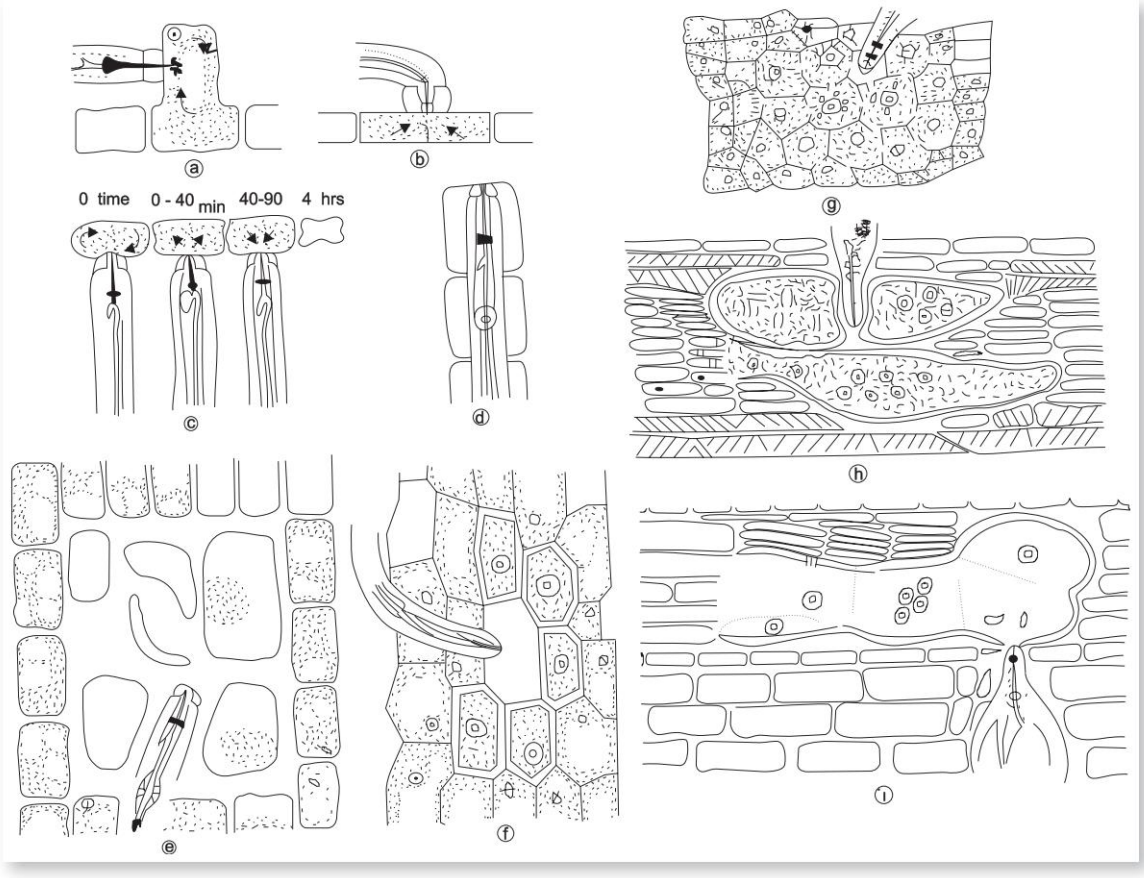


Figure 26.4 Effects of nematodes on plant cells: (a) no visible destructive effect; (b) immediate removal of cell contents; (c) delayed removal of cell contents (d) progressive penetration (e) cell lysis (f) nurse cells of *Tylenchulus* (g) syncytium of *Nacobbus* (h) syncytium of *Heterodera* (i) giant cells of *Meloidogyne* [Redrawn from V.H. Dropkin (1969), *Ann. Rev. Phytopath.*]

In certain other associations, such as *Dictylenchus myceliophagus* feeding upon *Botrytis cinerea,* there is delayed removal of contents (Fig. 26.4c). Progressive penetration is shown by the nematode *Rotylenchus uniformis* when feeding on the root hairs and cortical parenchyma cells of *Lolium perenne* as well as by the nematode *Pratylenchus crenatus* when piercing the epidermal cells of *Poa annua* rootlets (Fig. 26.4d). Many plant parasitic nematodes affect only those cells upon which they feed or

a limited number of cells in the immediate vicinity of the feeding site cells of infected tissues separate, undergo hypertrophy and lose chloroplasts (Fig. 26.4e). In certain host–parasite combinations, the pathogen stimulates changes in host cells, bringing about alterations in metabolism vital to the growth and development of the pathogen but do not bring about any cell destruction. The nematode *Tylenchulus semipenetrans* brings about an increase in the size of the nuclei and nucleoli, the cytoplasm of the host cells becomes dense, their wall thickens and the vacuoles of the infected cells disappear (Fig. 26.4f).

Anguina and *Nothanguina* species stimulate gall formation in leaves and flower parts of grasses and other plants. The galls develop here due to hypertrophy and hyperplasia of parenchymatous tissue with a central cavity harbouring the nematode. No syncytia develop here.

Members of the genera belonging to the family Heteroderidae show the greatest morphological adaptation to parasitism. At the site of the feeding of the nematode, a group of cells develop into characteristic syncytia around the head of the parasite. The cytoplasm becomes dense and the nuclei and nucleoli enlarge considerably. The cell walls are usually altered. Species of *Meloidogyne* induce hyperplasia in the pericycle, which results in the formation of galls. *Heterodera* species do not induce extensive hyperplasia. *Nacobbus batatiformis* forms galls on the roots of sugar beets and some other plants. Here, galls are formed as a result of hypertrophy of cortex and epidermal cells. The stele is not affected. Syncytial mass is formed by the alteration of cells immediately adjacent to the nematode's head and extends longitudinally along the root (Fig. 26.4g).

In *Heterodera,* infection syncytium is formed as a result of the enlargement of nuclei, incomplete dissolution of cell walls, disintegration of nucleoli, thickening of cell walls and by the cytoplasm becoming dense and granular (Fig. 26.4h). Giant cells of *Meloidogyne* are formed as a result of the enlargement of nuclei and the cells become polyploid and undergo synchronous mitosis. The cytoplasm of these cells becomes granular and new cells are incorporated by cell-wall dissolution. The walls of the syncytium become thickened and the cells of pericycle divide repeatedly (Fig. 26.4i).

Interrelationship between Nematodes and Other Plant Pathogens

The role of certain nematodes as vectors of plant viruses has been studied in recent years and there is an increasing awareness that they may also be involved in the transport and inoculation of other pathogens, notably bacteria and fungi attacking roots or other plant organs in the soil. Several types of nematode–pathogen interactions are suggested by Pitcher (1965), of which the following are examples:

The nematodes transport the pathogen from the soil to the plant or from one part of the plant to another, and the pathogen may fail to develop fully in the absence of the eelworm, as in twist of grasses and cereals caused by the fungus *Dilophospora alopecuri,* in association with *Anguina tritici.*

Much more important and more common are the interrelationships between nematodes and viruses. Brown *et al.* (1995) have reviewed the work done on transmission of viruses by plant nematodes. Several plant viruses, such as grapevine fan leaf virus, arabis mosaic virus, tomato ring spot virus, tobacco black ring virus, raspberry ring spot virus and tobacco rattle virus are transmitted by nematodes. Sixteen species of nematodes have so far been thought to be vectors of plant viruses.

Hewitt *et al*. (1958) made the first demonstration that eelworms are capable of transmitting plant viruses. They showed that the fan leaf disease of grapevines was spread by the eelworm *Xiphinema index.*

Several nematode fungus disease complexes are known. Fusarium wilt of several plants increases in incidence and severity when the plants are also infected by nematodes. Similar effects have also been noted in disease complexes involving nematodes and *Verticillium* wilt, *Pythium* damping off, *Rhizoctonia* and *Phytophthora* root rots.

Relatively few cases of nematode–bacterial disease complexes are known. In most of these, the role of the nematode seems to be that of providing the bacteria with an infection court and to assist bacterial infection by wounding the host.

In the yellow ear rot of wheat, the role of the nematode is to mechanically carry the bacterium *Clavibacter (Corynebacterium) tritici* inside the ovaries of the wheat flowers. In strawberry infection by the leaf nematode and the bacterium, a more involved interaction between the two pathogens seems to exist. Plants inoculated with both pathogens produce the so-called cauliflower symptom, quite distinct from either of the symptoms produced by each of the pathogens described above.

Ecology and Spread

Almost all plant-pathogenic nematodes lead part of their lives in the soil. Many of these live freely in the soil, feeding superficially on roots and underground stems, and although they may cause injury to plants, they are not strictly parasitic. Even in the most highly specialized sedentary parasites, the eggs, the pre-parasitic larval stages, and the males are found in the soil for all or part of their lives. Soil temperature, structure (porosity), moisture and aeration affect the survival and movements of nematodes in the soil. Nematodes occur in greatest abundance at a depth of 0-15 cm in soil. The distribution of nematodes in cultivated soils is irregular and is greatest in or around roots of susceptible plants. The greatest concentration of nematodes is in the region of host roots. This is primarily due to the greater amount of food available and also to the attraction of nematodes to the substances released into the rhizosphere. To these must be added the so called hatching factor – the effect of substances exuded from the root which diffuse into, or are carried to the surrounding soil and markedly stimulate the hatching of eggs of certain species; although, most nematode eggs hatch freely in water in the absence of any special stimulus.

Nematodes spread through the soil very slowly and by their own power. The speed of movement in the soil is dependent on pore diameter, particle size, water content of the soil and the diameter and relative activity of nematodes.

In addition to their own movement, however, nematodes can be spread by anything that moves and can carry particles of soil. Farm equipment, irrigation, flood or drainage water, animals and dust storms spread nematodes.

Temperature has a profound effect on the survival and multiplication of nematodes in the soil and also on their parasitism. The literature on the effect of the soil, temperature, moisture, aeration, soil texture, organic matter, rhizosphere, and various cultural operations on the activity of plant parasitic nematodes is quite substantial.

Meloidogyne spp. have been reported to develop most rapidly at about 27°C. Reproduction in *M. halpa* is extremely reduced at 35°C. The optimum temperature for the hatching of larvae in *M. incognita* is 30°C. The temperature also affects the resistance of resistant varieties and the size of the galls formed by root knot nematodes on the host.

Soil moisture is important for nematodes in many ways. *Meloidogyne* spp. can enter potato tubers through lenticels, which occur on tubers commonly in wet soils. Galling of tubers is less in dry soils. Other factors which influence the activity of plant parasitic nematodes are soil pH, soil texture, aeration, types of plants grown between the susceptible hosts, duration of cultivation of resistant varieties in the field, type of decomposable organic matter added to the soil, inorganic fertilizers, and type of nitrogen and potassium status in the soil.

Characteristics of Plant Pathogenic Nematodes

The phytopathogenic nematodes and their relatives comprise a large number of species placed in 15 families and 111 genera. The gross morphology of these nematodes, with few exceptions, is generally very similar. The morphological and anatomical features of typical male and female plant parasitic nematodes are given in Fig. 26.5. A cuticle covers the body. Internally, there are digestive, excretory and reproductive systems.

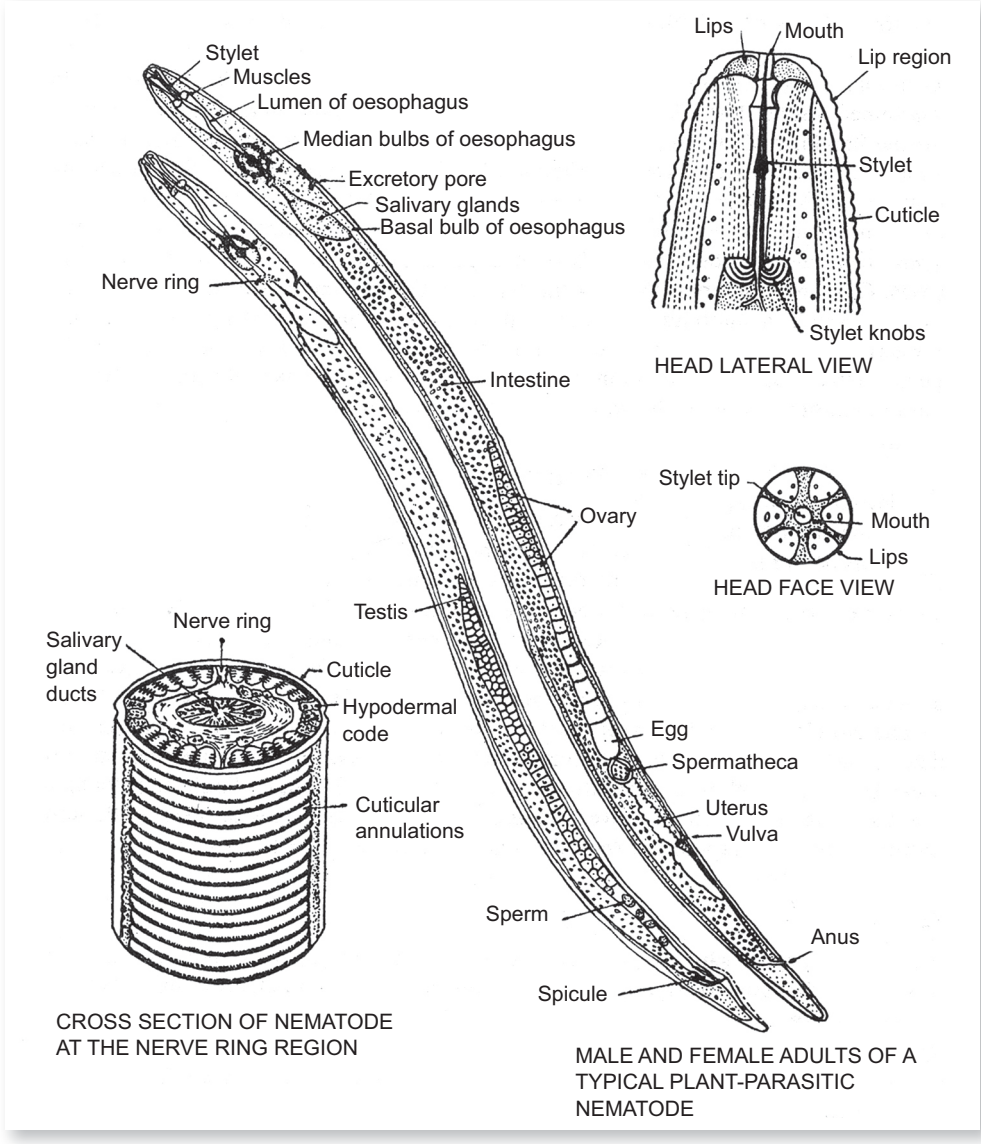


Figure 26.5 Morphological and anatomical characteristics of typical male and female plant parasitic plant nematodes [Redrawn from G.N. Agrios (1969), Plant Pathology, Academic Press, London, p. 500]

The nematode body is more or less transparent. It is covered by a colourless cuticle, which is usually marked by striations, or may show bristles, punctuations, warts, or other markings. The cuticle molts when the nematodes go through their successive larval stages. The cuticle is produced by the hypodermis, which consists of living cells and extends into the body cavity as four chords, separating four bands of longitudinal muscles. These muscles enable the nematode to move. Additional specialized muscles exist at the mouth and along the digestive tract and the reproductive structures.

Digestive System

The oesophagus (pharynx), which is the anterior end of the digestive system, consists in the Tylenchids, of a stylet (stomatostyle), corpus, isthmus and glandular posterior enlargement. Modifications in the morphology of the oesophagus are widely used at all levels of nematode taxonomy. Many of the groupings of higher taxa in the Tylenchida, as well as in those groups of Dorylaimida containing phytoparasitic nematodes, are based largely upon modifications of gross shape and internal morphology of the oesophagus. The digestive system is a hollow tube extending from the mouth through the buccal cavity, oesophagus, intestine, rectum and anus. Lips, usually six in number, surround the mouth.

All plant parasitic nematodes have a protrusible hollow stylet or spear. This is the main characteristic that suggests that the nematode is a plant parasite. This structure has been homologized in Tylenchida with the stoma of other nematodes and is used as a piercing organ through which nematodes acquire food. It is actually a three-part structure taxonomically; the stylet is used at all levels of classification.

The body cavity is rudimentary in its development and contains a fluid through which circulation and respiration take place.

The excretory system is not well developed in nematodes. However, the nervous system is well developed and consists of many nerves, ganglia and sensory structures.

Reproductive Structures

The reproductive systems are well developed. The female nematodes have one or two ovaries followed by an oviduct and uterus terminating in a slit-like vulva. The male's reproductive structures are similar to the females but have a testis, seminal vesicle and ejaculatory duct, and terminate in a common cloaca with the intestine. A pair of protrusible, copulatory spicules is also present in the male. Reproduction in the nematodes is through the eggs and may be sexual, hermaphrodite or parthenogenetic. Many species lack males.

Life Cycle

The life histories of most plant-parasitic nematodes are, in general, quite similar. There are six stages in the life cycle of a nematode – Egg, L_1 (Larval stage), L_2, L_3, L_4, and Adult. Eggs hatch into larvae, whose appearance and structure are usually similar to those of the adult nematodes, except for the development of the reproductive systems. Larvae grow in size and each larval stage is terminated by a molt. After the final molt, the nematodes differentiate into adult males and females. The females can then produce fertile eggs either after mating with a male or in the absence of males, parthenogenetically, or they can produce sperm themselves.

The time for the completion of the life cycle is about 20-40 days under optimum environmental conditions, but takes longer in cooler temperatures. In some species of nematodes, the first or second larval stages cannot infect plants and they depend for their metabolic functions on the energy stored in the egg. When the infective stages are produced, however, they must feed on a susceptible host or else starve to death. If suitable hosts are not available, all the individuals of certain nematode species may die but in other species, the eggs may remain dormant in the soil for years. The disease cycle of a typical root knot nematode of the genus *Meloidogyne* is given in Fig. 26.6 and that of soybean cyst nematode (*Heterodera glycines*) in Fig. 26.7.

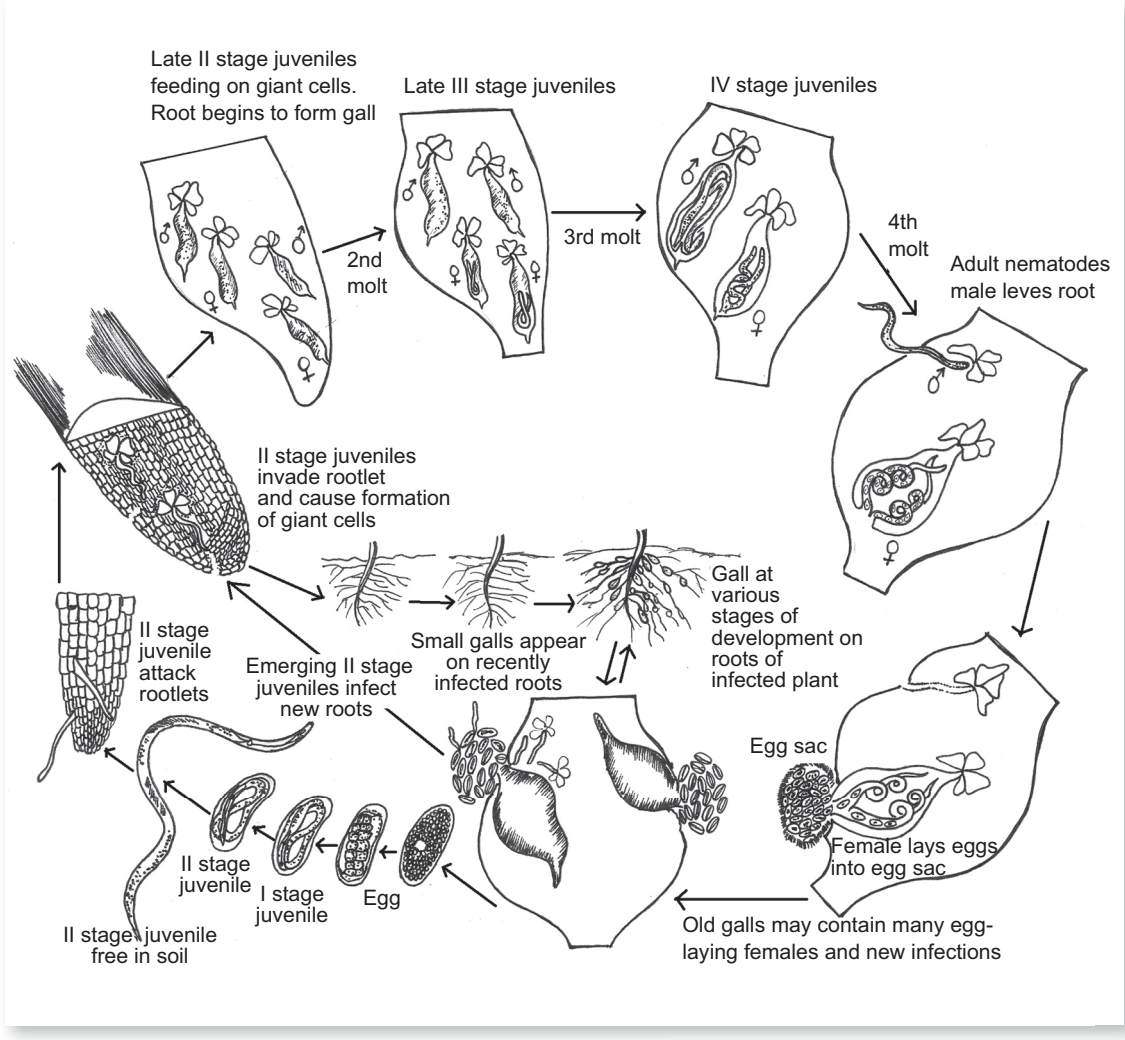


Figure 26.6 Disease cycle of root knot caused by nematodes of the genus *Meloidogyne* [Redrawn from G.N. Agrios (1969), Plant Pathology, Academic Press, London, p. 519]

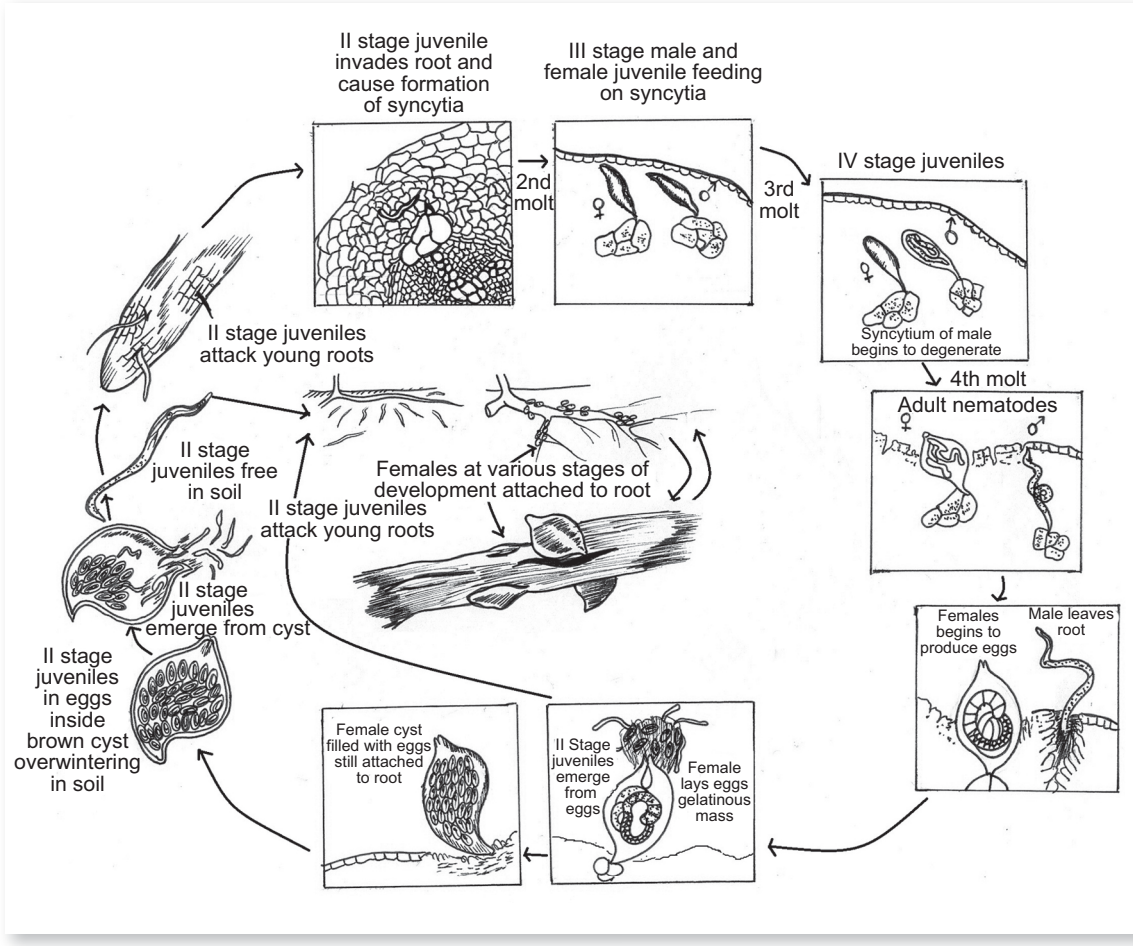


Figure 26.7 Disease cycle of the soybean cyst nematode *Heterodera glycines* Redrawn from G.N. Agrios (1969), Plant Pathology, Academic Press, London, p. 525]

General Methods of Control of Nematode Diseases

Many accounts have been published of the attempts to control plant nematodes in different countries and climates. These are discussed in brief here.

Chemical Control

Chemicals with nematicidal properties are known as nematicides. Many such chemicals are recommended for nematode control. Nematodes are diverse in their chemical and biological activities and in their behavior in soil. There are two general types of nematicides: fumigants and non-fumigants. Chemically, they can be categorized as halogenated aliphatic hydrocarbons, methyl isothiocynate liberators, organophosphates and organo carbamates. The common pesticides of nematodes are given in Table 26.1.

Table 26.1 Common Nematicides

	Common name	Class
1.	**Fumigants**	
A.	Halogenated aliphatic hydrocarbons	
	Bromomethane	Methyl bromide
	Trichloronitromethane	Chloropicrin
	1,3-Dichloropene	1,3 – D
	1,2-Dibromoethane	EDB (Ethylene dibromide)
	1,2-Dibromo-3Chloropropane	DBCP (Nemagon)
B.	Methyl isothiocynate liberators	
	Methyl isocynate	MIT
2.	**Non-fumigants**	
A.	Organophosphates	Ethoprop (Mocap)
	O EthylS,S- Dipropylphosphorodithioate	
	O,O-Diethyl-O- [(methylsulfinyl)phenyl]-Phosphorothioate	Fensulfothion (Dasanit, Terracur P)
	Ethyl 4-(methylthio)-m-tolyl-Isopropylphosphoramidate	Phenamiphos
B.	Organocarbamates	Carbofuran (Furadone)
	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate	Aldicarb (Temik)
	2-Methyl-2 (methylthio) propionaldehyde	
	O- (methylcarbamoyl) oxime	Oxamyl (Vydate)
	Methyl N, N-(dimethylcarbamoyl)-N-(methylcarbamoyl) Oxy)-1-thioformimodate	

Use of nematicides is one of the most important and reliable means for management of root knot nematodes. Nematicides are principally applied to control nematode population in the soil before the crop is grown (Khan, 1997). As the number of nematicides available for safe control of nematode diseases is declining drastically, the other methods of control are becoming increasingly important. Among the fumigants, Carbon disulphides, Chloropicrin, DD, DBCP (Nemagon), Vapam, methyl bromide, etc. have been banned now due to their polluting effect. Now, more stress is on systemic, non-fuming nematicides such as Carbofuran, Aldicarb, Oxamyl, Phenamiphos and Phorate etc. In addition, there are a number of highly effective contact nematicides/ insecticides, which are being used increasingly for chemical control of nematodes in soil.

Chemical control of nematodes has not made much headway in India mainly due to the prohibitive cost of the nematicides, difficulty of application over large areas and the lack of awareness of the nematode problem. Inspite of high initial costs of nematicides, there is increasing evidence that a judicious use of these chemicals would be economical on selected crops, such as cotton, sugarcane, tobacco, citrus, grape vine, potato, vegetables and even wheat and barley in heavily infested fields.

Cultural Practices

They result in partial or complete control of nematodes, and include:

Crop Rotation of Non-host Plants Several nematode species can infect only a few crops. Since all plant pathogenic nematodes are obligate, the absence of susceptible hosts from the soil for two to three years will result in the elimination of nematodes from that area through starvation and inability to reproduce. The value of crop rotation has long been recognized in the control of *Heterodera* spp. Thus, continuous cropping with potatoes increases the cyst population of *H. rostochiensis* more that three-year rotations with corn, green beans, red clover or perennial rye grass. Brown (1961) gave some idea of the effect of crop rotation on the yields of potatoes growing in soil infected with *H. rostochiensis.* Potato yield, after three years of continuous cropping was 1,631 kg/hectare, and with a non-host in the second year, it was 4,515 kg/hectare.

In recent years, a new term "cropping system", which is defined as a sequence of growing crop, may be spatial or temporal. Arrangement of different crops such as mixed cropping, row intercropping and strip cropping is known as spatial sequence. Distribution of crop cover a period of time such as crop rotation, relay cropping, discontinuous cropping and fallowing is called a temporal sequence. Both spatial and temporal sequencing have been found to be effective in controlling root knot nematodes on vegetable crops (Khan, 1997). A number of non-hosts or poor hosts of root knot nematodes like wheat, rice, onion and maize are known to increase yield of vegetable crops in appropriate temporal sequence. Economic consideration is however a serious demerit of crop rotation. It can be successfully used when all the crops grown in the sequence are of economic value. Rotation of crops or cultivars with different genes for resistance against the species or races of root knot nematodes has been suggested.

Some plants appear to be antagonistic to nematodes because they reduce populations to a greater extent than non-hosts do. *Crotalaria* is a good example of this. It has been suggested that the *Crotalaria* root system has a toxic effect on nematodes, and when planted before a crop, it may reduce subsequent damage.

There is evidence from Dutch nematologists that *Tagetes* spp. may reduce the population of some plant-parasitic nematodes. Oostenbrink and his colleagues (1957) tested 16 varieties of *Tagetes patula* and *Tagetes erecta,* placed between the rows or around other plants. They found that all varieties suppressed the populations of *Pratylenchus* in the roots of other plants as well as in the soil, resulting in a better growth of perennials in the second year of the succeeding crop. The *Tagetes* had to be grown for three to four months and it was suggested that the roots had a nematicidal action. Inspite of many criticisms, the use of cover, trap or enemy crops provides a potentially useful control measure in crop rotations. The evidence that *Asparagus* exudes a nematicidal chemical from its roots supported this opinion.

In addition to *Crotalaria* or *Tagetes,* there are some other plants such as some grasses, varieties of mustard, Margosa or neem (*Azadirachta indica*) root exudates and *Asparagus* have been tested as enemy plants to nematode population. In some vegetable plots and in green house-grown crops, mulching of soil with straw, weeds, grasses, old newspaper sheets, dry pine needles, the tree bark, etc., have been found effective in reducing nematode attacks.

Sanitary Practices They include the cleaning of all machinery thoroughly before moving into an uncontaminated area, taking care not to bring nematodes into a field by means of contaminated nursery stock, seed, containers, etc., keeping soil fallow free from host plants which deprive nematodes roots on which to feed. Thus, regulatory methods (quarantine) can be the least expensive methods to control nematode diseases.

Flooding Some species of nematodes have adapted to living in the normal soil with ordinary moisture content and depend on a certain amount of aeration present in it. Flooding the land for a period of several months results in the death of these nematodes and thus frees the land of these pathogens. However, it is possible or practical to flood the land for long periods in very few fields and, therefore, the applicability of this control method is quite limited.

Fallowing This is another method of controlling the soil-borne nematodes. The fields or plants are ploughed to keep them free from vegetation and exposed to dry summer heat of the sun. Starvation and desiccation kill the nematodes.

Predators Although simple observations have shown that predaceous species of nematodes occur in the soil and can be utilized to eat away parasitic forms, so far, very little work has been done along these lines. Nevertheless, it has been found that many nematophagous species of fungi are effective means of destroying the nematodes and these can be encouraged in the soil through organic soil amendments, such as the addition of decomposable vegetable matter.

Physical Methods

Plant-parasitic nematodes can be easily killed in the laboratory by the application of heat irradiation, osmotic pressure, etc. It is more difficult, however, to employ such methods on large quantities of soil, especially if they are under cultivation.

Irradiation There is strong evidence that nematodes are killed or seriously affected by irradiation. Fassuliotis and Sparrow (1955) demonstrated that irradiation of potato tubers with X-rays inhibited sprouting and *Heterodera rostochiensis*. Cysts of this developing nematode exposed to 20,000 roentgens contained only brown and dead eggs; at 40,000 roentgens, the eggs lost their contents completely. It is, however, difficult to irradiate nematodes. In fact, it is impracticable because of the duration of time needed to irradiate even small areas and because damage to plant roots occurs at levels of irradiation below those required to disrupt the nematode's life cycle.

Heat Heat treatment is probably the most successful physical control measure developed so far. It is widely used for the killing of nematodes within plant tissues before planting and has proved useful on nematode-infested bulbs, corms and tubers, and roots of plants such as chrysanthemums, strawberries, bananas and citrus. This process involves determining the correct time and temperature for killing the nematode but not the host. This entails immersing the nematodes (contained in as small volume of water as possible) into thermostatically-controlled water baths at different temperatures for different lengths of time. After such treatment, the nematodes are examined for activity. Blake (1961) suggested that banana setts less than 13 cm in diameter should be immersed in water at 55°C for 20 minutes in order to kill *Radopholus similis*.

A new approach to heat treatment is the application of dry heat (Thomason *et al.,* 1960). Sweet potato seed roots were treated at 45°C for 30 hours and these completely controlled root knot nematodes, with 60% of the roots surviving. The white tip nematode of rice *Aphelenchoides besseyi* has a widespread occurrence in India in most of the rice-cultivating areas. Hot water treatment of the seed at 52-55°C for ten minutes is the best way to ensure complete destruction of the nematodes.

Biological Control

According to Garrett (1965), "Biological control is any condition under which, or practice whereby, survival or activity of pathogen is reduced through the agency of any other living organism (except man himself), with the result that there is a reduction in incidence of the disease caused by the pathogen." Trap crops, resistant varieties and antagonistic plants that release nematicidal root exudates may be considered useful or potential biological controls for nematodes.

Biological control aims at increasing the parasites and predators of nematodes in the soil, to increase the mortality of plant nematodes. This can be done by changing the environment, adding organic amendments or introducing other organisms directly.

To increase the concentration of nematode-destroying organisms in the soil, it is necessary to alter the environment so that some of the forces restraining the multiplication of the organisms are weakened. The only serious attempt to biologically control plant nematodes has been concerned with the use of nematode-trapping fungi.

Oil cakes of margosa, castor and peanut at 0.2% (W/W), used three weeks before planting significantly reduce infestation of *M. javanica* on okra and tomato. In almost all these experiments, a soil amendment was used, thereby altering the environment in favour of nematode-trapping fungi.

In the late 1930's, Linford and his group successfully reduced the incidence of root knot of pineapple by incorporating green plant material into the soil. It is generally assumed that the addition of this material stimulates the population of organisms antagonistic to nematodes, which in turn, reduces the parasitic nematode population. There are several nematode-trapping fungi such as *Arthabotrys robusta, A. oligospora and A. superba.*

Duddington and Duthoit (1960) obtained statistically significant reductions in the mean number of larvae of *Heterodera avenae* on 60 cm square plots, to which 675 gm of chopped cabbage had been added as compared with untreated plots. Duddington *et al.* (1961), in later experiments, obtained further evidence that *Dactylaria thaumasia* reduces the infection of oat seedlings by *Heterodera avenae.* There is some doubt as to whether or not the fungi are entirely responsible for such reductions.

Resistance to nematodes may be due to the production of toxic root exudates as found in asparagus, lack of an attractant or a hatching factor in the exudates, barrier to penetration or failure of nematodes to develop within plant tissues.

Paecilomyces lilacinus has been found quite effective in controlling root-knot nematode under field conditions. *Pasteuria penetrans,* a bacterial biocontrol agent for the control of *Meloidogyne* spp., has also shown promising results.

The biology, ecology and potential of biocontrol agents for nematodes have been reviewed in recent years (Sikora, 1992; Trivedi and Sharma, 2012). It must be stressed that several organisms that are effective natural enemies of nematodes in the field may have limited potential as biological control agents for application by growers. The stage in the nematode life-cycle attacked by control agent has a profound effect on the damage to the control and the level of population control. Therefore, trapping fungi and rhizosphere bacteria that attack the second stage juveniles of cyst and root rot nematodes may significantly improve crop growth but is unlikely to prevent nematode populations from increasing, especially those species that have more than one generation in a growing season. Root-colonizing fungi such as mycorrhizae and endophytic species such as *Fusarium* may reduce both nematode invasion and development.

Soil amendments may also reduce nematode infestations and by providing an energy source, help to increase numbers of facultative parasites, pre-colonized substrates are most effective in establishing nematophagous fungi in soil. Soils that are naturally suppressive to some nematodes may be used

to shorten rotations of susceptible crops and improve the performance of nematicides and resistant cultivars.

It is established that many fungi are known to produce nematicidal or nematistatic compounds. Dababat and Sikora (2007) investigated the activity of *Fusarium oxysporum* against *Meloidogyne incognita.* The fungus inhibited juvenile penetration, gall formation and egg masses production within tomato plants. Olivares-Bernabeu and Lopez-Llorca (2002) studied the biological factors related with the development and performance of *Verticillium chlamydosporium, V. lecanii* and *Paecilomyces lilacinus* as biocontrol agents for *M. javanica*. Khan *et al.* (2004) could prove that, hatching of *M. javanica* eggs were reduced as a result of *Paecilomyces lilacinus* protease and chitinase enzymes either individually or in combination. The chitinase activity was also increased in culture filtrates of *Trichoderma harzianum*; this increment expresses the ability of this fungus to infect *M. javanica* eggs (Sahebani and Hadavi, 2008).

More than 100 fungus species have been designated as nematophagous, a term that they have applied to fungi that "feed" on nematodes either as predators or parasites. These nematophagous fungi are members of either the Chytridiomycota or Oomycota or the class Moniliales of the Fungi Imperfecti, are abundant throughout the world and are frequently encountered in soil samples.

Vesicular-arbuscular mycorrhizal (VAM) fungi are beneficial soil fungi, which form symbiotic association with roots of many plants. During colonization, VAM fungi can prevent root infections by reducing the access sites and stimulating host defence. VAM fungi have been found to reduce the incidence of root knot nematode. Various mechanisms also allow VAM fungi to increase a plant's stress tolerance. This includes the intricate network of fungal hyphae around the roots, which block pathogen infections. Higher amounts of amino acids, especially arginine, found in the root exudates of mycorrhizal plants, reduce chlamydospores production in *Thielaviopsis basicola*. Tomato roots inoculated with *G. fasciculatum* had increased concentration of phenylalanine and serine; these two amino acids being inhibitory to root knot nematode development. Increased peroxidase, localized mainly in the vacuoles and cell wall and phytoalexins (glyceollin, coumestrol) have been extensively documented in VAM positive plants. Thus, plants infected with VAM fungi are likely be more resistant to subsequent attack by pathogenic fungi and nematodes.

Nematodes in the soil are subject to infection by bacteria and fungi. This creates the possibility of using soil-microorganisms to control plant-parasitic nematodes. Bacteria are the most abundant organisms in the soil and some of the genera like *Pasteuria*, *Pseudomonas* and *Bacillus* have shown great potential for the biocontrol of nematodes. They affect nematodes by a variety of modes: for example, parasitizing, producing toxins, antibiotics or enzymes; interfering with nematode–plant host recognition; competing for nutrients; inducing systemic resistance of plants and promoting plant health. Spores of *Pasteuria* can attach to the cuticle of the second-stage juveniles and germinate after the juvenile has entered roots and begun feeding. The germ tubes can penetrate the cuticle and vegetative micro-colonies then form and proliferate through the body of the developing female. Finally, the reproductive system of the female nematode degenerates and the mature endospores are released into the soil. Numerous *Bacillus* strains can suppress pest and pathogens of plants and promote plant growth. Rhizosphere *Pseudomonas* strains also exhibit diverse pathogenic mechanism upon interactions with nematodes. The mechanism employed by some *Pseudomonas* strains is the production of antibiotics and the induction of systemic resistance. Other Rhizobacteria reported to show antagonistic effects against nematodes include members of the genera *Actinomycetes*, *Agrobacterium, Arthrobacter, Clavibacter, Clostridium, Corynebacterium, Enterobacter, Gluconobacter, Klebsiella.* Rhizobacteria reduce nematode populations mainly

by regulating nematode behavior, interfering with plant-nematode recognition, competing for essential nutrients, promoting plant growth and inducing systemic resistance. *Bacillus thuringiensis* (BT) produces one or more parasporal crystal inclusions (Cry or d-endotoxins), which are known to be toxic to a wide range of insect species. Cry protein exerts its effect by forming lytic pores in the cell membrane of gut-epithelial cells of an insect. After ingestion of toxin by target nematode larvae, the crystals dissolve within the gut of the nematode and this is followed by proteolytic activation. Cry toxicity is directed against the intestinal epithelial cells of the midgut and leads to vacuole and pore formation and eventually degradation of the intestine.

Breeding for Resistance

This is the best method of controlling any disease. However, breeding for resistance against soil nematodes is a difficult task. This is further made difficult by the presence of a large host range of the pathogens and the occurrence of several races or biotypes of the pathogens. Several varieties of crops resistant to nematodes have been developed in our country. Some important ones are: Karnataka Hybrid and VNF-8 in tomato against root knot nematode; Raj Kiran and RD-2052 in barley, against cereal cyst nematode; Anbeern-56 in cotton; Evergreen variety in onion; V-161 in cowpea against reniform nematode; TVAV 6464 in rice against rice lesion nematode; Troyer and Carrizo in citrus against citrus nematode; Gross Michel in banana against burrowing nematode; Branco muniero in garlic against stem and bulb nematode and SSM 591 in rice against *Aphelenchoides besseyi.*

Root Knot of Vegetables

The root knot nematodes, *Meloidogyne* spp., stand out as the most dominant group of plant parasitic nematodes in almost every vegetable field and cause enormous losses every year in the nursery as well as planted fields. The common species are *Meloidogyne arenaria, M. incognita* and *M. javanica.* They attack such crops as various cucurbits, potato, tomato, brinjal, chillies, lady's finger (Okra), groundnut, carrot, radish and *Colocasia. M. arenaria* is commonly associated with yellow decline disease of chilies in the district of Coimbatore. As a result of infestation, plant growth is reduced and the leaves turn yellow.

Symptoms

In potatoes, the disease is not easy to recognize in the field as often no above-ground symptoms are produced. Sometimes, plants are stunted, sickly, and even show signs of premature and sudden dying. The nematode infestation on the tuber appears as tiny tubercles but heavy and localized infestations stimulate excessive cell division leading to gall formation. The disease may be complex with brown rot or bacterial wilt caused by *Ralstonia (Pseudomonas*) *solanacearum* and in such cases plants usually die suddenly. In tomatoes, the symptoms are somewhat more pronounced. The plants develop slowly and appear stunted if the infestation has been early and severe. The leaves are yellowish-green to yellow, tend to droop and wilt suddenly if wilt organisms are present. Sometimes, the leaves get scorched from the margin inwards. The main root and the laterals in all cases bear spherical to elongated galls. The presence of these galls is the most characteristic symptom.

Symptoms similar to those on tomato are also noticed on brinjal (eggplant), chillies, lady's finger (Okra), cow pea, and French beans. In the case of chilies, the plant growth is reduced and the leaves turn yellow.

Morphology and Life Cycle

The eggs of *Meloidogyne* spp. are ellipsoidal and measure 67-128 × 30-52 microns. Differentiation of the larvae takes place within the eggs. The eggs are laid in the body of the female or in a gelatinous matrix extruded from the vulva. Up to 600 eggs are laid by each female. Numerous females of the nematodes are present in the root galls. These survive in the soil and leaf debris.

The second-stage larvae, liberated after the hatching of the eggs, move about in the soil but their movement is very slow. During their movement in the soil, various physical, chemical and biotic soil environments influence the larvae. High temperatures in the range of 40-50°C kill these larvae quickly. Hence, during hot summer months, most of them in the top 5-7 cm layer of soil are killed.

The movement of these larvae is slowed down tremendously by the presence of excess soil moisture, clay content of the soil, and lack of aeration; sandy, light soils favour their movement best. Consequently, the disease is common in light-textured soils. When these larvae approach the roots of plants, they are attracted by the root exudates and are held in a mass around the roots. Finally, these second-stage larvae cause root infection.

The invading larvae first penetrate the meristematic tissue and migrate until the head becomes established in an intercellular space near the endodermis. The females remain in this position permanently. The males soon die. Around the mouths of the feeding females, giant cells are formed due to host-parasite interaction. These giant cells are a characteristics feature of root knot infection. Roots are infected over a temperature range of 12-35°C. In general, a temperature of 25-28°C is best for infection, rapid multiplication and increased size of galls. The disease cycle of a typical root knot nematode of the genus *Meloidogyne* is given in Fig. 26.6.

Disease Management

Because of the widespread and serious nature of this problem in vegetable crops, much attention is being given to control these nematodes by the use of chemicals and resistant varieties.

The use of chemical fumigants has been attempted in certain cases with marked success. Nematicides, such as DD, DBCP, Phorate and Carbofuran have been found to be very effective in experiments, but their use is still restricted due to the high cost of the chemicals.

The addition of neem cake and similar non-edible oil cakes at the rate of 25 quintals per hectare has been found to reduce root knot incidence in repeated field trials.

Application of sawdust, at the rate of 25 quintals per hectare, along with standard NPK fertilizers is cheaper and equally effective in reducing the disease.

Soil solarization has been found effective in the control of root knot of tobacco. Krishna Rao and Krishnappa (1995) reported that solarization by covering the soil with clear transparent polythene sheet for 6 weeks during hot summer months showed an increase in soil temperature by 8°C and concentration of moisture (5% more) when compared to non-solarized soils. This resulted in decrease in population densities of *H. incognita* and *Fusarium oxysporum* in chick pea fields.

It is essential that all the sources of nematodes, such as weed hosts, should be eliminated from the field. The soil should be deep ploughed during summer and allowed to dry.

Flooding has been found to effective in eliminating root knot nematodes from the soil. Lowland rice culture preceding the vegetable crop reduced the incidence of root knot in the latter. Resistant varieties to various species of root knot nematodes do occur in many vegetable crops and these should be cultivated. The biocontrol agent, *Trichoderma harzianum* and *T. lignorum* have the potential to suppress the disease (root knot) by colonizing the second-stage larvae and even penetrating the eggs.

Molya Disease of Barley and Wheat

This disease (Molya disease or cereal cyst nematode) was first reported on wheat and barley in Rajasthan. Prasad *et al.* (1959), and Swarup and Singh (1961) described the disease. It is now known to be widespread in the states of Rajasthan, Haryana and Punjab. The damage may be as much as 50% in certain infected areas. Barley is more susceptible than wheat. This disease is known as cereal root eelworm in England and occurs on wheat, oats and barley.

Symptoms

The disease occurs in patches in the field. If the same crop is cultivated year after year the diseased patch increases until the whole field is infected. The infected plants become dwarfed and pale, with a matted root system. Mild swelling occurs hear the root tips. Glistening white bodies are seen adhered to the roots. These bodies (cysts) become brown and may remain attached to the roots or fall off in the soil after the roots decay.

Morphology and Life History

Prasad *et al.* (1959), and Swarup and Singh (1961) have identified the nematode causing this disease to be *Heterodera avenae* (*H. major*). The lemon-shaped, brown cysts of the nematode lying in the soil measure about 400-500 microns in width and 600-700 microns in length. The number of eggs in each cyst is variable. On attaining its full length, the embryo undergoes the first moult within the egg and gives rise to the second-stage larvae. At the anterior end, the stylet starts to form by this time. Inside the cyst, the larvae become fully developed. These escape via the vulva and other apertures in the cyst wall. The free second-stage larvae thus released migrate through the soil in search of a suitable host.

The second-stage larvae are attached to the root usually by their necks, with most of their bodies outside the root. The sexes can be distinguished in the third stage of larvae. The male develops a single testis while the female develops paired ovaries.

The male larva, when fully formed, is of a rather narrow and slender structure, tapering slightly at the anterior end and has a short, rounded tail. It has a short stylet. Within a short time the fourth moult occurs and the fully developed male larvae of the fifth stage wander in the soil for some time and then die.

Penetration of the host occurs just behind the root tip. After the fourth moult, adult female larvae are formed, which grow into lemon-shaped structures. Over the surface of the adult female (cyst), a subcrystalling layer is secreted. The cyst helps the nematode to survive in the soil. In the adult female larva, the body cavity is entirely filled by the ovaries, which tend to obliterate other structures.

The host range of *Heterodera avenae (H. major)* is confined to the family Gramineae, including some grasses, and these may serve as alternate hosts (collateral hosts). The main method of survival of the nematode in the soil is by means of cysts.

Disease Management

A long rotation of at least four years is recommended. The rotation is more effective if the soil is kept fallow and dry during summer.

Green manuring of infested soil with chopped cabbage leaves heavily reduces the larvae in the soil (Duddington and Duthoit, 1960; Duddington *et al.,* 1961). The activity of some nematode-trapping fungi such as *Arthrobotrys oligospora* and *Dactylaria thaumasia* is increased as a result of green manuring.

Soil fumigation with D-D mixture has been found to be quite efficacious in controlling the nematode population (90% kill) but the remaining larvae flare up in the soil in the presence of susceptible plants. Bhatti *et al.* (1980 a, b) had reported that early sowing of the crop (i.e. early November) and soil application of Aldicarb at the rate of 2 kg a.i/ha resulted in significant control of the disease. None of the wheat varieties had been found to be resistant to the cereal cyst nematode. However, in barley, resistance is reported.

Citrus Nematode Disease

The citrus nematode *Tylenchulus semipenetrans* is invariably present in citrus plantations all over India, especially with trees in various stages of decline and dieback. The population level in the soil and on the roots is often directly correlated with the stage of decline of the trees (Chona *et al.,* 1965). Experimental evidence is, however, lacking on the exact role of the nematode in the citrus decline complex under Indian conditions. Siddiqui (1961) reported that about 80% of the citrus trees at Aligarh, Uttar Pradesh were infested with the nematode.

Symptoms

The affected trees are generally stunted with a general reduction in citrus tree growth, leaves and undersized fruits with little bearing and exhibit dieback of twigs. The roots of such trees are dark in colour with shortened rootlets, swollen and irregular in appearance.

Morphology and Life History

Saccate, sedentary females are root parasites. The larvae, which survive in the soil, infect the roots. The females swell and deposit eggs in a gelatinous secretion. Swellings are not formed. Eggs hatch in 12-14 days at about 24°C and sex differentiation was possible at the second stage. The second-stage male larvae developed to maturity within 7 days, without feeding. The second-stage female larvae require 14 days to locate the roots, feed on the epidermal cells and moult. The life cycle from egg to egg takes 6-8 weeks. There are at least four biotypes of citrus nematode. The widespread distribution of *T. semipenetrans* in India has been mainly through the infested planting material. Infested soil causes more local and short distance spread of the free-living stages of the nematode. Agricultural implements, animals, man, wind and water are common agents in spreading the nematode. It is believed that the nematodes may remain viable in the absence of the host for as long as 10 years in soil.

Disease Management

The citrus nematode can be controlled by bare-root dips in hot water at 45°C for 25 minutes or alternatively at 46.7°C for 10 minutes.

Singh and Sitaramaiah (1973) reported that castor cake was highly effective whereas, neem, mahua, groundnut and mustard cakes also reduced the nematode population in the citrus rhizosphere. It has also been reported that irrigation of citrus orchards with sewage water reduces the nematode population in the soil.

Since citrus nematode is easily transmitted to newer sites through the infested seedlings and budded plants, nurseries should never be established near the old citrus orchards. Soils in the nurseries should be properly sterilized before planting.

Use of resistant rootstocks or highly tolerant rootstocks to nematodes is recommended. Trifoliate orange (*Poncirus trifoliata*) and its hybrids (*Citrumelo*) were highly resistant to citrus nematode. At IIHR, Bangalore, breeds obtained by crossing Rangpur lime (*Citrus limonia*) with trifoliate orange and its hybrids viz., CRH3, CRH 5 and CRH-41 were quite resistant.

Pasteuria penetrans (bacterium) can be multiplied readily on the citrus nematode. Similarly, inoculation of *Paecilomyces lilacinus* significantly reduced nematode population in soil. Therefore, biological control with the help of these two biocontrol agents is a distinct possibility. There are several other important nematode diseases, such as the reniform nematode on castor, the root knot nematode in groundnut and *Heterodera cajani* on sesame amongst the oil seed crops.

Root knot nematodes constitute another problem in plantation crops, such as cardamom, pepper vine, ginger and turmeric in the state of Kerala. Amongst the nematodes affecting tobacco are species of *Meloidogyne, Pratylenchus, Tylenchorhynchus* and *Trichodorus* and *Rotylenchulus reniformis*..

The lesion and burrowing nematodes pose the most serious problems to the coffee areas in South India. *Radopholus similis is* another nematode in banana in Tamil Nadu, Kerala and some parts of Karnataka. *Tylenchorhynchus brassicae* has been reported by workers in Aligarh, which causes damage to cauliflower in Uttar Pradesh.

Ear-Cockle of Wheat

The ear-cockle of wheat is a well-known disease caused by *Anguina tritici.* This is known in India as "sehun" disease and is common in different parts of northern India (Uttar Pradesh, Punjab and western part of Bihar). The nematode of this disease was first noticed in England in 1743 but its significance was realized only in 1775-76. It is often present in association with the yellow ear rot (tundu or tannan disease), caused by the bacterium *Clavibacter tritici.* However, it has now been renamed as *Rathayibacter tritici.* In Uttar Pradesh, Singh *et al.* (1953) reported that the disease causes an annual loss of 30%. Apart from loss in yield, the disease has been known to have toxic effects on humans and animals, when quantities of galls are consumed along with the flour.

Though this disease has been completely eradicated from Europe and the American continents, this nematode still persists in developing countries like India.

Symptoms

The disease does not produce galls but its effects are visible on the stems, leaves and floral organs. Affected plants may be dwarfed and their leaves twisted and crinkled which prevents the normal emergence of the younger leaves from within, causing them to be buckled. The infected heads are partially or completely replaced by cockles that are hard, dark brown or black. These stony structures vary in size from region to region. They are filled with nematode larvae, which can be seen by soaking the gall in water and then macerating it.

Severely affected plants may even die (Gupta, 1966). Even if seedling symptoms are absent, plants may bear galls in their ears. The plants show a spreading nature and tend towards more tillering. Early ear formation has also been noticed. Basal enlargement of the stem and formation of galls on awns, glumes and staminate tissues have been reported by Gupta (1966).

Causal Organism

The nematode causing this disease is known as *Anguina tritici* (Steinbuck) Filipjev. Each nematode larva is slender, with a cylindrical to spindle-shaped body, slightly blunt at the anterior end and tapering to a point at the posterior end to form the tail. The body consists of an outer covering enclosing an inner tube, the alimentary canal, which ends at the mouth. The mouth opens into a buccal cavity containing a buccal spear, 9-11 microns long, which is hollow and pointed. This spear helps the larva to get out of the egg and also to pierce the host tissues. Below the buccal cavity, the digestive canal continues as the oesophagus with an anterior and a posterior bulb, the latter joining the intestine. The intestine opens into the rectum and then into the anus. There is too much variation in the size of larvae from samples collected at different places. The adult males measure 3-5 mm in length while the adult females are 2-2.5 mm long and wider than the males. The eggs are, on an average, 87 × 44 microns in size. The second-stage larvae, which emerge from the eggs, are about 0.75 microns long.

The larvae in the wheat galls have extreme longevity. They can survive up to 28 years under dry conditions and up to 8-9 or even 14 years under moist conditions. In soil, the galls become moist and the larvae break free of the softened walls of the galls. The liberated larvae make their way to the growing point of the wheat plant while it is still near the soil base. These larvae around the growing point feed ectoparasitically and are carried upwards with the lengthening of the culms, until the embryonic flower tissues are formed, when they enter the endoparasitic mode of life by invading these tissues. There, they undergo rapid metamorphosis, becoming adult males and females. Copulation follows and after laying the eggs, the adults soon die. The eggs soon hatch into second-stage larvae, which remain inside the gall to carry on the life cycle. Occasionally, the larvae enter the leaves and mature there to form galls. The nematode is susceptible to desiccation and high temperature under moist conditions.

Plants can become infected with nematodes in the initial stages of growth only, that is, before the seedlings have emerged from the soil. Larvae free from bacteria have been found to produce only the cockle disease. Bacteria are unable to produce the rot independently. However, in combined infection, the nematode larvae are completely killed by the yellow rot bacterium (Gupta, 1966).

As already mentioned, there is a biological relationship of the nematodes with the bacterial ear rot of wheat. Infection of the plant with nematode can occur in the initial stages of plant growth (before the emergence of seedlings out of the soil). The nematode is essential for development of symptoms like basal enlargement of the stem, twisting and crinkling of the leaves, formation of gall and development of bacterial yellow rot. Larvae, which are devoid of bacterial contamination, cause only ear cockle disease.

Disease Management

Since the parasite is introduced into new areas mostly through galls mixed with seeds, proper seed selection is the best method of controlling this disease.

The diseased ears should be picked out and burnt. Healthy seeds can be picked out from a mixed lot by using a sieve that can retain normal plump grains and allow the small round galls to pass through. Immersing in water or brine solution (20% salt solution) can also separate infected and healthy seeds. The galls do not sink but instead float on the surface and can be easily removed.

In fields where the disease has occurred once, cultivation of wheat should be stopped for two to three years. The disease does not occur on barley and oats. Either of these crops can be grown in such fields. Nematode infection of wheat plants is influenced by temperature. The incidence of ear cockle is higher at lower temperatures. Hence, crops sown early usually escape infection. In areas where the disease has established itself, it is better to resort to early sowing.

Gupta (1966) tested certain nematicides against the disease. Nemaphos (granular) proved to be most effective when used at the rate of 4.5 kg per acre. Aldicarb or Thionazin can be used for soil treatment. Jain and Sehgal (1980, 1982) had reported that seed treatment and soil application of Aldicarb sulfone at the rate of 2 kg a.i./hectare gives good control of the diseases (both ear cockle and tundu disease).

Soybean Cyst Nematode

Distribution

Cyst nematodes cause a variety of plant diseases, mostly in the temperate regions. The soybean cyst nematode has been found in northeastern Asia, Japan and USA. The most severely affected host is soybean but many other legumes such as common bean, pigeon pea, black gram, chickpea, mung bean, hyacinth bean and cowpea are also affected by cyst nematode. Depending on the degree of infestation, it can cause losses varying from slight to complete destruction of the crop. In heavily infested areas, yield is reduced up to 75%. Wrather and Rao–Arelli (1992) have reviewed the work done on this disease till that period. This is a disease of international importance.

Symptoms

The main feature of cyst nematode infections is the presence of cysts on the roots and usually the proliferation of roots and production of shallow, bushy root systems. The adult *Heterodera* female is swollen and flask-shaped and is normally found attached to the root. When the female dies, the body wall becomes brown and hardens into a tough cyst, which contains embryonated eggs. The infected second stage juveniles hatch from the eggs and after leaving the cyst, penetrate suitable host roots and find a feeding site in the stele before developing further. On severely infested plants the mature, swollen white females are clearly visible covering the surface of the roots. The infected plants show stunted growth and their foliage turns yellow and fall off prematurely. The infected plants bear only a few flowers and a few small seeds. The root system of infected plants appears smaller than that of healthy plants and infected roots have considerably fewer bacterial nodules than those of healthy plants.

Causal Organism

The soybean cyst nematode is *Heterodera glycines.* Other common cyst nematodes and their hosts are *H. avenae* on cereals, *H. schachtii* on sugar beets, crucifers, and spinach and *H. tabacum* on tobacco and *H. trifolii* on clover. A cyst nematode severe on potato, tomato and eggplant is *Globodera rostochiensis.*

Disease Cycle

Brown cysts containing viable eggs can survive in the soil for many years. The infective larvae penetrate young primary roots or apical meristem of secondary roots directly. The larvae often pass through the cortex and pierce their stylets into cells of the endodermis or pericycle. Two days after penetration, larvae come to rest and feed on cells of the cortex and stele, causing the enlargement of these cells. Such enlarged cells are called syncytia. Development of syncytia and its degeneration is restricted to the peripheral part of the stele and to regions in the cortex that are invaded and stimulated by infective larvae.

Disease Management

Cultural Methods Many cyst nematodes have a limited host range and crop rotation can be successfully employed for their control. Planting the more resistant soybean varieties, which do not allow a quick and excessive buildup of nematode populations, increases the effectiveness of crop rotation. A two or three year rotation with cotton, maize or other non-host plants has been recommended to control *H. glycines* on soybeans.

Chemical Control The infective juveniles of *Heterodera* are well protected in cysts and chemical control is difficult and expensive. There are few specific examples of chemical control of cyst nematode on tropical legumes, but the carbamate group of nematicides, namely Aldicarb, Oxamyl and Carbofuran have given good control of cyst nematode at the rate of 2-11 kg a.i./ha. Soil fumigation of field with ethylene dibromide (EDB) temporarily increases plant growth and soybean yield. Even when used, effective and economic control can only be achieved when chemicals are applied in conjunction with crop rotation.

Biological Control Cysts and eggs of soybean nematode are often seen infected with many fungi such as *Verticillium, Fusarium* and *Nematophthora.* In the near future, it may be possible to use these fungi as biological control agents against cyst nematodes.

Quarantine regulations can be enforced to prevent the parasite from spreading into nematode-free areas by means of contaminated soil, products and machinery.

Plant Diseases Caused by Flagellate Protozoa

Certain trypanosomatida flagellates, belonging to the kingdom Protista, phylum Euglenozoa, order Kinetoplastida, family Trypnosomatidae, have now been known to be parasitic to plants since the beginning of the 19th century. Flagellate protozoa were first found to be associated with plants in 1909 in Mauritius, when Lafont reported that they parasite the latex bearing cells (the laticifers) of the laticiferous plants belonging to the family Euphorbiaceae specially *Euphorbia*.

Protozoa (singular, protozoan), from the Greek ‘protos’ and ‘zoon’, meaning “first animal” are eukaryotic protists. They generally occur singly and may be distinguished from other eukaryotic protists by their ability to move at some stage of their life cycle and by their lack of cell walls. Organisms of the order Kinetoplastida are grouped together because of the presence of a kinetoplast (an extranuclear region of DNA associated with mitochondrion). It is the members of the family Trypnosomatidae that are known to be parasitic to plants. It may be mentioned that African trypanosomiasis or sleeping sickness is caused by *Trapanosoma gambiense*, which is a human disease. To distinguish them from protozoa parasitizing humans, plant-parasitizing protozoa have been placed in a separate genus *Phytomonas*. Dllet (1984) has reviewed the work done on plant diseases caused by flagellate protozoa. Some of the diseases caused by flagellates are given in Table 26.2.

Table 26.2 Some of the Important Diseases Caused by Flagellates

Disease	Causal Organism
Phloem necrosis of coffee (*Coffee liberica* and *C. arabica*)	*Phytomonas leptovasorum*
Hartrot of coconut palm (*Cocos nucifera)*	*Phytomonas* species
Sudden wilt (Marchitez sorpresiva) of oil palm (*Elaeis guineensis*)	*Phytomonas* species
Empty root rot of Cassava (*Manihot esculenta*)	*Phytomonas francai*

In addition to these diseases, flagellates have been reported from milkweed (*Asclepias* sp.). *Phytomonas elmassiani* is the pathogen on milkweed. *Phytomonas bancrofti* has been reported from *Ficus* species.

Although flagellates have been associated with the diseases mentioned above, Koch's rules of pathogenicity have not been provided so far against any of these diseases.

Phloem Necrosis of Coffee

The disease has been reported from Surinam, Guyana, San Salvador, Columbia and probably Brazil. It has not been reported from India so far.

Symptoms

As a result of infection, trees show sparse yellowing and dropping of the leaves and in advanced cases of infection, only the top most leaves remain on the branches. The roots begin to die and the trees infected begin to die. It has also been seen that at the beginning of the dry season, trees wilt and die within 20-45 days.

Causal Organism

The pathogen is a flagellate, *Phytomonas leptovasorum*. As a result of the pathogen, internally, the roots and trunks of the trees show multiple divisions of cambial cells and production of a zone of smaller and shorter phloem elements of disorderly structure right next to the wood cylinder.

Histopathology of Infected Plants

As already indicated, the roots and trunks of the trees show phloem element of disorderly structure. Thus, abnormal phloem production takes place. The flagellates can be traced from the roots upward into the trunks where they seem to migrate vertically in the phloem and laterally through the sieve plates into healthy sieve tubes. They also move downwards into unaffected roots.

Disease Cycle

The disease is transmitted through root grafts but not through grafts of the aerial parts. The disease spreads in the field from one tree to another and healthy trees often become infected if transplanted into areas where diseased trees have been removed. In nature, the insect vector is the genus *Lincus* (pentatomid insect).

Disease Management

In order to control this disease it is absolutely essential that nurseries should be free from pathogenic flagellate protozoans. Healthy plants should be planted far away from the infected plants.

Hartrot of Coconut Palms

Hartrot disease has been reported from Surinam since the beginning of the nineteenth century, under the names lethal yellowing or bronze-leaf wilt. It has also been reported from Columbia, Ecuador and Trinidad.

Symptoms

The first symptoms are the yellowing and browning of the tips of the older leaves followed by the younger leaves. Unripe fruits (nuts) of the diseased trees fall off. The recently developing and opening inflorescences are blackened. Petioles of the older leaves break and the spear becomes necrotic. The root tips of the infected plants start dying. In later stages of infection, the apical region of the crown rots and produces a foul odor.

Causal Organism

The pathogen associated with this disease is a species of *Phytomonas*. The flagellates occur in the sieve elements of young leaves and inflorescence of hartrot-infected plants. In later stages of the disease, the flagellate virtually plugs the sieve elements.

Disease Cycle

Insects of the genera *Lincus* and *Ochlerus* transmit the disease.

Disease Management

Planting healthy seedlings from nurseries that are free of the pathogen is the first step in protecting plants from infection. Controlling the insect vectors by using insecticides will go a long way in managing the disease.

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Questions

1. Name some important plant pathogenic nematodes.
2. Name some important nematicides used for controlling plant nematode diseases.
3. What is the rationale in growing trap crops? How could it be utilized for controlling plant nematodes?
4. Name some important trap crops used to control plant nematodes.
5. What is a syncytium?
6. Give some suitable examples of controlling nematode diseases by means of biological agents.
7. What is soil solarization? How can it be used to control nematode population in soil?
8. What is Integrated Pest Management strategy?
9. Why are some of the earlier recommended nematicides abandoned now?
10. Name some important diseases caused by flagellates. How will you control them?

Diseases Due to Angiospermic Parasites

27

Parasitic Flowering Plants

We usually think of parasites as tiny plants without flowers, seeds or chlorophyll, such as fungi and bacteria or the scarcely visible eelworms (nematodes), which live at the expense of other plants. A number of flowering plants are, however, totally or partially parasitic on economic plants and cause considerable damage. Among the most important families containing parasites are Loranthaceae, Convolvulaceae, Scrophulariaceae, Orobanchaceae, Lauraceae, Santalaceae and Balanophoraceae. Kuijit (1977) had critically reviewed the subject of phanerogamic flowering plants. In India, Kumar (1940) had described flowering plants, which attack economic crops. Balasundaram and Ghosh (1986) have discussed the mistletoe problem of trees in India. A lot is known about the biology, embryology, haustorial system and host range of mistletoes, but little attention has been given to plant pathological problems posed by these parasites. Some important literature on parasitic higher plant is that of Hawksworth and Wiens (1970), *Biology and Taxonomy of Dwarf Mistletoes*; Musselman (1980), *The Biology of Striga, Orobanche and other Root Parasitic Weeds;* and Musselman (1987), *Parasitic Weed in Agriculture.* Thoday (1991) has discussed the histological relation between *Cuscuta* and its host.

Parasitic flowering plants tend to be more numerous in the tropics and subtropics where they take varied forms. *Rafflesia arnoldii* is parasitic on *Vitis* roots and produces flowers upto 0.9 m in diameter. The flower of *R. arnoldii* is said to be the largest in the world. In *Apodanthes casearia*, which is more or less a systemic parasite, small flowers and stalks are seen, often less than 0.6 cm high (Wellman, 1964).

Tarr (1972) has described several types of parasitism:

1. The parasite is rooted in the soil and is attached to the roots of the host plant by haustorial discs, through which the vascular tissue of the host and parasite become joined, such as in witchweed (*Striga*). In some cases, green aerial shoots and flowers are produced while in other cases, the host plant seems to suffer little damage.
2. The parasite has perennial underground stems, which are attached to the host roots and produce aerial flowering shoots each year, such as *Lathraea squamosa,* popularly known as toothwort. The leaves are without chlorophyll.
3. The tissues of the parasite merge with those of the host root to form a swelling from which aerial flowering shoots arise. There may be varying numbers of rudimentary or scale-like leaves or the host may be leafless, for example, broomrape, *Orobanche.*

4. The parasite invades the branches of the host, often a tree, and develops a bushy perennial plant with green leaves but does not root in the soil, for example, mistletoe, *Viscum*.
5. The seeds of parasite germinate in the soil and produce a hair-like shoot, which makes circular movements until a suitable host plant is found, after which it twines around the host stem, obtaining nutrients through haustoria. The stem of the parasite dies at its lower end, so that the connection with the soil is lost. Chlorophyll is absent although there may be small, rudimentary scale leaves. The best known of this group are the dodders, *Cuscuta* spp.
6. There may be more complete integration of the parasite and host, the former developing as a kind of hollow root or shoot. The flowers eventually burst through the cortex to the exterior. This situation is found in the family Rafflesiaceae, for example *Rafflesia arnoldii,* where the vegetative organs of the parasite are reduced to a network ramifying in the tissue of the host, somewhat suggestive of mycelium.

There are various degrees of parasitism among flowering plant pathogens. The most independent ones are referred to as hemiparasites. All the hemiparasites possess chlorophyll and are dependent on the host only for water and mineral nutrients. The hemiparasites look like normal green plants but they differ from them; their modified roots are anchored in the stems and branches of a host plant. The parasites damage the host by reducing the amount of water and minerals transported to the upper, non-infected portions of the host, sometimes resulting in the death of these parts. Then there are the angiosperm parasites, which lack chlorophyll (but may contain other pigments such as xanthophylls and carotenoides) and are dependent on the host for elaborated nutrients. All these are obligate parasites; they cannot grow when they are apart from the host. The leaves of these parasites are reduced to scales and their root systems show marked modification. Examples of these complete parasites are dodders (*Cuscuta* spp) and broomrapes (*Orobanche* spp).

Some important angiospermic parasites in India and their host ranges and distribution are given in Table 27.1.

Angiosperm parasites frequently invade the host through appresorium-like bodies from where pointed infection structures, modified roots or outgrowths of the stem arise. The mechanism of penetration is not clearly understood. It is believed that penetration is mainly by mechanical pressure but cell-wall degrading enzymes may also be involved. After gaining entry into the host tissue, the infected structure produces haustoria, which grows through the host tissue and establishes contact with the vascular tissue of the host. The pectolytic and cellulolytic enzymes perhaps facilitate the progress of the haustoria. The haustoria of the parasitic flowering plants have a higher osmotic pressure than those of the host tissue. This helps in the absorption of nutrients from the host tissue. Information about many aspects of the parasitism of flowering plants is still in its rudimentary stage. Kuijt (1977) had discussed the haustoria of phanerogamic parasites.

The infected host plant is affected to varying degrees. These may be slight to severe stunting and chlorosis, and in severe attacks, the host plants die. As a result, there may be losses in yields.

Dissemination of Angiosperm Parasites

Certain angiosperm parasites produce fleshy mucilaginous berries, which contain a viscous substance (viscin). Birds eat berries and the seeds are distributed in the droppings. The feet and beaks of birds may also distribute the seeds.

Table 27.1 Important Angiospermic Plant Parasites in India

	Family	Genus	Stem or Root Parasite	Important host	Distribution
1.	Loranthaceae	1. *Elytranthe*	Stem Parasite	*Emblica, Dalbergia* and other forest trees	Peninsular and North Eastern India
		2. *Macrosolen*	Stem Parasite	*Citrus,* Jackfruit, *Terminalia* and other forest trees	Peninsular India and Tropical Himalayas
		3. *Dendrophthoe*	Stem Parasite	A large number of hosts (More than 343 hosts)	Throughout India excepting temperate Himalayas
		4. *Helicanthes*	Stem Parasite	Mango, rubber, cashew, citrus, sapota, jackfruit, *Casuarina*	Peninsular India (Kerala)
		5. *Helixanthera*	Stem Parasite	*Myristica* and several forest trees	Khasi Hills and Peninsular India
		6. *Scurrula*	Stem Parasite	*Citrus* and several forest trees	Throughout India except temperate Himalayas
		7. *Taxillus*	Stem Parasite	*Emblica,* several trees and *Cordyline*	Peninsular India
		8. *Tolypanthus*	Stem Parasite	Citrus	Peninsular and North eastern India
2.	Viscaceae	1. *Arceuthobium*	Stem Parasite	Conifers	Temperate Himalayas
		2. *Viscum*	Stem Parasite	*Citrus*, several forest and fruit trees	Throughout India
3.	Orobanchaceae	1. *Orobanche*	Root Parasite	Tobacco, brinjal, tomato, cabbage, cauliflower, turnip and many solanaceous and cruciferous plant	Throughout India
4.	Convolvulaceae	1. *Cuscuta*	Stem Parasite	A very large number of hosts	Throughout India
5.	Scrophulariaceae	1. *Striga*	Root Parasite	A large number of plants including sugarcane, cereals, maize and millets	Throughout India

In the case of dwarf mistletoes, the seeds are produced in extremely large numbers and their size is minute and small. They are almost like dust or powder and are easily transported by air currents to other places.

The minute or tiny seeds of some parasites contain little food material and may show progressive maturation over a number of years. This is advantageous to the parasites as they have better chances

of successful infection under these conditions. Under normal conditions, these tiny seeds may remain dormant for a number of years and germinate when stimulated by the root exudates of certain plants.

The cuscuta plant is spread from infected to healthy plants by means of long, thin aerial shoots which wave about in the air and search for new hosts. Pieces of the broken stem may act as organs of vegetative reproduction.

Control of Angiosperm Parasites

1. Only seeds or planting material free from contamination or vegetative parts of parasites should be sown.
2. Whenever possible, the infected portions of the plants should be cut out and destroyed and if the trees or plants are severely infected, the whole plant should be destroyed.
3. Suitable crop rotation, if followed, may not allow the build-up of seeds of the parasites.
4. Selective herbicides, which kill the parasite and not the host, may be tried.
5. For example, in Sudan, spraying the contaminated soil with weed killer 2-4 dichlorophenoxyacetic acid at about 1.1 kg/ha, 2-3 weeks after *Sorghum* seed had been sown, was found quite efficacious in the control of witchweed (*Striga*) in the *Sorghum* fields.
6. Resistant or tolerant varieties may be developed.

Loranthus or "Banda" or Giant Mistletoe

Loranthus, now known as *Dendrophthoe,* is a common angiosperm stem parasite of fruit, wasteland, avenue and forest trees (Fig. 27.1). The most common host is the mango tree, and in northern India, 60-90% of the mango trees and some other trees such as *Ficus* spp., *Albizzia* spp. and *Dalbergia* spp. are infected by the parasite. Johri and Bhatnagar (1972) had listed 343 hosts, which included all the common horticultural and forest trees. Since then, a few more hosts have been added. *Dendrophthoe* species are semi-parasites of trees trunks and branches. In the central part of India, the most common host is *Madhuca latlifolia*.



Figure 27.1 *Dendrophthoe falcata*

The ancient Indians knew of this partial parasite and its Sanskrit name is 'Vrikshabhaksha', which means 'the eater of trees'. *Dendrophthoe* spp. are semi-parasitic on trees as a dense cluster of small twigs bearing smooth broad leaves and long, tubular, orange-colored flowers with red berries. *Dendrophthoe falcata* is the most common species in India. The giant mistletoe does not have a root system of its own but develops root-like absorbing organs called haustoria, which penetrate deep into the tissue of the host. Through these organs, water and minerals flow from the food conducting system of the host to the parasite. There is thus a continuous drain of nourishment from the host to the parasite and in course of time, the attached branch withers as a result of the interference in the flow of the sap to its leaves, beyond the point of attack.

The green leaves of the parasite manufacture sugar and starch and thus it is not completely dependent on the host plant. Sometimes, a parasite produces a creeping branch, which grows closely along the host stem and forms haustoria at intervals. The parasite is disseminated mainly through the seeds, carried by birds. The birds are attracted by the brilliant color of the berries, the pulp is sticky and viscous and so birds easily carry the seeds. The parasite causes damage by preventing the production of new growth by the host.

Eradication

The most common method of control is by hand-picking the parasite in the early stage of the growth or by sawing it off from the branches of its hosts, so that entire haustorial system is removed.

Injections of $CuSO_4$ and 2,4-D has been found effective on many hosts. One spray with 30-40% diesel oil emulsion in soap-water has also been found quite efficient in eradication of the parasite from mango trees.

Ghosh *et al*. (1984) reported successful control of *D. falcata* var. *pubescens* growing on teak by the hyperparasite *Viscum capitellatum.*

Other potential agents for biological control are the caterpillars of the butterfly *Delias eucharis,* widely seen in teak plantations in Kerala (Ghosh *et al.,* 1984). They feed on *D. falcata* leaves and tender branches, causing heavy defoliation. *Delius eucharis* has also been found to be a potential agent for biological control of *D. falcata* on cotton plants.

Boomrape (*Orobanche* spp.)

Orobanche spp. are total root parasites affecting brinjal, tomato, cauliflower, turnip and other solanaceous and crucifer plants, especially tobacco. In India, *Orobanche aegyptica* parasitizes many crop plants.

Broomrapes appear as clumps of whitish, yellowish, brownish, or purplish annual stems arising from the ground at or near the base of their host plants. The parasite consists of a stout fleshy stem, 15-45 cm high and covered by small, thin and brown scaly leaves. Flowers appear in the axil of leaves and are white and tubular. The fruits are capsular and contain numerous tiny seeds, which germinate in the soil under the influence of the root exudates of certain plants or hosts.

Broomrapes have no green tissue and are parasitic by their roots upon the roots of other green plants of hosts. A large number of parasitic stems may be seen breaking the soil around the host plants. When the host is carefully uprooted, the parasitic roots are seen intertwined with the host's root system. As a result of the drain on food supply, the growth of the host plant is checked and in severe attacks, the plants may even die.

When the primary roots of a germinating broomrape seed contacts the fibrous roots of its hosts, it forms a nodule of tissue, which fuses with the tissue of the host. New roots and a stem of the parasite develop at this point. While the stem emerges above the ground, the roots of the parasite form new contacts with other hosts roots and thus a large number of parasitic plants appear all over the field at the base of the affected crop plants.

Eradication

The best protection against broomrape is to separate the seeds of the parasite mechanically from the crop before sowing.

Another way of controlling the parasite is to destroy it before seed formation. If seeds are formed and shed on the soil, it becomes difficult to eradicate the parasite for several years. Soil below 22.5 cm depth is free from seed. Very few seeds are present at a depth of 15-22 cm. The population of seeds of the parasite increases with decrease in soil depth and maximum number of seeds are present in the top 5 cm of soil. Drying of soil for a period of 3 months at 15-30°C considerably reduce the population of seeds. It is also recommended that contaminated lots of seeds be treated with 25% $CuSO_4$ solution. Spraying the soil with $CuSO_4$ solution has also been reported to have successfully destroyed the parasite. The herbicide Glyphosate also effectively controls the parasite.

Krishnamurthy and Chandwani (1975), working on *Orobanche* on tobacco, reported that the seeds of the parasite are stimulated to germinate in presence of roots of several crops, which do not support its growth. Sorghum, chilli, and mothbean can be used as trap crops to stimulate seed germination and destruction of the parasite. In resistant carrot, the parasite germinates and attaches to the host but became necrotic before emergence. The mechanism involved in resistance has been identified as cell wall deposition and finally broomrape cellular disorganization. In non-host plants like maize, thickening of xylem vessels and formation of an encapsulation layer were associated with restricted development of haustoria.

Striga spp. (Witchweed)

This is commonly known as witchweed. It is a root parasite (semi-parasitic) on several angiosperm hosts (Fig. 27.2). Numerous species of *Striga* parasitize many important plants such as corn, sugarcane, cereals, and tobacco in Africa, Australia, India and Indonesia. Three species of *Striga: S. densiflora, S. asiatica* and *S. euphrasiodes,* are well-known semi-root parasites of sugarcane. Rao *et al.* (1935) reported *S. euphrasiodes* to be parasitic on paddy. *Striga* spp. are well known semi-root parasites.

Striga spp., although they are obligate parasites, do not obtain all their nutrients from their host's roots. They possess leaves which bear chlorophyll. Many of the *Striga* plants are fairly high (25-60 cm) and usually develop in clusters in and around each stool of sugarcane. The seeds of *Striga* spp. are very minute and are produced in great abundance. The seeds remain viable for a number of years. The stimulus provided by the root exudates of specific host enables the seeds to develop. After germination, the parasite grows below the soil surface for about 1-2 months and produces underground stems and roots. The underground portion of the stem contains buds in the axils of the leaves. The roots of the parasite form haustoria, which penetrates the host roots drawing nourishment from the host and eventually wasting and destroying the host.



Figure 27.2 *Striga* sp. on roots of another plant

Bhargava and Mathur (1992), while working with *Striga asiatica,* have observed that the parasite is common in light sand, sandy loam and loam soils than heavy clay.

Eradication

Weeding can be successful only when undertaken in the early stages of parasitic growth. Where water is easily available, flood irrigation is quite effective in eradicating the parasite. Catch crops consisting of host plants may be grown to force the germination of *Striga* seeds, and then ploughing or use of weedicides such as 2, 4-D can destroy the parasite. Trap crops (host plant), may be used to stimulate seed germination. The parasite cannot infect these plants and therefore is starved to death. Crop rotation with non-host cultivars, selected to stimulate parasite seed germination, is a successful control against *Striga.* Like *Celosia argentea*, a non-host stimulates suicidal seed germination.

Spray application with a selective weedicide, for example, Agroxone (MCPA) and Fernoxone (2,4-D) is effective in killing the parasite. The recommended concentration is 0.45 kg of Fernoxone in 100 gallons of water. Where there is scarcity of water, 1% Tetrachlorodimethyl phenoxyacetic acid kills the parasite outright. Aggarwal and Naqvi (1954) have reported the control of *S. euphrasiodes* by *Precis orithya.* Uttaman (1949) has discussed the biological control of *Striga*.

Soaking the soil upto a depth of 10-15 cm with 2-3% copper sulphate solution was once recommended for the control of *Striga* weed. Sodium salts of 2,4-D are toxic to many crops used in rotation with hosts of *Striga*. Such 2,4-D-sensitive crops are cotton, tobacco and soybean.

Soil fumigation with methyl bromide under polythene cover has been found effective to eliminate witchweed. Application of 2-Chloroethyl phosphonicacid (Ethephon) at the rate of 1 kg/ha into light sandy soils at about 25°C, which have been dampened, stimulated seed germination of *Striga* weed. The germination of seed is fatal since the parasite cannot survive after germination in the absence of nourishment from a host. Strigol, a natural stimulant, also works in the same fashion as ethylene. An acetolactate synthase inhibitor, Imazaquin, is effective as seed soak in protecting the host from witchweed infection (Berner *et al.,* 1994).

Berner *et al.* (1999) reported that ethylene-producing bacteria (*Pseudomonas syringae* var. *glycinea*) promote germination in *Striga* spp. The germination of *Striga* seeds is suicidal since the parasite can not survive after germination in the absence of nutrients from a host and further the parasite can be destroyed by ploughing or by using some weedicides such as 2,4 D. Ahonsi *et al.* (2003) have evaluated ethylene-producing *Pseudomonas* in combination with nitrogen-fixing *Bradyrhizobium japonicum* for *Striga hermonthica* control in cowpea or soybean crops. Bacterization of maize seeds with either *Pseudomonas fluorescens* or *Pseudomonas putida* reduced the number of *Striga* plants and increased maize growth. *Fusarium nygamai* is a hyperparasite on *Striga hermonthica,* which enters the seed coat and kills the endosperm.

Cuscuta

Cuscuta is called dodder and there are about 100 species of *Cuscuta,* which attack trees, shrubs, clovers, alfalfa, flax, sugarbeets, onion but not cereal plants. It is a stem parasite wholly dependent on the host (holo parasite). *C. gronovii* is the most common dodder in India and it attacks ornamental and hedge plants. It is an extremely fast-developing parasite and within 2-3 seasons may destroy a complete tree. *Cuscuta* spp. have no chlorophyll, and bear leafless, twinning, parasitic seed plants, which attach their

yellow, orange or pink thread-like stems to the stems or other parts of the host plant. On account of this entwining and strangling habit, it is also called strangleweed, devil's ringlet, devil's guts, gold thread, pull-down, hellvine, hairweed, devil's hair and love vine.

There are various species of *Cuscuta* and a description of one will represent all the entire species. It commonly appears as a dense, tangled mat of leafless, orange or yellow strands on its host plant. The strands originate from seeds that have germinated in the soil and eventually made contact with a host plant. When the contact is made, haustoria from the parasite penetrate the host and begin to supply the parasite with nourishment. Ultimately, the original strand coming from the soil dries up and the parasite becomes solely supported by its hosts. The growth of *Cuscuta* on one plant can become so extensive as to spread to the neighboring plants.

Cuscuta spp. produces flowers in clusters or racemes. The flowers are white, yellowish or pinkish in color. The calyx is colored and is shorter than the corolla. The latter is tubular, with short, triangular lobes. The stamens are attached near the mouth of the tube, which has five small sessile or terminal stigmas on short, diverging styles. The fruit is a capsule which opens by a horizontal line near the base (Fig. 27.3).



Figure 27.3 *Cuscuta* sp. with its host

The seeds germinate on the ground in spring and the young seedlings are able to lead an independent existence only as long as the stored food in the seed is available. If the young *Cuscuta* vine is not able to get a suitable host, it soon perishes. On a susceptible host plant, the stem of the parasite soon produces haustoria, which establish contact with the host. The vascular system of the parasite now has direct contact with the host and the parasite draws it nourishment entirely from its host.

The parasite is able to perpetuate through its seeds very easily. These are produced in large numbers and fall into soil.

Eradication

Dodder prevention can be achieved by avoidance and control by mechanical means or hand removal, resistant varieties and herbicides. None of these methods work 100% most of the time, but all of them offer some tools for managing this parasitic weed (Lanini and Kogan, 2005).

Sanitation

Avoidance and prevention are the most effective and economical methods to reduce dodder infestation. Dodder seeds are likely to spread by human, through seeds, international movement of equipments, and in the mud on tyres and shoes (Cudney and Lanini, 2000). Thus, it is recommended to avoid using seed from fields where dodder is present or use seed certified free of dodder. To prevent new dodder infestation, clean equipment after working in dodder infested field, prior to moving to another field. Similarly, do not allow animal feeding in a dodder-infested field to move to a dodder-free field, as

dodder seeds are known to survive passage through the digestive system of many animals. Many broad leaf weeds have been observed to host dodder. Hence, proper weed control in and around cultivated areas will prevent dodder from parasitizing weeds and would reduce the potential for dodder seed production and dispersal. Fluctuations in soil moisture and temperature near the surface may initiate breaking the dormancy of superficially buried dodder seeds. Parker and Riches (1993) observed that a flush of dodder seed germination often occurs in the early spring season and they attributed this to dodder seed loosing its dormancy during the cold winter months. Delayed planting can reduce dodder infestation but that is not always practical due to marketing considerations for certain crops. Crop rotation can be an effective method to reduce dodder infestations. However, the large weed and crop host range makes it difficult to avoid this pest by crop rotation; thus, careful crop selection is essential. Dodder is often observed coiling around grasses, but cannot form haustoria connections and will die unless a suitable host is found. Thus, growing cereals or other grass crops (which are not parasitized by most dodder species) continuously for several years may facilitate the exhaustion of dodder seed bank in the soil (Dawson, 1987). Lanini (2004) found that growing wheat followed by corn in a field heavily infested with *C. pentagona* reduced the number of dodder plants infesting tomato by 90%. Thus, two years of growing a non-host crop was effective in reducing the population. In addition, tillage may hasten drying the soil surface, thus preventing further dodder germination and emergence. However, the tillage only controls the dodder growing between the rows and not in the rows. Hand-removal of the dodder-infected crop remains a viable but expensive option when infestations are small patches. When infestations are extensive, hand removal is prohibitively expensive, not to mention the loss in the crop stand. If done, hand removal should be done when dodder is first detected to prevent spread and seed production.

Resistance

Although not much effort has been made to assess varietal differences to dodder attachment or damage, some resistance has been observed among sensitive crops. Different levels of resistance to *C. pentagona* were observed within four wild *Lycopersicon* species (Al-Menoufi and Ashton, 1991). Loffler *et al*. (1995) reported incompatible interactions between *Cuscuta* species and resistant/tolerant tomato plants. They described incompatible interactions between 30 tomato varieties and *C. reflexa*. This resistance was characterized by enlargement of epidermis, hypodermis and collenchyma of tomato cells, necrotic tissue developing around the pre-haustoria and hardening of cell walls adjacent to necrotic tissue, preventing haustoria formation. Ihl and Miersch (1996) observed a similar resistance phenomenon in 22 tomato varieties and four wild tomato species to four *Cuscuta* species, *C. reflexa*, *C. japonica*, *C. odorata* and *C. europaea*. In all cases, no functional haustoria were formed due to hypersensitive response of external cell layers of the tomato stems or petioles after pre-haustoria contact. The layer of dead tomato cells prevented intrusion of initial haustoria, followed by an increase in peroxidase activity.

Biological Control

The potential of using insects and pathogens for dodder control has been reviewed (Parker, 1991). In spite of the optimism, there is still much research to be done before any commercial biological agent for dodder control can be released (Bewick *et al*., 1987). Several fungi have been found to damage dodder, including *Fusarium tricinctum* and *Alternaria* spp., which attack swamp dodder (*C. gronovii*) and *A. alternata* and *Geotrichum candidum,* which both attack field dodder (*C. pentagona*). Additionally,

researchers in China have found that a conidial suspension of *Colletotrichum gloeosporioides* has provided selective control of *C. chinensis* and *C. australis* in soybeans. In field-tests with *Alternaria destruens*, Bewick *et al*. (2000) reduced *C. gronovii* attack by 90% in cranberry (*Vaccinium macrocarpon*) and carrot (*Daucus carota*) but it was not effective against *C. pentagona* under California conditions, possibly due to the dry climate and the different dodder species. Sari and Boži (2009) and Sari *et al.* (2009) reported the influence of soil microorganisms (PGPR- Plant Growth Promoting Rhizobacteria) on the germination of field dodder (*Cuscuta campestris*) and used the bacterial cultures of *Bacillus licheniformis, B. pumilus and B. amyloliquefaciens* isolated from manure; *B. megatherium* isolated from maize rhizosphere. The results inferred that bacterial cultures of the genus *Bacillus* had different degrees of inhibitory effects on field dodder germination.

Chemical Control

Dodder Control Prior to Attachment This approach attempts to control dodder before it attaches to the host and eliminates any possible damage (Parker, 1991). Fumigants such as methyl bromide, or metam sodium control many weeds prior to vegetable planting, but species with a hard coat seed such as *C. pentagona* are not affected (Lanini, 2004).

There are many pre-emergence herbicides that have been shown to affect germinating dodder seedlings. Dodder seeds germinate but initial growth is reduced and attachment to host fails when these herbicides are used. Chlorpropham, a carbamate herbicide was used for dodder control in various crops. Chlorpropham is no longer used; however, Pronamide, a benzamide herbicide, is now used to prevent dodder attachment in alfalfa, sugarbeet and cranberry.

Dinitroaniline herbicides have been examined for their effectiveness to control dodder in alfalfa. Although they differ in their persistence of dodder control, all dinitroanilines reduced dodder infestations. Pendimethalin and Prodiamine were more effective than Trifluralin and controlled dodder for a longer period of time. Pendimethalin is also recommended for selective dodder control in carrot, onions and alfalfa. Various herbicides including Ethofumesate were also reported to control dodder selectively in sugarbeet. Dichlobenil is used to prevent dodder growth in ornamentals and blue berries. Thiazopyr (thiazole herbicide), which is recommended in tree and vine crops, has been shown to stunt dodder seedlings and prevent attachment to the host crops such as alfalfa.

Post Attachment Dodder Control Parker (1991) stated that post-attachment control of a parasite is very important in order to reduce its seed production and further spread, although irreversible damage was already done. The nature of attachment in association between host and parasite requires a highly selective herbicide to destroy the attached dodder without inflicting damage to the crop. Post-emergence applications of herbicides such as Ethofumesate, Pronamide and Pendimethalin can suppress the parasite. Studies have been conducted using phloem mobile herbicides such as Glyphosate for selective post-attachment control of dodder in alfalfa. Several Acetolactate synthase inhibiting herbicides have been shown to control or suppress dodder. Imazethapyr at 100-150 g/ha, applied pre-emergence reduces dodder infestation in seedling alfalfa and suppression of more established dodder stems (Cudney and Lanini, 2000).

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Questions

1. Name some important angiospermic plant parasites of your area.
2. Give examples of total and semi-parasitic angiospermic parasites.
3. Discuss in brief some general methods of controlling phanerogamic parasites.
4. What types of chemicals are generally used to control the phanerogamic weeds parasites on economic plants?
5. Name angiospermic parasites that attack stems or branches of trees and those which attack roots of their host plants.

Non–Infectious or Abiotic Diseases

28

Non–Infectious or Abiotic Diseases of Plants

Non-infectious or abiotic diseases have been recognized as a major source of crop failure ever since man resorted to cultivation. For a long time, such failures were believed to be largely due to unfavourable climatic and edaphic conditions. During recent years, environmental pollution and debris from nuclear weapons and devices have added greatly to the problem of disorders of this type. Major disorders of non-pathogenic origin are given in table 28.1. Agrios (1997, 2005) has discussed these diseases and has provided useful information.

Table 28.1 Major Non-infectious or Abiotic Disorders of Plants

I. Disorders resulting from unfavourable soil environment	
(a) Nutritional imbalance:	Deficiency of essential elements, toxicity of essential and non-essential elements, multiple and induced deficiencies.
(b) Adverse water relation:	Drought, water logging, impeded aeration, etc.
(c) Adverse physio-chemical:	Salinity, alkalinity, acidity, calcareousness, etc.
II. Disorders resulting from climatic stress*	
(a) Temperature:	High and low temperatures.
(b) Light:	Duration and quality of light.
III. Disorders caused by atmospheric impurities (environmental pollution)	
(a) Harmful gases:	Sulphur dioxide, hydrogen fluoride, ozone, ethylene, chlorine, nitrogen dioxide, other harmful gases and dusts-smog (peroxyacetyl nitrate), fly ash, brick kiln fumes, etc.
(b) Pesticides:	Herbicides, insecticides, nematicides, rodenticides, molluscacides, etc.
(c) Ionization radiation:	Naturally occurring radio-nucleides and ratio-active fall out.

*Excluding injury due to climatic factors: lightning, high wind, heavy rain, hail, sleet, ice, mechanical injury, etc.

Nutritional Deficiencies

Green plants deficient in essential mineral nutrients are most susceptible to diseases.

These nutrient deficiencies are non-infectious. Twelve such elements and the symptoms of plants deficient in them are discussed below.

Nitrogen (N)

Nitrogen is a constituent of important compounds such as proteins, amino acids, enzymes, hormones, chlorophyll, and vitamins. Plants deficient in nitrogen are stunted and woody and the leaves become yellow or light brown. The ratio of the root tissue to the shoot tissue in such plants is high. Sugar accumulates in the tissues because deficiency of nitrogen affects respiration. Red leaf of cotton is caused by nitrogen deficiency. This deficiency can be corrected by side dressings of nitrogen rich fertilizers, such as ammonium nitrate or urea.

Phosphorus (P)

It is a constituent of phospholipids, nucleic acids and many proteins. It is essential in carbohydrate transformation and respiration. Phosphorus is involved in high-energy bonding, as in the conversion of high-energy bonds (i.e. in ATP andADP). This element is essential in the timely differentiation and maturation of plant tissues. The leaves of phosphorus-deficient plants become bluish-green with purple tints. Such plants grow and mature slowly and sugar accumulates in the tissues. Cotton is particularly susceptible to diseases caused by phosphorus deficiency. The deficient cotton plants are dwarfed and their foliage is dark green. Other highly susceptible plants are peaches, citrus fruits, and flax. The application of phosphatic fertilizers controls the disease.

Potassium (K)

Plants need potassium in large amounts. It is essential as a catalyst in nitrate reduction and in reactions that convert starch to sugar. It is also essential in photosynthesis and cambial activity. Potash deficiency is particularly drastic to potatoes, tobacco, cotton, forage legumes, and other crop plants that require large quantities of potassium. Mild chlorosis and browning of the tips of leaves may be seen in potash-deficient plants. Yellowing of interveinal tissues usually follows this. Browning of the laminar tissue and necrotic sports are seen as a result of potash deficiency in alfalfa and clover. The specific diseases caused by a deficiency of potassium include brown spot of cotton and little spot of alfalfa. The addition of potassic fertilizers controls the diseases caused by a deficiency of potassium.

Boron (B)

Boron increases the mobility of sugars and calcium and is important in cell division and protein synthesis. It is essential for pollination and affects flower formation, fruit set, and seed production. Boron is essential for the plant growth. Pea and lucerne are more sensitive to boron deficiency, than cereals are. As a result of boron deficiency, the growing points die, roots are thick and stunted, cambial activity is checked, stems are brittle, internodes are shortened, and seed production is reduced. Heart rot of beet is an important disease caused by boron deficiency. Some other diseases are, cracked stem of celery,

internal cork of apple, brown heart of cabbage and turnip, internal brown spot of sweet potato, terminal bud breakdown of tobacco, fruit pitting and die-back of olive, and rosetting and anthocynescence of alfalfa. Hollow stem of *Brassica* is also caused by boron deficiency. The root nodule bacteria may also become parasitic on the legumes in case of boron deficiency in soil. Soils with high lime content are usually deficient in boron.

It was previously thought that brick kiln fumes cause the black tip of mango. But later, this view was not accepted since spraying with borax reduced the disease. Boron deficiency was then believed to be the cause of this disease.

Boron deficiencies can be corrected by the application of 4-5 kg of boron per acre. Fritted boron can be applied to the soil, or borax or fertilizer-borate sprays can be applied to the plants or to the soil.

Calcium (Ca)

This is often a limiting factor in cell-wall formation and consequently, it is indirectly involved in cell division. As calcium pectate, it is perhaps the principal constituent of the middle lamella. The activity of several enzymes is influenced by this element. Calcium acts as an activator of the enzyme phospholipase arginine kinase and several other enzymes. In the affected plants, the tips of the leaves curl back with irregular ragged margins. The leaves have a distorted appearance. They become brittle due to the accumulation of starch. Blossom end rot of tomato, black heart of celery, and wither tip of flax are some of the specific diseases, which develop as a result of calcium deficiency. Some other crop plants such as peaches, tobacco, and peas seem particularly sensitive to calcium. Calcium deficiency is generally treated by the application of lime to the soil.

Copper (Cu)

Copper catalyses certain reactions in respiration and is a co-factor of several oxidative enzymes. Symptoms of copper deficiency appear only when copper concentration drops below 2-10 ppm. In the case of cereals, the deficient plants bear young leaves with chlorotic margins and withered tips. The heads become chlorotic. The cupping of young leaves is not unusual. In citrus, gum pockets form in the twigs, which also bear multiple buds. Die-back of citrus, reclamation disease of oats and wither tip of apples are some of the diseases caused by the deficiency of this element. Grasses, legumes, fruit and nut trees and solanaceous plants suffer from copper deficiency. The application of copper sulphate to the soil is generally recommended for copper-deficiency diseases.

Iron (Fe)

Iron is essential for the synthesis of chlorophyll, although it is not a constituent of chlorophyll. It is an electron carrier in the oxidation–reduction of respiration and is a constituent of certain enzymes and proteins. Iron-deficient plants bear leaves, which become chlorotic with the main veins remaining green in color. The leaves of grasses suffering from iron deficiency have a striped appearance. The disease due to iron deficiency in citrus is termed as green netting. Iron deficiency often occurs in soil that is calcareous. The addition of iron salts is rarely effective in remedying iron deficiency. Iron-chelates-organic compounds have been successfully used as foliar sprays and also in soil treatment.

Magnesium (Mg)

It is a metabolic constituent of chlorophyll. The leaves of magnesium-deficient plants become chlorotic in patches between the veins. Older bases are affected first. Necrosis often occurs within the yellow blotches, and as in potatoes, along the margins of leaves or leaflets. In tobacco, the disease 'sand drown' is caused due to magnesium deficiency. The application of magnesium sulphate or of magnesium limestone to the soil is generally recommended for magnesium deficiency in plants.

Manganese (Mn)

This element is a co-factor of several oxidative enzymes of cellular respiration, nitrogen metabolism and photosynthesis. The leaves of deficient plants show chlorosis. The smallest veins remain green. The symptoms appear first on the older leaves of plants with manganese deficiency. The gray speck disease of oats and marsh spot of garden peas are due to manganese deficiency and can be corrected by spraying $MnSO_4$.

Molybdenum (Mo)

A concentration of less than 0.5 ppm of molybdenum is required. It is necessary for protein synthesis and is a constituent of the enzymes that are responsible for the reduction of nitrates to nitrites. It is essential for symbiotic nitrogen fixation in the nodules due to *Rhizobium* spp. Molybdenum-deficient plants become stunted and have pale yellow leaves with marginal chlorosis. The leaf lamina becomes thin and dry. The growing tips also become distorted and die. The whiptail disease of cauliflower is a disease caused by molybdenum deficiency. Malformed leaves characterize it. Corn, legumes, sugar beets, flax, tomatoes, and some other crop plant suffer from molybdenum deficiency can be corrected by liming the soil. This releases molybdenum that remains chemically bound in an acid medium. Ammonium molybdate or Sodium molybdate may also be applied to the soil or to the foliage of deficient plants.

Sulphur (S)

Certain vitamins, enzymes, and amino acids contain sulphur. A deficiency of this element causes the leaves to become light yellow in color. The dwarfing and yellowing disease in cotton has been attributed to sulphur deficiency. Citrus plants also suffer from sulphur deficiency. The addition of ammonium sulphate to the soil is recommended for remedying sulphur-deficiency diseases.

Zinc (Zn)

This element is a component of many enzymes of auxin synthesis and carbohydrate oxidation. Zinc is important for the formation of chlorophyll and in photosynthetic activity. Deficient plants have chlorotic and necrotic leaves and the internodes are stunted. Zinc deficiency also results in diseases such as white bud of corn, rosette of fruit trees, bronzing of twigs, little leaf of stone fruits, mottle leaf of citrus, and Khaira disease of rice. The deficiency can be corrected by spraying zinc sulphate. An excessive supply of micronutrients and certain non-essential elements like aluminium, cobalt, nickel, chromium, selenium, arsenic, lead, and mercury have long been known to produce toxic effects in plants. Several workers have described these. The ecological aspects of heavy metal toxicity and plant adaptation to such conditions were discussed in a symposium at Sheffield (Rorison, 1969). Heagle (1989) has reviewed the work on ozone and crop yield. Katterman (1990) has edited a book *Environmental Injury to Plants,* while Benett (1993) has edited the book *Nutrient Deficiencies and Toxicities in Crop Plants.*

Tip Burn of Paddy or Pansukh of Rice

The names, tip burn, dakhina, chatra, ukra, kadamara or pansukh, have been given to leaf-drying disease of rice known in India for quite some time. Dastur (1937), and Mishra and Chakravarty (1955) have described it.

Symptoms

The first symptom of the disease is the drying of the leaves. They become less lustrous to bronze and later become scorched at the top. The drying progresses down along the margin resulting in the drying of the complete leaf. In advanced stages, the entire plant dries and wilts. The leaves and stalks turn brown. This spreads rapidly in the field.

Causal Factor

The cause of the disease is not properly understood. The disease is common in wet soils or in poorly drained fields where the water is allowed to remain stagnant for more than a fortnight. This is because water reduces the supply of oxygen to the roots.

Disease Management

The use of ammonium sulphate had been recommended by Dastur (1937) to control this disease. According to him, the disease occurs due to the lack of nitrogen availability. He recommended that the fields be drained during the early stages of plant growth and then ammonium sulphate be applied at the rate of 15-25 kg/acre. According to Sinha and Singh (1956), green manuring reduces the incidence of tip burn and increases the yield.

Blossom End Rot of Tomato

This disease of tomato fruit is a nutritional disorder that is caused due to derangement of the water balance between the leaves and fruits during the development of the plant. A lesion at the blossom end of the fruit, which occurs, characterizes it while it is ripening on the vine. A water-soaked spot appears at the point of attachment of the senescent petals. It enlarges quite rapidly until it is about 2 cm or more in diameter, and at the same time, the diseased tissue shrinks, depressing the surface of the lesion. The color of the fruit becomes darker and secondary organisms generally invade the tissues. The diseased portion of the fruit becomes delimited as a sunken, leathery, dark-colored area. If tomatoes are grown at relatively constant low moisture content, the disease does not develop. If plants are grown at relatively high moisture content, succulent growth is rapid, the rate of transpiration is high and disease incidence is greater. Sandy soils are more favourable to blossom end rot. Foster (1937) found that increased amounts of nitrogen favour the occurrence of the disease when other conditions are also conducive, while higher doses of phosphorus reduce the incidence of disease. Robbins (1937) had studied the relation of nutrient salt concentrations to the growth of tomato and to the incidence of blossom end rot of the fruit. At the lowest concentration where growth is greatest, no disease occurs; while at the highest concentration where there is greatest fluctuation in the rate of transpiration, 80% of the fruits become diseased. Schroeder (1949) proved that transpiration has an influence on the blossom end rot of tomato. Schroeder was

able to control the disease with emulsified hydrocarbon sprays. Raleigh and Chucka (1944) found that the incidence of disease was greater when the nutrient solution was high in nitrogen, sulphur, magnesium, potassium or chlorine and when it was low in calcium. Lyon *et al*. (1942) found that the blossom end rot has a correlation with the calcium content.

Disease Management

The most obvious method of controlling blossom end rot is to maintain an equable environment and to keep the soil uniformly moist. This should be taken care of, especially when the fruits begin to develop.

Heat Canker of Linseed

It is a non-parasitic disease caused by high soil temperatures near the soil surface.

Symptoms

Heat injury of the cortical tissues of the hypocotyl and stem near the soil line is common in flax (Reddy and Brentzel, 1922) and other succulent herbaceous plants. If the soils are dark colored and the surface temperature becomes high enough to kill the cells of young cortical tissues before the plants are larger enough to shade the surface, the cortical tissues collapse, which results in the death of the young seedlings. As a result of the collapse of tissues, sunken brown lesions develop on the stems. There is an enlargement of the cortical tissues above the cankers.

Disease Management

High temperatures can be avoided by planting early. The rows should be drilled from the north to the south. This results in maximum shedding. Damage from this disease can be reduced by higher rates of seeding using a nurse crop, mulching the soil surface, and irrigation.

Black Heart of Potato

Black heart is an important storage, transit and market disease of potatoes as a result of poor oxygen relation (Singh, 1998). The conditions under which this disease develops vary greatly. It is known that a number of rather distinct sets of environmental conditions lead to practically the same result or cause this disease—such as high temperature in transit, poor ventilation in storage and high soil temperature during the cultivating season. Bartholomew (1913) revealed the fundamental nature of black heart and made a pathological study of the black heart of potato tubers (1915). He found that he could induce black heart in tubers by exposing them to high soil temperatures for 24-48 hours in an oven heated to 40-42ºC. Mann and Joshi (1920) made a chemical study of heart rot or black heart in potato tubers. They could induce this disease by first coating the tubers with collodion or paraffin or replacing the air in container with carbon dioxide or nitrogen. Placing tubers in sealed containers can also produce black heart. This shows that oxygen is crucial to prevent the occurrence of black heart on potato tubers.

The centers of potato tubers attacked by black heart show black portions when sliced open; or after being sliced open, the potato tubers undergo a fairly rapid change in color from white or pink to brown

to dark brown or black (Fig 28.1). Bartholomew (1915) has studied the progress of the disease. When a tuber exposed to 40ºC for 24 hours is cut open, the interior tissue appears normal in the beginning, but upon exposure to air, pink discoloration occurs which gradually becomes dark brown or black. This was shown to be an enzymatic effect in which some of the amino acids, particularly tyrosine, pass through a series of intermediate compounds to the deeply colored, relatively stable and insoluble compounds known as melanins.

High temperatures bring about sub-oxidation by stimulating respiration under conditions in which cells in the interior of the tubers cannot secure an adequate supply of oxygen. Poorly ventilated storage over long periods predisposes potatoes to black heart. Davis (1976) showed that a disease similar to black heart could be caused by electric current, freezing, immersion in toluene or glycerin and by certain pathogens.



Figure 28.1 Black heart disease of potato (physiological), (courtesy of Dr. B.B. Nagaich).

Disease Management

Avoiding extremely high or low temperatures, properly storing potatoes in well-ventilated storage warehouses, avoiding piling of the bags of tubers upon one another can significantly control the disease. In the fields, if the temperature goes up very high, the fields should be irrigated.

Mango Necrosis or Black Tip

The disease was first reported from Bihar and is now well known in Bihar, Uttar Pradesh, West Bengal, and Punjab. It is generally found near brick kilns in Uttar Pradesh, Bihar, West Bengal, Punjab, and Haryana in northern India.

Symptoms

The necrosis of one third or half of the fruit characterizes black tip of mango. The fruit become abnormally long at the tip. Premature ripening of the affected fruit occurs. The most susceptible varieties are 'Daseri', 'Safeda' and 'Langra'. The disease starts as a small, circular, pale patch at the tip of the mango fruit and soon enlarges, becomes black and covers the tip of the mango completely. The outer skin of the affected portion becomes flat and hard. Internally, in the vessels of the mesocarp, certain brown deposits are found, which have been provisionally identified as belonging to the group of tannins, flavotannins, flavophenols or their derivatives.

Causal Factor

Previously, it was believed that black kiln fumes are the direct cause of the disease. Sen (1943), Das Gupta and Verma (1939), Das Gupta and Sinha (1944), working on the disease in Bihar and Uttar Pradesh, suggested that the smoke of these gases polluted the air with toxic gases such as sulphur

dioxide and that these gases caused the necrosis of the fruit tissues. In laboratory experiments, exposing mango fruit to smoke and sulphur dioxide could lead to the disease. Later on, Das Gupta and Sen (1960), in their final report, stated that there is a deficiency of boron in the mango fruit exposed to brick kiln fumes. The disease was, however, not attributed to a deficiency of boron in the soil but to disturbances in boron metabolism due to the interaction of brick kiln fumes with certain cell metabolites of mango.

Disease Management

The disease can be controlled by spraying borax at the rate of 2.7 kg/100 gallons, three times in the season: pre-flowering, flowering and immediately after fruit setting. However, Das Gupta and Sen (1960) recommended only two sprays.

Khaira Disease of Rice (Zinc Deficiency Disease of Rice)

Dr. Y.L. Nene and his associates first discovered Khaira disease of rice in the Tarai region of the then Uttar Pradesh (now Uttarakhand). The disease has also been reported from Mysore (Rangaswami, 1972) and Haryana (Ahuja *et al.,* 1989). It also occurs in Punjab. Khaira disease of rice is an excellent example of a disease due to non-availability of zinc to the plants in an otherwise normal soil (Singh, 1996). There is an interesting paper by Nene *et al*. (1965) on this disease.

Symptoms

The disease symptoms usually appear 10-15 days after the transplantation of the paddy crop. This period also coincides with the decomposition of the last year's stubbles in the flooded fields. The first symptoms seen are the chlorosis of the leaves starting from the base of the leaves. After this, a large number of small brown or bronze colored spots appear on the leaf surface which later on coalesce become bigger in size and cover the whole surface of the older leaves. The leaves become brown or bronze colored and ultimately dry and die. The infected plants show stunted growth. The underground portion of the plants also shows symptoms. The roots are poorly developed and usually the main roots turn brown. In severe cases, plants fail to grow further and produce ears with few grains. Sometimes, natural recovery may take place six weeks after transplantation.

Disease Management

Two sprays of a mixture of zinc sulphate and slaked lime (2 kg zinc sulphate + 1 kg of slaked lime) in 400 liters of water per acre of the plot can manage the disease effectively. Care should be taken that the mixture is first sprayed as soon as the disease is first seen in the field. The next dose should be sprayed 10 days after the first spray.

Zinc Deficiency Disease of Citrus

This disease is very common in many citrus gardens in India. It is also known as mottling or foliocellosis.

Symptoms

The main symptom is the production of alternative chlorotic regions and dark green patches in the leaf. The portions near the leaf veins are usually brown and the rest of the leaf is chlorotic. As a result of continual deficiency of zinc in the soil, there is severe stunting of the plant, reduction in leaf size, narrowing of the leaf, and severe chlorosis. The fruit size is also reduced. The plants become week and are exposed to root infections and this slowly results in their death.

Disease Management

The application of zinc sulphate to the soil gives a very slow response. Spraying zinc sulphate and lime mixture (zinc sulphate 2.268 kg, and quick lime 2.268 kg in 100 gallons of water) is recommended for a quick response.

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Questions

1. What are deficiency diseases?
2. Give examples of diseases in which excess of an element can cause a disease.
3. How is proximity to brick kilns responsible for mango necrosis or black tip?
4. What is Khaira disease of paddy? Who discovered this disease for the first time in India? What are its causes? Also, suggest the control measures.

Application of Biotechnology in Plant Pathology

29

According to Agrios (1988, 2005), biotechnology is defined as the manipulation, genetic modification and multiplication of any living organism through novel techniques such as tissue culture and genetic engineering, resulting in the production of improved or new organisms and products that can be used in a variety of ways. The term genetic engineering can be broadly defined to include all techniques designed to manipulate the genetic constitution of organisms. Jain (1993) reviewed the recent advances in plant genetic engineering. Verma and Duttamajumdar (1986) have discussed genetic engineering strategies for the management of bacterial diseases of plants. Willmitzer (1993) discussed genetic engineering for plant disease resistance. Nagarajan *et al.* (1992) reviewed the use of tissue culture and genetic engineering in crop pest control. Fuchs and Gonsalvas (1996) reviewed genetic engineering for controlling plant diseases, while Mandahar and Paul Khurana (1998) discussed the role of biotechnology in controlling plant diseases. Lopez *et al*. (2003) reviewed innovative tools for detection of plant pathogenic viruses and bacteria have and Sharma (2003) critically analyzed the molecular diagnosis and application of DNA markers in the management of fungal and bacterial plant diseases. Plant biotechnology impinges or helps plant pathology in many ways.

1. Biotechnology helps to obtain pathogen-free mother plants through rapid clonal propagation.
2. New plants to which genes have been incorporated through genetic engineering are likely to show instability towards environmental conditions and towards the pathogenic microflora of their habitats. Here, plant pathology plays its part.
3. The main vehicle for transferring genes from donor to recipient plants are plant pathogens, particularly the bacterium *Agrobacterium tumefaciens* and the cauliflower mosaic virus.
4. Biotechnology helps to control plant diseases by inserting resistance genes into plants by genetic engineering techniques.
5. The plant genes for resistance to disease and of pathogen genes for virulence to pathogen have been considerably utilized by genetic engineering techniques.

The Use of Tissue Culture Techniques of Importance to Plant Pathology

Almost all tissue culture techniques are used in plant pathology. Some of the important tissue culture techniques of importance to plant pathology are briefly discussed.

Callus Culture

Generalized protocol for the screening of callus tissue for the isolation of toxin-resistant plant is given below (Jones, 1990).

This technique is commonly used to study:

1. The behaviour of pathogens, particularly biotrophs such as rusts, powdery mildews, viruses, nematodes and so on. This potential of callus was first recognized by Morel (1944) who infected callus tissue from vine (*Vitis vinifera*) with zoospores of the downy mildew fungus, *Plasmopara viticola*. Since then, methods have been devised for growing a number of other biotrophs or symbionts (Mycorrhizal) in callus/organ culture.
2. The effect of toxins secreted by pathogens and phytoalexins produced as defence mechanism and is compared to the reaction of susceptible varieties. The plantlets that survive the toxin are apparently resistant to the particular pathogen and are therefore selected for incorporation into the breeding programs. Phytoalexins have been detected in the tissue cultures derived from a number of plant species. Examples are pisatin from *Pisum sativum* (Bailey, 1970), glyceollin from *Glycine max* (Ebel *et al*., 1976), phaseollin, phaseollidin, and phaseolinisoflavan from *Phaseolus vulgaris* (Dixon and Fuller, 1978).
3. These infected calli are used for *in vitro* testing of chemicals such as fungicides and antibiotics used for control of pathogenic microorganisms. Table 29.1 lists plants of some crops that have been regenerated from fungal toxin-resistant tissue (callus) culture.

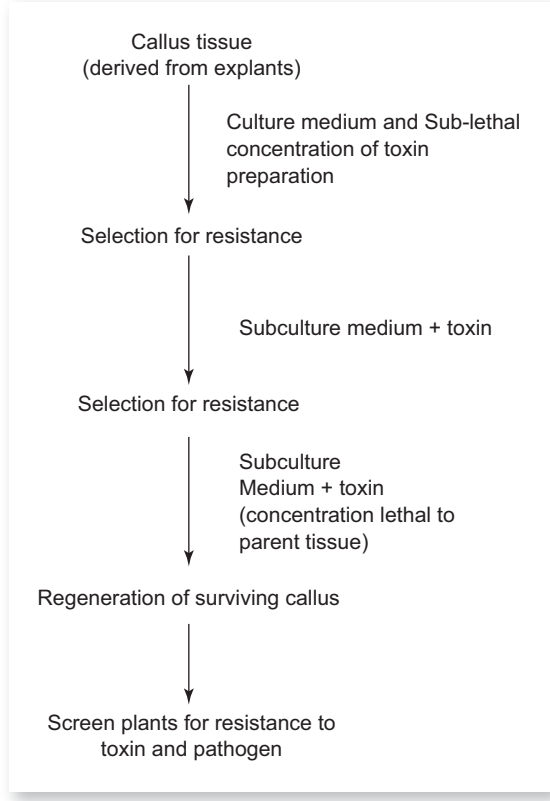


Figure 29.1 Generalized protocol for the screening of callus tissue for the isolation of toxin resistant plant

Table 29.1 Plants of Some Crops Regenerated from Fungal Toxin-Resistant Cultures

S. No	Crop	Toxin obtained from
1.	Alfalfa	*Fusarium oxysporum* f.sp. *medicaginis*
2.	Maize	*Helminthosporium maydis*
3.	Oats	*H. victoriae*
4.	Onion	*Pyrenochaeta terrestris*
5.	Potato	*Phytophthora infestans, F. oxysporum* f. sp. *solani*
6.	Rape	*Phoma lingam*
7.	Tomato	*Alternaria solani*, *Phytophthora infestans*

Apical or Meristem Tissue Culture

Morel and Martin (1952) published an important paper, in which they excised shoot apical meristem of infected dahlia variety and grew them *in vitro* tissue culture medium. This differentiation of the meristem led to the production of plantlets, which upon transferring to soil gave rise to adult plants, which were then multiplied. Most of such plants are found free of the virus, because majority of viruses are unable to attack or reach the apical and axillary meristem tissues of systemically infected plants. If meristem tissue culture technique is combined with thermotherapy, the combinations are very useful. The technique has been used by a number of workers for curing infected valuable cultivars of a wide variety of plants. Most vegetatively propagated plants and many perennial plants are infected by viruses that remain latent in the plant without displaying major visible symptoms. Hence, meristem tip culture technique is a very useful and valuable asset in cultivating vegetatively propagated plants. Raychaudhuri and Verma (1977) have reviewed the work on therapy by heat, radiation and meristem culture.

Some of the advantages of this technique are**:**

1. Some valuable ornamental and vegetable varieties that were previously lost completely have been saved.
2. Virus-free clones of a particular variety can be built up.
3. Production of disease-free clones and storage of valuable germplasm.

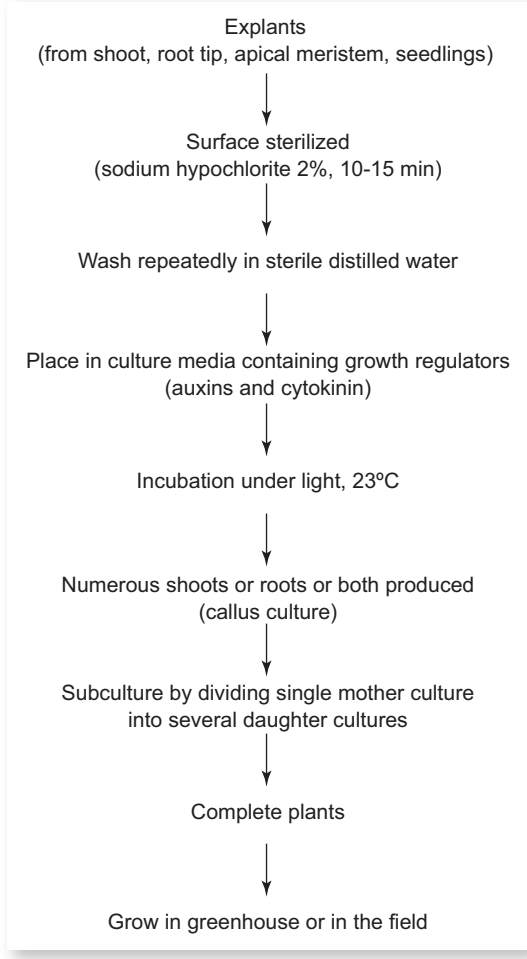


Figure 29.2 Protocol for the apical or meristem tissue culture technique

Protoplast Fusion

Disease resistance in a breeding program may come either from closely related species or from more distantly related species. Problems are generally encountered if an effort is made in crossing distantly related species. Protoplast fusion is one of the methods that can be used to circumvent problems in introducing genes for resistance. By this method, factors that contribute to crossing barriers between species can be avoided and viable hybrids (Cybrids) have been recovered, even between distantly related species (Harms, 1985). Some of the disease resistance plants produced from protoplast fusion are given in Table 29.3.

Table 29.2 Crop Species Freed of Virus by Tissue Culture

S. No.	Host	Viruses/viroid eradicated (heat treatment if applied)
1.	Garlic	Mosaic
2.	Cauliflower	CaMV, TuMV
3.	*Chrysanthemum morifolium*	Virus-B, vein mottle, green flower (35-38^{0}C, 4-37 weeks)
4.	Taro	Dasheen mosaic
5.	*Dianthus barbatus*	Ring spot, mottle, vein mottle.
6.	Strawberry	Vein chlorosis, vein banding (3-40^{0}C, 4-7 weeks)
7.	Sweet potato	Internal cork, mosaic, mottle (27-28^{0}C)
8.	Banana	CMV (35-3^{0}C, 14-15 weeks).
9.	Tobacco	Tobacco ring spot, alfalfa mosaic (32-40^{0}C)
10.	Potato	Potato virus A (37^{0}C, 2-4 weeks) Spindle tuber (33-38^{0}C, 4-8 weeks)

Table 29.3 Disease Resistant Plants Produced from Protoplast Fusion (After Eck and Smith, 1996).

Species used for fusion	Disease	Reference
Lactuca sativa and wild *Lactuca* sp.	Downy mildew (*Bremia lactucae*)	Maisonneuve *et al.,* 1995
Brassica oleracea and *Raphanus sativus*	Club root (*Plasmodiophora brassicae*)	Hagimori *et al*., 1992
Brassica napus and *Brassica nigra*	Black leg *(Phoma lingum)* club root *(Plasmodiophora brassicae)*	Gerdemann-Knorck *et al*., 1994
Brassica oleracea and *Brassica napus*	Black rot (*Xanthomonas campestris* pv. *campestris*)	Hansen and Earle, 1995
Solanum brevidens and *Solanum tuberosum*	Bacterial soft rot (*Erwinia* spp.)	Austin *et al*., 1988

An outline of the protocol for the isolation of plant protoplast from intact leaf tissue or from cell suspension culture is given in Fig. 29.3.

Tissue for protoplast liberation must be surface-sterilized and chopped to enable enzyme penetration or the lower epidermis may be removed to improve enzyme access. Enzyme mixtures normally make use of cellulase and pectinase activities to macerate wall polymers. Protoplasts are released into an osmotically stabilized solution to prevent lysis. It is harvested efficiently by gentle centrifugation. Washing in fresh stabilizer solution removes lytic enzymes. The protoplast may be separated from contaminating wall fragments by filtering or by layering in sucrose medium. The protoplast from cell suspension can also be liberated using the same methods.

For subsequent regeneration, protoplasts are placed on agar medium containing stabilizer solution, a carbon source and essential nutrients.

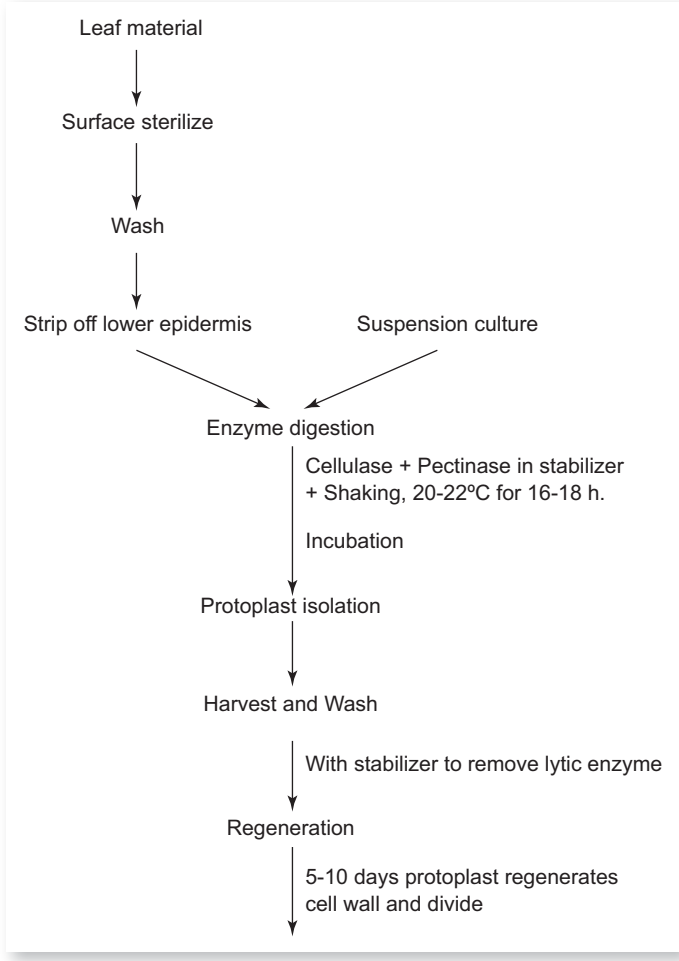


Figure 29.3 Protocol for the isolation of plant protoplast from intact leaf tissue or from cell suspension culture

Procedure for the chemically-induced fusion of plant protoplast shown in Figure 29.4.

Chemically Induced Fusion Isolated protoplasts are sticky, tend to aggregate in suspension and show fusion spontaneously during incubation. Chemicals tend to increase the fusion frequency. Fusion can occur in the presence of high Ca^{+2} and high pH (9-10) but the commonly used chemical (Fusogen) is polyethylene glycol (PEG). Due to addition of PEG, there is adhesion of protoplast to their neighbors, which can be assessed by a microscope. Subsequent dilution of the stabilized PEG, either stepwise or at once, results in fusion and mixing of the cytoplasm. PEG causes slight dehydration of the protoplasts and crinkling of the membrane. The level of fusion is usually 1-10% as chemical fusion agents are toxic and therefore damaging to the cell. Higher fusion has been reported for electrically stimulated fusion events (about 80%), than for chemically induced fusion (Zimmerman and Scheurich, 1981).

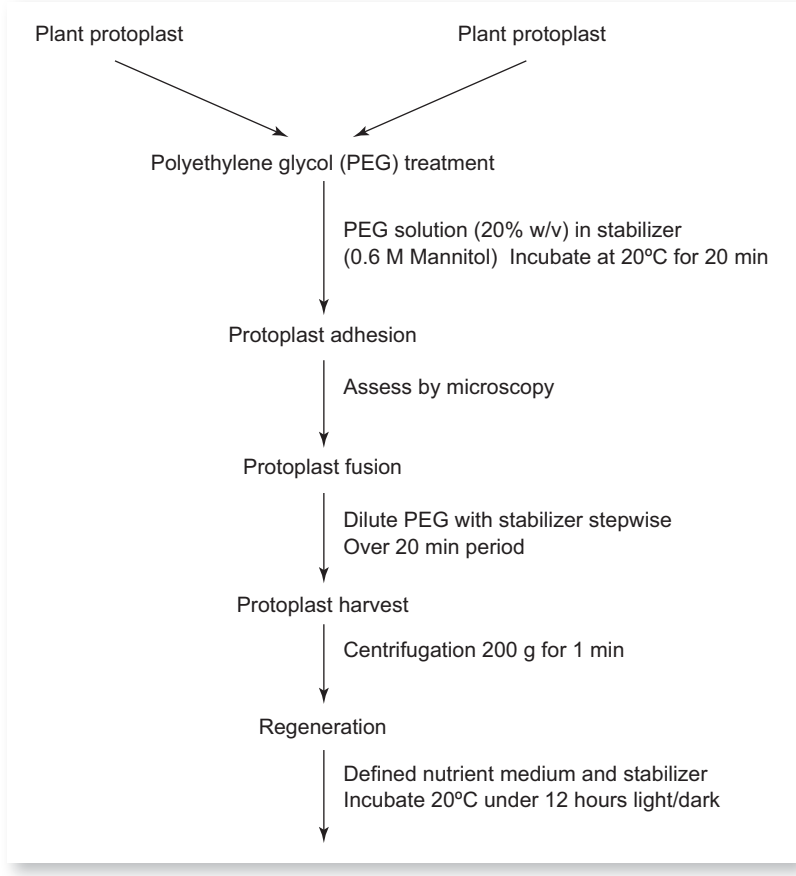


Figure 29.4 Protocol for the chemically- induced fusion of plant protoplast

The most important use of protoplast fusion is the transfer of genes between sexually incompatible plants. In plant pathology, protoplast fusion has been used for transmitting disease resistance genes into sexually incompatible hosts. For example, wild potato species *Solanum brevidens* is resistant to potato leaf-roll virus but is sexually incompatible with cultivated potato *S. tuberosum*. Here, protoplasts of *S. brevidens* were fused with protoplasts of a commercial and agronomically selected variety of potato (*S. tuberosum*) leading to regeneration of somatic hybrid potato plants which were resistant to potato leaf-roll virus (Austin *et al*., 1988; Helgeson *et al*., 1986).

It has been possible to inoculate the protoplast of plant with viruses and study their replication and physiology. The bacterium *Agrobacterium tumefaciens* or its modified Ti plasmid and the double-stranded DNA virus, cauliflower mosaic virus have been used to introduce foreign genetic material into plant cells. At present, this is the most likely method for introducing genes controlling disease resistance into important agronomic plants.

Selection for Disease Resistance

In vitro selection has a distinct advantage over other selection systems since it allows significant saving of space, time and money. For plant diseases that cause damage through toxins, cell selection for toxin resistance in cultures and regeneration of plants from descendants of the selected cell lines can give disease-resistant genotype. For example, disease-resistant crop plants have been produced through *in vitro* selection in potato against *Phytophthora infestans* (late blight of potato), in tobacco (*Nicotiana tabacum*) against *Pseudomonas tabaci*. At the biotechnology center, IARI, New Delhi, plants resistant to toxin produced by *Alternaria brassicae*, a blight-causing organism, have been isolated. Nagarathna *et al*. (1993) have selected for downy mildew resistance from the regenerants of pearl millet.

Recombinat-DNA Technology

Advances in molecular biology have opened up possibilities of identifying and isolating any gene of an organism, and mobilizing and expressing it in a different organism of one's choice.

Engineering Plants for Resistance to Disease

A notable success has been made with regard to viral diseases following the use of r-DNA technology. For example, a major achievement has been the transfer and expression of coat protein genes of tobacco mosaic virus (TMV) and alfalfa mosaic virus (AMV) in tobacco, resulting in protection against or delay of disease development in the transgenic plants (Beachy *et al*., 1990). The purpose of introducing coat protein genes to give resistance against the virus is that the coat protein synthesized in the plant cells somehow checks the multiplication of infecting viral RNA. With the help of r-DNA technology, the chitinase gene of *Serratia marcens* has been introduced into tobacco, potato, lettuce, and sugarbeet. Engineered plants synthesize chitinase, which breaks down the fungal cell wall and this kills the soil- borne pathogen, *Rhizoctonia solani* (Schell and Vasil, 1989).

Engineering Plants for Resistance to Pests

The best way of insect control has been the use of insecticides. These insecticides were effective but proved to be environmental hazards and forced development of resistant strains of insects.

There are genes in bacteria (*Bacillus thuringiensis*) that encode insecticidal proteins. *B. thuringiensis* strains toxic to dipteran, lepidopteran, and coleopteran insects have been identified and the insecticidal-protein gene cloned. Using Ti plasmid vectors of *Agrobacterium tumefaciens*, the gene encoding the insecticidal protein has been transferred to tobacco, potato, tomato, rice, and corn. Such transgenic plants incorporate resistance to specific insects that feed on these crops.

Another way of producing insect-resistant transgenic plants is to introduce genes that inhibit enzymes responsible for the breakdown of protein (protease inhibitors) into the plant. Transgenic plants with cowpea (*Vigna sinensis*) protease-inhibitor gene have shown resistance to tobacco budworm, corn earworm, armyworm, and hornworm of tomato and tobacco.

Use of Monoclonal Antibodies in Plant Pathology

Georges Kohler and Cesar Milstein developed the hybridoma technique in 1975 at the Medical Residential Council Laboratory in Cambridge, England. This technique was never patented but its commercial applications were recognized immediately. This discovery led to the production of monoclonal antibodies. Kohler and Milstein were awarded the Nobel Prize in 1984 in Medicine. In this technique, there is the fusion of myeloma cells, (cancer cells) with antibody-producing white blood cells (β lymphocytes). The resulting hybrid cell is called a hybridoma.

In the last few years, techniques have been developed to produce large quantities of identical antibodies. These new antibody-forming hybrid cells, hybridomas, can now be grown in culture indefinitely. Each hybridoma clone produces only one type of antibody. But via selection techniques, the clone that produces the desired antibody can be chosen. Monoclonal antibodies can be obtained from the liquid of hybridoma cultures and can be used to detect, identify and measure the antigens that induced their production. Monoclonal antibodies are however very specific and may not detect strains of the same virus. It is for this reason that mixtures of several monoclonal antibodies are often used in the detection of viruses and in screening tests.

A useful serological technique called ELISA, developed in 1970s, has been very widely used by all types of pathologists and has increased the ability of plant pathologists to detect and study plant viruses and other plant pathogens.

Technique for Production of Monoclonal Antibodies

1. Rats are immunized by injecting the selected antigen either subcutaneously or into the peritoneal cavity.
2. A few days later, the spleen is removed from the rat.
3. The antibody-synthesizing cells from the spleen are then mixed with fast-growing rat cancer cells, called myelomas in the presence of polyethylene glycol (PEG).
4. The result is a hybridoma—fusion of an antibody-making cell and a cancer cell.
5. All the hybridoma cells that occurred after a fusion were grown in selective culture medium, and then a mixture of antibodies would be released.
6. Individual hybridoma cells are grown as clones. In this way, all the cells in a clone will secrete only one type of antibody.
7. After the desired hybridoma cell lines have been identified and cloned, culture may be expanded and clones may be tested for the desired antibody.
8. Once the cells have been cloned, either selected clones are cultured and frozen or the hybridoma cell may be injected into peritoneal cavity of rat, to produce ascitic fluid.
9. From the ascitic fluid, monoclonal antibodies are purified.
10. The stored and frozen cells can be used at different times of the year and in different laboratories. This eliminates variation between laboratories since the same cells can be used to produce antibodies at different times in the same laboratories or in different laboratories. Enzyme-Linked Immunosorbent Assay (ELISA) makes it possible to screen a number of organisms.

Hybridoma Technology

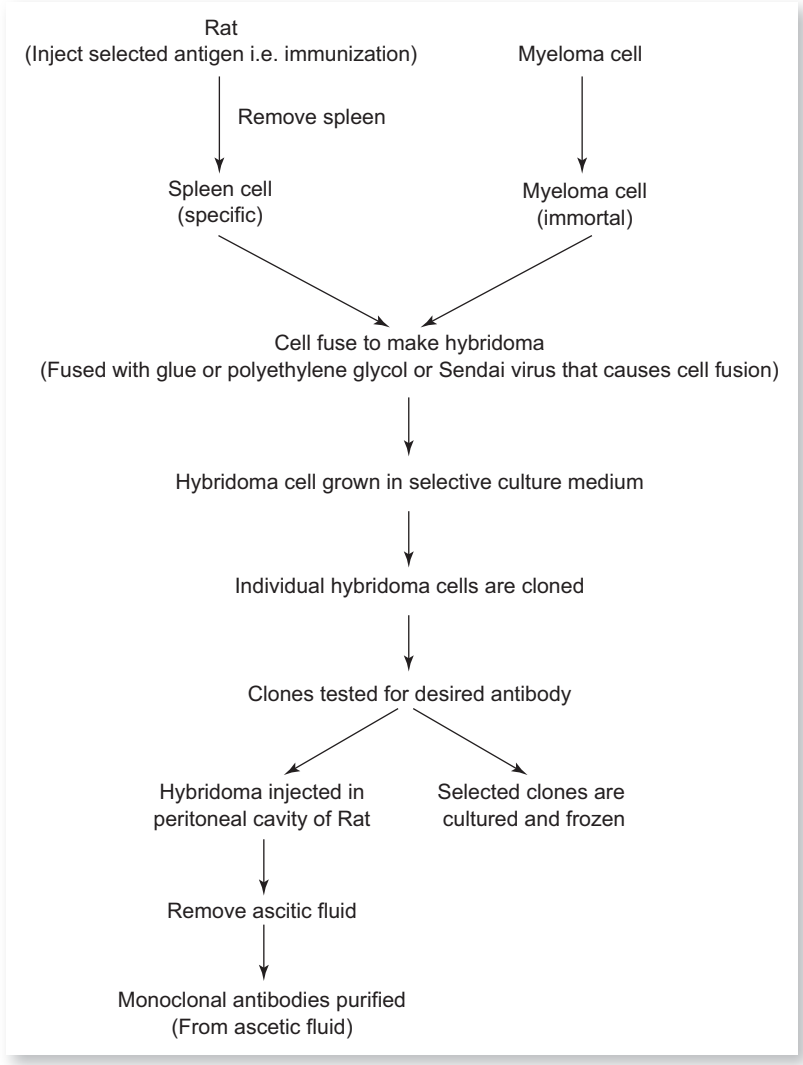


Figure 29.5 How monoclonal antibodies are made

Uses and Applications of Monoclonal Antibodies

1. The most effective application of monoclonal antibodies has been with plant pathogenic viruses. This technique has been used for accurate identification of viruses, for tracing the viruses during epidemiological studies, to distinguish between virus strains and in isolating and purification of viruses.

   Monoclonal antibodies, however, are very specific and may not detect even strains of the same virus that happen to lack the specific antigenic determinant responsible for the monoclonal

antibody. Due to this reason, mixtures of several monoclonal antibodies are often used in virus identification.

2. These procedures are now being used to study bacteria and fungi and will probably be useful in investigations of various populations of rhizosphere organisms. This technology is also very helpful in attempts to identify bacteria in mixed populations. Applications of this technology to fungi are in its initial stages, but the potential is great.

Somaclonal Variants as a Source of Disease Resistance

Plants regenerated from the callus, single cell or protoplast-derived plants, often show one or more different characteristic than the other or parent plants. Plants derived from any form of cell culture are called 'somaclones' and the variations shown by such plants, 'somaclonal variation'. These variations are due to the genetic instabilities displayed by non-meristematic tissue explants, cell or protoplast, once they are removed from stabilizing environment of intact plant and cultured *in vitro*. Somaclonal variations are widespread: being found not only in asexually propagated species but also in seed-propagated, self-fertilizing species (Larkin and Scowcroft, 1981). It is a useful tool for incorporating additional traits into existing plant varieties.

Initial studies with sugarcane suggested that the clones with disease resistance could be regenerated from the callus obtained from susceptible plants. Many plants resistant to different diseases have been regenerated from susceptible parent in many crops (Table 29.4).

Table 29.4 Some Examples of Disease-Resistant Somaclonal Variants in Plant Species

	Plant	Disease/Pathogen	Reference
1.	Potato	Late Blight, Early Blight	Matern *et al*. 1978, Behnke, 1980, Shepard *et al*., 1980
2.	Sugarcane	Fiji virus, downy mildew, Smut	Heinz *et al.,* 1977 Larkin and Scowcroft 1981.
3.	Maize	Southern corn leaf blight	Brettel and Ingram, 1979.
4.	Tobacco	TMV, Potato virus Y, Wild fire due to *Pseudomonas syringae* pv. *tabaci*, *Alternaria alternata* leaf spots and chlorosis	Burk & Chaplin, 1978; Thautony *et al*., 1983
5.	Rapeseed	Black leg	Sachristan, 1982
6.	Pearl millet	Green ear	Nagarathna *et al*., 1993

In vitro Screening for Disease Resistance

It is an obligatory step in crop improvement programs using tissue culture methods that offer several advantages over conventional glass house and field-screening methods with the intact plant. It is quick, and consumes less space and energy. It has many advantages: growing a large number of single cells or cell clumps in a small space, exposing them to different mutagens, diploidization of chromosomes and regenerating plant populations from resistant material. Here, a callus supporting poor or no growth towards pathogen's toxin(s) is characterized as resistant *in vitro*.

One of the primary advantages of tissue culture techniques is that environmental conditions can be controlled precisely. Plant protoplasts can be used to study penetration and replication of plant viruses, screening of antiviral chemicals, cell organelles, symptomatology, and maintenance of virus culture. Explants can be used for screening antiviral compounds. Tissue culture techniques can be used to study host–pathogen interaction, for the production of pathogen-free clones, for *in vitro* clonal propagation and for generation of disease-resistant plants. Phytoalexins may be used as selection agents and various procedures can be used to select disease resistance, using specialized tissue culture procedures. The use of molecular genetic engineering involving isolation and transfer of genetic material offers exciting possibilities. Tissue culture techniques provide a means to accelerate crop improvement and the production of disease-resistant crop plants.

Innovative Tools for Detection and Diagnosis of Plant Pathogenic Microbes

Plant Pathogenic microbes are responsible for increasing economic losses worldwide. They cause a large number of symptoms in most cultivated plants, which can affect different parts with various agronomic impacts. Viruses and bacteria cause plant diseases that are difficult to control because of the lack of efficient products for chemical treatment under field conditions. As many pathogenic viruses and bacteria remain latent in the plant material and in very low numbers, methods of high sensitivity and reliability are required. The molecular techniques available have evolved significantly in the last few years to achieve rapid and reliable detection of pathogens, extraction of the target from the sample being important for optimizing detection. Currently, automation and electronic data management are vital to increase the productivity and efficiency of routine analysis for detection of plant pathogenic bacteria and viruses. Several genomes from causal agents of plant diseases, both viral and bacterial, have been completely sequenced and more are underway. In the following pages, a number of important developments and improvements in serological and molecular techniques are briefly described:

Monoclonal and Recombinant Antibodies

The detection of viruses and bacteria by well-known serological techniques such as IF (Immuno Fluorescence) and ELISA (Enzyme Linked Immunosorbent Assay) has improved greatly with the use of specific monoclonal and recombinant antibodies. Both allow the selection of specific target epitomes to avoid false positives. Specificity problems are frequent when analyzing bacteria in plant material, water and soil due to large quantities of other microorganisms present in the sample and to the difficulties in obtaining polyclonal antibodies specific for the target pathogen without cross reaction with other microorganisms. Commercial monoclonal antibodies for the detection of plant pathogenic viruses are now available from several companies. Several companies have developed a wide range of kits for rapid and/or *in situ* bacteria detection using polyclonal or monoclonal antibodies based on slide agglutination, IF and ELISA. The kits based on agglutination or ELISA are appropriate only for diagnosing plants already showing symptoms. In plant pathology, serological detection has been widely used and some recombinant constructs have been produced but only a few of them have been applied to routine ELISA tests.

Enrichment ELISA Protocols

Combining ELISA, which uses specific monoclonal antibodies with a prior enrichment step greatly improves the sensitivity of detection of bacterial pathogen. This methodology has been developed because of the low sensitivity of ELISA for bacterial detection using specific monoclonal antibodies and the need to improve this sensitivity, especially to detect latent infection of Quarantine bacteria.

On-site Testing: Tissue Print ELISA and Lateral Flow Devices

Simple commercial methods of rapid detection are required for testing large number of samples by non-experienced technicians. For this purpose, tissue print ELISA and lateral flow devices have been designed for several plant bacteria and viruses. Although detection specificity is very high when using the appropriate monoclonal antibodies, the sensitivity of these methods, although good for virus detection, is relatively low for bacteria, and they are more appropriate for analyzing plants with symptoms. In some cases, tissue print ELISA has demonstrated potential in, for example, CTV (Citrus tristeza virus) detection in plant tissues imprinted on nitrocellulose membranes. Lateral flow devices use specific antibodies and the tests give results in a couple of minutes, but such kits are available only for few viruses and bacteria.

Flow Cytometry

It is a technique for rapid identification of cells or other particles as they pass individually through a sensor in a liquid stream. Bacterial cells are identified by fluorescent dyes conjugated to specific antibodies and detected electronically using a fluorescence-activated cell sorter, which measures several cellular parameters based on light scatter and fluorescens. Flow cytometry has excellent potential as research tool and possibly for routine use in seed health testing and other fields. Several parameters can be analyzed simultaneously including total particle count, distinction between living and dead cells, and differentiation of target and non-target bacterial populations associated with the seeds or other plant material. This technique has also been adapted to the analysis of viability, metabolic state and antigenic markers of bacteria.

Flow cytometry is a well-known tool for the detection and physiological status assessment of microorganisms in drinking water, marine environments, food and fermentation processes. However, flow cytometers are seldom used in plant pathology, despite its major advantages as both a detection method and a research tool. Potential uses of flow cytometers include the characterization of genome sizes of fungal and oomycete populations, multiplexed pathogen detection and the monitoring of the viability, culturability and gene expression of plant pathogens, and many others.

Fluorescence in situ Hybridization (FISH)

It is a technique applied for bacterial detection that combines the simplicity of microscopic observations and the specificity of hybridization. Its use in detection of plant pathogenic bacteria is recent and is dependent on the hybridization of DNA probes to species-specific regions of bacterial ribosomes. They are particularly suitable as diagnostic targets because ribosomal RNA contains functional sequences that are common to all species but also sequences that are very specific to individual species and FISH only needs to recognize this specific information.

Cucumber green mottle mosaic virus (CGMMV), which belongs to the genus Tobamovirus, is a major pathogen of cucurbit crops grown indoors and in open fields. Currently, immunology (e.g., ELISA) and molecular amplification techniques (e.g., RT-PCR) are employed extensively for virus detection in plant tissues and commercial seed lots diagnostics. But now, a fluorescent *in situ* hybridization (FISH) technique, using oligonucleotides, whose 5′-terminals were labeled with red cyanine 3 (Cy3) or green fluorescein isothiocyanate (FITC), was developed for the visualization of the pathogen *in situ.* This simple and reliable method allows detection and localization of CGMMV in the vegetative and reproductive tissues of cucumber and melon. When this technique was applied in male flowers, other tissues were found to be infected; whereas, the pollen grains were found to be virus-free. These results have meaningful epidemiological implications for the management of CGMMV, particularly with regard to virus transfer via seed and the role of insects as CGMMV vectors.

Polymerase Chain Reaction (PCR)

PCR is a very rapid and sensitive method for the molecular diagnostics of plant pathogens, which are otherwise difficult to identify morphologically. Pathogen detection by using PCR is mainly dependent on the design of primers. Specific, semi-specific and arbitrary primers can be used for the PCR. However, DNA sequences of plants and other organisms are also amplified with random primers, which lead to ambiguous results. Thus, plant pathogens must be isolated from the host and purified before the DNA extraction. Hence, RAPD (Random amplified Polymorphic DNA) analysis may not be useful for the direct detection of plant pathogens in the infected tissues. However, longer and species-specific PCR primers allow detection of target sequences in crude specimens of infected tissues. Primers have been used to develop PCR-based diagnostic assays for *Xanthomonas campestris* pv. *phaseoli*, *Erwinia amylovora* and *X. campestris* pv. *citri.* Genes controlling the specific properties (e.g. toxin production and pathogenicity) of pathogens have also been used as target sequences for specific detection of plant pathogens. PCR amplification of *Tox* gene region (phaseolotoxin producing gene) for the detection of *Pseudomonas syringae* pv. *phaseolicola* have been successfully used.

Serological techniques can also be combined with PCR; for example, immuno-capture PCR and immuno-PCR. In immuno-capture PCR, antigen is concentrated by use of specific antibodies, which is then subjected to PCR, while immuno-PCR enhances the antigen–antibody reaction. Immuno-capture technique was used for the detection of *Xanthomonas campestris* pv. *citri*.

The co-operational PCR technique has been used successfully for the detection of plant RNA viruses like cherry leaf roll virus, cucumber mosaic virus and Citrus tristeza virus. Multiplex-PCR allows the simultaneous and sensitive detection of different DNA or RNA targets in a single reaction. Multiplex-PCR is useful in plant pathology because different bacteria and/or RNA viruses frequently infect a single host and consequently, sensitive detection is needed for the propagation of pathogen-free plant material. One of the examples is the simultaneous detection of six major characterized viruses described in olive trees which belong to four different genera: Cucumovirus, Nepovirus, Necrovirus and Oleavirus.

In Multiplex-nested RT–PCR has been developed for simultaneous or sensitive detection of Cucumovirus, Nepovirus and the bacterium *Pseudomonas savastanoi* pv. *savastanoi* from olive plants using 20 compatible primers in a compartmentalized tube. It enables the simultaneous detection of several viral RNA and bacterial DNA targets in a single analysis performed with woody plants. It also saves time and reagent cost because it can be performed in a single reaction, although accurate design of compatible primers is needed.

DNA Microarrays

DNA microarrays or biochips consist of a surface on which multiple capture probes are linked, each one being specific for a DNA or RNA sequence of the targets. Their purpose is the detection of numerous sequences in a single assay. Various supports are currently in use for the elaboration of microarrays including glass, nylon and different polymers. Up to 30,000 DNA probes (gene sequences) can be arrayed on to a single chip. The probes arrayed can be PCR products amplified to high concentrations or relatively short oligonucleotides probes. Once arrayed, the chip can be exposed to fluorescently labeled DNA/RNA from the sample to be tested. The detection system uses one or several fluorophores that can be read with laser technology to reveal the targets presence in the samples. Microarrays can generate fast results for several pathogens but their cost is still very high.

Gene expression profiling using DNA microarrays have been extensively used from developmental biology of model organisms such as *Drosophila*, *Saccharomyces cerevisiae* and *Arabidopsis.* Recent reports on initiating research on the detection of plant viruses and other plant pathogens by DNA microarrays have been limited. Research works have been initiated toward detection of plant viruses, which included potato viruses. Similarly, investigations have been initiated toward detection of bacteria, mycotoxins-producing *Fusarium* spp., and aflatoxin-producing *Aspergillus* spp.

Agilent technologies of Palo Alto, California, in collaboration with North Carolina State University, Raleigh, North Carolina, have developed the first commercial DNA microarrays to include DNA probes of the rice blast fungus, *Magnaporthe grisea*, and rice plant on one chip. By having two genomes on one microarray, researchers will have a unique opportunity to study host–pathogen interactions between plants and pathogens in a way that was never before possible.

In addition to understanding gene regulation and processes in an infected host plant, microarray technology has been used to explore such processes in pathogenic organisms under different conditions. Examples include investigating gene expression of *Fusarium graminearum* in infected barley (Güldener *et al.,* 2006) and the response of chestnut blight fungus (*Cryphonectria parasitica*) to hypovirus infection (Allen *et al.,* 2003). Arrays have even been used to investigate parasitoid–host interactions (wasp/white-fly) and to investigate how genes affect virulence properties in bacteria such as *Xylella fastidiosa* (Pierce's disease of grapevine).

Characterization of Genetic Variability in Plant Pathogens using DNA Markers

Traditional markers are used to study the variability in pathogens and are based on the use of differential hosts, cultural characteristics, morphological markers and biochemical tests. These markers distinguished pathogens on the basis of their physiological characters i.e. pathogenicity and growth behavior and can group them according to their similarity for these particular characters. Moreover, differential hosts are not available in most of the host pathogen systems, thus variability cannot be assessed. In such cases, molecular markers are used for the characterization of genetic variability of the plant pathogens. Using PCR, very close strains of a pathogen can be distinguished without prior knowledge of the nature of polymorphic region by the use of RAPD. In India, 20 isolates of *Alternaria brassicae,* collected from geographically distinct regions of the world and different host species with RAPD markers have also been analyzed and identified.

Molecular Mapping of Disease Resistance Genes

The process of locating genes of interest via a linkage to markers is refereed as gene tagging. The tagging of disease-resistant genes with molecular markers involves the evaluation of classical phenotype of resistance and molecular marker genotype on the same individuals and the data is analyzed to determine if any of the markers co-segregate with the target phenotype (resistance phenotype). The basic requirement of tagging plant disease-resistant genes with molecular markers is the development of a mapping population.

NILs (Near Isogenic Lines) differening for single gene for resistance are good material for use in R gene mapping experiments. NILs are developed by crossing resistant individuals with susceptible plants and generally back-crossed for 6 to 7 generations with an objective to get homozygous resistant lines, which differ in only one resistant gene. In those cases where NILs are not available, BSA (Bulk Segregant Analysis) method is used. Here, polymorphism is conducted in DNA bulks made from resistant and susceptible F2 plants. Sharma (2003) has reported that molecular tagging and cloning of blast resistance genes and resistant gene analogous from rice line *Tetep,* which possesses durable resistance to the *Magnaporthe grisea* population of India, has been developed. Five blast resistant gene analogues (RGAs) have been isolated, cloned, and sequenced from rice blast-resistant lines, *Tetep*.

Gene Pyramiding

Gene pyramiding is the technique of combining more than one gene for resistance, in a common genetic background, by repeated back-crossing and the selection with the virulent races of the pathogen. The technique is highly effective in getting durable resistance to the target pathogen. Gene pyramiding is being used in many host–pathogen systems. Hittalmani *et al*. (2000) pyramided three rice blast resistance genes by using MAS (Marker assisted selection).

There are three main benefits to agriculture and crop improvement programs with the use of biotechnology. The first of these is the reduction of the duration of breeding programs, with the use of tissue culture. Plant tissue culture also allows creation of new genetic variations from which useful new varieties can be obtained. New methods of hybridization can be combined with cell and tissue culture for selection of plants with desired traits. The last and most promising benefit is from the use of recombinant-DNA technology. Transgenic plants, engineered for resistance to disease, pests and herbicides and for better nutritional quality, have already been produced in the laboratory (Chopra and Sharma, 1991).

The panel of techniques for rapid, specific and sensitive detection of plant pathogenic viruses and bacteria has improved and increased during the last few years. The future will bring more novel tests for detecting plant pathogens and novel demands will be introduced. However, only some of these will be accepted by plant pathologists, with not only the analytical quality of the test results but also their sensitivity and specificity contributing to selection of the best cost effective diagnostic strategies. These new detection tools will be used not only for rapid, sensitive and specific diagnosis but also in understanding host–pathogen relationships and the structure and function of pathogens and their communities.

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Questions

1. What is biotechnology? Give at least two examples where this technology has helped in the production of resistant genotypes.
2. Define the followings terms:
   (i) r-DNA
   (ii) Hybridoma
   (iii) Transgenic plants
   (iv) Monoclonal antibodies
   (v) Somaclonal variation
3. Why does apical or meristem tip culture generally free the plantlets from viruses or other pathogens?

4. List the main contributions of the following:
   (i) P. R. White
   (ii) G. Morel
5. Is biotechnology the alternative to traditional resistant plant breeding?
6. Give a brief account of innovative tools for detection and diagnosis of bacteria and viruses.
7. Define the following terms:
   (i) ELISA
   (ii) FISH
   (iii) PCR
   (iv) DNA microarray
   (v) Gene Pyramiding

Phytopathological Techniques in Plant Pathology

30

Isolation of Pathogens

Most of the plant diseases can be diagnosed by observation with the naked eye or with the microscope, in which case isolation of the pathogen is not necessary. But if the pathogen is mixed with one or more contaminants and has not produced spores or fruiting bodies or if the disease is caused by a new, unknown pathogen, then the isolation of the pathogen is necessary.

Requirements for Isolation of Pathogens

To isolate the microorganism from the diseased plant tissue, the following things are required:

Common Culture Media Used in Laboratories

There are a large number of natural, semi-synthetic and synthetic media that can be used to grow plant pathogenic microorganisms.

Natural Media A natural medium is one, which is composed entirely of complex natural materials of unknown composition. Among natural substances are plant parts, malt, yeast, peptone, fruits and vegetables. The following are few natural media used in isolation of fungi.

(i) Cooked Vegetable Agar Broth or a decoction of the desired vegetative plant parts can be prepared by placing 10-20% of the tissue in water, steaming for 30 minutes and then mashing and squeezing the contents through muslin cloth. To this broth, the required quantity of agar-agar (2%) may be added. It may be required to adjust the pH of the medium to desired level, before autoclaving.

(ii) Lima Bean Agar Used to cultivate *Phytophthora* spp. Blend 50 g lima beans, previously soaked in water for 10 h with 500 ml of water, containing 15 g of melted agar. Adjust the final volume to 1 L and then autoclave.

(iii) Oatmeal Agar Used for general cultivation of fungi. Blend, in a commercial blender, 50 g of rolled oats in 600 ml of water and then heat to 45-55^{o}C, add 12 g agar dissolved in 400 ml of water. Autoclave it for 90 minutes.

Semi-Synthetic Media Semi-synthetic medium is made up of natural substances of unknown composition, and by adding some chemical compounds of known composition.

(i) Potato–Dextrose Agar (PDA) Numerous variations occur in the quantity of potato and dextrose; the standard preparation is to boil 200 g of peeled and sliced potatoes in 1 L of water until the potatoes are soft. Strain through a cheesecloth and adjust the filtrate to 1 L. Add 20 g dextrose and 17 g agar. Autoclave it.

(ii) Malt Extract Peptone–Dextrose Agar Used to cultivate fungi and bacteria. Malt extract = 20 g; Dextrose = 20 g; Peptone = 1 g; Agar-agar = 20 g and Distilled water = 1000 ml.

(iii) Martin Rose Bengal Streptomycin Agar Dextrose = 10 g; Peptone = 5 g; KH_2PO_4 = 1 g; $MgSO_4$ = 0.5 g; Rose Bengal - trace; Streptomycin = 0.03 g; Agar-agar = 20 g; Distilled water =1000 ml. Dissolve 1 g of streptomycin sulphate in 100 ml sterile distilled water, after opening the vial aseptically, add 0.3 ml of streptomycin in solution to each 100 ml of the basal medium after it is cooled.

(iv) Soil-Extract Agar Used to isolate and enumerate bacteria from soil. To 1 L of soil extract, add 0.2 g K_2HPO_4 and 15 g agar. Adjust pH to 6.8 and autoclave. To prepare soil extract, autoclave 500 g of fertile soil in an amount of water that will yield 1 litre of extract. After cooling, filter through filter paper.

(v) Yeast Extract Glucose =10 g; Peptone = 1 g; Yeast extract = 3 g; Distilled water = 1000 ml.

Synthetic Media A synthetic medium is one of known composition and concentration. Compounds that are used should be 'chemically pure'.

(i) Brown's Medium Glucose = 2 g; K_2HPO_4 = 1.25 g; Asparagine = 2 g; $MgSO_4$. 7 H_2O = 0.75 g; Agar-agar = 20 g; Distilled water = 1000 ml;

(ii) Czapek-Dox Agar Used to cultivate many fungi. $NaNO_3$ = 2 g; K_2HPO_4 = 1 g; $MgSO_4$. 7 H_2O = 0.5 g; KCl = 0.5 g; $FeSO_4$ = 0.01 g; Sucrose = 30 g; Agar-agar = 20 g; Distilled water = 1000 ml.

If glass distilled water is used, add 1 ml of 1% $ZnSO_4$ and 0.5% $CuSO_4$. Heat the full chemical solution with sucrose in a water bath for 15 minutes. After cooling, add sucrose and agar-agar, melt the agar and autoclave. Do not filter.

(iii) Galactose-Nitrate Agar Used to isolate *Fusarium oxysporum* from soil. Galactose = 10 g, $NaNO_3$ = 2 g; KH_2PO_4 = 1 g; $MgSO_4$. $7H_2O$ = 0.5 g; $K_2S_2O_5$ = 0.3 g; Agar- agar = 15 g; Distilled water-1 litre. Autoclave for 20 minutes.

Special Media for Cultivation of Pathogens

Media for Actinomycetes

(i) Basal Mineral Salts Agar Glucose = 10 g; $(NH_4)_2SO_4$ = 2.64 g; KH_2PO_4 anhydrous = 2.38 g; $K_2HPO_3.3H_2O$ = 5.65 g; $MgSO_4.7H_2O$ = 1 g; Distilled water = 1000 ml; Agar-agar = 15 g.

Adjust pH to 6.8-7.0 with 1N NaOH or 1N HCl. Used for culture of *Streptomyces* with addition of sterile carbon source at a concentration of about 1%.

(ii) Oatmeal Agar Oatmeal = 20 g; Agar-agar = 18 g; Distilled water = 1000 ml; Steam oatmeal in a litre of water for 20 minutes, filter through cheesecloth, add agar and steam for 15-20 minutes. Add trace salts ($FeSO_4.7H_2O$ = 0.1 g; $MnCl_2.4H_2O$ = 0.1 g; $ZnSO_4.7H_2O$ = 0.1 g. Dissolve these in 100 ml and out of this solution, add one ml to oatmeal agar solution), and bring to a volume to 1 L. Adjust to pH 7.2 with NaOH.

Media for Bacteria

(i) Asparagine Lactose Medium This medium is used to isolate *Rhizobium* sp.

Lactose = 10 g; Asparagine = 0.5 g; Sodium faunocholate = 5 g; K_2HPO_4 = 1 g; $MgSO_4$ = 0.2 g; Neutral red = 3.5 ml (2/40 ml ethyl alcohol); Agar-agar = 20 g; Distilled water = 1000 ml; pH = 7.5.

This medium is suitable for the growth of all the Rhizobial species and especially for isolating *Rhizobium* from the nodules of groundnut. This medium can be used for distinguishing the *Agrobacterium* and *Rhizobium. A. tumefaciens* shows quick growth within 24 hours with pink color colony on Congo red (10 ml of 1/4000 aqueous solution) incorporated in the medium, while *Rhizobium* strains grows late, after 72 hours showing no absorption of Congo red.

(ii) Ashby's Mannitol Agar Medium Used to cultivate *Azotobacter* sp.

Potassium acid phosphate = 1 g; $MgSO_4$ = 0.2 g; Ammonium sulphate = 0.2 g; NaCl = 0.2 g; Mannitol = 10 g; Calcium sulphate = 0.1 g; Calcium carbonate = 5 g; Agar-agar = 20 g; Distilled Water = 1000 ml.

Dissolve the phosphate in 500 ml and make alkaline to phenolphthalein. Melt agar and other ingredients in the remaining 500 ml, mix both the lots and sterilize for 20 minutes at 15 lbs.

Media for Fungi

(i) V-8 Juice Agar Used to support growth and induce sporulation of many fungi. It can be used instead of PDA. V-8 juice = 200 ml; $CaCO_3$ = 3 g; Agar-agar = 15-20 g; Distilled water = 1000 ml; pH = 7-7.5, which can be varied by changing the amount of juice or $CaCO_3$.

(ii) Cassava-Dextrose Agar Used in place of PDA for routine work of fungi. Peel cassava (*Manihot utilissima*) tubers and cut into chips. Dry overnight at 55°C and grid to 30 mesh. Soak 135 g of the powder in 500 ml of water for 15 minutes at 60°C. Filter through cheesecloth. Add 20 g glucose and 12 g agar-agar. Complete the volume to 1 L and autoclave.

Apart from the above-mentioned media for fungi, there are some basic points which are to be kept in mind while preparing media.

(a) Fungi usually grow best in a rich carbohydrate medium.
(b) Fungi usually prefer a slightly acid reaction, pH 6-6.5.
(c) Agar is slow to dissolve thoroughly. It is advisable to dissolve the agar in half the water and nutrients in the other half and then mix. Agar does not solidify satisfactorily in very acid or alkaline solutions.
(d) Peptone may generally be omitted from fungus culture.
(e) Tap water is often preferable to distilled water as it contains useful trace elements.

Selective Media for the Isolation of Plant Pathogens

There are several selective media for the isolation of plant pathogenic fungi. For example, P10VP medium of Ocana and Tsao (1969) are very useful in isolating *Phytophthora* species from plant tissues and soil. The medium contains Difco cornmeal agar-agar (17-20 g/L), which is autoclaved (cooled to 45°C) and amended with low dosage of Pimaricin (10 ppm), Vancomycin (200 ppm) and Pentachloronitrobenzene (100 ppm). Plates are stored in the dark because of sensitivity of Pimaricin to light. After usual sterilization procedure of the diseased tissues, the tissues are plated on this selective medium. Infested soil or suspected soil containing the *Phytophthora* propagules or mycelium can also be plated on this medium to isolate the pathogen. Hymexazole can be added after autoclaving to suppress species of *Pythium.* There are several other selective media for the isolation of *Phytophthora* species.

Nash and Snyder's PCNB medium (Nash and Snyder, 1962) It is a selective medium for the isolation of *Fusarium* species from field soils. In the same way, there are several selective media used for the isolation of plant pathogenic bacteria and other pathogens. *Plant Pathological Methods-Fungi and Bacteria* by Tuite (1969); *Plant Pathologist Pocket Book,* published by Commonwealth Mycological Institute, England (1968); *Basic Plant Pathology Methods* by Dhingra and Sinclair (1968); and *Laboratory Exercises in Plant Pathology,* edited by Boudoin *et al.* (1988) and published by the American Phytopathological Society are some of the books that can be referred on this topic.

Sterilization

Sterilization is achieved by exposing materials to lethal agents, which may be chemical, physical, or ionic in nature or in the case of liquids, physical elimination of cells from the medium. Selection of a method depends upon the desired efficiency, its applicability, toxicity, case of use, availability and cost, and effect on the properties of the object to be sterilized.

Red Heat Inoculating needles etc. can be sterilized by heating to redness.

Hot Air (Oven) Glassware, metal instruments resistant to high temperature can be sterilized in a hot air oven. Sterilization should be complete after the time and temperature shown.

Temp (°C)	Time (hours)
120	8 h
140	3 h
160	1 h
180	20 min

Steam Under Pressure (Autoclaving) Most media can be sterilized by heating at 10-15 psi for 15 minutes in an autoclave or domestic pressure cooker.

Pressure (psi)	Temperature (°C)	Time (hours/min)
5	107	
7	110	2.5 h
10	115	51 min
15	121	15 min
20	126	6.4 min

Gas Sterilization It is used on objects that cannot be sterilized by heat or liquid filtration. Advantages are that the process can be carried out at low temperature and low relative humidity. The process can be carried out using simple equipment such as rubber or plastic bags and metal or plastic drums. Major disadvantages are: longer time is required for sterilization over that of heat; materials used are flammable and highly toxic and the cost is higher than heat. Some gases used are ethylene oxide, propylene oxide, formaldehyde, methyl bromide, β-propiolactone and ozone. The first three are commonly used as alkylating agents.

Filtration Filtration physically separates microorganisms, cells and debris from liquids but not viruses or metabolic byproducts. Aqueous solutions, oils and organic solutions can be sterilized by

filtration. Filters used are sintered glass, asbestos pads, unglazed porcelain, diatomaceous earth discs or candles, and cellulose ester membrane. Microorganisms and other large particles are retained on the filter in part by the small size of the pores and dry adsorption onto pore walls.

Surface Sterilization Some of the liquids used in removing microorganisms from bench tops, instruments and the surface of plants are given below.

	Chemical	Concentration	Uses
1.	Ethanol	70-95%	Disinfection of instruments and bench tops.
2.	Mercuric Chloride	0.1%	Disinfection of the plants surface, Surface for isolation.
3.	Sodium hypochlorite	1%	Isolation of microorganisms.
4.	Formaldehyde alcohol	8% formaldehyde in 70% isopropyl alcohol	Sterilizing of instruments
5.	Formaldehyde	1 part of formaldehyde to 10 parts of water	Elimination of plant pathogens from green house tools
6.	Propylene oxide	1 ml in 1 L	Filter papers are sterilized
7.	Ultra violet light 210 nm to 300 nm	15-30 min	Surface sterilization

Isolation Techniques

From Soil Here, the method involves the transfer of microbial propagules from soil to the culture medium with the aim of obtaining a pure culture. Because of the diversity of growth requirement of soil microbes, various procedures are developed. Therefore, any isolation technique is relative and can be adopted on the basis of its applicability.

(i) The Soil Dilution Plate Method This is the classical and most widely used method in soil microbiology and has been adopted from the method originally developed for the general isolation of soil bacteria (Waksman, 1927). The main modifications are the use of lower dilution on account of lower number of soil fungi, and development of different isolation media adjusted to lower pH (4.5) for supression of bacteria.

There are many variations of this method. In its simplest form, place 10 g soil (own dry) in a sterile flask with 90 ml sterile water and stir with a magnetic stirrer for 20 to 30 min. From this solution, withdraw 10 ml soil water and add to 90 ml of sterile water in a sterile bottle. Shake for 1 min and transfer 10 ml of the suspension to another 90 ml sterile water. Repeat the process until the desired dilution is obtained. A proper dilution allows 50 to 150 colonies per culture plate. In general, suitable dilutions are 10^{-3} to 10^{-4} for actinomycetes, 10^{-5} to 10^{-6} for bacteria and 10^{-4} to 10^{-5} for fungi. Spread 1 ml of a dilution on a suitable agar surface by an inclined rotary motion of the plate.

(ii) Soil Plate Method A less complicated method, which tends to yield more kinds of fungi than those obtained from the dilution plate technique. Suitable small amounts of soil (0.005-0.15 g) are taken from the mean soil sample, using a sterile needle with a flattened tip. Each small sub-sample is placed in a drop of sterile water in a Petri dish and dispersed. 8-10 ml of molten agar medium is added to each Petri dish, the dish being rotated gently to allow dispersion of the soil particles in the nutrient medium (Warcup, 1950).

(iii) The Direct Inoculation and Soil Desiccation Method Lumps of soil, about 1 cm in diameter are transferred onto the sterile plates of Czapek's solution agar. After incubation for 24 hours, hyphal tips are removed on a slate. The organisms thus isolated are believed to come from the mycelium that is actually found in soil (Waksman, 1961). Warcup (1960), however, observed that while mucorales were isolated from soil lumps, direct microscopic examination of lumps failed to reveal lower fungi hyphae thus suggesting that mycelia developing on the agar plate are derived from the spores.

(iv) The Immersion-tube Method This method is described by Chester (1948). This consists of introduction of glass tube, with 4-6 spirally arranged invaginated capillaries filled with agar into soil. After 7-14 days, the tube is removed and fungi are isolated by removing the soil core and then cutting it into portions, which are plated out. The limitation of this method is that it easily isolates only active spreading mycelium or active localized mycelium, which happens to come in contact with capillaries.

(v) Screened Immersion Plate Thornton (1952) devised screened immersion plate method, which permits isolation of microorganisms capable of growing from the soil on water agar, placed behind a perforated screen.

A glass slide that fits closely into a shallow perspex box carries the medium. A 1 mm thick plastic sheet covers the slide and box without touching the slide. Two rows of five 5 mm diameter holes each are made in the corner. All components are sterilized. After sterilization, the slide in the box is placed in a large culture plate and 8 ml of water agar is poured over it. After solidification, it is covered with the perforated plastic sheet. After a suitable period of burial in the soil, the plates are removed, examined and fungal growth is transferred to PDA.

(vi) Isolation by Baiting A variety of microorganisms may be isolated from soil by the use of some preferential substrate. The desired organism is able to develop on the bait to the exclusion of other organisms, thus allowing isolation by ordinary method. *Agrobacterium tumefaciens* and *Thielaviopsis basicola* are isolated from soil by the use of carrot discs. *Rhizoctonia* with cellophane, a number of water molds are isolated by the addition of boiled hemp seeds to pond water or soil water mixtures. The insertion of diseased materials or soil inside the bait—apple is used to isolate many but not all species of *Phytophthora.* Planting susceptible plants in infested soil is often considered a form of baiting.

From Diseased Plant Tissues Tissues sampled during the active stage of an infection are likely to have only the pathogen responsible for the infection within them; the surfaces of such tissues however, are usually contaminated with saprophytic organisms. Thus, surface disinfestation of tissues selected for isolation work is a prerequisite to successful isolation. Washing diseased tissues with soap or detergent and then rinsing them for 1-2 hours under running tap water can remove many surface contaminants. Tender tissues such as seedling stems containing damping-off fungi, are cut into small pieces and planted directly on acidified PDA, immediately after they have been rinsed under running tap water.

Additional surface disinfestation of less tender tissues is usually accomplished with 0.5-1.0% calcium hypochlorite (10-20% commercial bleach) or with a 0.1% aqueous solution of mercuric chloride. Tissues treated with bleach can be planted directly on PDA and those treated with mercuric chloride, however, must be rinsed in sterilized water before they are planted.

To isolate plant-pathogenic fungi by the tissue-plating method, the following procedure is recommended:

Pieces of root, stem, leaf and other diseased tissue showing distinct symptoms are washed and placed in a beaker, which is then covered with a cheesecloth and left under running water for 1 or 2 hours. Squares of tissues, approximately 0.5 cm across, are cut from the advancing margins of lesions and

placed in 0.5-1.0% calcium hypochlorite; after 3 to 5 minutes, the small pieces of tissues are lifted with alcohol-flamed forceps onto hardened agar medium in Petri dishes. Later, the predominating organisms can be transferred to fresh agar slants for growth in pure culture.

When sclerotia are associated with diseased tissue, they can be surface-sterilized, cut in half and planted on solidified PDA.

Bacteria and fungi that sporulate in or on diseased tissues can be isolated by a dilution method. Bits of diseased tissues are chopped with a flamed scalpel in a few drops of sterilized water. After a few minutes, a loopfull of the water containing the bacteria or fungal spores are transferred to a drop of sterilized water in a Petri dish. Then serial dilutions are made to several other dishes. Melted agar medium at 37°C is then poured into the dishes. After a day, colonies of bacteria or fungi are visible, and single colonies can be transferred to an appropriate medium in tubes or in other Petri dishes.

Inoculation Techniques

Soil Infestation Bits of mycelium are often used as inoculum when the infection material is a root or seedling stem. The mycelium from liquid or solid media can be chopped in water in a commercial blender.

To test the pathogenicity of damping-off, root rotting, or wilt-inducing fungi, a small square of PDA infested with the appropriate mycelium can be buried in soil near the center of a clay pot and seeds of the suscept can be planted around the center. Within about a week, damping-off fungi will have induced diseases; more time is required by the other pathogens. A better method is to grow the pathogen on sand maize meal or sand oatmeal medium. The constituents of the medium are: washed sand = 100 g; oatmeal or maize meal = 3 g; water 20-25 cc. The sand is taken in a 250 cc conical flask, oatmeal or maize meal is sprinkled on the surface of sand in the flask and sufficient water is added to wet the sand and oatmeal. The flasks are corked with non-absorbent cotton and sterilized at 15 lb psi for 15-20 minutes. The pathogen is inoculated in these flasks and allowed to grow for 2-3 weeks and then added to soil in pots at the rate of 10% to the soil (w/w). In zoospore-forming fungi or those, which produce conidia abundantly, zoosporic suspension can be prepared of the pathogen and similarly conidial suspension can be prepared. The healthy seedlings can be inoculated by dipping the root system in zoosporic or conidial suspension for varying periods of time and planted in healthy soil.

Root-invading pathogens (including bacteria, fungi) can be mixed with potting soil into which seeds are transplanted and placed. Suspensions of bacterial cells or fungal spores may be used. Fungal mycelium may be chopped in water in a blending machine and added to the soil; or the fungus may be grown on sterilized cereal grains, which should then be added to soil in which the suspect is to be planted.

Inoculation of Leaves and Stems with Fungi or Bacteria Leaves and stems of susceptible plants can be inoculated with suspensions of fungal spores or bacterial cells in the following manner. Culture dishes or tubes are flooded with sterilized water, to which a drop of wetting agent has been added, and the spores or bacteria are gently scraped off the substrate into the water. The suspension is then placed in an atomizer, from which it is sprayed on the susceptible plants. When the fungus is inoculated, the sprayed plants should be kept in a moist atmosphere for 12-48 hours. With leaf-attaching bacteria, however, best results are usually experienced when the plants are inoculated in the greenhouse, preferably between 9:00 AM to 3:00 PM, when their stomata are most likely to be open. It is preferable to inoculate the plants in the afternoon.

When dry spores, such as those of cereal rusts, can be collected in mass, dry inoculum can be prepared by mixing one part of the spores with 3-4 parts of talc power. After plants to be inoculated have been sprayed with water containing a little wetting agent, the spore-talc mixture is dusted onto the plants. Inoculated plants should be kept in a moist chamber for 12-24 hours.

Another method of inoculating pathogen is to cut agar discs with the help of sterilized cork borer and transferring them to healthy leaves in injured (needle pricked) or healthy plants under moist conditions, covered with glass cages.

Verma and Singh (1982) have described several techniques of inoculation of bacterial pathogens. These are spray inoculations, smear/swab inoculation, leaf infiltration inoculation (The bacterial suspension is injected into the leaf tissue with the help of a hypodermic syringe), pin prick/ needle inoculation, root dip inoculation and some special techniques. The special techniques include: (a) clipper with bacterial suspension mounted on it in a plastic bottle allowing continuous release of phytopathogenic bacteria; (b) use of insect vectors in a special cage; and (c) immersion of whole seedling in bacterial suspension before transplanting.

Maintenance and Preservation of Microbial Cultures

After successful isolation and growth of microorganisms, it is important to maintain the viable cultures free from contamination for a long time period. With the passage of time, many important cultures are lost due to negligence or some other reason and thus, it became important to maintain and preserve so that they could serve as repositories for microbial cultures. These cultures are used for teaching, research and for industrial purposes.

There are many methods of maintaining microbial cultures, as all microbial cultures do not respond in a similar manner to a given method. Some of the commonly used methods are described in this section.

Substratum

Fungal culture can be maintained on a sterile medium, which can be in liquid or solid form containing agar-agar. Maintenance on agar medium is more satisfactory. There are a large numbers of media known but a few are more suitable for a particular fungus or bacterium than other and one has to select a medium according to the known requirements for a particular fungus or bacterium.

A synthetic medium is generally employed for biochemical or physiological studies but for the maintenance of a culture, a natural medium is more appropriate. Vegetable/ fruit decoctions are helpful for plant pathogenic and some protein in the medium for animal and human pathogens.

A few parasitic fungi appear to be obligate parasites and will only grow in tissue cultures or in their host. If the host is a microfungus as in the case of *Piptocephalis*, it is possible to grow one fungus on the other. *Dictyostelium* requires bacteria and these can be grown in mixed cultures. PDA (Potato-Dextrose Agar) and Malt Agar are rich and contain most important nutrients for the growth of fungus cultures. Other substrates such as soil, parts of plant tissues, cereals, wheat straw etc., are also used for growing microbes.

Factors Affecting Microbial Growth

Light Most of the cultures respond well to normal daylight but some sporulate better in dark.

Temperature Majority of fungi grow well at temperatures in the range of 22-25°C while a few like Chytrids prefer below 20ºC and thermophiles above 45°C.

Methods of Transfer

Mass spore transfer, wherever possible, remains the most reliable and effective technique. When spores are not available, mycelium from the growing edge of culture colony can be used. As far as possible, single spore cultures should be raised. Cultures are generally maintained in sterilized glass test tubes having plugs. If plastic tubes are available they have the advantage, as they are unlikely to break if dropped. Culture tubes can be labeled with glass marking pencil or permanent markers. However, stickers can carry more information and are of more permanent nature.

Storage

Periodical Transfer to Fresh Media or Sub-culturing or Micro-gardening The most traditional and simplest method of maintaining is by periodically preparing fresh culture from the previous stock culture. The culture medium, the storage temperature and the time interval at which the transfer are made vary with the species and must be ascertained beforehand. The temperature and the type of medium selected should support a slow growth so that the time interval between transfers can be as long as possible.

This method has following advantages:

- It is cheap and requires no specialized equipment.
- Culture can be maintained for a long time.
- Retrieval is very easy.

This method is not recommended as a long-term preservation method because of the following disadvantages:

- Mutations (Genetic changes) are likely to seen when culture are frequently transferred. There is danger in loss of morphological, physiological, or pathogenicity characters.
- It is time-consuming and laborious for maintaining large number of pure cultures for a long time period.
- There are chances of contamination by air-borne spores or mite-carried infections.

Refrigeration or Cold Storage Microbes can be preserved for a short period of time in the refrigerator or in the freezing chamber of refrigerator at –4°C.

Some of the common fungi survive well at this temperature. A domestic type refrigerator is quite satisfactory. Under refrigeration conditions, the rate of growth and drying out of cultures are reduced, mites become immobile and interval between transfers can be extended up to six months. For slants, it is recommended to use screw capped test tubes and for cultures on Petri plates, the plates should be sealed with paraffin wax. Sealing the plate not only helps to prevent molds from entering into the plates, but it also slows the drying of agar-agar.

Culture tubes or Petri plates should not be tightly packed in the refrigerators. As condensation may occur on the outside of cooled tubes or plates due to refrigeration, it will lead to the growth of unwanted fungus on the surface of tubes/plates. Such fungi may finally penetrate the culture through the space

between the lids or through cotton plugs and contaminate them. Packing the culture tightly and opening the refrigerator less frequently could prevent this. In general, ordinary freezing is not recommended for preservation for a long time because the freezing processes damage the microbial cells.

Deep Freeze It is a good method to store bacteria and most of the fungi. Generally, the colder the storage temperature, longer the culture will remain viable. Deep-freezing at -20ºC is superior to ordinary freezing. However, ice crystal formation is the major problem when bacteria stored at low temperatures. Ice crystals can damage cells by dehydration caused due to localized increase in salt concentration. As water is converted into ice, solutes accumulate in the residual free water and thus high concentration of solutes can denature biomolecules. To protect from the negative effects of freezing, a cryoprotective agent such as glycerol or Dimethyl-sulfoxide (DMSO) is often used. Cryoprotectants are of two types: penetrating agents such as DMSO and Glycerol, which pass through the cell membrane and protect intracellularly and extracellullary; and non-penetrating agents such as Sucrose, Dextrose, Lactose, Sorbitol, Mannitol, which exert their protective effect external to cell membrane.

Mineral Oil or Liquid Paraffin Storage Well-grown and sporulating cultures can survive up to 10 years if preserved in mineral oil. Lumiere and Chevrotier originally described this technique in 1914 to keep the Gonorrhea agent *(Neisseria gonorrhoeae*). In this procedure, the established growth of the culture as a colony or as a slant is layered with sterile, neutral, white paraffin oil to a depth of about 1 cm above the top of agar. The aim is to limit the oxygen supply that reduces the microorganism metabolism and growth, as well as to restrict the cell drying during preservation. Retrieval of culture is done by draining of as much oil as possible and streaking the inoculum into agar plates or slants. It may be necessary to sub-culture the colony several times to get a vigorous oil-free culture. The oil cultures can be stored at room temperature or in a refrigerator. This method is cheap and easy. A wide variety of fungi can be preserved in this way and mites don't penetrate the cultures. However, oil is messy to handle, liable to contamination and tubes and bottles must be stored in upright position.

Soil Storage Many fungi can be preserved well in soil. In this method, spore suspension in sterile water is added to sterile soil and allowed to grow at 25ºC for about 10 days. After proper growth of the microorganism has taken place, the soil cultures are then stored in a refrigerator. This has proved useful for the preservation of *Fusarium* sp.

Water Storage It is an inexpensive, simple and low maintenance method for storing members of Oomycota, Ascomycota, Basidiomycota, Hyphomycetes, human pathogens, water molds and yeasts. In this method, 6 mm diameter of agar discs are cut from a fungal colony and placed in sterile distilled water and stored at 15ºC. Revival is done by removal of agar disc and placing the mycelium on a suitable medium. The cultures can be stored for 2-3 years.

Cryopreservation Storage in liquid nitrogen is an effective way of long-term preservation and is considered superior to other methods. Cryopreservation refers to the storage of a living organism at ultra low temperature (–196ºC). During cryopreservation, dehydration of cells occurs and osmotic imbalance is created due to the changes in the concentration of salts and other metabolites. In this method, actively growing cells are harvested and suspended in cryoprotective agents i.e. DMSO and Glycerol. The cell suspension is sealed into small vials and then frozen at –150ºC and these vials are then stored in liquid nitrogen by immersion (–196ºC). Most of the microbes can remain viable under these conditions for up to 30 years.

For revival of cultures, the frozen vials are removed from the liquid nitrogen. For partial thawing, these are immediately immersed in water bath at 37ºC. The thawed contents of the vials are immediately transferred to fresh growth medium to dilute the cryoprotectant, which otherwise is lethal at higher temperature. The DMSO, which is toxic, is diluted 100-200 times in the culture medium to a non-inhibitory concentration. The inoculated growth medium is incubated under appropriate growth conditions.

As mentioned earlier, cryopreservation in liquid nitrogen seems to be the most reliable, safe and perspective method of long-term maintenance of most fungal species, especially those not amenable to freeze-drying. It probably ensures genomic, phenotypic stability, time saving, and preservation of culture loss from contamination and increased assurance of long-term availability of cultures.

Lyophilization (Freeze-Drying) It is a well-established and effective method for long-term storage of bacterial species, molds and bacteriophages and has remained viable for over 40 years. In this method, the culture is frozen at a very low temperature and placed under a high vacuum. Under these conditions, the water in the microbial cells goes directly from the frozen state to the gaseous state, thereby, drying the cells without disrupting them. This method is laborious and requires specialized apparatus but it has the advantage of generating cultures that are unaffected by power failure and empty liquid nitrogen tanks.

Lyophilization or freeze-drying is a process in which water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying). Freeze drying is a process of drying in which water is sublimed from the product after it is frozen.

The main principle involved in freeze drying is a phenomenon called sublimation, where water passes directly from solid state (ice) to the vapour state without passing through the liquid state. The material to be dried is first frozen and then subjected under a high vacuum to heat (by conduction or radiation or by both) so that the frozen liquid sublimes leaving only solid, dried components of the original liquid.

The fundamental process steps are:

(i) Freezing The product is frozen. This provides a necessary condition for low temperature drying.

(ii) Vacuum After freezing, the product is placed under vacuum. This enables the frozen solvent in the product to vaporize without passing through the liquid phase, a process known as sublimation.

(iii) Heat Heat is applied to frozen product to accelerate sublimation.

(iv) Condensation Low temperature condenser plates remove the vaporized solvent from the vacuum chamber by converting it back to a solid. This completes the separation process.

Advantages of Lyophilization include:

- Chemical decomposition is minimized.
- Removal of water without excessive heating.
- Enhanced product stability in a dry state.
- Ease of processing a liquid simplifies aseptic handling.
- More compatible with sterile operations than dry powder filling.

Disadvantages of Lyophilization include:

- Increased handling and processing time.
- Volatile compounds may be removed by vacuum.

Plant Disease Assessment Methods

Epidemiology is a quantitative science and hence calls for quantifying plant disease growth. The need for quantification hardly needs any emphasis. According to Tarr (1972), the objectives of plant disease appraisal are (1) to measure the amount of disease present in terms of its prevalence (percentage diseased plant or organs) and severity of the individual plants, (2) to relate this to loss of crop, and (3) to evaluate this crop loss in financial terms and to assess its effects of the economy on the farm or plantation. This is largely a problem for agricultural economists but is very relevant to plant pathologists.

Plant disease assessment is possible in the following three ways:

Visual Method in the Field

This is the most direct method, which is generally used in experiments. Direct methods measure diseases as incidence or severity. The term disease intensity is often used to denote either incidence or severity.

$$\text{Disease incidence (I)} = \frac{\text{Number of infected plant unit}}{\text{Total number (healthy and infected of units assessed)}} \times 100$$

$$\text{Disease Severity(s) (Area)} = \frac{\text{Area of plant tissue affected}}{\text{Total area}}$$

Recording of percentage of diseased plants, organs and tissues is particularly applicable to diseases which kill plants rather quickly or which cause plant diseases about the same amount of damage to all the infected plants such as damping off diseases and root rots, many viral diseases or when the diseased plants or parts thereof result in total loss, for example, cereal bunts or smuts.

Descriptive Keys or Scales for Estimating Disease Intensity The simplest type of descriptive scale is formulated by dividing the disease into three different categories: light, moderate, and severe. A descriptive scale may be inadequate if the description of any grade is not realistic, easily recognizable and usable in practice. The UDSA evolved a descriptive scale, which may be examined of its merits and demerits.

0 = No infection
1 = Very slight, 1 or 2 diseased specimens in an acre of field plot
2 = Slight, 8-10 diseased specimens in an acre
3 = Considerable, 30-40 diseased specimen in an acre
4 = Abundant, 25-50% in the field diseased
5 = Very abundant, more than 50% plants diseased

In the above, 1 to 3 grades are distinguishable. The gap between grades 3 and 4 is enormous. The grades developed by the British Mycological Society (1947) are given below:

0 = Not seen in the field
1.1 = A few spots (1 or 2 spots) in 10.8 m radius. Few plants are affected here and there
1% = Up to 10 spots per plant, or general light spotting
5% = About 50 spots per plant, or up to one leaflet in 10 attacked.
25% = Nearly every plant with lesions, plants still remain in normal form. Field may smell of blight but look green although every plant is infected.

50% = Every plant affected and about half of the leaf area destroyed by blight. Field looks green, flecked with brown.

75% = About three-quarters of the leaf area destroyed by blight. Fields look neither predominantly brown nor green.

95% = Only a few leaves left green, but stems are green

100% = All leaves dead, stem dead or dying

Grades of diseased incidence can be assessed by comparison with standardized diagrams, photographs or even preserved specimens. These are often used for leaf spotting fungi, blights, rusts, mosaics, fruit spotting, and other diseases. One of the earliest assessment diagrams was that of Cobb for leaf rust (*Puccinia triticina* now *Puccinia striiformis*) in Australia. A number of disease intensity scales for the measurements of cereal rusts have been developed and used by workers all over the world such as Melchers and Parker (1922), and Rusakov (1926) and James (1971). These scales are primarily percentage scales of disease incidence and disease severity and are superior to most of other methods. The advantages of these percentage scales are: (1) the upper and lower limits are well defined; and (2) the scale is flexible and can be divided and sub divided conveniently.

However, these scales are not without drawbacks. For example, in the modified Cobb's scale developed by Melchers and Parker (1922), the maximum rust incidence is indicated as 100% but the actual total area occupied by the pustules is only 37%. To overcome this difficulty, James (1971) developed a series of standard area diagrams where the percentage of the disease infection recorded always represents the area occupied by leaf rust pustules.

Disease assessment keys are subjective to some extent because of visual observation. Horsfall and Barratt (1955) pointed out that grades detected by human eye are approximate. Equal divisions on a logs scale generally follow Weber-Fechner law, which states that visual acuity (power of observation) depends on the logarithm of the intensity of stimulus.

They described the following twelve grades 1 = 0%, 2 = 0-3%, 3 = 3-6%, 4 = 6-12%, 5 = 12-25%, 6 = 25-50%, 7 = 50-75%, 8 = 75-87%, 9 = 87-94%, 10 = 94-97%, 11 = 97-100% and 12 = 100%. From the standpoint of epidemiology and disease appraisal, logarithm scale is satisfactory because most pathogens multiply at a logarithmic rate while time advances linearly.

Models for Estimating Yield Losses

Mathematical models for estimation of the relationship between disease yield loss comprise of two kinds: (1) Critical point models and (2) Multiple point models.

Critical Point Models These are based on presumption that measuring disease at a critical (single) stage of crop's growth can drive the yield–loss relationship.

(a) Rice Blast: Losses had been estimated with the equation $Y = 0.57x$, where Y is the percentage loss and x is the percentage of blast of neck nodes 30 days after heading.

(b) Stem Rust of Wheat Losses can be estimated given disease severity at a particular growth stage. Urediniologists developed the equation $Y = 35.33 + 27.171 \log x$ (where x is the percentage severity of the disease when the caryopsis had reached ¾ of its final size); the regression line in this case does not pass through the origin.

(c) Late Blight of Potato E.C. Large (1952, 1966) related loss to the time when 75% of the foliage was killed by the blight pathogen. Losses can be estimated given the time when a particular disease is reached.

(d) Late Blight of Potato Losses can be estimated given the time of onset of the epiphytotic. This method is based on the number of blight-free days.

65 blight free days	15% tuber loss
80 blight free days	12% tuber loss
95 blight free days	09% tuber loss

(e) Purple Blotch of Onion Gupta and Pathak (1988) estimated yield loss in onion crop due to purple blotch caused by *Alternaria porri*. The disease's severity was computed in terms of coefficient of disease index (CODEX). A linear relationship was found between yield and CODEX. The amount of infection was quantified by recording the percentage of infected plants (PIP) and the percentage disease index (PDI) one week before the crop was harvested.

It may be mentioned that the critical point model will probably be applicable if: (1) the disease is a late epidemic, (2) the infection rate is fairly stable and (3) the accumulation the dry matter covers only a short period.

Multiple Point Models The limitation of critical point models can be overcome by the multiple point models, which measure disease at many points during the epiphytotic. James *et al*. (1972) developed such a model for estimating losses due to late blight of potato in Canada. The technique of multiple regression analysis was used to drive an empirical equation using the increase in disease during 9 weekly periods as the independent variable and the loss in yield as the dependent variable. The general form of equation is

$$Y = bx + bx + bx \ldots\ldots bx$$

Where, Y = % yield loss and x, x, x, are disease increments for different weeks.

Similarly, Burleight *et al.* (1972) developed a multiple point model for estimating loss in yield due to wheat leaf rust.

Remote Sensing Techniques

Remote sensing has been successfully used to monitor disease development and in the estimation of losses for some epiphytotics. The disease assessment is aided by the use of sophisticated instruments such as microdensitometer and electronic scanners to quantify the disease intensity as exhibited on infrared aerial photographs. This technique can monitor large areas in a short period of time and generate objective quantified data. In case of potato crop and late blight, the healthy crop shows a chlorophyll canopy, which reflects infrared (IR). Late blight destroys chlorophyll and the decrease in reflectance is a measure of disease intensity. The disadvantage in this method is that the exposed soil also led to decrease in reflectance due to disease. Then late blight starts on the lower leaves and is covered by a healthy canopy and therefore is not detectable on IR film.

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Questions

1. Describe the natural, semi-synthetic and synthetic media with suitable examples.
2. Describe some special media for cultivation of plant pathogens.
3. Why are so many types of microbiological media employed in laboratories for the culture of microorganism?
4. Describe briefly the various sterilization techniques used in microbiology/plant pathology.
5. What is aseptic transfer technique? Why must you master this technique to work in a plant pathology laboratory?
6. Discuss the various inoculation techniques used in plant pathology.
7. What is pure culture? Why do we place such importance on obtaining and maintaining pure cultures?
8. Describe the methods employed for the maintenance and preservation of microbial cultures.

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