Fundamentals of Plant Pathology

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LECTURE 1

INTRODUCTION TO THE SCIENCE OF PHYTOPATHOLOGY: ITS IMPORTANCE, SCOPE AND CAUSES OF PLANT DISEASES

SCIENCE OF PHYTOPATHOLOGY OR PLANT PATHOLOGY AND ITS IMPORTANCE

Science of Phytopathology or Plant Pathology and Its Importance

- Plant protection has been accepted as broad area of research and technology at the national level by the Indian Council Agricultural Research, New Delhi; and Plant Pathology is an important discipline of Plant Protection.

Plant Pathology- Definition

- Plant Pathology, also known as Phytopathology is a branch of agricultural, biological or botanical science which deals with the study of diseases in plants - their causes, etiology, epidemiology, resulting losses and management.

Relation to other Sciences

- Plant pathology is related to many other sciences such as virology, mycology, bacteriology, microbiology, physiology, chemistry, genetics, biotechnology etc., all of which provide the knowledge required for the correct diagnosis and management of plant diseases.

Objectives of Plant Pathology

- To study living, non-living and environmental causes of diseases or disorders of the plants.
- To study the mechanism of plant disease development.
- To study interaction between host/susceptible and the pathogens.
- To develop systems of management of plant diseases and reducing losses caused by them.

Importance of Plant Diseases or Plant Pathology

- Losses they cause.
- About 34% of the crop produce is lost annually due to diseases, insect-pests and weeds on the global basis (Cramer, 1967); out of which, 12% is lost due to diseases (caused by fungi, bacteria or viruses), 11% due to nematodes, 7% due to insect-pests and 3% due to weeds.
- When plant protection measures are not implemented, annual loss of 30-50% are common in major crops including horticulture (Encyclopedia Britannica, 2002)

Epidemics

- Late blight of potato caused by Phytophthora infestans was responsible for causing Irish famine in 1845 by destroying the potato crop, the staple food of the people.
- Hundreds of thousand people died of hunger and disease, and there was a large scale migration of the population to other countries including North American continent.
- The population of Ireland was 8 million in 1940, which was reduced to 4 million after the famine.
- This single disease forced man to realize the importance of plant diseases, and brought the science of Plant Pathology to lime light.

Other Famines

- Wheat rust epidemics occurred from time to time in many countries. Wheat rusts forced farmers to change their cropping pattern and wheat was replaced by corn or maize or rye.
- Brown spot of rice caused by Helminthosporium oryzae was responsible for Bengal famine in 1943, which many people think one of the reasons for the division of Bengal
- Coffee rust caused by Hemileia vastatrix forced to cut down the coffee plants in Sri Lanka in 1867.
- Powdery mildew of grapevines caused by (Uncinula necator), by 1854, reduced the French wine production by 80 per cent.
- In 1878, the downy mildew caused by Plasmopara viticola ultimately led to the discovery of Bordeaux mixture.

Losses in India

- Wheat rusts cause a loss of Rs. 400 crore annually.
- In the years of epidemics, losses are Rs. 5000 crore or more.
- Loose smut of wheat is estimated to cause an average loss of 3 per cent (about Rs. 500 crore) every year.
- Other plant diseases such as red rot of sugarcane, potato viruses, rice blast and blight, Karnal bunt of wheat, root knot of tomato, eggplant and cucurbits, apple scab, mango malformation, bunchy top of banana and sandal spike are responsible for huge losses.

Effect on Society

- Infected grains or the fruits may contain toxins (such as aflatoxin, fumonosin) which cause insanity, paralysis, stomach disorder and liver cancer.
- The money spent on the management of plant diseases is also a loss because in the absence of diseases this money could be saved.
- There are many other implications on the transport and agro-based industry in the event of plant disease inflicted yield loss.
- There is restriction on the movements of food grains and other agricultural produce due to the threat of quarantine pathogens and pesticide residues in the produce causing further loss.

CAUSES OF PLANT DISEASES

Causes of Plant Diseases

- Plant diseases are caused by a variety of pathogens.
- The word pathogen can be broadly defined as any agent or factor that incites 'pathos' or disease in an organism. Thus in strict sense, the pathogens do not necessarily belong to living or animate groups.

Abiotic (Inanimate) factors

- They include mainly the deficiency or excess of nutrients, light, moisture, aeration, abnormality in soil condition, atmospheric impunities etc. Examples are: Black tip of mango (due to SO_2 toxicity), khaira disease of rice (due to Zn deficiency), whiptail of cauliflower (Mo deficiency), hollow and black heart of potato (due to excessive accumulations of CO_2 in storage), bitter pit of apple (due to Ca deficiency).

Mesobiotic causes

- These are the disease incitants which are neither living nor non-living. They are considered to be on the threshold of life. They are:
- Viruses: They are infections agents made up of one type of nucleic acid (RNA or DNA) enclosed in a protein coat. Examples of viral diseases of plants are: potato leaf roll, leaf curl of tomato and chillies, and mosaic disease of many plants.
- Viroids: They are naked, infectious strands of nucleic acid. They cause diseases like potato spindle tuber, citrus exocortis, chrysanthemum stunt, cadang cadang of coconut palm, star crack of apple, etc.

Biotic (Animate) causes

This category includes the pathogens which are animate or living or cellular organisms. They are:

- Prokaryotes like bacteria which are unicellular prokaryotic microorganisms lacking true nucleus. Examples of diseases caused by true bacteria are: brown rot or wilt of potato, soft rot of potato and vegetables, , citrus canker, etc.

i) Phytoplasma are wall-less prokaryotes and cause diseases like peach X. ii) Fastidious bacterium, Xylella fastidiosa causes almond leaf scorch, Pierce’s disease of grapevine.

- Eukaryotes are the organisms with true nucleus.

i) Fungi: Potato wart, powdery mildew, rust, smuts, red rot of sugarcane (nearly 80% of plant diseases are caused by fungi).
ii) Straminopiles (Oomycetes): Downy mildews, late blight of potato, white rust of crucifers, damping off etc.
iii) Protozoa: Hart rot of coconut palm and phloem necrosis of coffee.
iv) Algae: Red rust of mango or papaya or litchi
v) Metazoan animals (Nematodes): Root knot of vegetables, ear cockle of wheat, citrus decline etc.
vi) Parasitic flowering plants (Phanerogamic plant parasites): Dodder, Striga, Orobranche, Loranthus, Phoradendron, etc.

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LECTURE 2

HISTORY OF PLANT PATHOLOGY (EARLY DEVELOPMENTS AND ROLE OF FUNGI IN PLANT DISEASES)

ANCIENT HISTORY

- Since organized agriculture developed 4000 years ago, special attention was given to plant diseases and pests. Symptoms such as blight, wilt, root rot, etc. were known to the people at that time.
- In Rig Veda, germ theory of disease was also advocated and the men of learning in Vedic period (Ca. 1500-500 BC) were aware that the diseases were caused by microorganisms. While this fact was not perceived by majority of the scientists in Europe only until 120 years ago.
- Vraksha Ayurveda, a book written by Surapal in 11th century in Ancient India is the first book in which much light has been thrown on plant diseases. The diseases have been divided in 2 groups: i). Internal (probably physiological disorders), and ii) External (probably infectious diseases).
- Symptoms of plant diseases are also mentioned in old testaments like Bible, Shakespear's poems and dramas and other Christian literature. Rust, smut, mildew and blights are very often quoted in Bible.
- In his book, "Enquiry into Plants", Theophrastus (286 BC), a Greek philosopher, recorded his experiences about plant diseases in a book. His experiences were based on imagination and not on experimentation.
- After Theophrastus, no definite opinion could be formed about plant diseases for the next 2000 years, although diseases continued to harm the crops and puzzle scientists of those days.
- During this period, the plant diseases were attributed to many causes which included divine power, religious belief, superstitions and effects of stars and wrath of God, etc.
- The Romans used to celebrate a festival called 'Robigalia' to ward off rust. It is reported to be initiated by the king Numa Pompilus around about 700 BC to please the 'Rust God' Robigo and continued with modifications in the Christian era.
- The association of barberry with stem rust of wheat has been recognized by the farmers for centuries. In 1660, the farmers in France secured passage of law which required destruction of barberry bushes in that area. Similar laws were made and executed strictly in United States in early 18th century.
- Antony van Leeuwenhoek observed the microorganismic cells for the first time under microscope in 1674.
- The Italian botanist Pier Antonio Micheli, also known as Father of Mycology first observed fungi and saw their spores. He also demonstrated that if spores of these fungi are placed on the pieces of fruit, they grew into new thalli of the fungus. Though this was a successful experiment, it was not accepted universally.
- He wrote a book entitled, 'Nova Plantarum Genera' in 1729. Many of such early researches were not accepted because of the strong belief of the people in the 'Theory of Spontaneous Generation' in which microorganisms spontaneously or automatically originate from inanimate or non-living matter.
- In 1743, Needham, an English Clergyman and naturalist upheld strongly this theory. To prove his point, he boiled meat broth in flask which was corked.

- In 1775, Lazarro Spallanzani, an Italian investigator challenged Needham and set out to prove that decay of meat broth by bacteria and other lower forms of life could be prevented by heating the material in a flask which was sealed off in such a way as to exclude contamination from the air. He, however, did not receive recognition and was refuted by opponents who did not get the results due to their faulty techniques.
- Carl von Linne (Linnaeus), a Swedish scientist wrote a book entitled, “Species Plantarum” in 1753 and is credited with giving binomial nomenclature.
- C.H. Persoon’s “Synopsis Methodica Fungorum” (1793) is the chief starting point for the nomenclature of the Uredinales, Ustilaginales and Gasteromycetes.
- E.M. Fries (1821-1832) was a Swedish scientist and is regarded as ‘Linnaeus of Mycology’. His Systema Mycologicum is the chief starting point for the nomenclature of fungi.
- In 1775, Tillet, a French botanist published a paper on bunt or stinking smut of wheat. He proved that such wheat seeds that contained a black powder on their surface produced more diseased plants than the clean seeds. He believed that the disease was caused by some poisonous substance produced by the black powder. He also observed a reduction in disease incidence when the seeds were treated with salt and lime. Therefore, it can be said that he was an experimenter who lived ahead of his times.
- Felice Fontana, an Italian physicist and naturalist published a paper in 1767 in which he expressed the view that grain rust was a distinct parasitic entity. He distinguished the red and black stages of wheat rust and made microscopic drawings.
- Benedict Prevost, who was Swiss Professor of Philosophy at the Academy of France, in 1807 discovered the life cycle of bunt fungus. He studied germination of wheat bunt fungus spores 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 microorganisms in the causation of the disease. He also demonstrated the control of smut by steeping seeds in a solution of copper sulphate.
- Tulasne brothers (R.L. and C. Tulasne) of France who have produced illustrated descriptions of rust and smut fungi, had also confirmed the findings of Prevost.
- In 1845, late blight devastated the potato crop in Ireland and other parts of Europe which attracted the attention of mycologists and plant pathologists to plant diseases. Much of early literature on this disease is found in ‘Gardener’s Chronicle’.
- M.J. Berkeley who was the most prominent British mycologist was at first somewhat conservative in supporting of the parasitic theory, but advocated it strongly in 1846.
- Montague in France in 1845 described the causal fungus as Botrytis infestans. However, the fungus was real cause of this disease and not the result was proven experimentally by Speers and Schneider in 1857 and Anton de Bary (1861, 63).

MODERN EXPERIMENTAL PLANT PATHOLOGY

- The foundation of modern experimental plant pathology was laid by the German scientist Heinrich Anton de Bary (1831-1888). He made a great contribution to the understanding of science of Plant Pathology and is suitably regarded as Father of Modern Plant Pathology. His major contributions are:
- He confirmed the findings of Prevost in 1853.
- In 1861, he experimentally proved that Phytophthora infestans was the cause of late blight of potato. He is credited with the ultimate proof of the organisms being plant pathogens.
- He studied other diseases like rusts, smuts, downy mildews and rots.
- He reported the heteroecious nature of rust fungus in 1885.
- He also reported the role of enzymes and toxins in tissue degradation caused by Sclerotinia sclerotiorum in 1886.
- de Bary was first to report that lichen consists of a fungus and an alga, and coined the term symbiosis.
- He studied in detail the life cycle of downy mildew fungi and their parasitism.
- His well known text book “Morphologie und Physiologie der Pilze Flechten und Myxomyceten” written in 1866 and 1884 records the broad classification of fungi.
- He trained a large number of students from all over the world who came to his laboratory.
- More than 60 of them became prominent in field and carried his techniques.
- Famous among them were M.S. Woronin of Russia, who studied club root of crucifers caused by Plasmodiophora brassicae; P.A. Millardet of France; HM ward of England, who studied coffee rust in Sri Lanka and gave ‘bridging host theory’, Fallow of U.S.A., who spent his long active career as cryptogamic botanist, and provided leadership in study of parasitic fungi; and A. Fisher of Switzerland worked on bacterial Plant Pathology and is known for the infamous Fisher –Smith controversy regarding the role of bacteria in causing plant diseases.
- J.G. Kuhn, who was a contemporary of de Bary and his countryman, was, initially a farm manager. He contributed significantly to the studies on infection and development of smut in wheat plant and development and application of control measures, particularly seed treatment for cereals.
- He wrote the first book on Plant Pathology “Diseases of Cultivated Crops, Their Causes and Their Control” in 1858 in which he recognized that plant diseases are caused not only by an unfavourable environment, but can also be caused by parasitic organisms such as insects, fungi and parasitic plants.
- The theory of spontaneous generation was a major impediment in the development of science of Plant Pathology, which was finally disproved by Louis Pasteur who established the ‘germ theory of the disease’ in case of anthrax in relation to man and animals. It changed the way of thinking of scientists and led to a tremendous progress.
- Significant impetus to this progress was added by Robert Petri, who developed artificial nutrient media for culturing of microorganisms and Brefeld (1875,1883,1912) who contributed greatly to Plant Pathology by introducing and developing modern techniques for growing microorganisms in pure culture.
- Robert Koch (1876) who was a Germen Physician and co-worker of Pasteur established that for proving that a certain microorganism was the cause of some infectious disease, certain necessary steps (Koch’s postulates) must be carried out and certain conditions must be satisfied. They are:
- A specific organism must always be associated with the disease.

- The organism has to be isolated in pure culture.
- The organism must produce specific disease in a healthy susceptible host when the latter are inoculated with it.
- The organism must be re-isolated from the experimental (diseased) host in pure culture and its identity be established and it must have the characteristics as the organism in step 2.

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LECTURE 3

HISTORY OF PLANT PATHOLOGY (ROLE OF OTHER PLANT PATHOGENS)

ROLE OF OTHER PLANT PATHOGENS

Bacteria as Plant Pathogens

- In 1882, T.J. Burrill of USA for the first time reported that a plant disease (fire blight of apple and pear) was caused by a bacterium (now known as Erwinia amylovora).
- Wakker (1883) showed that yellows disease of hyacinth was also caused by a bacterium.
- E.E. Smith of USA is regarded the most outstanding and main contributor to the discovery of most of plant diseases due to bacteria since 1895. He is considered Father of Phytobacteriology for his discoveries and methodologies.
- Smith's name is still remembered as he resolved the controversy with the German scientist A. Fischer (1897, 99) who did not agree that bacteria were the causes of diseases in plants.
- Smith was also among the first to notice and study the crown gall disease (1893, 1894). He considered crown gall similar to cancerous tumors of humans and animals.
- Later in 1977, it was demonstrated by Chilton and his team that the crown gall bacterium, Agrobacterium tumefaciens transforms the normal plant cells in tumour cells by introducing into them a part of plasmid which becomes inserted into the plant cell chromosome DNA.

Viruses

- Virus diseases of plants have a long history. Among many diseases of unknown cause, potato leaf roll, as 'leaf curl' gave concern in the 2nd half of 18th century and broken tulips were illustrated by painters 200 years before that.
- There were many methods of transmission of leaf mottling of jasmine and passion flower by grafting.
- In 1886, Adolf Mayer, a Germen Director of Agricultural Experiment Station at Wageningen, Netherland, introduced the term 'mosaic' and showed that the mosaic was infectious and the juice from infected plants if applied to the healthy plants could reproduce the disease.
- In 1891, Smith showed that the peach yellows was contagious disease and could be bud transmitted.
- In 1892, Dimitri Ivanowski proved that the causal agent of tobacco mosaic disease could pass through bacteria proof filters.
- In 1898, Beijerinck, (Father of Plant Virology) a distinguished Dutch microbiologist, demonstrated that the causal agent of tobacco mosaic could diffuse through an agar-agar membrane and concluded that the tobacco mosaic was caused by a non-corpuscular "contagium vivum fluidum" (or contagious living fluid) and called founder of virology and it's a virus.
- Stanley (1935) obtained a crystalline protein by treating the juice of the tobacco mosaic infected leaves with ammonium sulphate, which when placed on the healthy leaves could produce the disease symptoms. It was the first major contribution regarding the nature of the viruses and was awarded Noble Prize for it.

- In1936, Bawden and Pirie discovered the real nature of the Tobacco mosaic virus and demonstrated that the crystalline preparations of the virus actually consisted of not only proteins but also small amount of nucleic acid (RNA).
- In 1939, Kausche et al. viewed first Tobacco mosaic virus particles under electron microscope.
- Finally in 1956, Gierrer and Schramm showed that the nucleic acid fraction of the virus is actually required for infection and multiplication in the host and protein coat provided the protective covering to it.

Viroids

- In 1971, Diener and Raymer reported that the potato spindle tuber disease was caused by a small (250-400 base pair long), single stranded circular molecule of infectious RNA, which he called a viroid.
- Viroids seem to be the smallest nucleic acid molecules to infect plants but no viroid has so far been found in animals.
- Since then a dozen more viroids have been reported.
- In 1982, a circular single stranded viroid-like RNA (300-400 base pairs long) was found encapsidated together with the single stranded linear RNA (about 4500 base pairs long) of velvet tobacco mottle virus. This small circular RNA was called Virusoid which seems to form an obligatory association with the viral RNA in many plant viruses.

Phytoplasma and Rickettsia like Organisms

- Doi et al. (1967) and Ishiie et al. (1967) independently observed Mycoplasma Like Organisms (MLOs) now called as phytoplasma in the phloem of plants exhibiting yellows and witches' broom symptoms (earlier thought to be caused by viruses).
- The number of plant diseases of phytoplasma etiology is large. Some examples are aster yellows, mulberry dwarf, potato witches' broom and sandal spike.
- These organisms resembling mycoplasma could not be isolated and cultivated on artificial cell free media and they have shown more relatedness to acholeplasma than the mycoplasma, and are called phytoplasma.
- Later in 1973, some of the mycoplasmas such as the causal agent of citrus stubborn and corn stunt diseases could be grown in cell free media, and were helical in morphology and had motile stages and were named as spiroplasma. The agent Spiroplasma citri causing citrus stubborn is the type species of the genus and S. kunkelli causes corn stunt.

Fastidious Vascular Bacteria

- Some organisms were also observed in grapevines infected with Pierce's disease, in peach infected with phony peach and others.
- More recently such diseases have been reported to be caused by fastidious vascular xylem limited bacteria Xylella fastidiosa, and phloem limited bacteria Candidatus librobacter.
- Examples of xylem inhabiting fastidious bacteria causing diseases: Pierce's disease of grapevine, citrus variegated chlorosis, almond leaf scorch.
- Examples of phloem inhabiting fastidious bacteria are: Club leaf of clover, citrus greening, yellow vine disease of watermelon, bunchy top of papaya.

Flagellate Protozoa

- In 1909, Lafont observed flagellate protozoa in the latex bearing cells of laticiferous plants of Euphorbiaceae family without causing any harm to their hosts.
- However, in 1931, Stahel found the flagellates infecting phloem of coffee plants and causing abnormal phloem formation and wilting of trees.
- Vermeulen in 1963 presented additional and more convincing evidence of the pathogenicity of flagellates to coffee trees and in 1976, flagellates were also found in the phloem of coconut palm trees infected with the hart rot disease.

HISTORY OF PLANT PATHOLOGY IN INDIA

- The development of science of Plant Pathology in the modern era in India as in other countries followed the development of mycology. The study of fungi in India was initiated by Europeans in the 19th century. They used to collect fungi and send the specimens for identification to the laboratories in Europe.
- During 1850-1875, D.D. Cunningham and A. Barclay started identification of fungi in India itself. Cunningham made a special study of rusts and smuts.
- K.R. Kirtikar was the first Indian scientist who collected and identified the fungi in the country.
- E.J. Bulter who is also known as the ‘Father of Plant Pathology’ in India, initiated an exhaustive study of fungi and diseases caused by them in 1901 at Imperial Agricultural Research Institute at Pusa (Bihar).
- During his stay of 20 years in this country, he made a scientific study of mostly fungal plant diseases known in India at that time. The diseases studied by him for the first time included wilt of cotton and pigeon pea, different diseases of rice, toddy palm, sugarcane, potato and rusts of cereals.
- He wrote a monograph on ‘Pythiaceous and Allied Fungi’; and a classic text book, ‘Fungi and Diseases in Plants’ in 1918.
- J.F. Dastur (1886-1971), a colleague of Butler, was the first Indian Plant Pathologist who is credited with a detailed studies of fungi and diseases in plants.
- He studied the genus Phytophthora and diseases caused by it in castor and potato. He is internationally known for the establishment of Phytophthora parasitica from castor.
- G.S. Kulkarni published exhaustive information on downy mildew and smuts of sugarcane and pearl millet.
- B.B. Mundkur started work on control of cotton wilt through varietal resistance.
- He was also responsible for the identification and classification of large number of Indian smut fungi.
- His most significant contribution to plant pathology will be remembered through the ‘Indian Phytopathological Society’ which he started almost single handedly in 1948 with its journal ‘Indian Phytopathology’.
- He also authored a text book entitled, ‘Fungi and Plant Diseases’.
- Dr. K.C. Mehta of Agra College, Agra investigated the life cycle of cereal rusts in India during the first half of 20th century.
- Dr. R. Prasada trained by Dr K.C. Mehta continued the work on rusts and added to the knowledge of linseed rust.
- Luthra and Sattar (1953) developed the solar heat treatment of wheat seed for the control of loose smut. SN Dasgupta carried out exhaustive studies on black tip of mango.
- T.S. Sadasivan worked out the mechanism of wilting in cotton due to Fusarium oxysporum f. sp. vasinfectum.
- M.K. Patel, V.P. Bhide and G. Rangaswami pioneered the work on bacterial plant pathogens in India.
- M.J. Thirumalachar conducted exhaustive studies on rusts and smuts, and developed a number of antibiotics for controlling plant diseases in India.
- Afterwards, Plant Pathology became a major subject in various agricultural colleges and universities and organized research was conducted on major plant diseases affecting crop plants in India.

- Notable contributions included the works of B.L. Chona on sugarcane diseases and Agnihothrudu in tea diseases, R.K. Agrawala on apple diseases and G.S. Saharan on oilseed plant diseases to name a few.

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LECTURE 4

GENERAL CONCEPTS AND CLASSIFICATION OF PLANT DISEASES

DEFINITIONS AND CONCEPTS

Disease: According to Horsfall and Diamond (1959), disease may be defined as a malfunctioning process that is caused by continuous irritation by a pathogen and/or environmental factor resulting in some suffering producing symptoms.

Disorder: The diseases caused by the deficiency of nutrients or unfavourable environmental are sometimes termed as disorders or physiological disorders.

Pathogen: It is the agent responsible for inciting 'pathos' i.e. ailment or damage.

Parasite: These are the organisms which derive the food materials needed for their growth from other living organism (the host). All the pathogens are parasites but all the parasites are not pathogens. As some of the parasites live on their hosts without causing any damage to them as symbiotic relationships, e.g., Rhizobium bacterium in legume roots, mycorrhizae and lichens.

Biotrophs are the organisms which regardless of the ease with which they can be cultivated on artificial media obtain their food from living tissues only in nature in which they complete their life cycle). They were earlier also called **obligate parasites**, e.g., rusts, smuts, powdery mildews etc.

Saprophytes/saprobes are the organisms which derive their nutrition from the dead organic matter. Some parasites and saprophytes may have the faculty or (ability) to change their mode of nutrition.

Facultative saprophytes are ordinarily parasites which can grow and reproduce on dead organic matter under certain circumstances. They are also called **hemibiotrophs** which attack the living tissues in such a way as biotrophs but continue to grow and reproduce after the tissues is dead.

A parasite is called **necrotroph** when it kills the host tissue in advance of penetration and then lives saprophytically, e.g. Sclerotium rolfsii and Pythium species. Similar to **necrotrophs** are **facultative parasites** which live as saprophytes but under favourable conditions they can attack living plants and become parasites. The necrotrophs are also known as **perthotrophs** or **perthophytes**.

Pathogenicity is the ability of a pathogen to cause disease under a given set of environmental conditions. Whereas, **pathogenesis** is the chain of events that leads to development of a disease in the host.

Parasitism is a phenomenon by which a plant parasite becomes intimately associated with the plant; it draws nutrition and multiplies and grows at the expense of the plant host.

Virulence is a measure or degree of pathogenicity of an isolate or race of the pathogen. The term **aggressiveness** is often used to describe the capacity of a pathogen to invade and grow in the host plant and to reproduce on or in it. This term like virulence is used as measure of pathogenicity.

Immunity of a plant against a disease is absolute quality. It denotes the freedom of plant from disease, when the pathogen cannot establish parasitic relationship with the host. High resistance and low susceptibility approach immunity.

Disease resistance is the ability of an organism to overcome completely or in some degree the effect of a pathogen or other damaging factor; whereas susceptibility in the inability of the plant to resist the effect of the pathogen or other damaging factor.

Hypersensitivity is the extreme degree of susceptibility in which there is rapid death of the cells in the vicinity of the invading pathogen. It halts the further progress of the pathogen. Thus, hypersensitivity is a sign of very high resistance approaching immunity.

Infection is the establishment of the parasitic relationship between the pathogen and host following entry or penetration.

Incubation period is the time elapsing between penetration and completion of infection i.e. development of the disease symptoms.

Invasion and colonization is the growth and multiplication of the pathogen through the tissue of the host varying extent.

Effects of Disease

- The diseased plants do not function or look normal showing structural abnormality and / or physiological disorder and can not grow, develop and reproduce to its genetic potential.

CLASSIFICATION OF PLANT DISEASES

Based on plant part affected

- **Localized**, if they affect only specific organs or parts of the plants.
- **Systemic**, if entire plant is affected. or

They can be classified as root diseases, stem diseases, foliage/foliar diseases, etc.

Based on perpetuation and spread

- **Soil borne** -when the pathogen perpetuates through the agency of soil.
- **Seed borne** -when the pathogen perpetuates through seed (or any propagation material).
- **Air borne** -when they are disseminated by wind e.g. rusts and powdery mildews.

Based on the signs and symptoms produced by the pathogens

- Diseases are classified as rusts, smuts, powdery mildews, downy mildews, root rots, wilts, blights, cankers, fruit rots, leaf spots, etc. In all these examples, the disease are named after the most conspicuous symptom of the disease appearing on the host surface.

Based on the host plants affected
They can be classified as cereal crop diseases, forage crop diseases, flax diseases, millet diseases, plantation crop diseases, fruit crop diseases, vegetable crop diseases, flowering plant diseases, etc.

Based on major Causes
They can be classified as fungal diseases, bacterial diseases, viral diseases, mycoplasmal diseases, etc.

Based on Infection Process

- **Infectious** -All the diseases caused by animate causes, viruses and viroids can be transmitted from infected host plants to the healthy plants and are called infectious.
- **Non-infectious**- Non-infectious diseases can not be transmitted to a healthy plant. Also referred as non-parasitic disorders or simply physiological disorders, and are incited by abiotic or inanimate causes like nutrient deficiency or excess or unfavorable weather conditions of soil and air or injurious mechanical influences.

Classification of Animate Diseases in Relation to Their Occurrence

- **Endemic diseases** -which are more or less constantly present from year to year in a moderate to severe form in a particular geographical region, i.e. country, district or location.
- **Epidemic or epiphytotic diseases** - which occur widely but periodically particularly in a severe form. They might be occurring in the locality every year but assume severe form only on occasions due to the favourable environmental conditions occurring in some years.

- **Sporadic diseases** occur at irregular intervals and locations and in relatively few instances.
- **Pandemic diseases:** A disease may be endemic in one region and epidemic in another. When epiphytotics become prevalent through out a country, continent or the world, the disease may be termed as pandemic.

Disease triangle

- The interaction of the host, the pathogen and the environment results in disease development. It is generally illustrated by a triangle, also called a disease triangle.

Disease Development in Plant Population
This is determined by:

- **Host:** All conditions in host that favour susceptibility.
- **Pathogen:** Total of virulence, abundance etc.
- **Environment:** Total of conditions that favour the pathogen and predispose the host plants to pathogen attack.
- **Time:** Specific point of time at which a particular event in disease development occurs and the duration or length of time during which the event takes place.

'Effective disease control or measures aim at breaking this E-H-P triangle'.

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LECTURE 5

SYMPTOMS AND SIGNS OF PLANT DISEASES

DISEASE SYNDROME

Symptoms and signs

Symptoms- External expression or the evidence of the abnormalities in the appearance of the diseased plants brought about by the pathogens after host-pathogen interaction.

Sign- When the pathogen itself becomes visible on the host surface in the form of its organs or structures. eg. sclerotia, mycelium etc.

Disease syndrome- A sum total of variety of symptoms produced by the disease.

I. Symptoms of Plant Diseases Due to the Character and Appearance of Visible Pathogen, its Structures and Organs

i. Mildews

- Mildews consist of white, grey, brownish or purplish pathogen growth on the host surface.
- Downy mildew is characterized by a tangled cottony or downy growth mostly on the lower surface of the leaves or other plant parts.
- Powdery mildew consists enormous number of spores are formed on superficial growth of the fungus giving a dusty or powdery appearance on the host surface. Black minute fruiting bodies may also develop in the powdery mass.

Fig.5.1 Grapevine downy mildew

Fig.5.2 Pea powdery mildew

ii. Rust

- Rust appears as relatively small pustules of the spores, usually breaking through the host epidermis.
- Pustule is a small blister-like elevation of the epidermis, often opening to expose spores. The pustules may be dusty or compact, and red, brown, yellow or black in colour.

Fig.5.3 Pea rust

iii. Smut

- Smut means a sooty or charcoal like powder.
- The affected parts of the plants show black or purplish black dusty areas.
- Symptoms usually appear on floral organs, particularly the ovulary areas.
- The pustules on the leaves and stems are usually larger than those of rusts.

iv. White Blister

- **White blister**-like pustules appear on the leaves and other parts of cruciferous plants which break open the epidermis and expose powdery masses of spores.
- Such symptoms are called ‘white rust’, although there is nothing common with them and the rusts.

Fig.5.4 White blisters on a crucifer

v. Blotch

- It consists of superficial growth giving the affected plant parts i.e., fruits and leaves smoky (blotched) appearance, e.g. sooty blotch of apple.

Fig.5.5 Sooty blotch of apple

vi. Sclerotia

- A **sclerotium** is a compact, often hard mass of dormant fungus mycelium.
- **Sclerotia** are mostly dark in colour and are found mixed with the healthy grains as in the case of ergot of wheat and rye.

vii. Exudation

- Mass of bacterial cells ooze out on the surface of the affected organs where they may be seen as a drop or smear in several bacterial diseases such as bacterial blight of paddy, gummosis of stone fruits and fire blight of apple and pear.
- They form crusts after drying.

viii. Mycelial growth

- Appearance of white cottony, mycelial growth of the fungi like Dematophora necatrix on affected roots of apple is an important diagnostic feature of white root rot in the field.

Sclerotium rolfsii sclerotia

White root rot of apple

Mango gummosis

II. Symptoms Resulting from Internal Disorders in the Host Plants

i. Colour change

- **Discolouration** is change of colour from normal. It is one of the most common symptoms of plant diseases. The green pigment of leaves disappears entirely and is replaced by yellow pigments.
- **Etiolation** is yellowing due to the lack of light.

- **Chlorosis** is yellowing due to low temperature, lack of iron, excess of the lime or alkali in soil and infection by viruses, fungi and bacteria.
- **Albilinism** is the phenomenon in which the leaves become devoid of any pigment and look bleached or white.
- **Chromosis** is change of colour to red, purple or orange.

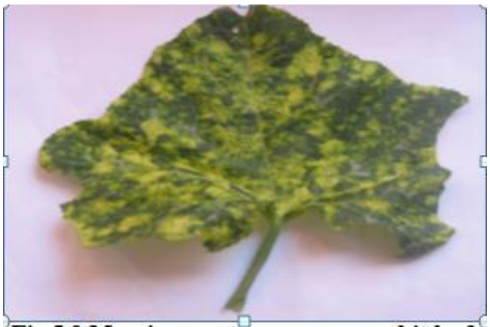

Fig.5.9 Mosaic symptoms on a cucurbit leaf

ii. Overgrowths or hypertrophy

- **Hypertrophy** is the abnormal increase in the size of the plant organs due to increase in the size of the cells of a particular tissue, whereas
- **Hyperplasia** is the abnormal increase in the size of the plant organs due to increase in the number of cells of which the tissue or organ is composed, owing to increased cell division.
- The overgrowths cause galls, curl, pockets or bladders, hairy root, witches' broom, intumescence etc.

Fig. 5.10 Crown gall of peach

iii. Atrophy or Hypoplasia or Dwarfing

- **Atrophy** is inhibition of growth and thereby showing stunting and dwarfing effectmon the plants.
- The whole plant may be dwarfed or only certain organs are affected. e.g. rice dwarf, phony peach etc.

III. Necrosis

- Death of the cells, tissues and organs occurs as a result of parasitic activity.
- The characteristic appearance of the dead areas differs with different hosts, host organs and with different parasites.

- Necrotic symptoms include spots, streaks or stripes, canker, blight, damping off, burn, scald or scorch and rot.


Fig.5.11 Colocasia blight


Fig.5.12 Brown rot of pear

IV. Wilt

- Characterized by drying of the entire plant.
- Leaves and other green or succulent parts loose their turgidity, become flaccid and droop down.
- Usually seen first in some of the leaves.
- Later, the young growing tip or the whole plant may dry up.
- May be caused by injury to the host system or the conducting vessels.
- Wilting due to disease is different from the physiological wilting where the plant recovers as soon as the supply of water is retained.


Fig.5.13 Fusarium wilt of pea


Fig.5.14 Bacterial wilt of capsicum

V. Die-back or Wither Tip

- Symptoms are characterized by drying of plant organs, especially stems or branches, from the tip backwards.
- It is also a form of necrosis caused directly by the pathogen or its toxins.


Fig.5.15 Die-back symptom on mango

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LECTURE 6

GENERAL CHARACTERISTICS OF FUNGI AND FUNGAL-LIKE ORGANISMS CAUSING PLANT DISEASES

FUNGI

Fungi are eukaryotic, spore bearing achlorophyllous organisms with absorptive nutrition that generally reproduce both sexually and asexually and whose somatic structures known as hyphae are surrounded by cell wall containing chitin and glucans (but no cellulose) as the skeletal components.

Oomycetes

- A group of fungal like organisms, the Oomycota generally referred to as (Oomycetes), until about 1990 were called considered as lower fungi.
- Majority have cell wall composed of glucans and small amount of cellulose, but not chitin.
- Now regarded as members of the kingdom Chromista (also known as Straminopila) rather than Fungi; but continued to be discussed with fungi because of their many other similarities to them, especially the way they cause disease in plants.

Habitat

- Most of the more than 1,00,000 known fungus species are strictly saprophytic and they live on dead organic matter.
- About 50 species cause diseases in humans
- About 50 species cause diseases in animals.
- More than 10,000 species of fungi can cause diseases in plants.
- All plants are affected by some kinds of fungi and each of the parasitic fungus can attack one or many kinds of plants.
- Some fungi like those causing rusts, smuts, powdery mildews and downy mildews can grow and multiply on their host plants during their entire life, and therefore called as obligate parasites or biotrophs.
- Fungi like *Venturia inaequalis*, the apple scab fungus, pass a part of their life on the host as parasites and a part on the dead tissues of the same host on the ground as saprophytes in order to complete their life cycle in nature, and therefore are called hemibiotrophs.
- **Facultative saprophytes** generally grow parasitically on the hosts but continue to live, grow and multiply on the dead tissues of the host and may further move out of the host debris into the soil or other decaying plant material to grow and multiply strictly as saprophytes, e.g., Phytophthora infestans.
- **Facultative parasites** live perfectly well in the soil or elsewhere as saprophytes but can parasitize or cause disease in the plants when they get opportunity under favourable conditions to do so, e.g., Alternaria alternata.

Morphology

- **Mycelium:** A filamentous vegetative body.
- **Hypha (pl. hyphae):** Individual branch of mycelium which are generally uniform in thickness, usually about 2-10 µm in diameter. The hyphae may be septate or aseptate
- **Coenocytic hyphae** - The aseptate or non-septate hyphae having the nuclei scattered in the cytoplasm.
- **Septate hyphae**- The hyphae have septa having perforations through which cytoplasmic strands, containing nuclei can migrate from one cell to the other.
- A characteristic dolipore septum is formed in certain basidiomycetes.
- Diameter of hyphae may be as narrow as 0.5 µm and as wide as 100 µm.
- The vegetative thallus may consist of only one cell or may even be naked, amoeboid, multinucleate plasmodial mass without cell wall or a system of strands of varying diameter called rhizomycelium.
- In some fungi, hyphae form aggregates and develop various kinds of structures. These may be:
- **Rhizomorphs:** thicker root like aggregates.
- **Sclerotium:** a hard roundish or amorphous structure and has a hard rind surrounding a soft interior i.e. medulla.
- **Stroma:** some fungi also develop mat like structures which contain the fruiting bodies.
- Rhizomorphs and sclerotia help the fungus to survive from one cropping season to the other and also function in initiating the disease as a primary inoculum.
- **Pseudoparenchyma:** Sometimes the hypahe aggregate to form tissue like structure called plectenchyma. In cross section, it appears like parenchymatous cells of the higher plants. This is called pseudoparenchyma and consists of rounded fungal cells.
- **Prosenchyma:** Less compact structures consisting of hyphae made of elongated cells. These are found mostly in the stroma or fruiting bodies of Ascomycota or Basidiomycota.

FUNGAL CELL STRUCTURE

A typical fungal cell consists of protoplasm which is surrounded by a cell membrane, and cell wall being its outer most covering. The protoplasm typically contains nucleus, mitochondria, ribosomes, golgi bodies and endoplasmic reticulum among others.

Cell wall

- Made up of chitin and ß-glucans in the members belonging to the kingdom Fungi.
- Chitin is a polymer of N-acetyl glucosamine units which is also found in the exoskeleton of insects.
- Made up of cellulose in kingdom Straminopila (including Oomycota).
- Cellulose is a polymer of ß D-glucose units and is also found in the cell wall of plants and algae.

Nucleus

- The nuclei of fungi are extremely small and lie near the limit of resolution power of light microscope.
- Electron microscopic studies have revealed that the nuclear membrane does not disappear but constricts in the centre like a dumb-bell during nuclear division. This type of division is known karyochoresis, term given by Moore in 1964.
- In meiosis, however, the nuclear membrane disappears.

Life Cycle

- The fungi are mostly haploid organisms, i.e. their nuclei are haploid.
- The thallus multiplies by asexual methods.
- After sexual reproduction, a diploid zygote is formed.
- The zygote represents the diploid phase which is terminated by meiosis forming haploid spores.
- The spores on germination form the haploid somatic phase.
- In Ascomycota and Basidiomycota, plasmogamy is not immediately followed by karyogamy.
- The two nuclei of opposite strains remain as paired nuclei called dikaryon and give rise to dikaryotic hyphae.
- These are of limited duration in Ascomycota, as only a small portion of the mycelium, viz., the ascogenous hyphae become dikaryotic.
- In Basidiomycota, these extend through the major portion of the life cycle. The monokaryotic mycelium is of short duration and its function is only to establish dikaryotic mycelium as soon as possible.
- The dikaryotic phase, which is of short duration in Ascomycota is extensive in the life cycles of Basidiomycota.

Basic Types of Life Cycles Found in Fungi (depending on the extent of haploid, diploid or dikaryotic phases of the mycelium)

- **Asexual cycle**: Found in all anamorphic (=imperfect fungi)
- **Haploid cycle**: Mucor , Rhizopus, etc.
- Haploid cycle with restricted dikaryophase: Eurotium, Talaromyces

- **Haploid dikaryotic cycle**: Ustilago maydis
- **Dikaryotic cycle**: Ustilago tritici, Puccinia graminis
- **Haploid**- diploid cycle: Saccharomyces cerevisiae
- **Diploid cycle:** Pythium, Phytophthora, Albugo

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LECTURE 7
REPRODUCTION IN FUNGI AND FUNGAL LIKE ORGANISMS CAUSING PLANT DISEASES

REPRODUCTION

Fungi may reproduce in three ways:

- Vegetative reproduction
- Asexual reproduction
- Sexual reproduction

I. Vegetative Reproduction
It occurs through:

- Fragmentation of hyphae
- Production of thin walled spores such as oidia or arthrospores
- Production of swollen, thick walled spores with storage of rich food, i.e. chlamydospores which are formed to tide over the adverse environment.
- In Fragmentation, a bit of broken hyphae establishes a new colony. Fragmentation occurs in nature and is usually employed in the laboratory to keep the fungus growing by transferring small portions of hyphae to new culture tubes.
- In Budding, small soft portion of the cell wall bulges out and a daughter nucleus migrates into it. Then the bud is pinched out by a constriction at the point of its origin. Sometimes the budding is so quick that a chain of buds is formed due to non-detachment of the daughter buds, and is called pseudomycelium.
- In Fission, a cell divides in a transverse plane into two cells. Although, it is a characteristic of bacteria and it also occurs in fission yeasts.

II. Asexual Reproduction

- It occurs through internally or externally produced spores which also act as agents of dissemination, survival and infection.
- In Straminopila (Oomycota) and some Fungi (Zygomycota), asexual spores are produced endogenously inside a sac like structure called sporangium and are released either by rupture of sporangial wall or through a pore or opening in its wall.
- They are either motile with one or two flagella called zoospores or non-motile aplanospores.
- Sporangia are formed on specialized hyphal branches called sporangiophores.
- Conidia are another type of asexual spores which are cut off terminally or laterally from specialized hyphal branches called conidiophores.
- Conidiophores may be unbranched or may branch variously, both monopodially or sympodially and conidia are produced singly or serially in chains on these branches.
- While conidiophores of most fungi remain free, in some they appear to be aggregated and often compactly arranged to form a variety of characteristic structures such as coremia, synnema, sporodochia, acervuli and pycnidia. These are primarily designed to provide large number of spores within a small space available to the fungus.
- The locomotory appendages or flagella of zoospores are of two types, i.e. whiplash and tinsel.

- The whiplash flagella are much thinner at the tip.
- The tinsel type flagella, which are found only in the members of kingdom Straminopila (Oomycota) have large number of small hair like outgrowths called mastigonemes or flimmers on their entire length.

Structure of Flagella

- The flagellum of fungi has a typical 9+2 structure as in case of other eukaryotes. In 9+2 structure, the flagellum is composed of 9 peripheral pairs of fibrils surrounding the two central fibrils (hence the name 9+2). The two central fibrils are attached to the blepharoplast lying inside the motile cell. The membrane surrounding the 9+2 fibrils is continuous with the plasma membrane of the cell. Sometimes, a threadlike rhizoplast connects the blepharoplast to the nucleus.
- The bacterial flagella lack this 9+2 structure and are made of 8 rows of flagellin (protein) molecules twisted around each other like a rope.

III. Sexual Reproduction
The sexual reproduction in fungi and other similar micro-organisms involves:

- **Plasmogamy-**. fusion between two sexual cells.
- **Karyogamy-** fusion of the nuclei. It results in the formation of a diploid nucleus, which immediately or later undergoes meiosis to form 4 haploid nuclei.

Fungi achieve plasmogamy by a variety of methods:
i. Gametogamy
ii. Gametangiogamy,
iii. Spermatization
iv. Somatogamy.

i) Gametogamy: It is the fusion (or copulation) between gametes.

- Gametes are naked wall- less sex cells which copulate to form a zygote.
- If two gametes are similar in size, they are called isogametes and their copulation is called isogamy.
- Copulation between two dissimilar gametes, one smaller (male) and the other bigger (female) is called anisogamy.
- The fusion between motile male gamete and non- motile female gamete (oosphere or egg) lying in the oogonium is called heterogamy.

ii) Gametangiogamy: It is the fusion between gametangia (or the sex organs) when gametangia are similar in shape and size, these are called isogametangia and are designated as (+) and (-) gametangia rather than male and female.

- When the gametangia are different in shape and size, they are called heterogametangia.
- The male is usually smaller and club shaped while the female is bigger and globular.
- Fusion between two similar gametangia results in a zygote which is called a zygospore.
- The zygote formed by the fusion between morphologically distinct gametangia is called oospore and the process oogamy.
- The plasmogamy between them is called gametangial copulation or contact.

Gametangial copulation is of two types:

- The entire gametangia fuse, the intervening wall disappears and their contents come to lie in the common cell formed by their fusion, e.g., Mucor.
- The contents of the male gametangia migrate into the female gametangium through a pore or fertilization tube and the male gametangium is left empty, e.g., Rhizophidium.
    - **Gametangial contact:** The male nuclei and not the cytoplasm of antheridium migrate into the oogonium through a pore dissolved at the point of contact or through a fertilization tube formed by the antheridium. e.g. Pythium, Phytophthora, Albugo.

iii. Spermatization: It occurs in Ascomycota and Basidiomycota.

- Spermatia (sing. Spermatium), minute male gametes, are formed like conidia on spermatiophores.
- The spermatiophores may be formed exogenously or inside a spermogonium e.g. Puccnia.
- The spermatium when comes in contact with the female gametangium (or the receptive hyphae) releases the male nucleus into the female gametangium through a pore.

iv. Somatogamy:
In this, sex organs are not formed and somatic cells as such act as gametangia and fuse together. e.g. Agaricus. Somatogamy may occur between cells of the same hypha (in a homothallic fungus) or between cells of the different thalli (in a heterothallic fungus). Anastomosis, which is the fusion of hyphae is frequent in Ascomycota and Basidiomycota.

IV. Parasexual Reproduction

- The anamorphic (=imperfect) fungi lack sexual reproduction involving karyogamy and meiosis. But the genetic recombination in this case is achieved through the parasexual method.
- In this, the haploidization takes place by aberrant mitosis, whose frequency is, however, very low. It was first discovered by Pontecorvo and Roper in 1952 in Aspergillus nidulans.

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LECTURE 8

CLASSIFICATION OF FUNGAL PLANT PATHOGENS

INTRODUCTION

- **Traditionally**, fungi were classified under Thallophyta division of Plantae Kingdom under 4 classes, viz., phycomycetes (also called lower fungi including Oomycetes, Chytridiomycetes and Zygomycetes), ascomycetes, basidiomycetes and deuteromycetes (including fungi imperfecti).
- **Ainsworth's classification** (1966, 1973) included kingdom Fungi with Myxomycota and Eumycota divisions. The Eumycota division was divided into 5 sub-divisions, viz., mastigomycotina, zygomycotina, ascomycotina, basidiomycotina and deuteromycotina which were further sub-divided into (classes, orders and so on).
- Classification system based on information on phylogenetic relationships of fungi obtained by small submit (18 S) ribosomal RNA gene comparison are now used. **The classification given by Hawksworth et al. (1995) and partially modified by Kirk et al. (2001) is now universally accepted.** According to it, the fungi are placed in three kingdoms: Fungi, Protozoa and Straminopila
- All the taxa (kingdom to species) are now italicized in print and underlined when hand written.

There are standard endings for the various taxa. Phylum ends in -mycota, class in –mycetes, sub-class in -mycetidae, order in –ales, family in –aceae, and genus and species have no fixed endings.

- Genus is always written with the first letter in capital (proper noun).
- Species in small letters as adjective qualifying the noun.

Kingdom Fungi
They are characterized by:

- Unicellular or filamentous somatic phase
- Cell wall made of chitin and glucans
- Absorptive mode of nutrition
- Presence of only whiplash type of flagella
- Mitochondria with flattened cristae, and
- Presence of peroxisomes and golgi bodies

There are four Phyla in Kingdom Fungi: Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota

- **The imperfect fungi that reproduce only asexually and were earlier placed in Deuteromycotina (Ainsworth, 1973) are no more accepted as a distinct taxonomic category.** They are not monophyletic unit, but are fungi which are anamorphs (asexual stages) of Ascomycota and Basidiomycota.
- Those which have not been shifted to Asco- or Basidiomycota are placed under **mitosporic or anamorphic** fungi.

Kingdom Straminopila
These are characterized by:

- Unicellular or filamentous somatic phase
- Cell wall made of cellulose
- Absorptive mode of nutrition
- Presence of tinsel type of flagella
- Tubular mitochondrial christae
- Presence of peroxisomes and golgi bodies

They showed closer genetic relationship to brown algae and diatoms rather than the Fungi in 18 S r RNA phylogenetic studies.
There are 3 phyla: Hyphochytridiomycota, Labrynthulomycota, Oomycota.

Kingdom Protozoa
These are characterized by:

- The somatic phase is plasmodial or amoeboid.
- There is no cell wall in the somatic phase.
- Nutrition is by ingestion except in plant parasites (e.g., Plasmodiophoromycota), where the plasmodium obtains nutrition by absorption.
- Mitochondrial cristae are tubular.

There are 4 phyla: Acrasiomycota, Dictyosteliomycota, Myxomycota, Plasmodiophoromycota.

OUTLINE CLASSIFICATION OF FUNGI

Pseudofungi

Kingdom 1: Protozoa (uni-cellular microorganisms)
Phylum i: Myxomycota
Class: Myxomycetes (plasmodial slime moulds, do not infect plants)
Order: Physarales
Genera: Mucilago, Fulago, Physarum causing slime moulds in low lying plants)

Phylum ii: Plasmodiophoromycota (endoparasitic slime moulds)
Class : Plasmodiophoromycetes
Order: Plasmodiophorales
Genera: Plasmodiophora, P. brassicae causing club root of crucifers, Polymyxa, P. graminis on wheat and other cereals, and can transmit viruses, Spongospora, S. subterranea causing powdery scab of potato tubers

Kingdom 2: Chromista/ Straminopila (microorganisms unicellular or multi-cellular)
Phylum i: Oomycota (zoospores flagellate)
Class: Oomycetes (mycelium non-septate, sexual spores- oospores, asexual spores- zoospores)
Order: Peronosporales
Family: Peronosporaceae, (the downy mildews, sporangia borne on sporangiophores of determinate growth, obligate parasites)
Genera: Plasmopara, P. viticola causing downy mildew of grapes, Peronospora, P. tabacina causing downy mildew/ blue mould of tobacco), Bremia, B. lactucae causing downy mildew of lettuce, Pseudoperonospora, P. cubensis causing downy mildew of cucurbits; Sclerospora, S. graminicola causing downy mildew/ green ear disease of pearl millet
Family: Pythiaceae (sporangia, usually zoosporangia are produced along somatic hyphae, or tips of the hyphae of indeterminate growth and set free. OSogonia thin walled. Facultative parasites)
Genera: Pythium, P. debaryanum causing damping off of seedlings; Phytophthora, P. infestans causing late blight of potato
Family: Albuginaceae (the white rusts, sporangia borne in chains)
Genus: Albugo, A. candida causing white rust of crucifers

True Fungi

Kingdom 3: Fungi (produce mycelium, the walls of which contain glucans and chitin)
Phylum i: Chytridiomycota (produce zoospores with single posterior flagellum)
Class: Chytridiomycetes
Order: Chytridiales
Family: Olpidiaceae
Genera: Olpidium, O. brassicae being parasitic on roots of cabbage and other plants, and can transmit plant viruses; Physoderma, P. maydis causing brown spot of corn; Synchytrium endobioticum causing potato wart

Phylum ii: Zygomycota (produce non-motile spores in sporangia. Sexual spores are zygospores)
Class: Zygomycetes (bread moulds)
Order: Mucorales
Family: Mucoraceae

Genera: Rhizopus, R. stolonifer causing soft rot of fruits and vegetables; Choanephora, C. cucurbitarum causing soft rot of squash; Mucor causing bread mould and storage rots of fruits and vegetables)
Order: Glomales (the endomycorrhizae)
Genera; Glomus, Acaulospora, Gigaspora, Scutellospora

Phylum iii: Ascomycota (the sac fungi, produce ascospores)
Class i: Archiascomycetes
Order: Taphrinales (asci naked arising from binucleate ascogenous cells)
Family: Taphrinaceae
Genus: Taphrina, T. deformans causing peach leaf curl
Family: Protomycetaceae
Genus: Protomces, P. macrosporus causing stem gall of coriander
Class ii: Saccharomycetes (the yeasts, mostly unicellular fungi that reproduce by budding, asci naked, no ascocarps produced)
Order: Saccharomycetales
Family: Saccharomycetaceae
Genera: Galactomyces causing citrus sour rot; Saccharomyces, S. cerevisiae, the bread yeast
Class iii: Plectomycetes (ascomycetes with cleistothecia)
Order: Eurotiales
Family: Eurotiaceae
Genera: Eurotium, teleomorph of Aspergillus, causing bread mould and seed decay; Talaromyces, teleomorph of Penicillium, P. expansum causing blue mould rot of fruits
Order: Erysiphales (the powdery mildew fungi, asci formed in cleistithecia. mycelium, conidia, and cleistothecia produced on the host surface, obligate parasites)
Family: Erysiphaceae
Genera: Blumeria, B. graminis causing powdery mildew of cereals and grasses; Erysiphe cichoracearum causing powdery mildew of cucurbits; Leveillula, L. taurica causing powdery mildew of tomato and capsicum; Podosphaera, P. leuchotricha causing powdery mildew of apple; Sphaerotheca, S. pannosa causing powdery mildew of rose and peach; Uncinula, U. necator causing powdery mildew of grapes
Class iv: Pyrenomycetes (ascomycetes with perithecia)
Order: Hypocreales
Genera: Hypocrea rubra is the teleomorph of Trichoderma viride; Nectria galligena causing European canker of apple; Gibberella fujikuroi causing bakane/ foolish disease of rice; Claviceps purpurea causing ergot of grain crops
Order: Microascales
Genera: Ceratocystis, C. fagacearum causing oak wilt; C. fimbriata causing root rot of sweet potato, C. paradoxa causing butt rot of pineapple
Order: Phyllachorales.
Genera: Glomerella, G. cingulata (teleomorph of Colletotrichum sp.) causing many anthracnose diseases and bitter rot of apple; Phyllachora graminis causing leaf spot of grasses
Order: Ophiostomatales
Genera: Ophiostoma, O. novo-ulmi causing Dutch elm disease
Order: Diaporthales
Genera: Diaporthe, D. citri causing citrus melanose, D. vexans causing fruit rot of eggplant, Gnomonia leptostyla causing leaf blotch of walnut, Cryphonectria parasitica causing chestnut blight; Leucostoma (formerly Valsa) causing canker disease of peach and other trees
Order: Xylariales

Genera: Hypoxylon, H. mummatum causing severe canker of poplars; Rosellinia, R. necatrix causing root rot diseases of trees and vines; Xylaria, causing tree cankers and wood decay; Eutypa, E. armeniacae causing apricot canker
Class iv: Loculoascomycetes (ascomycetes with ascostroma or pseudothecium)
Order: Dothidiales
Genera: Mycosphaerella, M. musicola causing Sigatoka of banana, M. fragariae causing leaf spot strawberry; Elsinoe, E. fawcetti causing citrus scab, E. ampelina causing grape anthracnose
Order: Capnodiales
Genera: Capnodium, being one of the many fungi causing sooty moulds on the plants
Order: Pleosporales
Genera: Cochliobolus, the teleomorph of Helminthosporium or Bipolaris causing leaf spot diseases on many plants including C. miyabeanus causing brown spot disease of rice leading to the infamous Bengal famine, Pleospora, teleomorph of Stemphylium) causing black mould of tomato, Leptosphaeria, the teleomorph of Phoma causing black leg and foot rot of cabbage, Venturia, V. inaequalis causing apple scab and V. pirina causing pear scab, Guignardia bidwelli causing black rot of grapes
Class v: Discomycetes (ascomycetes with apothecia)
Order: Rhytismatales
Genera: Lophodermium, L. seditiosum causing pine needle cast, Rhytisma acerinum causing tar spot of maple leaves
Order: Helotiales
Genera: Monilinia, M. laxa, M. fructigena, M. fructicola causing brown rot of pome and stone fruits, Sclerotinia, S. sclerotiorum causing white mould and watery soft rot of vegetables, Stromatinia, S. gladioli causing corm rot of gladiolus, Diplocarpon, (the teleomorph of Marssonina), D. maculatum causing black spot of quince and pear, D. rosae causing black spot of rose, D. mali leaf blotch and premature leaf fall of apple
Class vi: Deuteromycetes or mitosporic fungi (imperfect fungi): Sexual reproduction or structures rare, lacking or unknown.

Phylum iv. Basidiomycota (basidiomycetes, the club and mushroom fungi)
Class i: Urediniomycetes (rust fungi)
Order: Uredinales
Family: Pucciniaceae
Genera: Puccinia, P. graminis tritici causing black rust of wheat, P. recondita causing brown rust of wheat, P. striiformis causing yellow rust of wheat, P. dianthii causing rust of carnation. Gymnosporangium, G. juniperi-virginianae causing cedar apple rust; Cronartium, C. ribicola causing rust of pine; Hemileia, H. vastatrix causing coffee rust; Melampsora, M. lini causing flax rust; Phragmidium, P. mucronatum causing rose rust; Uromyces, U. appendiculatus causing rust of bean
Class: Ustilaginomycetes (smuts and bunts)
Order: Ustilaginales
Family: Ustilaginaceae
Genera: Ustilago, U. tritici causing loose smut of wheat, U. maydis causing corn smut, U. nuda, causing smut of barley; Urocystis, U. cepulae causing smut of onion
Family: Tilletiaceae
Genera: Tilletia, T. caries and T. foetida causing hill bunt of wheat, T. (Neovossia) indica causing Karnal bunt of wheat
Class: Basidiomycetes (earlier grouped under hymenomycetes and gasteromycetes) produce basidiospores, basidia and basidiocarps)

Order: Exobasidiales (no fruiting bodies or basidiocarps)
Family: Exobasidiaceae
Genera, Exobasidium, E. vexans causing blister blight of tea
Order: Ceratobasidiales
Genera: Athelia, the teleomorph of Sclerotium, S. rolfsii causing southern blight and root rot of many plants, S. cepivorum causing the white rot onion; Thanatephorus, T. cucumrris , the teleomorph of Rhizoctonia solani, causing root rot and web blight of many plants; Typhula, causing Typhula blight or snow mould of turf grasses
Order: Agaricales (the mushrooms)
Family: Agaricaceae
Genera: Agaricus, A. bisporus , the white button mushroom; Armillariella (Armillaria) mellea causing root rot of trees; Marasmius, causing fairy ring disease of turf grass, Pleurotus, causing white rot on logs, tree stumps and living trees; Pholiota, causing brown wood rot in deciduous forest trees
Family: Pluteaceae
Genus: Amanita, A. muscaria, the fly agaric is a poisonous mushroom
Family: Lycoperdaceae
Genus: Lycoperdon, the puff balls
Order: Phallales
Family: Geastraceae
Genus: Geastrum, the earth stars
Order: Aphyllophorales
Genera: Chondrostereum, C. purpureum causing the silver leaf disease of trees, Corticium, C. salmonicolor causing pink disease in many fruit and other trees; Heterobasidion, H. annosus causing root and butt rot of many trees; Ganoderma, G. lucidum causing root and basal rot in many trees; Phelinus, causing tree root rot and cubical rots in buildings; Peniophora, P. gigantea causing decay in coniferous logs and pulpwood; Polyporus, P. squamosus causing heart rot of many forest trees

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LECTURE 9

GENERAL CHARACTERISTICS AND REPRODUCTION OF BACTERIAL PLANT PATHOGENS

GENERAL CHARACTERISTICS

Bacteria are second most important organisms which cause plant diseases.

- They are prokaryotic single celled mostly achlorophyllous organisms whose body is surrounded by cell wall and nuclear material is not surrounded by membrane.
- They lack membrane bound organelles such as mitochondria or plastids and also a visible endoplasmic reticulum.
- Most of the bacterial species are saprophytes living on dead organic matter. There are about 200 bacterial species which are plant pathogenic.
- Morphologically the bacteria are rod shaped (bacilli), spherical (cocci), spiral (spirilli), coma shaped (vibrios) or thread like (filamentous).
- Streptomyces has a filamentous branched hypha-like structure, sometimes mistakenly called as ray fungi; and mycoplasma have no definite shape due to lack of cell wall.
- In young cultures the rod shaped bacteria range from 0.6 to 3.5 µm in length and from 0.5 to 1µm in diameter (0.6-3.5 x 0.5-1 µm size).
- Single bacterium mostly appears as hyaline or yellowish white under the compound microscope, when grown on a medium, soon a colony is formed.
- The colonies of most of bacteria have a whitish or greyish appearance but some of them develop yellow, red or other colours.

Bacterial Cell Structure

- A bacterium has a thin, relatively tough, rigid cell wall, and a distinct three layered but thin cytoplasmic membrane.
- Most bacteria have a slime layer made up of viscous gummy material. Slime layer has bacterial immunological property.
- When the layer is thick and firm, it is called capsule.
- Generally plant pathogenic bacteria lack capsule but some of them like Pseudomonas and Xanthomonas produce slime.
- Slime layer is mostly composed of polysaccharides but may rarely contain amino sugars, sugar acids, etc.

Flagella

- Most of the plant pathogenic bacteria have delicate thread like flagella, which are usually longer than the cell
- They are the organs of locomotion.
- The arrangement of flagella on bacterial cell is an important taxonomic character that aid in bacterial classification.
- This arrangement may be
- **Monotrichous-** with one polar flagellum
- **Lophotrichous** -tuft of flagella at one end
- **Amphitrichous**- at both the ends

- **Peritrichous** - distributed all around the cell or surface.
- **Atrichous**- bacteria lacking flagella.

Gram Staining

Bacterial species are often distinguished from one another by Gram staining.

- In this process, a bacterial smear is heat fixed on glass slide, stained with crystal violet and mordanted with iodine and finally rinsed with ethanol.
- When the bacteria retain the crystal violet stain after rinsing, the bacteria are called gram positive; and those which do not retain the stain are called gram negative.
- The later are then counter stained with pink colour safranin.
- The ability of bacteria to retain crystal violet stain or not, depends upon fundamental structure of cell wall.

Gram positive vs Gram negative bacteria

Gram Positive bacteria	Gram Negative bacteria
1. Cell wall is thicker and homogemous.	Cell wall is thinner and usually thin layered.
2. Contains lower content of lipids (5-10%)	Contains higher content of lipids (up to 40%)
3. Peptidoglycan comprises up to 90% of the cell wall and hence maximum lipid.	Peptidoglycan comprises only 10%.
4. Techoic acid present.	Techoic acid absent.
5. Cell wall has higher amino sugar content (10-20%)	Low content of amino sugars
6. Cell wall is simple in shape and is single layered.	Varying cell wall shape and is tripartite (3-layered).
7. Mesosomes more prominent.	Mesosomes less prominent.
8. Retains violet dye	Retains red dye
9. Examples: Bacillus, Clavibacter, Streptomyces	Examples: Erwinia, Pseudomonas, Xanthomonas, Agrobacterium, Xylella

BACTERIAL CELL STRUCTURE

Bacterial Cell Structure

- The bacterial cell is surrounded by a cell wall composed of peptidoglycan consisting of chain of alternating N-acetyl muramic acid and N-acetyl glucosamine units cross linked by tetrapeptide and pentaglycine units.
- The cell wall allows the inward passage of nutrients and the outward passage of waste matter and digestive enzymes.
- All the material inside the cell wall constitutes the protoplast.
- The protoplast consists of a cytoplasmic or protoplast membrane, which determines the degree of selective permeability of various substances into and out of the cell.
- The cytoplasmic membrane of bacteria resembles those of eukaryotes, but also contains respiratory and other enzymes located in the bacteria.
- The cytoplasm, which is a complex mixture of proteins, lipids, carbohydrates, many other organic compounds, minerals and water.
- The nuclear material consists of large circular chromosome, composed of DNA.
- The chromosomal DNA makes up the main body of genetic material of the bacterium and appears as a spherical, ellipsoidal, dumb-bell or Y-shaped body in the cytoplasm, but without any membrane.
- Such nuclear material does not show meiosis and mitosis.
- Some species also have additionally single or multiple copies of smaller circular genetic material called plasmids.
- Plasmids can move from one bacterium to another and even from the bacterium to plants as in crown gall disease. This special property is being utilized with much success in genetic engineering for transformation of some desired genes from one plant to another by using it as vector.

Flagella

- In bacteria, flagella are the organs of locomotion.
- They are very delicate and fragile and cultures are to be handled carefully for their staining.
- The flagella vary from 10-12 nm in width which is smaller than wavelength of light, therefore, cannot be seen by ordinary staining.
- Mordants like potassium sulphate and mercuric chloride are generally precipitated on flagella making the width more for making them visible under light microscope.

Parts of a Flagellum

- **Filament:** It is the outermost region of flagellum, and is helical, composed of flagellin with a molecular weight of 30000-40000 and is synthesized in the cell, which moves to the hollow core of the flagellum to the tip. Flagellin is a protein with 14 amino acids and is characterised by higher content of aromatic amino acids and absence of cysteine in many cases.
- **Hook:** Filament is attached to hook which is wider than the flagellum. This is 45 nm wide and made up of different types of protein. The hook of gram positive bacterium is longer than that of gram negative bacteria.
- **Basal body:** The third part called basal body consists of small central rod which is inserted into a system of rings. The gram positive and gram negative bacteria are

different in the number of rings. The inner pair of rings (S and M) are embedded in cell membrane and are formed in both gram positive and gram negative bacteria. L and P rings are formed only in gram negative bacteria. S and M rings are important for movement of flagella.

Pili

- In some bacteria, small hair like structures are also present which are called pili.
- These are shorter than the flagella and are thicker (3-15 nm in diameter).
- The term fimbriae is sometimes also used for pili, but the term pili is reserved for those which are involved in conjugation.
- They are made up of protein sub-units pilin of molecular weight of 70000.
- It consists of a helically coiled fibre with a central hole of 2 nm in diameter.
- Fimbriae may be involved in attachment, whenever there is infection. Both flagella and pili originate from cell membrane and extend outward through the cell wall.

Reproduction

Bacteria multiply at a phenomenal rate by the process of fission or binary fission.

- As the cytoplasm and cell wall undergo division into two, the nuclear material is organized into a circular chromosome like structure which ultimately duplicates itself and gets distributed equally into 2 newly formed cells.
- Similarly, plasmids also duplicate and come into 2 daughter cells.
- The duplication occurs rapidly, once every 20 minutes.
- As a result a bacterium like Eschersia coli, starting from one bacterium may produce 1 million bacteria in 10 hours.
- However, this number is not reached because of gradual limitations of nutrients and toxic metabolites. Still what is achieved normally is phenomenonal.
- Such prolificacy in multiplication must be of great advantage both in survival of bacterial pathogen, and also for successive plant infections.

Recombination

The genetical recombination in bacteria has been noticed by the following sexual-like processes:

- **Conjugation:** Conjugation occurs when two compatible bacteria come into contact and part of the chromosomal or non-chromosomal genetic material of one is transferred to the other and incorporated into the genome of later through conjugal zygote formation and breakage and reunion. It was first observed by Lederberg and Tatum (1956) in E. coli.
- **Transformation:** It occurs when the bacterium is genetically transformed by absorption of genetic material of another compatible bacterium, secreated by or released in a culture during the rupture, and its incorporation into the genome of the former. It was first observed by Griffith (1928) in Enterococcus pneumoniae.
- **Transduction:** When genetic material from one bacterium is carried by its phage (virus) to another bacterium that it visits next and the later is genetically transformed. It was first discovered by Zinder and Lederberg (1952) in Salmonella.

Mycoplasma/PPLO’s

Mycoplasma, earlier known as ‘Pleuro Pneumonia Like Organisms’ (PPLO’s) were discovered to be associated with the disease bovine pleuro pneumonia and were described in one of the orders mycoplasmatales under Eubacteria.

- Mycoplasma represent a group of organisms that lack cell wall and contain a very small genome.
- Phylogenetically, they are closely related to clostridia, the gram positive bacteria.
- As per the requirement for their growth, they can be divided into those which require sterol (mycoplasma and spiroplasma); and those which do not require sterols (acholeplasma and thermoplasma).
- The mycoplasma cells are small, pleomorphic (of different shapes) and divide by budding. The colonies of mycoplasma on agar exhibit a characteristic ‘fried egg’ appearance because of the formation of dense central core surrounded by lighter circular spreading area.
- The growth of mycoplasma is not inhibited by penicillin or other antibiotics that inhibit cell wall synthesis. But they are sensitive to tetracycline.
- The Spiroplasma genus is important plant pathogenically and has cork screw shaped cells. They are motile and exhibit undulating or rotating movement. Spiroplasma citri has been associated with citrus plants, where it causes citrus stubborn disease and corn plants which causes corn stunt.

Phytoplasma

They were earlier called MLO’s and were found to be associated with several yellows and witches’ broom diseases after their discovery by Doi et al. in 1967.

- They are different from mycoplasma in the sense that they can not be cultured on synthetic media.
- The change in terminology from MLO’s to phytoplasma occurred since the studies of DNA homology in the highly conserved genes encoding ribosomal RNA and ribosomal protein.
- It showed that the phytoplasma comprise a coherent group distinct from other prokaryotes. Their closest relatives are in the genus Acholeplasma.
- As they have not been cultured on artificial medium in vitro and characterized apart from their host, they are referred to Candidatus status.
- They are associated with about 200 plant diseases including aster yellows, apple proliferation, peanut witches’ broom, peach-x-disease, rice yellow dwarf and elm yellows.
- They are phloem inhabiting organisms and are graft transmissible in nature, and can also be transmitted by leaf hoppers.

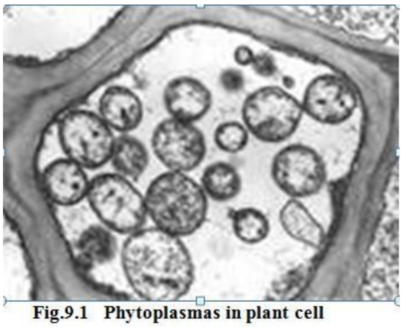

Fig.9.1 Phytoplasmas in plant cell

Bacteria as Plant Pathogens

- Bacteria are known to grow in a wide range of habitat.
- All the plant pathogenic bacteria are mesophilic (they can grow at a temperature of 20-35ºC); and remain in the host plants as plant parasites and only partly in plant residues or as saprophytes in soil.
- They enter the plants either through natural openings such as stomata, lenticels or hydathodes or through wounds.
- The presence of free water is essential for bacterial infection. Once inside the plant tissues, they multiply only if there is water or at very high humidity.
- They multiply in the intercellular spaces and produce pectolytic and other cell wall degrading enzymes, thereby creating more space to move inside the host tissue.
- They kill the host cells by the action of extracellularly released enzymes and toxins and subsequently invade the dead cells. Most of the bacterial pathogens are necrotrophs.
- Some are apparently biotrophs.
- Some species colonize the xylem vessels and because of their physical presence or the slime ultimately cause the plugging of the water conducting tissues and cause wilt symptoms.
- Plant pathogenic bacteria produce various types of symptoms in plants as are caused by fungal pathogens. They cause soft rot of vegetables and fruits, wilts, cankers, scabs and also over-growths.

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LECTURE 10

CLASSIFICATION OF BACTERIAL PLANT PATHOGENS

CLASSIFICATION

Traditionally bacteria have been included in Plantae kingdom under Thallophyta; however, Haeckel in 1966 proposed the kingdom Protista to include all unicellular organisms and placed various organisms of Thallophyta plants and Protozoa animals in Protista. Later, the nucleus character was given more importance. Chatton proposed the most appropriate conceptual basis for taxa at the highest level by recognizing two general patterns of cellular organelles as prokaryotes and eukaryotes in 1937. Stanier (1969) considered prokaryotes as lower protists including blue green algae, myxobacteria and eubacteria; and eukaryotes as higher protists including algae, fungi and protozoa. Prokaryotae was recognised a separate kingdom. However, the correct concept is that of 5 kingdoms according to Whittaker (1969) including Plantae, Animalia, Fungi, Protista and Monera (Prokaryotes).

- In 'Bergey's Manual of Determinative Bacteriology' the phytopathogenic bacteria have been classified into three divisions:

Division I – Gracilicutes
They include prokaryotes with thin cell walls consisting of outer membrane with fatty acid glycerol ester-type lipids and are usually gram negative. They do not form endospores.

Division II – Firmicutes
It included prokaryotes with thick (firm) cell wall consisting of peptidoglycan and unit membrane but without any outer membrane. Some of them produce endospore. They are gram positive.

Division III – Tenericutes
They lack cell wall and cells are enclosed by a unit membrane only. They include mollicutes or mycoplasma like organisms (now called phytoplasma).

Detailed Classification of Phytopathogenic Bacteria
Kingdom: Prokaryotae
Division I: Gracilicutes
Class: Proteobacteria (mostly single-celled, non-photosynthetic)
Family 1: Enterobacteriaceae (They are peritrichous bacteria)
Genus: Erwinia
E. amylovora causing fire blight of apple and pear
E. carotovora pv. *carotovora* causing soft rot of vegetables
E. carotovora pv. *atroseptica* causing black leg of potato
Family 2: Pseudomonadaceae
Genus: Pseudomonas
P. syringae pv. *syringae* causing stone fruit bacterial canker
P. syringae pv. *tabaci* causing wild fire disease of tobacco
Genus: Ralstonia
R. solanacearum causing bacterial wilt of solanaceous crops
Genus: Xanthomonas

X. campestris pv. *campestris* causing black rot of cabbage, *X. campestris* pv. *phaseoli* causing common bean blight
X. campestris pv. *vesicatoria* causing tomato bacterial spot
X. oryzae pv. *oryzae* causing bacterial leaf blight of rice
X. axonopodis pv. *citri* causing citrus canker
Family 3: Rhizobiaceae
Genus: Agrobacterium
A. tumefaciens causing crown gall of stone fruits
A. rhizogenes causing hairy root of apple
Family : Still unnamed
Genus: Xylella
X. fastidiosa [earlier called RLO's rickettssia like organisms] xylem- inhabiting causing Pierce's disease of grapevines, phony peach, almond leaf scorch
Candidatus liberobacter asiaticus, phloem-inhabiting causing citrus greening
Unnamed, latex-inhabiting, causing bunchy top disease of papaya

Division 2: Firmicutes
Class 1: Firmibacteria (Simple gram positive bacteria)
Bacillus subtilis – biocontrol agent
Class 2: Thallobacteria (Gram positive, branching bacteria)
Streptomyces scabies causing common scab of potato
Clavibacter michiganense pv. *sepedonicum* causing ring rot of potato
Clavibacter michiganense pv. *michiganense* causing bacterial canker of tomato
Curtobacterium (Corynebacterium) flaccumfaciens causing bacterial wilt of bean

Division 3: Tenericutes
Class: Mollicutes (wall less prokaryotes)
Family: Spiroplasmataceae
Spiroplasma citri causing citrus stubborn
Spiroplasma kunkelii causing corn stunt

Several organisms called phytoplasma have been reported to cause various yellows and witches' broom type diseases are included in this group and have been given Candidatus status for the time being due to the inability of their culturing.

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LECTURE 11

GENERAL CHARACTERISTICS AND CLASSIFICATION OF VIRAL PLANT PATHOGENS

GENERAL CHARACTERISTICS AND STRUCTURE

Characteristics of viruses which separate them from other causes of plant pathogens are:

- They are acellular.
- They are sub-microscopic and intracellular.
- They lack lipid membrane system and energy production.
- They use host machinery for their replication.

Sturcture of virus

- Virion is a technical term used for the virus particle. A virion consists of nucleic acid surrounded by a protein coat.
- The nucleic acid is called 'nucleoid' which may be either de-oxyribonucleic acid (DNA) or ribonucleic acid RNA (mostly RNA in plant viruses), but never both; and forms the genome.
- The protein coat is called 'capsid'. It consists of many subunits which are similar and occasionally dissimilar, and these subunits are called capsomeres.
- The combined genome and the capsid are called 'nucleocapsid'.
- Some viruses possess an envelop around the protein coat which is made of virus proteins and host cell lipids. These viruses are called 'enveloped viruses'.
- In many groups of viruses, there is an additional protein layer between the capsid and the nucleoid. This is called 'virus core'.
- In addition to the typical nucleoprotein composition, some viruses have carbohydrates/ lipids / enzymes.

Nucleoid

- The nucleoid (nucleic acid component) is located internally within a protein coat.
- Only one type of nucleic acid, i.e. either RNA or DNA is found in a virus.
- The amount of nucleic acid in a virion varies from 1 to 50 per cent.
- Higher percentage of nucleic acid is associated with larger DNA viruses like bacteriophages; while low content is found in animal viruses.
- The nucleic acid is infectious part and contains the genetic information for the synthesis of proteins and its own replication; and their assembly into the virion.
- Most of the plant viruses contain RNA, with exceptions like Cauliflower mosaic virus.

Capsid
The capsid is a protein coat surrounding the nucleoid and has the following functions:

- It protects nucleic acid from unfavourable extracellular environment.
- It facilitates nucleic acid entry into the host cells.

- It is antigenic.
- As compared to nucleoid, the protein coat shows a complex structure and provides shape to the virus particles.
- It interacts with the vector for specific transmission.

Morphology of Viruses

Viruses are of different shapes and sizes. They may be:

i. Elongated (rigid rods or flexuous threads)

ii. Spherical (isometric or polyhedral)

iii. Cylindrical (bacillus-like rods)

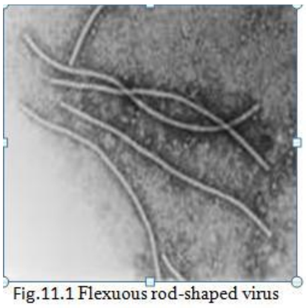
Fig.11.1 Flexuous rod-shaped virus

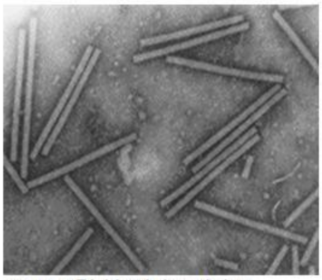
Fig.11.2 Rigid rod-shaped virus

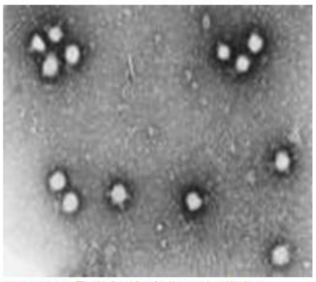
Fig.11.3 Polyhedralvirus particles

- Some elongated viruses are rigid rods about 15 x 300 nm in size, but most appear as long, thin, flexible threads that are usually 1-10 nm wide and 480-2000 nm in length.

- Rhabdoviruses are short bacillus-like cylindrical rods approximately three to five times as long as they are wide (52-75 x 300-380 nm in size).

- Most spherical viruses are actually polyhedral, ranging in diameter from about 17 nm (Tobacco necrosis satellite virus) to 60 nm (Wound tumor virus).

- Tomato spotted wilt virus is surrounded by a membrane and has a flexible, spherical shape about 100 nm in diameter.

- Many plant viruses have spilt genome consisting of two or more distinct nucleic acid strands encapsidated in different-sized particles made of the same protein subunits. For example: Bipartite, Tobacco rattle virus consisting of two rods, a long one (195 x 25 nm) and a shorter one (43 x 25 nm) and Multi-partite Alfalfa mosaic virus, consisting of four components of different sizes.

Composition and structure of viral proteins

- Viral proteins, like all proteins, consist of amino acids.
- The sequence of amino acid within a protein, which is encoded by the sequence of nucleotides in the genetic material, determines the nature and properties of the protein.
- For example, the protein subunit of tobacco mosaic virus consists of 158 amino acids in a constant sequence and has a mass of 17,600 daltons.

Composition and structure of viral nucleic acids

- Nucleic acid of most plant viruses consists of RNA, but now, a larger number of viruses have also been shown to contain DNA as its genome.
- Both RNA and DNA are long chain-like molecules consisting of hundreds or most of them thousands of units called nucleotides.
- Each nucleotide consists of a ring compound called the base attached to a five-carbon sugar (ribose in RNA, de-oxyribose in DNA), which in turn is attached to phosphoric acid.
- The sugar of one nucleotide reacts with the phosphate of another nucleotide, which is repeated many times, thus forming the RNA or DNA strand.
- In viral RNA, only one of four bases, adenine, guanine, cytosine and uracil can be attached to each ribose molecule. The first two, adenine and guanine, are purines and interact with the pyrimidines, uracil and cytosine.

VIRUS INFECTION AND TRANSMISSION

Virus Infection and synthesis

- Plant viruses enter cells only through the wounds made mechanically or made by vectors, or are deposited into an ovule by an infected pollen grains.
- In a RNA replication of an RNA virus, the nucleic acid (RNA) is first freed from the protein coat.
- It induces the host cell to form the viral RNA polymerase.
- The RNA polymerase utilizes the viral RNA as a template and forms complementary RNA.
- The first new RNAs produced are not the viral RNAs but are the mirror images (complementary copies) of that RNA.
- As the complementary RNA is formed, it is temporarily connected to the viral strand. Thus, the two form a double-stranded RNA that soon separates to produce the original virus RNA and the mirror image (-) strand, with the latter then serving as a template for more virus (+) strand RNA synthesis.
- In dsDNA viruses, the viral ds DNA enters the cell nucleus and appears to become twisted and supercoiled and forms a minichromosome.
- The latter is transcribed into two single-stranded RNAs.
- The smaller RNA is transported to the cytoplasm, where it is translated into virus-coded proteins
- The larger RNA is also transported to the same location in the cytoplasm, but it becomes encapsidated by coat protein subunits and is used as a template for the reverse transcription into a complete virion dsDNA.

Spread of viruses

- For successful infection, viruses move into the adjacent cell through plasmodesmata intracellularly with cytoplasmic streaming.
- Some viruses move through host parenchyma cells, causing mostly local lesions.
- Many viruses move over fairly long distances through phloem specifically through sieve tubes and spread systemically and often quite rapidly in their host plant through vascular streams.
- Such movement is mostly through phloem but rarely xylem transport also occurs, e.g., Lettuce necrosis yellows virus and Southern bean mosaic virus.
- Once the virus enters the phloem, it moves rapidly into the sieve tubes towards the growing regions and other food utilizing parts of the plant.
- Virus movement is mostly upwards. Once the virus reaches the phloem, it spreads systemically throughout the plant and then moves to adjacent parenchyma cells through the plasmodesmata. This systemic distribution of viruses affects all parts of host plant except the apical meristem in some cases.

Transmission of Viruses

- Viruses must be brought in contact with the contents of living host cells. They achieve this quite effectively by transmission from an infected plant to a healthy plant in a number of ways.

Through vegetative propagation

- The viruses are transmitted from the infected plant parts to the healthy ones of the same generation and it results in only primary infection and occurs in monocyclic diseases.
- Examples are mosaics and leaf roll of potato, and sugarcane viruses.

Mechanical transmission through sap

- Some viruses can transmit from diseased to healthy plants through the mechanical transmission of the infected sap by touch.
- Virus infected sap adhering to tools, implements, insect mouth parts, and body, hands, clothes of man etc. can transmit viruses to the wounded plant cells.
- This type of mechanical transmission is wide spread in Tobacco mosaic virus (TMV), Potato virus X, etc.

Seed transmission: Majority of seed transmitted viruses are carried internally.

- Virus may be carried to the seed from the infected ovule or the pollen.
- A small percentage show seed transmission.
- Examples are muskmelon mosaic virus in watermelon, barley stripe mosaic virus, tobacco ring spot virus in soybean, common bean mosaic virus.

Pollen transmission

- Pollen transmission of virus occurs in sour cherry infected with Prunus necrotic ring spot virus.

Insect transmission

- Aphids, leaf hoppers, white flies, mealy bugs and scale insects constitute the insect vectors. However, aphids and leaf hoppers transmit a larger number of viral diseases and are most important .
- The viruses can be classified as:

a) **Stylet borne viruses** (mostly aphid transmitted), which do not go into the system of insects and remain near the tip of the stylet and are lost after one or two visits. They are also called 'non-persistent viruses, e.g., Cucumber mosaic virus, Papaya ring spot virus, etc. b) **Circulative viruses**, mostly vectored by leaf hoppers, which take them into their system and after circulation, they are returned to the stylet, mixed with the saliva and are transmitted to healthy plants they visit, e.g., Maize streak virus

- Some viruses of circulative nature may multiply inside the insect body and are called as 'propagative viruses'. Leaf hoppers transmitted viruses are mostly circulative and propagative in nature, e.g., Tomato spotted wilt virus

c) **Persistent viruses**, which are acquired by leaf hoppers and are incubated for 1-2 weeks and become viruliferous. Once they start transmitting viruses, they remain infective for long periods or even for rest of their life, e.g., Banana bunchy top virus.
d) **Transovarial transmission**, in which the viruses once acquired are transferred to their following generations.

Examples of other Insects transmitting viruses:

- Green peach aphid transmits potato virus Y (Potyvirus) and potato leaf roll virus (PLRV)
- Leaf hoppers transmit rice stunt virus, aster yellow virus etc.
- White flies can transmit Papaya leaf curl virus, Yellow vein mosaic virus, Tomato leaf curl virus
- Mealy bug transmits Swollen shoot of cocoa
- Thrips transmit Tomato spotted wilt virus
- Beetles transmit Squash mosaic virus, Cowpea mosaic virus and Turnip yellow mosaic virus
- Grass hopper transmits Tobacco mosaic virus (TMV), Potato virus X (PVX) and Tobacco ring spot virus.

Mite transmission
Mites transmitting viruses have piercing and sucking mouth-parts.
1. Examples are Wheat streak mosaic virus, Peach mosaic virus and viruses of sterility disease of pigeon pea.

Fungus transmission: Three major classes of fungi can transmit viruses.
(a) Chytridiomycetes- Olpidium brassicae transmits Lettuce big vein virus; and Synchytrium endobioticum transmits Potato virus X and Potato mop top virus .
(b) Plasmodiophoromycetes- Polymyxa graminis transmits Wheat soil borne mosaic virus, Spongospora subterranea transmits Potato mop top virus.
(c) Oomycetes- Pythium ultimum transmits Pea false leaf roll virus. Nematode transmission:

2. NEPO viruses- Longidorus and Xiphinema species are shown to transmit several polyhedral viruses, such as Grapevine fan leaf virus, Tobacco ring spot virus, Cherry leaf roll virus and Tomato black ring virus.

3. NETU viruses- Species of Trichodorus and Paratrichodorus transmit tubular viruses, like Pea early browning and tobacco rattle virus.
Dodder transmission:

4. Plant viruses are also transmitted from one plant to another through the bridge formed between two plants by twining stems of the parasitic plant dodder (Cuscuta sp.)

5. Most commonly involved species are C. campestris transmitting Cucumber mosaic virus,Tomato bushy stunt virus and Tobacco mosaic virus; and C. subinclusa is known to transmit Sugar beet curly top virus.

Symptoms of viral diseases

- Viruses cause a number of symptoms on plants, like mosaic, mottle, vein clearing, vein banding, ring spots, enations, yellow vein mosaic, etc.

NOMENCLATURE AND CLASSIFICATION OF PLANT VIRUSES

Nomenclature and Classification of Plant Viruses

- Traditionally, viruses are named after the most conspicuous symptom they produce on the first host. A virus causing mosaic on tobacco is called Tobacco mosaic virus, whereas the disease itself is called as tobacco mosaic.
- There have been frequent changes in the nomenclature and classification of viruses and generic names have been adopted.
- A genus is usually considered as a population of virus species that shares common characteristics and are different from other population of species.
- Currently 70 genera of plant viruses have been recognized.
- The genera are named either after the type species (Caulimovirus after Cauliflower mosaic virus) or are given a descriptive name often from a Greek or Italian word for a major feature of a genus, e.g., Closterovirus from the Greek word ‘kloster’ meaning ‘a spindle or thread’ – descriptive of virus particle shape; Geminivirus from the Latin word ‘geminous’ meaning twins to describe the particles.
- Secondly, genera are grouped together into family on common characteristics.
- There are 14 families recognized for plant viruses, such as Reoviridae and Rhabdoviridae, which are common with animal viruses. However, 22 genera have not yet been assigned any family and are called ‘floating genera’.
- The family is either named after type member genus (e.g., Caulimoviridae named after Genus Caulimovirus) or given a descript to be named associated with genus for a major feature of family, e.g., Geminiviridae descriptive of virus particles.
- Only three orders have been accepted so far by International Committee for Taxonomy of Viruses (ICTV). The mononegavirales contains, among others the Rhabdoviridae in which there are two plant virus-families

Use of virus names

- The ICTV sets rules which are regularly revised on virus nomenclature and the orthography of taxonomic names.
- The last word of the species is ‘virus’; and suffix word for a genus is ‘virus’, for a subfamily is ‘virinae’, for a family is ‘viridae’, for an order is ‘virales’.
- In formal taxonomic usage, the virus order, family, genus and species names are printed in italics or underlined with first letter being capitalized.
- Other words in species names are not capitalized unless they are proper nouns or parts of proper noun.
- Also in formal usage, names of taxons should proceed the name being used e.g. Family Caulimoviridae, the Genus Closterovirus, the species Potato virus Y.
- However, in less formal instances which are widely used, the taxonomic unit is omitted.
- The plant viruses are classified on the basis of structure, physico-chemical properties, serological relationships, activities in the host plants and transmission.

Latest Classification
The plant viruses are classified in five major groups based on:
– Nature of the genome (RNA or DNA)
– Strandedness (single or double stranded)
– Method of replication

- Each group (not a recognized taxon) has orders, families, genera and species.
- The five groups are:

i) Single stranded positive sense RNA [(+) RNA] viruses
ii) Single stranded negative sense RNA [ss (-) RNA] viruses
iii) Double stranded RNA (ds RNA) viruses
iv) Double stranded DNA virus [ds DNA (RT)] viruses
v) Single stranded DNA [ss DNA] viruses

I. Single stranded positive sense RNA [(+) RNA] viruses:

- **Order:** Nidovirales

i) Family: Bromoviridae, e.g., Bromovirus (Brome mosaic virus-BMV), Alfamovirus (Alfalfa mosaic virus-AMV), Cucumovirus (Cucumber mosaic virus-CMV) and Ilarvirus (Tobacco streak virus-TSV).
ii) Family: Closteroviridae, e.g., Closterovirus (Beet yellows virus-BYV), Ampelovirus (Grapevine leaf roll associated virus GLRaV).
iii) Family: Comoviridae, e.g., Comovirus (Cowpea mosaic virus), Fabavirus (Broad bean wilt virus), Nepovirus (Nematode transmitted polyhedral virus, like Tobacco ring spot virus).
iv) Family: Flexiviridae, e.g., Potexvirus (Potato virus X), Carlavirus (Carnation latent virus).
v) Family: Luteoviridae, e.g., Luteovirus (Barley yellow dwarf virus-BYDV) and Polerovirus (Potato leaf roll virus- PLRV)
vi) Family: Potyviridae,
This family is largest single group of plant viruses and has been studied more extensively. Members of genus Potyvirus are one of the most successful plant viral pathogens. e.g., Potyvirus (Potato virus Y-PVY), Ipomovirus (Sweet potato mild mottle virus-SPMMV) and Bymovirus (Barley yellow mosaic virus).
vii) Family: Sequiviridae, e.g., Sequivirus (Parsnip yellow fleck virus-PYFV) and Waikavirus (Rice tungro spherical virus- RTSV).
viii) Family: Tombusviridae, e.g., Tombusvirus (Tomato bushy stunt virus- TBSV), Carmovirus (Carnation mottle virus), Necrovirus (Tobacco necrosis virus-TNV)
ix) Family: Tymoviridae, e.g., Tymovirus (Turnip yellow mosaic virus- TYMV).

  - Some of the very important viruses like Tobamovirus (Tobacco mosaic virus-TMV), Tobravirus (Tobacco rattle virus-TRV), Potexvirus (Potato virus X-PVX) etc. have not been assigned any family yet.

II. Single stranded negative sense RNA [ss (-) RNA] viruses: Members of this group are only enveloped plant viruses.
Order: Mononegavirales

- **Family:** Rhabdoviridae, e.g., Cytorhabdovirus (Lettuce necrotic yellows virus-LNYV) and Nucleorhabdovirus (Potato yellow dwarf virus- PYDV).
- **Family:** Bunyaviridae, e.g., Tospovirus (Tomato spotted wilt virus-TSWV; Groundnut bud necrosis virus- GBNV)

III. Double stranded RNA (ds RNA) viruses : There is no order assigned.

- **Family:** Rheoviridae, e.g., Fijivirus (Fiji disease virus-FDV) and Phytorheovirus (Wound tomur virus- WTV).
- **Family:** Partiviridae, e.g., Alphacryptovirus (White clover crypto-virus 1) and Betacryptovirus (White clover crypto-virus 2)

IV. Double stranded DNA virus [ds DNA (RT) virus]: No order has been assigned.

- **Family:** Caulimoviridae, e.g., Caulimovirus (Cauliflower mosaic virus- CaMV)

V. Single stranded DNA [ss DNA] virus: No order has been assigned.

- **Family:** Geminiviridae, e.g., Mastrevirus (Maize streak virus- MSV), Curtovirus (Beet curly top virus-BCTV), Begomovirus (Bean golden mosaic virus- BGMV), Bhendi yellow vein mosaic virus- BYMV and Cassava latent virus- CLV.
- **Family:** Circoviridae, e.g., Nanovirus (Subterranean clover stunt virus; Banana bunchy top virus-BBTV).

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LECTURE 12

ALGAE AND FLAGELLATE PROTOZOA CAUSING PLANT DISEASES

INTRODUCTION

Algae

- Parasitic algae are green in colour.
- Cephaleuros is the best known genus, and is a plant parasite living under leaf cuticle.
- It was first reported from India in the 19th century, causing damage to tea and coffee plantations.
- Now, over 400 hosts of Cephaleuros are recorded all over the world infecting hibiscus, orchids, euphorbias, citrus and forest trees, and 90 percent of its hosts are dicot.
- Cephaleuros belongs to phylum Chlorophtya, class Ulvophyceae, order Trentepohliales, and family Trentepohliaceae.
- There are 13 species of Cephaleuros, but 6 are more common. These are: *C. expansa, C. .henningsii*, *C. karstenii*, *C. minimus*, *C. parasiticus, and C. virescens.* Among these, *C. parasiticus* and *C. virescens* are most common and cause maximum damage.
- *C. virescens* causes red rust of tea and mango.

Red rust

- Fluffy, bright-orange red spots occur on leaves and stems that look very much like rust fungi.
- *C. virescens* has the misleading common name ‘red rust’.
- Species of Cephaleuros have fungus-like filaments, sterile hairs and produce sporangiophores and zoosporangia on the lower surface of leaves that look like downy mildew fungi. Necrosis may be limited to the epidermis or spread into the deeper tissues of the leaves. Severe damage usually occurs on older leaves leading to defoliation.

Lichens

- Fungi parasitize Cephaleuros to form lichens.
- The lichenized state of *C. virescens* is identified as *Strigula elegans*.
- Early literature suggests that the fungus portion of the lichen (mycobiont) was responsible for plant damage.
- Recent findings show that the fungus parasitizes the alga, not the plant. Plant injury is caused by the alga much before a fungus colonizes it.
- **Management of algal infections includes:**

i) Plant spacing and pruning to increase air circulation and light
ii) Sanitation
iii) Appropriate use of fertilizers and irrigation to promote plant growth.

Fig.12.1 Algal spot of magnolia

Fig.12.2 Lichen growing on apple tree trunk

Protozoa

- Certain protozoa, such as trypanosomatid flagellates belonging to class Mastigophora, order Kinetoplastida, family Trypanosomatidae are accepted as plant parasites even though Koch's postulates could not be established for them.
- However, evidence supporting their pathogenicity is more evident than that available for the fastidious bacteria and mollicutes, and so they are accepted as plant pathogens.
- The protozoa as such may be living freely, or living symbiotically or as parasites subsisting on organisms such as algae, yeasts, bacteria and other protozoa.
- Only the flagellates among the protozoa have been found to be associated with plant diseases.

CHARACTERISTICS

Characteristics

- The flagellates have a long, oval or spherical body with a thin, flexible covering membrane or it may be armoured.
- They have one or more slender flagella, which are used for both locomotion and food capture.
- Many species of the flagellates like Phytomonas have been reported from several plants belonging to the family Euphorbiaceae (P. davide on Euphorbia sp.), Asclepiadaceae (P. almassiani on milkweed), Moraceae (P. bancrofti on Ficus sp.), and Rubiaceae (P. leptovasorum on coffee).
- Some other unknown forms are parasitic on oil palm and coconut palm.
- These parasites seem to be insect- transmitted, though an insect vector has been reported only for P. almassiani.
- Since most of those associated with laticiferous plants do not produce any clear symptoms, there is a feeling that these are just parasites but not pathogens.
- The non-laticiferous plants like coffee, coconut palm and oil palm develop characteristic external symptoms, such as leaf yellowing, wilting and malformation of phloem tissue often leading to considerable damage to the plant and ultimately death.

Phloem necrosis of coffee

- It is caused by Phytomonas leptovasorum and occurs in Suriname, British Guyana, and probably Brazil and Columbia.
- It affects the tree of Coffea liberica and C. arabica. Infected trees show sparse yellowing and dropping of leaves, and, only the young top leaves remain on the otherwise bare branches, followed by death of the trees, sometimes within 3-6 weeks.
- The flagellates can be traced from the roots upward to trunk, where they seem to migrate vertically in the phloem and laterally through the sieve plates into healthy sieve tubes.
- This disease can be transmitted through root grafts but not through green branch or leaf grafts. Its insect vector is a pentatomid Lincus sp.

Hart rot of coconut palms

- Hart rot has been known in Suriname since 1906, sometimes under the more appropriate names of lethal yellowing or bronze-leaf wilt , Cedros wilt and unknown disease.
- Many of the symptoms of hart rot are similar to those caused by lethal yellowing disease of coconut palm in the Caribbean, West Africa, Florida, the cause of the two diseases seem to be unrelated.
- The hart rot symptoms include yellowing and browning of the tips of the older leaves which subsequently spread to the younger leaves.
- Recently opened inflorescences are black , and unripe nuts of the symptomatic trees fall off.
- Flagellates of the genus Phytomonas occur in mature sieve elements of young leaves and inflorescences of hart rot affected coconut palms.
- The number and spread of the flagellates in the sieve tubes increase proportionally with the development of the disease.

- They are also transmitted by the pentatomid insects of genera Lincus and Ochlerus.

Sudden wilt (Marchitez) of oil palm

- The symptoms begin as browning of the tips of the lower leaflets of the oil palms.
- The browning subsequently spreads to the upper leaves and eventually becomes ash grey.
- In the mean time, root tips also begin to die and the whole root system deteriorates.
- As a result, the plant growth slows down, fruit bunches discolour and rot or fall off; and within a few weeks, all leaves become ash grey and dry up and the whole tree dies out.
- Phytomonas flagellates occur widely in the phloem sieve elements of the roots, leaves and inflorescences of the infected plants.
- These flagellates are also transmitted by Pentatomid insects Lincus and Ochlerus.

Empty root of Cassava

- The empty root disease was observed affecting certain cultivars of cassava (Manihot esculenta) in the Espirito Santo state of Brazil.
- Affected plants show poor root development.
- They remain small and slender, and contain little or no starch.
- Above ground parts of the infected plants show chlorosis and decline.
- Diseased plants contain numerous Phytomonas like protozoa in the laticiferous ducts but not in phloem.
- The empty root disease can be transmitted by grafting.
- It also spreads rapidly in the field, probably by the above insect vectors.

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LECTURE 13

FLOWERING PARASITIC PLANTS

INTRODUCTION

Characteristics of Flowering Parasitic Plants

- The pathogenic flowering plants, also called parasitic angiosperms can be classified as root parasites or stem parasites.
- Root parasites (witchweed and broomrape) are more common and more diverse taxonomically.
- Stem parasites include the dodder (Cuscuta) and mistletoes (Arceuthobium).
- The angiospermic parasites can also be classified as holoparasites (total parasites) or hemiparasites (semiparasites).
- The holoparasites lack chlorophyll and are totally dependent on the host for nutrition. Thus, they are obligate parasites.
- The hemiparasites contain chlorophyll and make their own food, and absorb water and minerals from their host. But, in some cases, e.g., Arceuthobium, the photosynthesis is negligible and the parasite draws nutrition from the host. Practically, it is an obligate parasite.

Important Genera

- There are 277 genera and as many as 4100 parasitic plant species; but only 25 genera are recognized as plant pathogens.
- Out of these 25 genera, four are more damaging to crops viz., Striga (witcweed), Orobanche (broomrape), Cuscuta (dodder) and Arceuthobium (dwarf mistletoe).
- Striga is more prevalent in Asia and Africa, while Orobanche is worldwide, but more damaging in the Middle East.
- Both Striga and Orobanche produce microscopic seeds called “dust” seeds that persist in the soil for a long time, and are difficult to control.
- Dwarf mistletoes (*Arceuthobium* spp.) are the major pathogens of coniferous trees (belonging to families Pinaceae and Cupressaceae).
- Dendrophthoe (Loranthus) and Viscum species are parasitic on the forest, fruit and avenue trees; and are responsible for their die back and drying in Himachal Pradesh.

ROOT PARASITES

Striga (whichweed)

- Striga is an obligate root hemiparasite, although the seedlings above ground do form chlorophyll.
- Striga has made greater impact than any other parasitic angiosperm.
- It attacks important crops like maize, sorghum, pearl millet, rice, sugarcane and legumes (cowpea, groundnut, etc.).
- Two species, *S. asiatica* and *S. hermonthica* cause maximum damage to crops.
- Striga has a complex life cycle. It produces thousands of 'dust' seeds that are disseminated by wind and rain.
- The seeds after a dormant 'ripening' period of several months, respond to chemical signals exuded by the host.
- The chemical signals enable the Striga seeds to detect the type of host and its distance from the host.
- Seed germination of Striga, as in all obligate root parasites, is cryptocotyler i.e. the cotyledons remain within the seed when the radical comes out.
- The radical produces root hair like structures that glue it to the host.
- If the host is suitable, a haustorium is formed that penetrates and forms a link with the host vascular system.
- Once the parasite is established, the distinctive seedling of Striga is formed underground, which lacks chlorophyll, possesses scale-like leaves, and produces abundant adventitious roots that form additional haustoria, establishing more connections with the host.
- The seedlings exert great influence on the growth-regulating metabolism of the host, stimulating root production.
- Significant damage to the host occurs at this stage. The next stage is emergence of the seedlings above ground.
- Chlorophyll develops, and in due course, flower and seeds are formed. The life cycle is ready for a repeat.
- A major problem in control is persistence of the tiny seeds in the soil. Ethylene gas is introduced into the soil to induce seed germination, which becomes suicidal in absence of the host.
- Equipments and application methodologies have been developed to introduce the gas into the soil.
- Up to 90% seeds germinate by this method, and die in absence of the host.

Fig.13.1 Partial root parasite-*Striga*

Fig.13.2 Total root parasite-*Orobanche*

Orobanche (broomrape)

- This is an obligate root holoparasite, infecting legumes, solanaceous crops, carrot, cabbage, cauliflower, lettuce and sunflower.
- Total crop failure may occur in heavily infested soils. The parasite appears as whitish, yellowish or brownish stems, about 30 cm high that arise from the roots of infected host, and bear beautiful flowers, besides bracket-like leaves lacking chlorophyll.
- In general, Orobanche is a parasite of colder climate and need 10-20oC of temperature for seed germination.
- This is the reason why it attacks tobacco during winter in India, but fails to infect sunflower during summer in the same field.
- Seed germination requirements of Orobanche are different from those of Striga.
- It needs low temperature (10-20oC); the germinated seeds are geotropically neutral i.e. they do not grow downward in the soil and ethylene has no stimulatory effect.
- Its control is difficult due to the high longevity (more than 5 decades) of the seeds in the soil, their extremely small size (less than the thickness of human a hair), their production in extremely large number, and subterranean infection.

STEM PARASITES

Cuscuta (dodder)

- It is obligate stem holoparasite and is among the best known of all parasitic plants.
- Its slender, twining, orange-yellow, leaf less stems form conspicuous tangled mass on the host.
- The host range is large, though monocots are less preferred.
- Dodders are most important parasites of legumes.
- Cuscuta campestris is the most widely distributed among its 10 species that attack crops.
- It causes considerable damage to alfalfa, flax, sugarbeet, onion and other crops besides fruit, fodder and forest trees and shrubs . It also transmits viruses.
- The most effective means of control is seed sanitation. Several herbicides are effective on newly-germinated seeds.


Fig.13.3Dodder (*Cuscuta* sp.)


Fig.13.4 Showy mistletoe (*Dendrophthoe*)


Fig.13.5 Leafy mistletoe (*Viscum* sp.)

Mistletoes

- Mistletoes are stem holoparasites occurring in three families of the order Santalales as follows:
- Family Loranthaceae: Showy mistletoes [Loranthus (Dendrophthoe)]
- Family Santalaceae: sandalwood (Pyrularia, Santalum)
- Family Viscaceae: Dwarf mistletoe (Arceuthobium), leafy mistletoe (Viscum)
- The showy “mistletoes” produce large and beautiful flowers that are pollinated by birds.
- The co-evolution of these parasites and the birds is also suggested by the seed dispersal mechanism operating in the birds.
- Santalaceae, the sandalwood family have a few members (Pyrularia etc.) that cause negative impact on their hosts.
- Family Viscaceae is called “Christmas mistletoe family”, because their shoots with the white berries are used as door festoons during Christmas in temperate countries.
- The family has seven genera, and a large number (543) of species, most of which belong to three genera, Viscum, Phoradendron and Arceuthobium.
- The seeds are covered with a sticky substance, called ‘viscin’ that glues the seeds to the host surface.

Arceuthobium (Dwarf mistletoe)

- Arceuthobium is the most important mistletoe in terms of economic losses, especially to the coniferous trees belonging to families Pinaceae and Cupressacceae.
- 11.3 million cubic meters of wood is lost annually due to the ‘dwarf mistletoe’ Arceuthobium in the US.
- It is a small (1.0-2.5 cm long) plant having green to brown aerial shoots, without secondary branching.
- Leaves are small, scale- like leaves. The major function of the aerial shoot is reproduction.
- The flowers are small and unisexual, present on same (monoecious) plants or on different (dioecious) plants.
- Pollination is brought about by insects and wind.
- Male aerial shoots are shed soon after pollination, but the female shoots persist until the seeds are formed and dispersed.
- Remnants of the aerial shoots persist as basal cups, on the host tree, where once the aerial shoots were formed.
- The seeds, which are discharged explosively from the fruit at the rate of 27 metres per second, reach up to 16 metres.
- The seed sticks to the surface by the viscin coating.
- It slides to the base of the “needle” where it germinates.
- The radicle forms a hold fast from which the haustorium emerges and penetrates the host tissue.
- Thus endophyte is formed, but it takes one year to form the aerial shoots, and 3-10 years to complete the life cycle (infection to seed dispersal).
- This long life cycle is profitably used in disease management.
- Selective removal of infected trees has been highly effective in controlling Arceuthobium infestation.
- Chemical control has also been successful with ethephon, an environmentally safe chemical.

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LECTURE 14

NON-PARASITIC CAUSES OF PLANT DISEASES

INTRODUCTION

- Diseases caused by non- parasitic/ abiotic (nonliving) agents are not transmitted from one plant to another. Thus, they are not infectious, and also called non-infectious diseases or simply, disorders.
- Extremes of temperature and water (flooding or drought), deficiency or excess of essential nutrients, presence of toxic chemicals in air, water and soil, transplant shock and mechanical injury are the important causes of abiotic diseases.
- Surprisingly, the symptoms of non-infectious (non-parasitic) diseases resemble those produced by living agents viz., fungi, bacteria, viruses and nematodes. If no signs of these organisms are present, a nonliving agent may be the cause of the disease.
- Meteorological weather reports and plant and soil analyses for mineral elements is the next step to confirm the disease agent.

TEMPERATURE EXTREMES

Temperature Extremes

- Sudden rise and fall in temperature causes injury to plants.
- Harmful effects of chilling, freezing and sunburn are well known.
- The plants may get adapted to their climate and show chill or frost resistance.

Chilling injury: It occurs at temperature close to 0oC. Tropical plants begin to experience cold damage at 5-100C.

- Symptoms include wilting of the upper portions of stems and leaves, blackening or softening of the plant tissue, surface pitting, necrosis or failure of ripening of fruits.
- This injury is severe in some warm season fruits.

Freezing or Frost injury: It occurs at temperatures below 0oC.

- It is caused by formation of ice. Since water in the intercellular spaces is pure, ice crystals are first formed there, then inside the cells.
- The crystals formed inside the cells damage the cell organelles.

High temperature and dry winds: They cause rapid loss of water.

- Leaf margins turn yellow or brown and leaves fall off prematurely.
- Sun-scald injury occurs when shade loving plants are suddenly exposed to direct sun.
- Sunken brown areas on apples, water-soaked areas on tomatoes are examples of heat injury.
- High temperature also causes water core symptoms in apple.

Fig. 13.6 Frost injury in banana

Fig. 13.7 Water core of apple

SOIL MOISTURE EXTREMES

Low soil moisture, which occurs during drought, causes accumulation of toxic ions of manganese and boron, which damage tissues and cause stomatal closure.

- This adversely affects the plants. Wilting discoloured foliage, twig and branch ‘die back’ in the crown, and death of fine roots are the symptoms of water deficiency.

Excess soil moisture or flooding results in diminished oxygen supply in the soil water that kills the root.

- Symptoms of oxygen deficiency, during high moisture are reduced growth, small leaves and thin crowns, twig and branch ‘die back’ and plant death.

Unfavourable light

- Insufficient light causes etiolation, stunted growth, and reduction in flowering.
- High light intensity leads to scorching and rolling of leaves and drying of flowers.
- Enhanced photoperiod results in abnormal shape, erratic flowering etc.

Lack of Oxygen

- Apart from the asphyxiation of plant roots in waterlogged soils and its adverse effects on the plants as discussed above, lack of oxygen may also result from its failure to diffuse adequately both between and within fruits or the storage organs kept in bulky piles or under poor ventilation.
- In such stress conditions, the cells die of suboxidation and results in storage diseases such as black heart of potatoes, in which the cortex of the affected tuber is blackened; and internal browning in apple.

MINERAL NUTRIENT IMBALANCES

- Currently seventeen elements (nickel is the latest addition) are recognized as essential for plant growth. Among them, thirteen are found in soil mineral.
- The essential elements are divided into three categories: macronutrient elements, micronutrient elements (or trace elements) and beneficial elements.
- Carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium and sulphur are macronutrients.
- The micronutrients (needed less than 100µg per gram in dry tissue) include iron, manganese, zinc, copper (these are metal micronutrients) boron, molybdenum, chlorine and nickel.
- Beneficial elements, although not essential, do provide benefit to the plant.

Mineral deficiencies

- When the soil is deficient in any element, or the element is 'unavailable' due to, for example unsuitable pH, it shows up as typical symptoms in the plant.
- The common symptoms of nutrient deficiencies are: reduced growth, leaf chlorosis of different patterns, and necrosis.
- Symptoms are very similar and occasionally indistinguishable for different elements.
- Soil and plant tissue analyses are therefore, necessary for the symptoms. The deficiency may be acute or chronic. The former is due to sudden unavailability of the element, while he latter is a continuous insufficient supply.
- Nutrient mobility plays an important role in determining the site of the deficiency symptoms.
- For highly mobile elements like nitrogen and potassium, the deficiency symptoms appear predominantly in older and mature leaves.
- Deficiency of poorly mobile elements, like calcium, boron and iron show up in younger leaves, opposite of what happens with highly mobile elements.


Fig. 13.8 Bitter pit of apple due to Ca deficiency


Fig.13.9 Spray injury on pear fruits

- The symptoms of deficiency of moderately mobile elements like magnesium and sulphur, are uniformly spread all over the plant.
- The symptoms of mineral deficiencies is recorded in the following table:

Sr.No	Deficient element	Symptoms
1	Nitrogen	Poor growth, chlorosis of leaves, delayed flowering and fruiting
2	Phosphorus	Poor growth, blue/green, but not yellow, colour of leaves, oldest leaves affected first
3	Potassium	Brown scorching and curling of leaf tip, yellowing of leaf veins
4	Calcium	Curling and tip burn of leaves, stunted growth abnormal development of meristmatic tissues, and eventual death of buds and root tips. Also causes **bitter pit in apple**.
5	Magnesium	Interveinal chlorosis, giving a mottled appearance to leaves, similar to virus infection
6	Sulfur	Chlorotic leaves, petioles and veins distinctly red.
7	Iron	Chlorosis of leaves, young leaves appear bleached, symptoms similar to manganese deficiency
8	Manganese	Chlorotic leaves with green veins
9	Zinc	Younger leaves yellow, pitting on lower leaf surface, leaves and internodes shortened, giving a rosette-like look to plants; guttation increased
10	Copper	Leaves pale and curled; petioles droop down
11	Boron	Drying of growing tip, bushy stunted growth, and internal tissue breakdown.
12	Molybdenum	Chlorosis of leaves; symptoms similar to nitrogen deficiency. Also causes **whiptail disease in cauliflower**.
13	Chloride	Leaves of abnormal shapes, and with interveinal chlorosis.

Aluminum toxicity

- Aluminum is one of the most abundant element is the earth crust.
- It is toxic to several plants at 2-3 ppm concentration, when the soil pH is 5.5 and above.

Symptoms

- Symptoms of aluminum toxicity are not easily identifiable.
- The foliar symptoms resemble those of phosphorus deficiency, viz., overall stunting, blue/green colour of leaves.
- In some cases, aluminum toxicity appears as an induced calcium deficiency, showing curling, rolling and tip burn of young leaves, and collapse of growing points or petioles.

Injury By Air Pollution (SO2, F, Cl2, O3, PAN,C2H4)

- Injury caused by air pollution is often evident on plants before it affects human beings and other animals.
- Air pollutants may be inorganic (sulphur dioxide-SO2, fluorine-F , chlorine-Cl2, and ozone-O3; or organic (peroxy-acetyl nitrate- PAN, and ethylene- C2H4).
- The symptoms caused by air pollutants are very similar to those caused by biotic and other abiotic factors, like mineral deficiency and excesses, or the adverse effects of temperature, water and light.
- **In general, the visible symptoms are of three types:**

i) Collapse of leaf tissues, forming necrotic symptoms
ii) Yellowing or other colour changes
iii) Alterations in growth
iv) Premature defoliation

- Damage is severe during warm, still and humid weather, when the atmospheric pressure is high.
- In this condition the pollutants accumulate near the earth's surface, as the warm air above in the atmosphere traps the cooler air near the ground. This is called air inversion.
- The plants vary in their response to the pollutants, and are categorized into very sensitive and somewhat resistant plants for each type of pollutant.
- For control and disease management, resistant plants should be grown in SO2 prone areas, and plant vigor should be maintained.
- In good health, plants resist all types of injury better than weakened plants.
- The damage to plants by air pollutants is given in following Table.
- **Symptoms caused by air pollutants**

Air pollutant	**Symptoms**
Sulphur dioxide	Marginal and inter-veinal chlorosis, reduced growth and defoliation. Near brick kilns, SO_2 toxicity is known to **cause black tip or tip necrosis** of mango in India.
Fluoride	Yellowish mottle to scorching at margins and the tips of broad-leaf plants, tip burns in grasses and conifers
Chloride	Marginal and inter-veinal chlorosis, similar to that caused by sulphur dioxide and chloride.
Ozone	Chlorosis and necrosis, flecking, bronzing and reddening of leaves, stunted growth, reduction in yield more pronounced in dicots (soybean, cotton and peanut) than in monocots (sorghum, corn and wheat)
Organic	
PAN	Collapse and death of leaf tissues, typical leaf glazing, browning or silvering; stunted growth, early senescence and defoliation
Ethylene	Modifies activity of other hormones, affecting normal organ development, shoot and leaf epinasty, accompanied by stunted growth.

LECTURE 15

INFECTION PROCESS

INTRODUCTION

- In every infectious disease, a series of more or less distinct events occurs in succession which leads to the development of the disease. This chain of events is called Pathogenesis or disease cycle.
- A disease cycle sometimes corresponds fairly closely to the life cycle of the pathogen, and refers primarily to the appearance, development and perpetuation of the disease as a function of the pathogen rather than to the pathogen itself.
- The life cycle of a pathogen refers to the stage or successive stages in the growth and development of the pathogen (or any organism) that occurs between the appearance and reappearance of the same stage (e.g. spore) of the organism.
- Disease cycle involves changes in the plant and its symptoms as well as those in the pathogen; and spans periods with in a growing season and from one growing season to the other.

Primary Events in the Disease Cycle

i) Inoculation
ii) Penetration
iii) Establishment of the infection
iv) Colonization (invasion)
v) Growth and reproduction of the pathogen
vi) Dissemination of the pathogen
vii) Survival of the pathogen in the absence of the host i.e. overwintering and over-summering (over-seasoning) of the pathogen

  - In some cases, there may be several infection cycles within one disease cycle.

INOCULUM AND INOCULATION

Inoculum and Inoculation

- **Inoculum-** The infective pathogen propagules coming in contact with the host constitutes the inoculum.
- **Inoculation** is the initial contact of the pathogen with the site of the plant where infection is possible.
- In fungi, the inoculum may include the spores, sclerotia (compact mass of mycelium) or fragments of the mycelium.
- In bacteria, phytoplasmas, protozoa, viruses and viroids, the inoculum is always their whole individuals.
- In nematodes, it may be adult nematodes, juveniles or eggs. In parasitic flowering plants, the inoculum may be plant fragments or seeds.
- Primary inoculum -The inoculum that survives dormant in the winter or summer and brings about original infections in the spring and autumn is called primary inoculum, and the infections it causes are called primary infections.
- Secondary inoculum- The inoculum produced from primary infections is called secondary inoculums, and it, in turn causes secondary infections.
- Generally, the amount of inoculum and prevalence of favourable environmental conditions determine the success of infection.
- Inoculum potential has been defined as the ‘energy of growth of parasite available for infection of host at the surface of the host organ to be infected’.
- It is the resultant of action of the environment, the vigour of the pathogen to establish an infection, the susceptibility of the host and amount of the inoculum present.
- The inoculum, which survives whether on perennial plants, plant debris or soil, or on the propagative planting material is carried to the host plants mostly by wind, water, insects or man.
- Only a tiny fraction of the potential inoculum produced actually lands on the susceptible host plants.
- The bulk of the produced inoculum lands on the things that cannot become infected.
- Some types of inoculum, in the soil, e.g., zoospores and nematodes may be attracted to the host plant by chemical substances like sugars and amino acids diffusing out of the plant roots. This process is known as chemotaxis.
- Vector transmitted pathogens are usually carried to their host plants with an extremely high efficiency.

Pre-penetration Activities of the Pathogen on the Host Surface

- Almost all fungi, bacteria and parasitic higher plants, must be first attached to the host surfaces.
- This attachment takes place through their adhesive materials which are composed of water insoluble polysaccharides, glycoproteins, lipids and fibrillar materials, which when moistened become sticky and help the pathogens to adhere to the plant.
- Pathogens, such as phytoplasmas, fastidious bacteria, protozoa and most viruses are placed directly into the cells of the plants by their vectors.
- Many fungal pathogens first grow on the surface of the host to get proper mechanical, morphological and chemical strength to bring out the penetration of the barriers.
- In Rhizoctonia solani, the fungus first forms infection cushions and appressoria and from their multiple infections take place by means of infection pegs.

- In Armillariella mellea, the fungus hyphae form the rhizomorphs (aggregation of hyphae forming rope like/root like structures), which produce desired amount of enzymes required for direct penetration of the host.
- In other fungi, the spores landed on the host surfaces germinate producing germ tubes, which cause penetration, directly or indirectly or they first produce appressoria from which infection threads develop and penetrate the host.
- Appressoria are swollen structures formed on the tips of the germ tubes and facilitate in attachment and penetration of the host, which are produced by their thigmotropic (contact) response to the hard surfaces, and in turn produce infection hyphae or infection pegs and exert pressure to affect the direct penetration of the host.
- Seeds of parasitic flowering plants germinate by producing a radical which either penetrate the host plant directly or first produces a small plant that subsequently penetrates the host plant by means of specialized feeding structures called haustoria.
- Nematode eggs also require conditions of favourable temperature and moisture to become activated and hatch.

PENETRATION

Plant pathogens penetrate the plant surfaces by direct penetration of the cell walls, natural openings or through wounds.

Direct penetration

- Direct penetration through intact plant surfaces is probably the most common type of penetration by fungi, oomycetes and nematodes and only type of penetration through parasitic flowering plants. None of the other pathogens can enter plants by direct penetration.
- Hemibiotrophs or non-obligate parasitic fungi do so through a fine hyphae produced directly by the spore or the mycelium.
- The obligate parasites do so through a penetration peg produced by an appressorium.
- They are formed at the point of contact of the germ tube or mycelium with a plant surface.
- The fine hyphae growing towards the plant surface pierces the cuticle and the cell wall through mechanical force and enzymatic softening of the cell wall substances.
- Most fungi form an appressorium at the end of germ tube, it being bulbous or cylindrical with a flat surface in contact with the surface of the host plant.
- Then a penetration peg grows from the flat surface of the appressorium towards the host and pierces the cuticle and cell wall.
- The penetration peg grows into the small hyphae generally much smaller in diameter than the normal hyphae of the fungus and regains its normal diameter once inside the cell.
- Parasitic higher plants also form an appressorium and penetration peg and the point of contact of the radical with the host plant; and penetration is similar to that in fungi.
- Direct penetration in nematodes is accomplished by repeated back and forth thrusts of their stylets. Such thrusts finally create fine opening in the cell wall. It then inserts its stylet into the cell so the entire nematode enters the cell.

Indirect penetration (through wounds)

- All bacteria, most fungi, some viruses and all viroids can enter through various kinds of wounds.
- Some viruses and all mollicutes, fastidious vascular bacteria and protozoa enter plants through wounds made by their vectors.
- The bacteria and fungi may grow briefly on the lacerated or dead tissues before they advance in to the healthy tissues.
- The penetration of viruses, phytoplasmas, fastidious bacteria and protozoa through wounds depends on their deposition by the vector on fresh wounds created at time of inoculation.
- Some viruses and viroids penetrate through wounds made by human hands and tools.

Indirect penetration through natural openings

Stomata are natural openings and are more in number on lower leaf surfaces; measuring about 10-20 x 5-8 µm, and are open in the day time; but are more or less closed at night.

- Bacteria which are present in a film of water over stomatal openings, swim through it easily and reach the sub-stomatal cavity when they can multiply and start infection.

- Fungal spores generally germinate on the plant surface and germ tubes may grow through stomata.
- The germ tubes form an appressorium that fits tightly over one stomata and normally one fine hypha grows from it into the stoma.
- It enlarges through sub-stomatal cavity giving rise to several small hyphae that actually invade the cells of the host plants directly or through hasutoria.
- Although some fungi can penetrate through closed stomata, others penetrate stomata only while they are open, e.g., Puccinia graminis tritici, the cause of stem rust of wheat.

Hydathodes are more or less permanently open pores at the margins and tips of the leaves. They are connected to the veins and secrete droplets of liquids called guttation drops containing various nutrients.

- Some bacteria, e.g., the one which causes black rot of cabbage (Xanthomonas campestris pv. campestris) use these pores as means of entry into the leaves.
- *Erwinia amylovora* causing fire blight of apple and pear also enter blossoms through the nectarthodes or nectaries which are similar to hydathodes but are present on the receptacle or other parts of the flower.

Lenticels are openings on the fruits, stems and tubers that are filled with loosely connected cells to allow passage of air and seem to offer little resistance to pathogen entry.

- Lenticel and wound penetration are quite similar. Many lenticel invaders can also enter through wounds, particularly soil borne pathogens like Streptomyces scabies (causing potato common scab), Erwinia carotovora (soft rot of vegetables), Armillariella mellea (root rot), Spongospora subterranea (potato powdery scab), , Penicillium expansum (causing blue mould rot), Monilinia fructicola (causing brown rot of apple) and Nectria galligena (causing apple canker).
- The germ tubes or hyphae of invading fungi grow between the lenticel cells and enter the plant tissues.

INFECTION AND INVASION

Infection

Infection is the process by which pathogens establish contact with susceptible cells or tissues of the host and obtain nutrients from the tissues.

- Successful infection results in the appearance of symptoms, viz., discolouration, malformation or necrotization of the affected plant parts.
- Some infections do not produce symptoms right away, and remain latent but do so at a later time when environmental conditions and/or stage of plant become more favourable.
- The time interval between inoculation and the appearance of disease symptoms is called incubation period.
- It varies from few days to years with different pathogens-host combinations, stage of host and prevailing environmental conditions.
- During infection, some pathogens obtain nutrition from the living cells, often without killing them or at least not for a long time; others kill the cells and utilize their contents as they invade them; and still others kill cells and disorganize the surrounding tissues.
- During infection, pathogens release a number of biologically active substances (e.g., enzymes, toxins and growth regulators) that may affect the structural integrity of the host cells or their physiological processes.
- In response, the host reacts with a variety of defence mechanisms.
- For a successful infection, the host must be susceptible, the pathogen must be virulent and the environment must be favourable.
- When these conditions occurred at an optimum, the pathogen can further invade the host plant up to the maximum of its potential even in the presence of plant defenses; and in consequence the disease develops.

Invasion

Various pathogens invade hosts in different ways and to different extents.

- Some fungi, such as those causing powdery mildews produce mycelium only on the surface of the plants and send haustoria into the epidermal cells.
- Others such as those causing apple scab and black spot of rose produce mycelium that grows only in the area between the cuticle and epidermis showing sub-cuticular growth.
- Most fungi spread into the tissue of the plant organs either by growing directly through the cells as intracellular mycelium or by growing between the cells as intercellular mycelium.
- Fungi that cause vascular wilts invade the xylem vessels of the plants.
- Bacteria invade tissues inter-cellularly, although when parts the cell walls dissolve, they also grow intra-cellularly.
- Bacteria causing vascular wilts and fastidious bacteria (Xylella fastidiosa) invade the xylem vessels.
- Viruses and viroids invade all types of living cells, phytoplasma and protozoa invade phloem sieve tubes and a few adjacent phloem parenchymatous cells.

- **Local infections-** Many infections caused by fungi, bacteria, nematodes, viruses and parasitic flowering plants are local, i.e., they involve a single cell, a few cells or a small area of the plant. They may remain localized throughout the growing season or may enlarge slightly or very slowly.
- **Systemic infections-** Other infections enlarge more or less rapidly and may involve an entire plant organ, a large part of plant or even the entire plant.
- Infections caused by fastidious bacteria, phytoplasma, protozoa, and natural infections caused by viruses and viroids are systemic, i.e., the pathogen from one initial point spreads and invades most or all susceptible cells and tissues throughout the plant. For example, vascular wilts, some downy mildews, white rust of crucifers, loose smut and hill bunt of wheat.

Growth and reproduction (colonization) of the pathogen

- After infection, pathogens grow, multiply or both within the plant tissues and invade and colonize the plant to a lesser or greater extent.
- Growth and reproduction of the pathogen (also called colonization) in or on the infectious tissues are actually two concurrent sub-stages of disease development.
- Most of the fungi and parasitic higher plants generally invade and infect the plant tissues by growing on or into them.
- They continue to grow and branch out within the infected host and spread into more and more of the plant until, its spread is stopped or the plant is dead.
- All other pathogens, namely, bacteria, phytoplasmas, viruses, viroids and protozoa do not increase in size much if at all, as their size and shape remains relatively unchanged throughout their existence.
- They invade and infect the new tissues within the plants by reproducing at a rapid rate and increasing their number tremendously in the infected tissues.
- Their progeny may then be carried passively into the new cells and tissues through plasmodesmata (some viruses, viroids), phloem (viruses, viroids, phytoplasmas, some fastidious bacteria, protozoa) or xylem (some bacteria).
- Alternatively, as happens with bacteria they may move through cells on their own power.
- Plant pathogens reproduce in a variety of ways.

i) Fungi reproduce by means of spores, either asexually or sexually.
ii) Parasitic higher plants produce seeds.
iii) Bacteria and phytoplasma reproduce by fission in which one mature individual splits into two equal, smaller individuals.
iv) Viruses and viroids are replicated inside the host cells.

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LECTURE 16

ROLE OF ENZYMES AND TOXINS IN PLANT DISEASE DEVELOPMENT

ENZYMES

- Enzymes are generally large protein molecules that catalyze organic reactions in living cells and in solutions.
- Because most kinds of chemical reactions that occur in a cell are enzymatic, there are almost as many kinds of enzymes as there are chemical reactions.
- Each enzyme, being a protein, is coded for by a specific gene.
- Some enzymes are present in cells at all times (constitutive).
- Many are produced only when they are needed by the cell in response to internal or external gene activators (induced).
- Each type of enzyme often exists in several forms known as isozymes that carry out the same function but may vary from one another in several properties, requirements, and mechanism of action.

Plant Substances and Their Enzymatic Degradation

- Aerial plant part surfaces consist primarily of cuticle and/or cellulose, whereas root cell wall surfaces consist only of cellulose.
- Cuticle consists of cutin, wax and covering of layer of wax
- The lower part of cutin contains pectin, cellulose lamellae and a thin layer of pectic substances; below that there is a layer of cellulose.

Cuticular wax

- Plant waxes are found as granular, blade, or rod-like projections or as continuous layers outside or within the cuticle of many aerial plant parts.
- The presence and condition of waxes at the leaf surface affect the degree of colonization of leaves and the effect varies with the plant species.
- Electron microscope studies suggest that several pathogens, e.g., Puccinia hordei, produce enzymes that can degrade waxes.

Cutin

- Main component of the cuticle.
- The upper part is admixed with waxes.
- The lower part is admixed with pectin and cellulose.
- Cutin is insoluble polyester of 16- and 18-carbon hydroxy fatty acids.
- Many fungi and a few bacteria produce cutinases and/or nonspecific esterases, i.e., enzymes that can degrade cutin.
- Cutinases break cutin molecules and release monomers (single molecules) as well as oligomers (small groups of molecules) of the component fatty acid derivatives from the insoluble cutin polymer.

Pectic substances

- Main components of the middle lamella, i.e., the intercellular cement that holds in place the cells of plant tissues.
- Also make up a large portion of the primary cell wall in which they form an amorphous gel filling the spaces between the cellulose microfibrils.
- Are polysaccharides consisting mostly of chains of galacturonan molecules interspersed with a much smaller number of rhamnose molecules and small side chains of galacturonan, xylan and some other five carbon sugars.

Degradation of pectic substances

- Several enzymes degrade pectic substances and are known as pectinases or pectolytic enzymes.
- The pectin methyl esterases remove small branches off the pectin chains.
- Pectin methyl esterases have no effect on the overall chain length, but they alter the solubility of the pectins and affect the rate at which they can be attacked by the chain-splitting pectinases.
- Pectinases cleave the pectic chain and release shorter chain portions containing one or a few molecules of galacturonan.
- Some chain splitting pectinases, called polygalacturonases, split the pectic chain by adding a molecule of water and breaking (hydrolyzing) the linkage between two galacturonan molecules.
- Others, known as pectin lyases, split the chain by removing a molecule of water from the linkage, thereby breaking it and releasing products with an unsaturated double bond.

Cellulose

- Cellulose - a polysaccharide, but consists of chains of glucose (1–4) ß-d-glucan molecules.
- The glucose is produced by a series of enzymatic reactions carried out by several cellulases and other enzymes.
- One cellulase (C1) attacks native cellulose by cleaving cross-linkages between chains.
- A second cellulase (C2) also attacks native cellulose and breaks it into shorter chains.
- These are then attacked by a third group of cellulases (Cx), which degrade them to the disaccharide cellobiose.
- Finally, cellobiose is degraded by the enzyme ß -glucosidase into glucose.

Cross-linking glycans (hemicelluloses)

- The enzymatic breakdown of hemicelluloses requires the activity of many enzymes.
- Several hemicellulases seem to be produced by many plant pathogenic fungi.
- Depending on the monomer released from the polymer on which they act, the particular enzymes are called xylanase, galactanase, glucanase, arabinase, mannase, and so on.
- The non-enzymatic breakdown of hemicelluloses by activated oxygen, hydroxyl, and other radicals produced by attacking fungi also occurs.

Suberin

- Suberin is found in certain tissues of various underground organs, such as roots, tubers, and stolons, and in periderm layers, such as cork and bark tissues.
- Suberins are also formed in response to wounding and to pathogen-induced defenses of certain organs and cell types.
- Although plants obviously produce enzymes that synthesize suberin, it is not known whether or how pathogens break it down during infection.

Lignin

- Found in the middle lamella, as well as in the secondary cell wall of xylem vessels and the fibres that strengthen plants.
- Also found in epidermal and occasionally hypodermal cell walls of some plants.
- The lignin content of mature woody plants varies from 15 to 38% and is second only to cellulose in abundance.
- Lignin is an amorphous, three-dimensional polymer that is different from both carbohydrates and proteins in composition and properties.
- It is obvious that enormous amounts of lignin are degraded by microorganisms in nature, as is evidenced by the yearly decomposition of all annual plants and a large portion of perennial plants.

Cell wall flavonoids

- Flavonoids are a large class of phenolic compounds that occur in most plant tissues and, especially, in the vacuoles.
- Also occur as mixtures of single and polymeric components in various barks and heartwoods.
- Among the various functions of flavonoids, some act as signaling molecules for certain functions in specific plant-microbe combinations.

Cell wall structural proteins

- Cell walls consist primarily of polysaccharides, i.e., cellulose fibres embedded in a matrix of hemicelluloses and pectin, but structural proteins, in the form of glycoproteins, may also form networks in the cell wall.
- Four classes of structural proteins have been found in cell walls.
- hydroxyproline-rich glycoproteins (HRGPs)
- proline-rich proteins (PRPs)
- glycine-rich proteins (GRPs)
- arabino-galactan proteins (AGPs)

Enzymatic degradation of substances contained in plant cells

- Most kinds of pathogens spend all or part of their lives in association with or inside the living protoplast.
- These pathogens obviously derive nutrients from the protoplast.

- The great majority of fungi and bacteria -obtain nutrients from protoplasts after the latter have been killed.
- Some of the nutrients, e.g., sugars and amino acids are sufficiently small molecules to be absorbed by the pathogen directly.
- Some of the other plant cell constituents, however, such as starch, proteins and fats can be utilized only after degradation by enzymes secreted by the pathogens.

Proteins

- Plant cells contain innumerable different proteins, which play diverse roles as catalysts of cellular reactions (enzymes) or as structural material (in membranes and cell walls).
- Proteins are formed by the joining together of numerous molecules of about 20 different kinds of amino acids.
- All pathogens seem to be capable of degrading many kinds of protein molecules.
- The plant pathogenic enzymes involved in protein degradation are similar to those present in higher plants and animals and are called proteases or proteinases or, occasionally, peptidases.

Starch

- Starch is a glucose polymer and exists in two forms:
- amylose, an essentially linear molecule
- amylopectin, a highly branched molecule of various chain lengths
- Most pathogens utilize starch and other reserve polysaccharides in their metabolic activities.
- The degradation of starch is brought about by the action of enzymes called amylases.
- The end product of starch breakdown is glucose and is used by the pathogens directly.

Lipids

- Various types of lipids occur in all plant cells, with the most important being phospholipids and glycolipids, both of which, along with proteins, are the main constituents of all plant cell membranes.
- The latter form a hydrophobic barrier that is critical to life by separating cells from their surroundings and keeping organelles such as chloroplasts and mitochondria intact and separate from the cytoplasm.
- Oils and fats are found in many cells, especially in seeds where they function as energy storage compound.
- The common characteristic of all lipids is that they contain fatty acids, which may be saturated or unsaturated.
- Several fungi, bacteria, and nematodes are known to be capable of degrading lipids. Lipolytic enzymes, called lipases, phospholipases, and so on, hydrolyze the lipid molecules with liberation of the fatty acids.

Microbial Toxins in Plant Disease

- Toxins are extremely poisonous substances and are effective in very low concentrations.
- Fungi and bacteria may produce toxins in infected plants as well as in culture medium.
- Toxins injure host cells either by affecting the permeability of the cell membrane or by inactivating or inhibiting enzymes and subsequently interrupting the corresponding enzymatic reactions.
- Certain toxins act as antimetabolites and induce a deficiency for an essential growth factor.

TOXINS

Non-host specific or non-host selective toxins

- Several toxic substances produced by phytopathogenic microorganisms have been shown to produce all or part of the disease syndrome not only on the host plant, but also on other species of plants that are not normally attacked by the pathogen in nature. Such toxins, called non-host-specific or non-host-selective toxins.

Tabtoxin

- Tabtoxin is produced by the bacterium Pseudomonas syringae pv. tabaci, which causes the wildfire disease of tobacco.
- Tabtoxin is a dipeptide composed of the common amino acid threonine and the previously unknown amino acid tabtoxinine.
- Tabtoxin as such is not toxic, but in the cell it becomes hydrolyzed and releases tabtoxinine, which is the active toxin.
- Toxin-producing strains cause necrotic spots on leaves, with each spot surrounded by a yellow halo.
- Sterile culture filtrates of the organism, as well as purified toxin, produce symptoms identical to those characteristic of wildfire of tobacco not only on tobacco, but in a large number of plant species belonging to many different families.
- Strains of P. syringae pv. tabaci sometimes produce mutants that have lost the ability to produce the toxin.

Phaseolotoxin

- Phaseolotoxin is produced by the bacterium Pseudomonas syringae pv. phaseolicola, the cause of halo blight of bean and some other legumes.
- Phaseolotoxin is a modified ornithine–alanine–arginine tripeptide carrying a phosphosulfinyl group.
- Soon after the tripeptide is secreted by the bacterium into the plant, plant enzymes cleave the peptide bonds and release alanine, arginine, and phosphosulfinylornithine, which is the biologically functional moiety of phaseolotoxin.
- The localized and systemic chlorotic symptoms produced in infected plants are identical to those produced on plants treated with the toxin alone so they are apparently the results of the toxin produced by the bacteria.
- Infected plants and plants treated with purified toxin also show reduced growth of newly expanding leaves, disruption of apical dominance, and accumulation of the amino acid ornithine.

- Phaseolotoxin plays a major role in the virulence of the pathogen by interfering with or breaking the disease resistance of the host toward not only the halo blight bacterium, but also several other fungal, bacterial, and viral pathogens.

Tentoxin

- Tentoxin is produced by the fungus Alternaria alternata (previously called A. tenuis), which causes spots and chlorosis in plants of many species.
- Tentoxin is a cyclic tetrapeptide that binds to and inactivates a protein (chloroplast-coupling factor) involved in energy transfer into chloroplasts.
- The toxin also inhibits the light-dependent phosphorylation of ADP to ATP. In sensitive species, tentoxin interferes with normal chloroplast development and results in chlorosis by disrupting chlorophyll synthesis.
- An additional but apparently unrelated effect of tentoxin on sensitive plants is that it inhibits the activity of polyphenol oxidases, enzymes involved in several resistance mechanisms of plants.
- Both effects of the toxin, namely stressing the host plant with events that lead to chlorosis and suppressing host resistance mechanisms, tend to enhance the virulence of the pathogen.

Cercosporin

- Cercosporin is produced by the fungus Cercospora and by several other fungi.
- It causes damaging leaf spot and blight diseases of many crop plants, such as Cercospora leaf spot of zinnia and grey leaf spot of corn.
- Cercosporin is unique among fungal toxins in that it is activated by light and becomes toxic to plants by generating activated species of oxygen, particularly single oxygen.

Other non-host-specific toxins

- **Fumaric acid-** produced by *Rhizopus* spp. in almond hull rot disease
- **Oxalic acid** -*Sclerotium* and *Sclerotinia* spp. in various plants they infect and by *Cryphonectria parasitica*, the cause of chestnut blight
- **Alternaric acid, alternariol, and zinniol** -*Alternaria* spp. in leaf spot diseases of various plants
- **Ceratoulmin-** *Ophiostoma ulmi* in Dutch elm disease
- **Fusicoccin-** *Fusicoccum amygdali* in the twig blight disease of almond and peach trees
- **Ophiobolin** -several *Cochliobolus* spp. In diseases of grain crops
- **Pyricularin-** *Pyricularia grisea* in rice blast disease
- **Fusaric acid and lycomarasmin** -*Fusarium oxysporum* in tomato wilt

Host-specific or host-selective toxins

- **A host-specific or host-selective toxin** is a substance produced by a pathogenic microorganism that, at physiological concentrations, is toxic only to the hosts of that pathogen and shows little or no toxicity against non-susceptible plants.
- **Most host-specific toxins** must be present for the producing microorganism to be able to cause disease.
- **Host-specific toxins** have been shown to be produced only by certain fungi (Cochliobolus, Alternaria, Periconia, Phyllosticta, Corynespora, and Hypoxylon), although certain bacterial polysaccharides from Pseudomonas and Xanthomonas have been reported to be host specific.

Victorin, or HV Toxin

- Victorin, or HV-toxin is produced by the fungus Cochliobolus (Helminthosporium) victoriae, which causes Victoria blight of oats.
- This fungus appeared in 1945 after the introduction and widespread use of the oat variety Victoria and its derivatives, all of which contained the Vb gene for resistance to crown rust disease.
- *C. victoriae* infects the basal portions of susceptible oat plants and produces a toxin that is carried to the leaves, causes a leaf blight, and destroys the entire plant.
- Victorin is a complex chlorinated, partially cyclic pentapeptide.
- The primary target of the toxin seems to be the cell plasma membrane, where victorin seems to bind to several proteins.
- Victorin also functions as an elicitor that induces components of a resistance response that include many of the features of hypersensitive response and lead to programmed cell death.

T Toxin [HMT Toxin]

- T toxin is produced by race T of Cochliobolus heterostrophus (anamorph: Bipolaris maydis, earlier called Helminthosporium maydis), the cause of southern corn leaf blight.
- First appeared in the United States in 1968, it spread throughout the corn belt by 1970, attacking only corn that had the Texas male-sterile (Tms) cytoplasm.
- The ability of C. heterostrophus race T to produce T toxin and its virulence to corn with Tms cytoplasm are controlled by one and the same gene.
- T toxin does not seem to be necessary for the pathogenicity of C. heterostrophus race T, but it increases the virulence of the pathogen.
- The T toxin apparently acts specifically on mitochondria of susceptible cells, which are rendered nonfunctional, and inhibits ATP synthesis.

HC Toxin

- Race 1 of Cochliobolus carbonum (anamorph:Bipolaris(Helminthosporium) zeicola) causes northern leaf spot and ear rot disease in maize.
- It also produces the host-specific HC toxin, which is toxic only on specific maize lines.
- The mechanism of action of HC toxin is not known, but this is the only toxin, so far, for which the biochemical and molecular genetic basis of resistance against the toxin is understood.
- Resistant corn lines have a gene (Hm1) coding for an enzyme called HC toxin reductase that reduces and thereby detoxifies the toxin.
- Susceptible corn lines lack this gene and, therefore, cannot defend themselves against the toxin.

Alternaria alternata Toxins

- Several pathotypes of Alternaria alternata attack different host plants and on each they produce one of several multiple forms of related compounds that are toxic only on the particular host plant of each pathotype.
- Some of the toxins and the hosts on which they are produced and affect
- AK toxin causing black spot on Japanese peat fruit,
- AAL toxin causing stem canker on tomato,
- AF toxin on strawberry,
- AM toxin on apple,
- ACT toxin on tangerine,
- ACL toxin on rough lemon, the HS toxin on sugar cane.
- As an example of A. alternata toxins, the AM toxin is produced by the apple pathotype of A. alternata, known previously as A. mali, the cause of Alternaria leaf blotch of apple.

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LECTURE 17

HOST PARASITE INTERACTION

HOST PLANT INTERACTIONS

- After the pathogen enters the host until the disease symptoms appear, a series of interactions between host and pathogen take place.
- The disease symptoms may be considered as the signs of reaction of the host to infection by the pathogen.
- The severity of the symptoms varies depending upon the capacities of each of them to supersede the other.
- In that process, which may last for a few hours to many days, different biochemical reactions become involved.
- The establishment of the pathogen or colonization of the host by the pathogen after its entry initiates the symptoms.
- The hypersensitive reaction of the host is a mechanism to prevent such colonization by the pathogen.
- Many pathogens which cause disease in plants are highly specific in their nutritional requirements.
- They must reach the proper site inside the host tissue to obtain the required nutrients.
- The chances of a pathogen establishing itself in a host depend on:
- its entering the suitable host
- its reaching the proper location within the host tissue so that the required nutrients are obtainable
- A vascular pathogen may not establish itself inside a host if it is confined to sub-stomatal region.
- Similarly, wood rotting basidiomycetous fungus may not find food for its growth if it enters the leaf tissues.
- Tissue preference of parasites has been, for a long time, attributed to specific nutrients present in that particular tissue.
- Recent evidence indicates that tissue preference is solely influenced by inhibitory substances (prohibitins) present in the tissue.

Post Entry Stages

- The subsequent stages, after the pathogen has entered a susceptible host which is pre-disposed to the disease, are of two kinds:
- The pathogen may kill the host tissue in advance, drawing nutrients from the dead cells.
- The pathogen and host may develop an harmonious relationship or association in which neither is killed. The pathogen absorbs nutrients from the living host cells.
- Another biological relationship which exists is nature in the case of root nodule bacteria and legume plants is symbiosis, whereby two biological systems derive benefit from each other.

Effects of pathogens on photosynthesis

- The pathogen directly affects the photosynthetic capacity of the host.

- In many cases the pathogen causes chlorosis of the tissues, indicating through inhibition of certain enzyme activities.
- The Hill reaction, in which water is split into oxygen and hydrogen atoms, which in turn, is coupled with the production of adenosine triphosphate (ATP) through photosynthetic phosphorylation is adversely affected.
- Apparently the enzymes of CO2 fixation in photosynthesis are not affected, but the glycolic acid oxidases are affected, resulting in a reduction in protein synthesis.
- Because of the reduction in photosynthetic activity, other chain reactions in the plant result, causing increased water loss, wilting and reduction of plant vigour.

Effects of pathogens on respiration

- In general, plants infected by most pathogens react with an immediate increase in the respiratory rate.
- This increase is mostly non-specific, as even mechanical injuries to certain tissues also cause it.
- In incompatible and destructive host-pathogen relationships, the respiratory rate is high in the early stages of infection, whereas in compatible host-pathogen relationship, as in obligate parasitism, there is little change in the respiratory rate.
- In the destructive host-pathogen relationship, the pathogen destroys the normal physiological balance, the Pasteur effect, (which implies that in the presence of oxygen the fermentative degradation of carbohydrates is reduced and the energy release is more) is abolished, and hence, the respiratory efficiency is also reduced.

Effect of pathogens on Embden-Meyerhof-Parnas pathway

- There is an accumulation of carbohydrates, mostly starch, in the infected tissues, coupled with a shift from the Embden-Meyerhof-Parnas pathway to the pentose-phosphate cycle which causes the accumulation of reduced nicotinamide adenine dinucleotide phosphate (NADPH2).
- The pathogen causes tissue disintegration of the host, which is accomplished by the activity of several oxidative enzymes such as peroxidase, phenol oxidase and ascorbic acid oxidase, linked up with the oxidation of NADPH2 to NADP.
- The hypersensitive reaction in a plant that results in necrosis also causes a shift in the respiratory pathway from the Embden- Meyerhof-Parnas to the pentose phosphate system.
- There is also an increase in the oxidative enzyme activity to cause an enhancement of oxygen uptake. If certain oxidative enzyme inhibitors are produced in the host-pathogen system, the resistance of the host plant is reduced, and in the absence of oxidative enzyme inhibitors, the resistance of the host is increased.

Pathogenesis related proteins

- Infection by pathogens also interferes with the host nucleic acid and protein metabolism, especially enzymes.
- Most conspicuous changes are in peroxidase, ascorbic acid oxidase, cytochrome oxidase, phenol oxidase, etc.
- In addition, several proteins accumulate which were first called pathogenesis related proteins and are now referred to as stress proteins as they accumulate in response to physical and biological stresses.

- Both in virus infected tissues and galls, nucleic acids especially RNA accumulate.
- Hypertrophy of host cells, accompanied by increases in the size of nuclei has been demonstrated.
- There is redirection of protein synthesis towards accumulation of proteins of the pathogen, or a reduction in the protein and nitrogen level of the host, especially towards the degenerative stages of disease.
- The synthesis of virus protein markedly affects the host protein metabolism. In hypersensitive reaction, however there is no great change in the total protein content or nitrogen level in the affected tissues.

Phytoalexins

- The phenol metabolism of infected plants is profoundly altered.
- In incompatible host-pathogen combinations, there is more rapid accumulation of phenolic substances than in compatible combination.
- Certain phenolics and other aromatic substances with antimicrobial properties, named phytoalexins, are produced post-infectionally in host tissues, and this is believed to be a response to infection directed towards imparting resistance in the host.
- This response is greater in incompatible host-pathogen combination than in the compatible one.
- Production of phytoalexins may also be induced in the host in non-pathological conditions, such as physical stress and chemical stimuli.
- The production of leaf spots, necrosis and other types of lesions on the host are correlated with enhanced phenol oxidase activity. Oxidation of phenolic substances causes the accumulation of melanin pigments in the infection site, resulting in discoloration.

Growth regulators

- Due to pathological condition of the plant, the growth regulatory mechanism is upset and depends largely on the nature of host –plant relationship.
- Auxin level often increases in the host tissue as a result of infection by fungi and bacteria.
- This may be due to increased auxin synthesis by the host and /or pathogen, or the suppression of activity of enzymes indole acetic acid oxidase which degrades the auxins under normal conditions.
- Gibberellins or gibberellin-like substances have been found in many plants and are also produced by a few fungi.
- The auxin activity in meristematic tissues of plants is believed to be controlled by gibberellins.
- Infection of plants with some pathogens alters the level of gibberellin-like substances which result in the hyper-elongation of the plant.
- One such typical example is the Bakane disease of rice plants caused by Fusarium moniliforme (teleomorph: Gibberella fujikuroi).
- Dwarfism or stunting in some host-pathogen interactions is caused by a reduction in the gibberellins.
- Kinetin (cytokinin), 6-furfuryl aminopurine, an essential hormone, is involved in cell division in plant tissues.
- IAA and cytokinin act in a linked manner, the former responsible for cell expansion and the latter for cell division.

- In the absence of IAA, however, kinetin seems to be functionless.
- From plants and microorganisms, cytokinin has been isolated and characterized.
- It is a precursor of tRNA. Certain plant pathogens interfere with normal cytokinin metabolism in plants causing gall formation, senescence, fasciations, yellowing of leaves, green island formation, etc. which could be reversed by the exogenous application of kinetin.
- Ethylene, another well known growth regulator in plants plays a prominent role in causing epinasty, leaf yellowing and senescence in diseased plants.
- Many plant pathogens favour accumulation of high levels of ethylene in host tissues which contribute to symptom development like leaf drop, e.g., due to Diplocarpon rosae, the black spot of rose pathogen.

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LECTURE 18

VARIABILITY IN PLANT PATHOGENS

INTRODUCTION

Variability means the quality of being subject to variation or quality of being uneven or lacking uniformity. In plant pathogens, the variation takes place due to genetic variability. Genetic variability is the measure of the tendency of individual genotype in a population to vary from other or having more than one genetic state or allele at each locus.

- Variation is usually associated with sexual reproduction by fungal oospores, zygospores,
- a variant can be defined as an individual among a certain population that have deviated from the normal individuals genetically and possesses certain different characters which are not generally associated with that particular population previously.

Mechanisms of variability in plant pathogens

- General mechanisms of variability are:

- mutation, recombination, gene and genotype flow, genetic drift and selection, out-crossing etc.

- Specialized mechanisms of variabilityare:

- heterokaryosis, parasexuality, saltation etc. in fungi
- conjugation, transformation and transduction in bacteria
- recombination in viruses

GENERAL AND SPECIALIZED MECHANISMS OF VARIABILITY IN FUNGI

1. General mechanisms of variability in fungi

Mutation is more or less an abrupt change in genetic material of an organism or a virus, which is then transmitted in a hereditary fashion to the progeny.

- Mutations in nature are less frequent and are result of infrequent changes that take place during cell division and result in irregularities in replication or rearrangement of minute parts of genetic material of the cells.
- Mutation can be induced artificially with increased frequency by physical agents like ultra violet rays, X-rays, gamma-rays or by chemicals like alkaloids, phenols etc.
- Most mutations are recessive; therefore in diploid organisms mutation remains unexpressed until they are brought together in a homozygous condition.
- Although frequency of mutation is low, but given the great number of progeny produced by pathogen, it is possible that large number of mutants differ in virulence from their parents.
- Mutation has been reported in Cladosporium fulvum causing tomato leaf mould, Phytophthora infestans, Puccinia graminis and Melampsora lini, apart from the appearance of highly destructive race T of Heliminthosporium maydis.

Recombination occurs primarily during the sexual reproduction of plants, fungi, and nematodes whenever two haploid nuclei containing genetic material that may differ in many loci unite to form a diploid nucleus called a zygote.

- Recombination can be intraspecific, interspecific or even intergeneric; and the resulting hybrids may have different pathogenic abilities from 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.
- Recombination of genes occurs in autoecious rusts, such as Melampsora lini. Similarly, evolution of new physiological races through meiotic recombination is common in many pathogenic fungi, rusts, smuts, powdery mildews apart from potato blight fungus.

Gene flow is a process by which certain alleles move from one population to another geographically separate population.

- Population genetics, genetic drift and selection also bring about variability in the plant pathogens.
- Mating system is considered in terms of the amount of inbreeding that occurs in a population of sexual organism. Many smut fungi are forced to inbreed because a dikaryon must form for a successful infection and likely encounters are between the basidiospores arising from same psuedobasidia of the strains in the soil.
- Outcrossing of individuals put together new combination of genes rapidly leading to many different genotype within population.

2. Specialized mechanism of variability in fungal pathogens

Heterokaryosis is occurrence of dissimilar or genetically different nuclei in a vegetative cell or spore or hypha as in basidiomycetes.

- It provides haploid organisms the ability or somatic flexibility with changing environment.
- It increases diversity as genetic recombination is brought about by interchanges of whole chromosome or through mitotic crossing-over.
- It plays an important role in homothallic and imperfect fungi.
- Heterokaryosis can arise by

a) gene mutation
b) fusion of vegetative mycelium
c) at the time of spore formation in many fungi such as Neurospora tetrasperma, Podospora sp.

- Heterokaryosis is certainly a way in which avirulent strains may acquire virulence, for example in Thanatephorus cucumeris.
- Parasexuality is a process in which genetic recombination occurs in the vegetative thallus in absence of sexual stage.
- Sequence of events in parasexuality is as follows:

i) formation of dikaryotic mycelium
ii) fusion between two nuclei
iii) multiplication of diploid nuclei side by side the haploid nuclei
iv) occasional mitotic crossing over during the multiplication of diploid nuclei
v) sorting out of diploid nuclei
vi) occasional haploidization of the diploid nuclei
vii) sorting out of the new haploid strain.

- Parasexuality has produced new races of Fusarium oxysporum f. sp. pisi, Ascochyta, Verticillium albo-atrum etc.
- Saltation is appearance of morphologically different sectors in fungal colonies.
- It occurs frequently in fungal colonies of some isolates of Fusarium and Helminthosporium.
- Saltation may be influenced by compaction and thickness of culture media.

OTHER MECHANISMS

3. Sexual-like processes in bacteria

New biotypes of bacteria seem to arise with varying frequency by means of at least three sexual-like processes.

i) In conjugation, two compatible bacteria come in contact with each another and a small portion of the chromosome or plasmid from one bacterium is transferred to the other through a conjugation bridge or pilus.

ii) In transformation, bacterial cells are transformed genetically by absorbing and incorporating in their own cells genetic material secreted by or released during rupture of other bacteria.

iii) In transduction, a bacterial virus (phage) transfers genetic material from the bacterium in which the phage was produced to the bacterium it infects next.

- When the gene transfer is limited to members of the same species or even genus, it is called vertical gene transfer. Sometimes, gram-negative bacteria can transmit genetic material readily across species; as Agrobacterium transmits genes across kingdom barriers to plants, such events are called horizontal gene transfer.

4. Genetic recombination in viruses

- When two strains of the same virus are inoculated into the same host plant, one or more new virus strains are recovered with properties (virulence, symptomatology, and so on) different from those of either of the original strains introduced into the host.
- The new strains probably are recombinants, although their appearance through mutation, not hybridization, can not always be ruled out.
- In multipartite viruses consisting of two, three, or more nucleic acid components, new virus strains may also arise in host plants or vectors from recombination of the appropriate components of two or more strains of such viruses.

5. Loss of pathogen virulence in culture

- The virulence of pathogenic microorganisms toward one or all of their hosts often decreases when the pathogens are kept in culture for relatively long periods of time or when they are passed one or more times through different hosts.
- If the culturing of the pathogen is prolonged sufficiently, the pathogen may lose virulence completely. Such partial or complete loss of virulence in pathogens is sometimes called attenuation, and it has been shown to occur in bacteria, fungi and viruses.
- Loss of virulence in culture, or in other hosts, seems to be the result of selection of individuals of less virulent or avirulent pathogen strains that happen to be capable of growing and multiplying in culture, or in the other host, much more rapidly than virulent ones.
- After several transfers in culture or the other hosts, such attenuated individuals largely, or totally, overtake and replace the virulent ones in the total population so that the pathogen is less virulent or totally avirulent.
- On reinoculation of the proper host, isolates in which the virulent individuals have been totally replaced by avirulent ones continue to be avirulent, and therefore loss of pathogenicity is irreversible.

- However, on reinoculation of the proper host with isolates in which at least some virulent individuals survived through the transfers in culture or the other host, the few surviving virulent individuals infect the host and multiply, often in proportion to their virulence.
- The virulent individuals increase in number with each subsequent inoculation; while at the same time, non-virulent individuals are reduced or eliminated with each reinoculation.

Stages of variation in pathogens

- **Species:** The entire population of a particular organism on the earth, e.g. a fungal pathogen, has certain morphological and other phenotypic characteristics in common and makes up the species of pathogen, such as Puccinia graminis, the cause of stem rust of cereals.
- **Varieties or special forms:** Some individuals of this species, however, attack only wheat, barley, or oats, and these individuals make up groups that are called varieties or special forms (forma specialis) such as P. graminis f. sp. tritici or P. graminis tritici, P. graminis hordei, and P. graminis avenae.
- **Race:** Even within each variety or special form, some individuals attack some of the varieties of the host plant but not the others, some attack another set of host plant varieties, and so on with each group of such individuals making up a race. Thus, there are more than 200 races of P. graminis tritici (race 1, race 15, race 59 and so on).
- **Variant:** Occasionally, one of the off-spring of a race can suddenly attack a new variety or can cause severe symptoms on a variety that it could barely infect before. This individual is called a variant.
- **Biotype:** The identical individuals produced asexually by the variant make up a biotype. Each race consists of one or several biotypes (race 15A, 15B and so on).

Appearance of new pathogen biotypes

- The appearance of new pathogen biotypes may be very dramatic when the change involves the host range of the pathogen.
- If the variant has lost the ability to infect a plant variety that is widely cultivated, this pathogen simply loses its ability to procure a livelihood for itself and will die without even making its existence known to us.
- If, however, the change in the variant pathogen enables it to infect a plant variety cultivated because of its resistance to the parental race or strain, the variant individual, being the only one that can survive on this plant variety, grows and multiplies on the new variety without any competition and soon produces large populations that spread and destroy the resistance variety.
- This is the way the resistance of plant variety is said to be broken down although it was the change in the pathogen, not the host plant that brought it about.

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LECTURE 19

DISEASE RESISTANCE AND DEFENSE MECHANISMS IN PLANTS

DISEASE RESISTANCE

Disease resistance

- Resistance is the ability of a host plant to resist the growth or establishment of a pathogen.
- Many plant pathogenic microorganisms are specific to certain host plants.
- The plant species and cultivars vary widely in their capacity to resist the establishment of a pathogen.
- When a pathogen enters the host, it must overcome several barriers before establishing itself.
- Sometimes the host reacts to the entrance of the pathogen or alters growth of the pathogen and thus effectively checks the establishment of the disease.
- There is great variation in the degree of resistance of a plant to a given parasite.
- This is influenced by various factors, involving the host, parasite and environment.

Disease escape: When the plant, though genetically susceptible to the pathogen, may show resistance in the field; such a condition is termed as disease escape.

- It happens when the optimum conditions are not present for the disease development on the crop variety, or the crop may not be in the proper stage of growth when the pathogen is most active.
- The host plant may be susceptible to the pathogen when it is young and develop resistance at maturity or vice-versa.

Disease endurance: In some cases, the plant is capable of carrying on most of the normal metabolic processes in spite of the diseased condition without reducing the yield to a considerable extent. It is known as disease endurance or disease tolerance.

Hypersensitive reaction or hypersensitivity: In hypersensitivity, when the pathogen enters the host, the cells in the immediate vicinity react in such a manner as to delimit the spread of the pathogen through death reaction of the cell, or by forming other barriers.

- The symptoms of such reactions in the host appear as minute specks, which the indicative of high resistance in the plant.
- The plant may be called resistant to the disease, if it shows hypersensitive reaction to infection by the pathogen.

Types of resistance

Resistance to diseases is also a genetically controlled character. Plants possess two different types of resistance:

- **Monogenic-** It is controlled by specific single gene. This type possesses high resistance to a given strain or race of the pathogen but it susceptible to other races.

- **Polygenic-** It is controlled by many genes and is not so high but at the same time does not easily breakdown due to the evolution of new races. This is also referred to as durable resistance.

Vertical vs. horizontal resistance

- The concept of vertical and horizontal resistance was suggested by Vander Plank in 1968.
- Resistance is vertical (differential) when it is completely effective against some races of a pathogen but not against the others.
- Vertical resistance is complete but is not permanent.
- Horizontal resistance is effective against all races of a pathogen.
- Horizontal resistance, though incomplete, is of permanent nature.

Conceptual explanation of different terminologies used to explain vertical and horizontal resistance by research workers are illustrated hereunder:
Gene for gene hypothesis

- It was first proposed by Flor (1942, 1945) as the simplest explanation of the result of studies of the inheritance of resistance and pathogenicity flax-rust host-pathogen system, and now applies to most of the combinations.
- According to this hypothesis, "for a resistance gene in the host there is a complementary avirulence gene in the pathogen."
- The coexistence of host plant and pathogen side by side in nature indicate that they have co-evolved; and changes in virulence of pathogen have been continuously governed by the changes in host or vice versa.
- This concept has been shown to operate in many other diseases like rusts, smuts, apple scab, late blight of potato and many other fungal , bacterial , virus and higher parasitic plant diseases.
- Generally, but not always in the host, gene for resistance is dominant (R) and gene for susceptibility is recessive (r).
- In pathogen, however, gene for avirulence that have inability to infect is dominant (A) and for virulence it is recessive (a).
- Thus plant variety when carrying gene for resistance (R) for certain pathogen and other lacking gene (R) i.e. carrying gene for susceptibility (r) are inoculated with two races of pathogen, one of which carries a gene for avirulence (A) and other carrying the gene for virulence (a) against (R); it gives a 4 gene combinations as below:

(-) denotes incompatible (resistant reaction); (+) denotes (compatible) susceptible reaction

- Out of the four combinations as above; only AR interaction is resistant or incompatible i.e. host has certain gene for resistance(R) that recognizes the corresponding genes for avirulence (A), so there is incompatibility.
- In Ar combination, infection results because host lacks the gene for resistance so pathogen can attack with other gene for virulence.
- In aR combination, infection occurs, although the host has gene for resistance but the pathogen lacks the gene for avirulence (A) which is recognized by specific gene for resistance; hence no defence mechanism is activated.
- Finally in ar gene combination, as plant lacks the gene for resistance and have gene for susceptibility (r) and pathogen have the gene for virulence (a), it results in infection.

Genetics of resistance

- Gene for resistance appear and accumulate first in host through evolution and they coexist with non-specific genes for pathogenicity that exist in pathogen.
- Genes for pathogenicity exist in pathogen against all host plants that lack specific resistance.
- When a specific gene for resistance appears in the host or bred into the host, the gene enables the host to recognize the product of the particular gene for virulence in pathogens, the pathogen gene is then thought of as the virulence gene (avrA) of pathogen that correspond to plant resistance gene (R).
- The change in the function of the pathogenic gene is because of subsequent recognition of avrA product i.e. elicitor molecule by the receptor coded by R gene which triggers the hypersensitive response reaction in the plant that keeps the plant resistant.

Breakdown of resistance

- A new gene for virulence that attacks existing gene for resistance appears by mutation of an existing avr gene which then avoids gene for gene recognition and the resistance breaks down.
- Plant breeders introduce another gene for resistance (R) in plant which recognizes the protein of new gene for virulence of pathogen and extend the resistance of host beyond the range of new gene for virulence in pathogen.
- This produces a variety that is resistant to all races that have an avirulence gene corresponding to specific gene for resistance until another gene for virulence appears in pathogen.

DEFENCE MECHANISMS IN PLANTS

Defence mechanisms in plants

- Resistance to infection has been attributed to morphological and structural feature of plants.
- Evidences, however, are very much against their participation in resistance.
- The essential character of resistance is a result of physiological activity of plants and the resistance is displayed through substance called prohibitins.
- **Prohibitins** - inhibit and also inactivate extra-cellular enzymes and toxin of parasites, and prevent their spread.
- Most of the prohibitins are phenolic in nature; they react with enzymes and inactivate toxins by forming complexes.
- **Examples are:** allicin produced in garlic, catechol and protocatechuic acid in onions, chlorogenic acid and caffeic acid in coffee, mangiferin in mango, and phloretin in apple.
- Phytoalexins
- During infection, most plants synthesize toxic substances which form the basis of phytoalexin theory.
- Phytoalexins are "low molecular weight substances produced by plants relatively rapidly and in high concentration in response to infection by pathogens, and physico-chemical stress which are active in a wide range of pH, temperature and nutrient conditions; and confer protection against the infecting parasites.
- Ipomeamarone produced in sweet potato, rishitin in tomato, phaseollin in beans and pisatin in peas are some examples of such compounds.
- They are confined to the tissue colonized by the pathogen.
- The speed with which phytoalexins are produced depends upon the degree of resistance in the host.
- The sensitivity or capacity of the host to produce phytoalexins is governed by genetic factors; and a gene-for gene relationship in host parasite reactions appears to be present.

Structural defence

- A few host plants produce structures in response to infection which prevent the spread of the pathogen.
- These structures are cork layers, tyloses and abscission layers.
- Deposition of gums, resins, polymers of tannins and melanin, swelling of cell walls and their sheathing in columns are other defense reactions.
- Cork layers are produced by potato tubers infected by Rhizoctonia solani, which prevent the spread of toxic substances, produced by the pathogen and effectively check the flow of nutrients and water from the healthy to the infected area.
- Tyloses formation has been noted in tomato and sweet potato plants against wilt pathogens.
- Formation of abscission layer is a wide spread response of plants infected by fungi, bacteria or viruses.
- Gum deposits impregnated with toxic substances along the borders of lesions frequently check the advance of parasites; a typical example being the resistance of rice to blast pathogen Pyricularia oryzae.

- Sheathing of infecting hyphae by extension of the cell walls has been observed in a few instances. The sheath consists of cellulose, callose substances and lignin.

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LECTURE 20

DISSEMINATION OF PLANT PATHOGENS

METHODS OF DISSEMINATION

Dissemination is the spread of plant pathogens within the general area in which it is established is termed as their dissemination or dispersal or transmission.

Methods of dissemination

- Different methods of dissemination of the plant pathogens with in a crop season as well as to the next season after its survival are:

i) Direct methods

ii) Indirect methods.

- In direct transmission, the dispersal takes place along with the seeds and vegetative plant parts used for propagation.
- Indirect transmission may be active/autonomous or brought about passively by different agencies like wind, water, animals or human beings.

Direct transmission
It is further divided into:

- Adherent transmission
- Germinative transmission
- Vegetative transmission

- In adherent type, the pathogen propagules are carried over the surfaces of seed or other propagative materials. Bunt of wheat caused by Tilletia foetida and T. caries is carried through the seed externally. Similarly, spores of Synchytrium endobioticum which causes potato wart and Rhizoctonia solani (the black scurf pathogen) sclerotia are adherent to potato tubers used as seeds.
- In germinative type, the plant pathogens are carried through the seed or other propagules internally as in case of loose smut of wheat and barley. Similarly, bean mosaic is transmitted through pollen grains and is carried in the seeds.
- In vegetative transmission, a large number of fungal, bacterial, viral and phytoplasmal plant pathogens are carried in the vegetative plant parts used as seeds such as tubers, cuttings, runners, grafts etc.
- For example, ring rot of potato caused by Clavibacter sepidonicum and Ralstonia solanacearum causing brown rot is carried through infected potato tubers to new ones.
- Similarly, dormant mycelium of Phytophthora infestans is carried through potato tubers. A large number of viruses, e.g., potato virus X, potato virus Y, citrus tristeza and apple mosaic virus, etc. are transmitted through their propagative parts apart from red rot and whip tail of sugarcane.

INDIRECT TRANSMISSION

Indirect transmission
It can be:
a. Autonomous/Active transmission
b. Wind dispersal of pathogens (Anemochory)
c. Water dispersal of pathogens (Hydrochory)
d. Animal dispersal (Zoochory)
e. Insect dispersal (Entomochory)
f. Human dispersal (Anthropochory)

Autonomous transmission

- By this method, the plant pathogens are spread to short distances.
- It takes place by active growth of the hyphae or hyphal strands.
- It is characteristic of wood rotting fungi like Arnillariella, Fomes, Ganoderma, etc. which migrate independently through the soil from plant to plant or even from field to field by active growth of their strands.
- Zoospores of Phytophthora and Pythium can swim through the water film in soil although to limited distances, likewise nematodes.
- Spores of some fungi are expelled forcibly from the sporophores or sporocarps by puffing action.
- The seeds of some parasitic plants are also expelled forcibly and may reach over distances of several meters.

Dissemination by wind/air

- Many fungal spores and seeds of most parasitic plants are disseminated by wind or air currents that carry them as particles to various distances.
- Air current pickup spores and seeds of sporophores and carry them upward or horizontally.
- While air borne some of the spores may reach the wet surfaces and get trapped; and when air movement stops or it rains the rest of the spores land or may be brought down by rain drops on to the susceptible host surfaces.
- The spores of many fungi are too delicate to survive a long trip through air and are, therefore, successfully disseminated to a few hundred to a few thousand meters only.
- The spores of other fungi, particularly those of cereal rusts are very hardy and can be successfully transported over distances of several kilometers for causing widespread epidemics.
- Bacteria and nematodes present in the soil may be blown away along with soil particles on the dust storm.
- Wind also helps in the dissemination of bacteria, fungal spores and nematodes by blowing away rain splash droplets carrying them and wind also carries away insects that may contain or smeared with viruses, bacteria or fungal spores.

Disseminations by water

- Water is an important agency for disseminating pathogens in three ways:

- Bacteria, nematodes and spores, sclerotia and mycelial fragments of fungi present in soil are disseminated by rain or irrigation water that moves on the surface or through the soil.
- All bacteria and spores of many fungi are exuded in a sticky liquid and depend for the dissemination on rain or over-head irrigation water which either washes them downward or splashes them in all directions.
- Rain drops or drops from over-head irrigation pickup the fungal spores and any bacterial propagules from the air and wash them downward where some of them may land on susceptible plants.

Dissemination by insects

- Insects, particularly aphids and leaf hoppers are by far the most important vectors of viruses, phytoplasmas and fastidious bacteria.
- Each one of these pathogens is transmitted internally by only one or a few species by insects during feeding and movements of insect vectors from plant to plant.
- More than 50 species of aphids are known to transmit the viruses of plants. Some can transmit only a few kinds or other transmits 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, bunchy top of banana, cowpea mosaic, papaya mosaic, potato leaf roll.
- Most of the yellows and witches' broom type of diseases are transmitted by leaf hoppers including rice tungro.
- Other insects like white flies transmit some important diseases like leaf curl of cotton, tobacco etc. Bottle gourd mosaic is transmitted by red pumpkin beetle.
- Thrips transmit the spotted wilt virus; whereas squash mosaic, cowpea mosaic and turnip yellow mosaic viruses are transmitted by beetles while the turnip yellow mosaic virus (TYMV) is also transmitted by grass hoppers and ear wigs.
- In general, the viruses that require no incubation period in the insect vectors are called non persistent or stylet borne viruses; and those which enter into the system of the insects and require incubation period are called persistent or circulative viruses.
- Insects are also important in the dissemination of certain bacterial and fungal plant pathogens. Erwinia tracheiphila, the cucurbit wilt organism in completely dependent on cucumber beetle for its spread. Xanthomonas stewartii, the corn wilt pathogen overwinters inside the corn flea beetle. Insects are also important agent of dissemination and inoculation of Erwinia carotovora causing black leg of potato. Erwinia amylovora causing fire blight of pear and apple is known to be transmitted by ants, whereas Xanthomonas citri causing citrus canker is carried by leaf mite from diseased to healthy plants. The conidia of Claviceps are spread from malformed to healthy flowers by insects feeding on honey dew. The spores of Ceratocystis fagacearum (oak wilt) and Ceratocystis ulmi (Dutch elm disease) are carried by beetles.

Dissemination by Nematodes

- About 20 viruses are known to be transmitted by the members of 4 genera of soil borne ectoparasitic nematodes, viz., Longidorus, Xiphinema, Trichodorus and Paratrichodorus. Longidorus and Xiphinema spp. transmit polyhedral-shaped viruses (NEPO) such as tobacco and tomato ring spots, cherry roll, grape vine fan leaf and others. Xiphinema index transmits fan leaf disease of grapevine. Whereas, Trichodorus and Paratrichodorus spp. transmit tubular (NETU) viruses like and tobacco rattle and pea early browning.

- Some fungal and bacterial plant pathogens are also transmitted by nematodes, e.g., Corynebacterium fasciens causing leaf gall disease is transmitted by ectoparasitic nematodes Aphelenchoides. Anguinia tritici is believed to transmit fungal pathogen Dilophospora alopecuri causing leaf spotting and twist of grains and cereals.

Dissemination by mites

- Mites transmit both stylet-borne and circulative viruses, e.g., wheat streak mosaic virus, peach mosaic virus, sterility disease of pigeon pea.

Dissemination by animals

- Almost all animals small and large that move among plants and touch them along the way can disseminate pathogens such as fungal spores, bacteria, seeds of parasitic plants, nematodes and some viruses and viroids.
- Most of these pathogens adhere to the feet or the body of the animals, but some may be carried in contaminated mouth parts.

Dissemination by fungi and dodder

- Some plant pathogens, like the zoospores of some fungi (e.g. Olpidium brassicae) and certain parasitic plants (e.g. dodder) can transmit viruses as they move from one plant to the others (zoospores) or they grow and form a bridge between two plants.
- Olpidium brassicae can transmit lettuce big vein virus, tobacco necrosis and tobacco stunt viruses.
- Synchytrium endobioticum can transmit PVX and potato mottle virus whereas dodder can transmit many viruses including aster yellows.

Dissemination by human beings

- Humans are known to disseminate all kinds of pathogens over short and long distances in a variety of ways.
- Humans disseminate some pathogens such as tobacco mosaic virus by successively handling of diseased and healthy plants.
- Other pathogens are disseminated through farm tools such as pruning shears (pear fire blight), ploughs etc. by contaminating the healthy plants or plant parts after their contamination with spores and other pathogen structures.
- Humans also transport pathogens by contaminated soil on their feet or equipment and by using infected transplants, seed, nursery stock and bud wood.
- Humans disseminate plant pathogens by importing new varieties in to an area that may carry pathogens that have gone undetected by travelling throughout the world and by importing food or other items which may carry harmful plant pathogens.
- For examples, introduction of Dutch elm disease (caused by Ophiostoma ulmi) or citrus canker in USA or powdery mildew of grapes in Europe; and more recently rapid spread of ergot of sorghum through out the world.

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LECTURE 21

SURVIVAL OF PLANT PATHOGENS

SURVIVAL

To continue the infection chain, most of the plant pathogens have developed some efficient means of survival through the unfavourable part of the year. So that with the onset of the favourable season its infection may be renewed. Chief sources of survival of plant pathogens are:

- Infected living hosts
- Infected or contaminated planting organs
- Infected crop residues
- Resting structures
- Soil

Infected living hosts

- Infected plants are the most important sources/reservoir of inoculum for plant diseases.
- Bacteria, fungal spores and spore producing structures such as pycnidia, acervuli etc. may survive the unfavorable season in the infected twigs and branches of perennial plants, e.g., Erwinia amylovora in apple, Xanthomonas campestris pv. citri in citrus causing canker.
- When the favourable season returns, the resting bodies come out of their dormant state, become active and produce primary inoculum.
- Some crops like rice, chillies, brinjal or brassicas are grown throughout the year and provide continuity to the pathogens infecting them year after year.
- Also, the volunteer or self sown plants growing outside the fields get infected during the off-season and may act as a good source of inoculum to the new crop, e.g., Puccinia graminis on wheat, Helminthosporium oryzae and Pyricularia oryzae on rice or Alternaria solani on chillies.

Subsidiary hosts

- Many plant pathogens have a wide host range and attack different related or unrelated plant species grown in different situations and different season. These are called subsidiary hosts. These include:

a) Collateral hosts
b) Alternate hosts
c) Wild hosts of the same family
d) Weed hosts.

Collateral hosts

- Fungal pathogens like Alternaria solani and A. brassicicola mostly attack members of Solanaceae and Brassicae family, respectively, which are their collateral hosts.

Alternate hosts

- *Puccinia graminis tritici* which causes stem rust of wheat attacks and survives on barberry (Barberis vulgaris), the only other species it affects other than wheat.
- Such transfer of inoculum is obligatory and essential for completing the life cycle. So barberry called the alternate host.
- Similarly, Cronartium ribicola which causes blister rust of the white pine also attacks wild or cultivated currant or gooseberry plants as its alternate host,
- Cedar is an alternate host of Gymnosporangium juniperi-virginianae causing cedar-apple rust.
- Both Rhizoctonia solani and Sclerotium roilfsii have very wide host range spread over many families. As a result the inoculum of these pathogens is available almost throughout the year for infecting the same or different host species.

Alternative hosts
Such transfer of inoculum from one host species to another is not compulsory. There are called alternative hosts.

- Cereal rusts can infect their wild hosts and survive the winter crop periods in such hosts and also some self sown wheat plants at higher altitudes.
- Powdery mildew fungus Erysiphe cichoracearum and viral pathogens of cucurbits also survive the inter-crop period in wild cucurbits when their normal crop host is absent.
- Important rice pathogens, Helminthosporium oryzae and Pyricularia oryzae can survive through their weed hosts like Leersia heaxandra, Echinochloa colonum, and Setaria intermediata and Digitaria marginata y or some others.

Vectors as hosts

- Among fungi and bacteria, which are transmitted by insects, some over winter within the body of their vectors, e.g., Ceratocystis fagacearum (oak wilt) survives in nitidulid beetles, Xanthomonas campestris pv. stewartii (maize wilt) in flea beetles and rice stunt virus in leaf hoppers.

Infected seed or planting material

- Infected or contaminated seeds and other planting material may carry the inoculum of fungal, bacterial, viral or nematode pathogens through the inter-crop period or the unfavourable season.
- Some attack the floral organs and enter in to the ovary and others infect the seed through the seed coat.
- Some infect the embryo and others the tissue beneath the seed coat and remain there in a dormant state without causing any damage to the seed and affecting their germinability.
- Ustilago tritici and Alternaria triticina (in wheat), Alternaria brasscicola (in brassicas) survive in/on the seed.
- Phytophthroa infestans (causing late blight of potato), Peronospora destructor (downy mildew of onion), Pythium aphanidermatum (rhizome rot of ginger), red rot of sugarcane, leaf roll and mild mosaic viruses of potatoes survive in the planting material like tubers, bulbs, rhizomes etc of the respective crops.

- Pathogens surviving in or on the seed or vegetative planting material are already in contact with the potential host plants and are not exposed to the vagaries of the environment.
- When the seeds germinate, the pathogens also become active, resume their growth or infect the seedlings at the earliest opportunity.
- Some other pathogens which enter through the seed coat and do not affect the embryo are Septoria apicola in celery, Colletotrichum in chillies, Pseudomonas phaseolicola in bean and Clavibacter michiganese in tomato.
- Seeds may also get contaminated with the pathogen during threshing and storage. For example, covered smut of barely (Ustilago hordei), grain smut of jowar (S. sorghi), bunt of wheat (Tilletia caries and T. foetida) or fruit rot of chillies (Colletotrichum capsici).
- Longevity of fungal spores carried externally on seed varies considerably for different pathogens depending upon storage conditions.
- While thin walled spores perish early, those with thick walls survive longer.

Crop Residue

- Crop residue consisting of infected plant parts (leaves, stems, fruits etc.), roots and stubbles help in the survival of plant pathogens are a major source of primary inoculum for the next crop to be grown there.
- Examples are Colletotrichum falcatum in sugarcane, Cercosporidium personatum in groundnut, Alternaria brassicae in crucifers, Ascochyta pisi in pea, Xanthomonas campestris pv. campestris in cabbage, Xanthomonas campestris pv. oryzae in rice, bean mosaic virus in bean and barley mosaic virus in barely.

Soil

- Soil is the ultimate resting place for most of the plant pathogens and one of the major source of primary inoculum for fungal, bacterial or nematode pathogens.
- Most pathogens come back to soil after completing parasitic existence on the host plants. Some of them survive in soil as free living saprophytes and also reproduce and complete their life cycles.
- Others just survive there showing only limited growth and no reproduction. Those that attack aerial organs of the hosts mostly survive the inter crop period or adverse climates through resistant propagules.
- Garrett (1950) distinguished behavior of soil borne fungal pathogens and grouped them as soil inhabiting and soil invading fungi.
- Soil inhabiting fungi were characterized by their ability to survive indefinitely in soil as saprophytes and to complete their life cycles there, e.g., Pythium, Phytophthora, Rhizoctonia sp., Sclerotium rolfsii and Fusarium solani. They are mostly primitive, less specialized parasites.
- Soil invading fungi also known as root inhabiting fungi were characterized by an extended parasitic phase on living host plants, mostly restricted to host roots and a declining saprophytic phase in soil after death of the host plant.
- The root inhabiting fungi with characteristic local distribution in soil are: Gaeumannomyces graminis, Fomes annosus, Phymatotrichum omnivorum and Armillariella mellea.
- Vascular pathogens including Verticillium albo-atrum represent the intermediate group. They are no doubt somewhat specialized in their parasitic habits but can

survive also in soil saprophytically for a few years. Their ability to form perennating structures like chlamydospores (in Fusarium oxysporum) or micro sclerotia (Verticillium albo-atrum) may have a contributory role in this respect.

Resting structures

- Many soil borne fungal pathogens survive effectively in soil through adverse circumstances by forming resting structures.
- Basically, the resting structures are of two types:

i) resting spores of sexual or asexual origin
ii) resting organs made of vegetative hyphae.

- Thick-walled chlamydospores help in the survival of many fungi as Pythium spp., Fusarium solani and F. oxysporum.
- Resting spores of Plasmodiophora brassicae also act in perennation. The conidia of Diplocarpon rosae also participate in the act of survival.
- Smut spores some times also called as chlamydospores also function in survival of some rust, and smut and bunt fungi, termed respectively as teleutospores, smut spores and bunt spores.
- Some fungi form sclerotia which are black hardened structures made up of aggregations of vegetative hyphae and are able to survive through extremely unfavourable conditions.
- Sclerotium rolfsii, Rhizoctonia spp. and Phymatotrichum omnivorum are some of the common fungal pathogens with an important role for sclerotia in their survival.

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LECTURE 22

EFFECT OF ENVIRONMENTAL FACTORS ON DISEASE DEVELOPMENT

EFFECT OF ENVIRONMENTAL FACTORS ON DISEASE DEVELOPMENT

- Plant diseases are ubiquitous throughout the world wherever plants grow, but of more common occurrence in humid to wet area with cool, warm or tropical temperatures.
- Diseases most commonly occur during wet, warm days and night and on plants heavily fertilized with nitrogenous fertilizers.
- So, environmental conditions frequently determine whether a particular disease will occur or not.
- Most common environmental factors that have considerable influence on development of plant disease are temperature and moisture.
- Other factors include wind, light, soil pH, soil structure etc.

Effect of temperature

- Each pathogen has an optimum temperature for its growth.
- Different growth stages of fungus, such as the production of spores, their germination and the growth of the mycelium may have slightly different optimum temperature.
- Storage temperatures for certain fruits, vegetables and nursery stock are manipulated to control fungi and bacteria that causes storage decay, provided the temperature does not change quality of products.
- In temperate regions, low temperature during late fall, winter or early spring are not congenial for the development of pathogen, but as the temperature rises, these pathogens become active and when other conditions are favourable they can cause infection and thus disease.
- Pathogen differs in their preference for higher or lower temperature. For example, the fungi namely Typhula and Fusarium causing snow mould of cereals and turf grasses, late blight pathogen Phytophthora infestans are more serious in cold regions whereas fungus like Colletotrichum, Ralstonia are favoured by higher temperature.
- Rapid disease development occurs when temperature is optimum for pathogen development and is below or above the optimum for host development.
- For stem rust of wheat (Puccinia graminis tritici) completion of infection cycle is 22 days at 5°C, 15 days at 10°C and 5-6 days at 23°C.
- The minimum, optimum and maximum temperature for the pathogen, host and disease are same, the effect of temperatures in disease development is apparently through its influence on pathogen.
- Effects of temperature may mask symptoms of certain viral and mycoplasmal diseases and making them more difficult to detect.

Effect of moisture

- Moisture influences the initiation and development of infectious plant diseases in many interrelated ways.
- It may exist as rain or irrigation water on plant surface or around the roots, as relative humidity in the air and as dew.
- Moisture is indispensable for the germination of fungal spores and penetration of the host by germ tube.

- It is also indispensable for the activation of bacterial, fungal and nematode pathogens before they can infect the plant.
- Moisture in the form of splashing rain and running water also plats an important role in the distribution and spread of many of the pathogens on the same plant and on other plants.
- Moisture also increases the succulence of host plants and thus their susceptibility to certain pathogens, which affects the extent and severity of disease.

Effect of rainfall

- The occurrence of many diseases in a particular region is closely correlated with the amount and distribution of rainfall within year.
- Late blight of potato, apple scab, downy mildew of grapes and fire blight are found or are severe only in areas with high rainfall or high relative humidity during the growing season.
- In apple scab, continuous wetting of the leaves, fruits etc. for at least 9 hours is required for primary infection to take place even at optimum range (18 to 23°C) of temperature.
- At lower temperature the minimum wetting period required is higher.
- In powdery mildews, spore germination and infection are actually lower in the presence of free moisture on the plant surface than they are in its absence.

Effect of Relative humidity

- Relative humidity is very critical in fungal spore germination and the development of storage rots.
- Rhizopus soft rot of sweet potato (Rhizopus stolonifer) is an example of storage disease that does not develop if relative humidity is maintained at 85-90 %, even if the storage temperature is optimum for the growth of the pathogen. Under these conditions, the sweet potato root produces corky tissues that wall off the Rhizopus fungus.
- Moisture is generally needed for fungal spore germination, the multiplication and penetration of bacteria and the initiation of infection e.g., germination of powdery mildew spores occurs at 90-95 % relative humidity.

Effect of soil moisture

- Soil moisture influences the initiation and development of infectious plant diseases.
- High or low soil moisture may be a limiting factor in the development of certain root rot diseases.
- High soil moisture levels favours development of destructive water mould fungi, such as species of Aphanomyces, Pythium and Phytophthora.
- Overwintering by decreasing oxygen and raising carbon-dioxide levels in the soil makes roots more susceptible to root rotting organisms.
- Diseases such as take all of cereals (Gaeumannomyces graminis), charcoal rot of corn, sorghum and soyabean (Macrophomina phaseolina), common scab of potato (Streptomyces scabies) and onion white rot (Sclerotium cepivorum) are most severe under low moisture levels.

Effect of wind

- Most plant diseases that occurs in epidemic portions and spread in large areas are caused by fungi, bacteria and viruses that are spread either directly by wind or indirectly by insects which can travel long distances with the wind.
- Uredospores and many conidia are transported to many kilometers by wind.
- Wind becomes more important when it is accompanied by rain.
- Wind blown rain splashes can help in spread of bacteria from the infected tissues.

Effect of light

- Light intensity and duration may either increase or decrease the susceptibility of plants to infection and also the severity of disease.
- Light mainly cause production of etiolated plants due to reduced light intensity which in turn increases the susceptibility of plants to non-obligate parasites but decreases the susceptibility of plants to obligate parasites.
- It also enhances the plants' susceptibility to viral infections.

Effect of soil pH

- Soil pH is a measure of acidity or alkalinity and it markedly influences occurrence of soil borne pathogens.
- Growth of potato scab (Streptomyces scabies) pathogen is suppressed at a pH of 5.2 or slightly below but is more severe at a pH 5.2 to 8.0 or above.
- Club root of crucifers caused by Plasmodiophora brassicae is most severe at a pH of 5.7, whereas its development drops sharply between 5.7 and 6.2 and is completely checked at pH 7.8.

Effect of soil type

- Certain pathogens are favored by loam soils and others by clay soils.
- Fusarium wilt disease which attacks a wide range of cultivated plants causes more damage in lighter and higher soils.
- Nematodes are also most damaging in lighter soils that warm up quickly.

Effect of host-plant nutrition

- Nutrition affects the rate of growth and the state of readiness of plants to defend them against pathogenic attack.
- Nitrogen abundance results in the production of young, succulent growth, a prolonged vegetative period and delayed maturity of the plants.
- These effects make the plant more susceptible to pathogens that normally attack such tissues and for longer periods.
- In contrast, plants suffering from a lack of nitrogen are weaker, slow growing and faster aging.
- Such plants are susceptible to pathogens that are best able to attack weak, slow-growing plants.
- Large amounts of nitrogen increases the susceptibility of pear to fire blight (Erwinia amylovora), wheat rust (Puccinia) and powdery mildew (Erysiphe).

- Reduced availability of nitrogen may increase the susceptibility of tomato to Fusarium wilt and Alternaria solani, of sugar-beets to Sclerotium rolfsii and of most seedlings to Pythium damping-off.
- Severity of the disease caused by Fusarium spp., Plasmodiophora brassicae and Sclerotium rolfsii increases when an ammonium fertilizer is applied whereas the severity of diseases caused by Streptomyces scabies and Gaeumannomyces graminis increase when nitrate form of fertilizers are applied.
- Phosphorus has been shown to reduce the severity of potato scab but to increase the severity of cucumber mosaic virus on spinach and Septoria infection of wheat leaves and glumes.
- Phosphorus seems to increase resistance either by improving the balance of nutrients in the plant or by accelerating the maturity of the crop and allowing it to escape infection by pathogens that prefer younger tissues.
- Potassium has also been shown to reduce the severity of numerous diseases including stem rust of wheat and early blight of tomato, whereas high amounts of potassium increase the severity of rice blast and root knot.
- Potassium seems to have a direct effect on the various stages of pathogen establishment and development in the host and an indirect effect on infection by promoting wound healing.
- Calcium reduces the severity of several diseases caused by root and stem pathogens such as Rhizoctonia, Sclerotium, Botrytis and Fusarium oxysporum, but it increases the severity of common scab of potato (Streptomyces scabies).
- The effect of calcium on disease resistance seems to result from its effect on the composition of cell walls and their resistance to penetration by pathogens.
- In general, plants receiving a balanced nutrition, in which all required elements are supplied in appropriate amounts, are more capable of protecting them from new infections and of limiting existing infections than plants to which one or more nutrients are supplied in excessive or deficient amounts.

Effect of pollutants

- Air pollutants cause various types of direct symptoms on plants exposed to their high levels.
- Ozone may affect a pathogen and sometimes the disease it causes. For example in wheat rust fungus, ozone reduces the growth of uredia and of hyphal growth and also the number of uredospores produced on ozone injured leaves.
- Ozone increases the infection of potato leaves by Botrytis.

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LECTURE 23

PLANT DISEASE EPIDEMIOLOGY

PLANT DISEASE EPIDEMIOLOGY

- Epidemiology deals with the outbreaks and spread of diseases in a population.
- It is the study of rate of multiplication of a pathogen which determines its capacity to spread a disease in a plant population.
- It is the most important part of the study of plant diseases from practical point of view.
- Epidemiologically, the diseases have been described as

i) Simple interest diseases
ii) Compound interest diseases.
Simple interest diseases

- In these diseases, the rate of increase is mathematically analogous to the simple interest in money.
- There is only one generation of the pathogen in the life of the infected crop.
- The primary inoculum is seed or soil borne and secondary infection is rare.
- All the infections noticed in the field are from the pre-existing inoculum in the soil.
- Most important example is loose smut of wheat (U. tritici), where the inoculum is internally seed borne or is carried in the seed resulting in the infected ears in the season showing black powdery loose mass in place of grains.
- In wilt and root rot diseases, the primary inoculum is important and there are very remote chances of secondary spread, even if the pathogen sporulates, due to the soil barriers and other factors.

Compound interest diseases

- In these diseases, the rate of increase is mathematically analogous to compound interest in money.
- The pathogen produces spores at a very rapid rate which are disseminated by external agencies like air, thereby infecting other plants.
- The incubation and sporulation period is also very short.
- The infection cycle is repeated many times during the cropping season.
- Secondary inoculum plays a vital role in the development of epidemics in such diseases.
- The prevailing environmental conditions play an important role in such diseases.
- Wheat rust, late blight of potato and apple scab are some diseases of this type.

Mathematical model of disease spread

- Vander Plank in 1963 in his historical book "Plant Diseases- Epidemics and Control" suggested a model based on the infection rate 'r'.
- It is the rate at which the population of the pathogen increases. The 'r' is on average estimated from successive estimates of population of pathogen as proportion 'X' of the infected plants (in case of systemic diseases) or of infected susceptible tissues in case of local lesion diseases.
- The equation for describing the epidemic is:
- X= X 0ert

Where X = proportion of disease at any time
X0 = the amount of critical inoculum
r = average infection rate
t = time during which infection occurred

- The value of e in the equation is the base of natural logarithm = 1+ 1 n/n
- We are dealing here with an exponential function and the basic assumption here is that at a given time the rate of disease increase is proportional to the amount of disease at that moment. This assumption is true at the beginning where plenty of tissue is there to be infected. As the disease progresses, the amount of susceptible tissue left for infection declines and the rate of increase is determined by the amount of susceptible tissue left i.e. 1-x, but not the one which is present (x). The infection rate 'r' is very important and used to compare epidemics of diseases at different localities/cultivars /fungicide treatments. The comparative 'r' is derived by taking log and transposing:
- Log ex = log ex0 + rt
- rt = log ex – log ex0
- r = 1 log ex/x0
- t
- This 'r' can be assumed at any time during the epidemic but its use is simpler in early stages of development of the epidemics which is an important stage. For comparing two epidemics in cultivars or otherwise, 2 readings at the starting point i.e. t1 (x1) and the other time i.e. t2 (x2) are must.
- Average infection rate in them calculated as:
- r = 1 log e x2
- t2-t1 x1
- When the disease has reached very high intensity, the formula for calculating 'r' is more complicated because the factor (1-x) is introduced. In that case:
- R= 1 log e x2 (1-x)
- T2-t1 x1 (1-x)

Slow and rapid epiphytotics

- The form an epidemic can take is governed by
- the nature of pathogen

- its host
- the weather which works as a reference in the battle between the two.
- At one extreme are the epidemics which develop slowly (tardive) and at the other end are those which develop rapidly (explosive).
- Many intermediate types may also occur.
- Slow epiphytotics occur among populations of perennial, long lived plants such as fruit trees.
- The causal organisms are mostly systemic to varying extents e.g. Dutch elm disease or chestnut blight.
- Rapid epidemics are chiefly caused by non-systemic pathogens with high multiplication/ reproduction rate which have several generations within a short time.
- Mostly annual crops are affected by such epiphytotics.
- They are more affected by environments than the slow epiphytotics.
- The increase of disease is rapid rising to distinct peak in short time, then showing sharp decline as the weather turns unfavorable, host become resistant due to maturity etc.

Analysis of epidemics

- Epidemic is a system i.e. an inter-locking complex of processes characterized by many reciprocal cause- effect pathways.
- It is a structural and functional phenomenon having two or more separable components and some interaction between these components.
- However, each component of the system can be studied separately.
- Analysis of a system with 2 or a few components is easy but that with too many variable and non variable components with their own components and all interacting with each other is a tedious and time consuming job.
- Epidemics interact with other sub eco-systems of agro-ecosystem making it more complex.
- In such a complex scenario, it is rather difficult to pin point the individual effect of any of the parameters say temperature or rainfall or any cultural practice on development of epidemics and its overall contribution.
- However, there are some diseases in which the role of some critical parameters like temperature, leaf wetness and rainfall or RH has been studied.

Elements of epidemics

- The disease epidemics develop as a result of the timely combination of the same elements that result in plant disease:
- susceptible host plants,
- a virulent pathogen,
- favourable environmental conditions over a relatively long period of time.
- Humans may unwittingly help initiate and develop epidemics through some of their activities, e.g., by tipping or pruning plants in wet weather.
- Humans may also stop the initiation and development of epidemics by using appropriate control measures under situations favourable for epidemics.

- The chance of an epidemic increases when the susceptibility of the host and virulence of the pathogen are greater, as the environmental conditions approach the optimum level for pathogen growth, reproduction, and spread, and as the duration of all favourable combinations is prolonged or repeated.

The Disease Pyramid

- To describe the interaction of the components of plant disease epidemics, the disease triangle can be expanded to include time and humans factors.
- The specific point in time at which a particular event in disease development occurs and the length of time during which the event takes place affect the amount of disease.
- The interaction of four components can be visualized as a tetrahedron, or pyramid, in which each plane represents one of the components. This figure is referred to as the disease tetrahedron or disease pyramid.
- The effect of time on disease development becomes apparent when one considers the importance of the time of year, the duration and frequency of favourable temperature and rains, the time of appearance of the vector, the duration of the infection cycle of a particular disease, and so on.
- If the four components of the disease tetrahedron could be quantified, the volume of the tetrahedron would be proportional to the amount of disease on a plant or in a plant population.

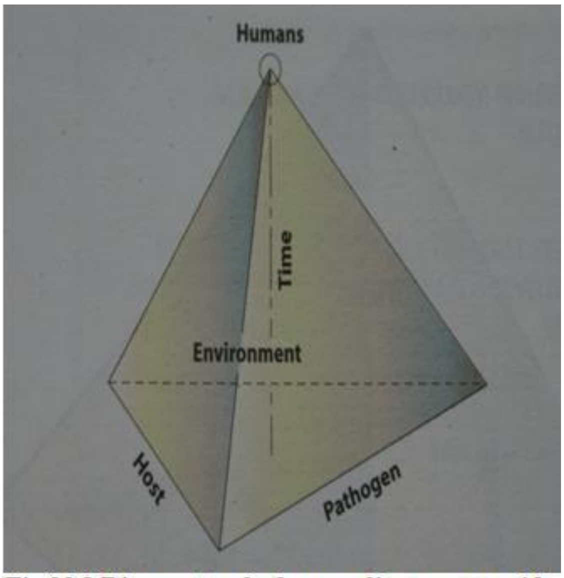

Fig.23.1 Disease tetrahedron or disease pyramid.

Effect of Humans

- Disease development in cultivated plants is also influenced greatly by a fifth component: humans.
- Humans affect the kind of plants grown in a given area, the degree of plant resistance, the numbers planted, time of planting and density of the plants.

- By the resistance of the particular plants they cultivate, humans also determine which pathogens and pathogen races will predominate.
- By their cultural practices, and by the chemical and biological controls they may use, humans affect the amount of primary and secondary inoculum available to attack plants.
- Humans also modify the effect of environment on disease development by delaying or speeding up planting or harvesting, by planting in raised beds by protecting plant surfaces with chemicals before rains, by regulating the humidity in produce storage areas, and so on.
- The timing of human activities in growing and protecting plants may affect various combinations of these components to a considerable degree, thereby affecting the amount of disease in individual plants and in plant populations greatly.
- The human component has sometimes been used in place of the component “time” in the disease tetrahedron, but it should be considered a distinct fifth component that influences the development of plant disease directly and indirectly.

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LECTURE 24

PLANT DISEASE FORECASTING

PLANT DISEASE FORECASTING

Plant disease forecasting involves all the activities in ascertaining and notifying the farmer in an area/community that the conditions are sufficiently favourable for certain diseases, that application of control measures will result in economic gain or that the amount of disease expected is unlikely to be enough to justify the expenditure of time, money and energy for its control.

- Plant disease forecasting requires complete knowledge of epidemiology i.e. the development of disease in plant population under the influence of the factors associated with the host, the pathogen and the environment.
- Forecasting is actually, the applied epidemiology.
- Plant disease forecasting is made more reliable if the reasons for a particular disease developing under certain conditions and not others are known.
- Experimental investigation is necessary to show that exactly what stage during the disease development is critical for variable incidence or intensity of disease.
- A timely and reliable forecast gives the farmer many options to choose from that he can weigh the risks, costs and benefits of his possible decisions.

Requirements or conditions of plant disease forecasting

- The disease must be causing economically significant damage in terms of loss of quantity and quality of the produce in the area concerned.
- The onset, speed of spread and destructiveness of the disease is variable mostly due to dependence on the weather which is variable.
- Control measures are known and can be economically applied by the farmer when told to so.
- Information on weather- disease relationship is fully known.

METHODS OF FORECASTING PLANT DISEASES

Plant disease forecasting has been applied in many diseases on the basis of:

- Weather conditions during the inter-crop period
- Weather during crop season
- Amount of disease in the young crop
- Amount of inoculum in the air, soil or planting material

Forecasts based on weather conditions during inter-crop period and amount of primary inoculum

- Weather during inter-crop period is closely related to the survival of many plant pathogens, mostly through the severe cold of winter months and in some cases to that of pathogen vectors also.
- *Erwinia stewartii* (causing Stewart wilt of corn) survives the winter in the bodies of flea beetles- its vectors.
- An assessment of vector population at the onset of spring gives an indication of the extent of survival of the vector through the cold months.
- Where the sum of mean temperatures for 3 winter months of December, January and February at a given location is less than -1oC, the most of vectors die and therefore no serious disease is expected.
- In contrast, mild winter permits good survival of vectors leading to severe disease outbreak. For curly top disease of sugar beet, severity of disease outbreak is correlated with the number of vectors that successfully overwinter.
- In California, USA, fire blight of apple and pear incited by Erwinia amylovora, became severe if the daily average temperature exceeded a 'disease prediction line' obtained by drawing a line from 16.7oC on March, 1 to 14.4oC on May, 1. This pathogen multiplies very slowly at temperatures below 15oC, and this makes the initial inoculum inadequate for any strong attack.
- Some fungal pathogens affecting temperate region crops like those causing apple scab, brown rot, ergot of rye, etc. survive winter by developing resistant structures. The forecasters look for them to get hints about their prospects in the coming season.
- For some soil borne fungal pathogens like Verticillium and Sclerotium spp. and cyst nematodes Heterodera and Globodera spp., greater the amount of propagules (sclerotia and cysts, respectively) more severe is the disease.

Weather conditions during the crop season and the production of secondary incoculum

- The temperature and moisture levels are very critical during the crop season for the development and spread of some air borne diseases.
- Severe outbreaks are likely to occur if certain combinations of temperature and moisture levels are available for a certain period of time.
- This requirement is different for different diseases.
- Several leaf spot diseases of fungal origin for example tikka disease of groundnut, turcicum blight of corn, apple scab and paddy blast can be predicated by taking into account the number of spores trapped daily over the cultivated field, the temperature and relative humidity over a certain period of time.

Amount of disease in the young crop

- Extent of disease developed in the young crop may occasionally provide a reliable indication of the likely severe development of the disease in the mature crop, e.g., leaf rust of wheat.
- The young crop mostly gets infected from overwintering local infections.
- The amount of infection at onset of spring often determines the subsequent development of this disease as the weather following is generally favourable.
- The crop is, therefore, periodically assessed to keep track of the infection and control measures are recommended accordingly.

Amount of inoculum in soil, planting material and air

- In many diseases, the primary inoculum comes from last years infected crop residues lying in the field.
- Overwintering fungal pathogens produce spores at the onset of the growing season which function as primary inoculum and initiate initial infections in some plants of the new crop.
- Examples are apple scab, brown rot of stone fruits or ergot of rye.
- The amount of such residues lying in the field or the plantation floor gives an indication of the availability of inoculum at the start of the season and if the level is high a forecast can be made.

Amount of inoculum in soil, planting material and air

- In the seed-borne plant diseases, the extent of infection and contamination in the seeds or planting material can be easily estimated in the laboratory.
- This can be useful for predicting seed-borne smut, bacterial and viral diseases.
- Depending on the level of infection or contamination, the seed or planting material can be rejected out rightly or made free from the pathogens by chemical or thermal treatments.
- Some soil borne fungal pathogens may survive in soil as hyphae; but most of them survive in soil as resting structures like sclerotia, chlamydospores and stromata.
- The soil borne inoculum can be approximately determined and if exceeds certain limits, a susceptible variety may not be grown in such fields.
- Blight and root rots caused by Sclerotium rolfsii are good examples.

SOME SUCCESSFUL EXAMPLES OF PLANT DISEASE FORECASTING

Late blight of potato

- Holland pioneered the development of forecasting and spray warning services for the control of late blight of potato.
- Van Everdingen (1926) analyzed the combined effect of several weather conditions on the development of Phytophthora infestans and evolved four rules, popularly known as Dutch rules, on which the appearance of blight was observed to depend.

i) Night temperature below dew point at least for 4 hours

ii) A minimum temperature of 10oC or above

iii) A mean cloudiness on the next day of 0.8 or more, and

iv) At least 0.1mm rainfall during the next 24 hours.

- When all these four conditions were obtained in Holland, potato blight was expected after the next 7 days and therefore the control measures were immediately recommended to the farmers.
- Beaumont and Hodson (1930) added the fifth rule to above four rules to apply them in England. The additional rule called for periods of not less than 2 days in which the relative humidity (at 3 PM) was higher than 75 percent.
- Staniland (1937) devised a system known as Beaumont period and reduced these five rules to two, according to which when a minimum temperature of 10oC or over and a relative humidity 75 per cent were available, the situation was right for forecast.
- The Beaumont period was later replaced by Smith period (Smith, 1961) which comprised the minimum temperature of 10oC for two consecutive '24 hour periods' in each of which there was at least 11 hours with more than 89 per cent relative humidity; and its fulfillment prompted forecast.
- Krause et al. (1975) devised for USA a complete forecasting system known as BLITECAST.
- It is a computer programme written in Fortran IV. In this system, local temperature, relative humidity and rainfall data is fed in to the centrally based computer system, which then provides the forecast.
- Later, addition of data on cultivar resistance or fungicide to be used corrected the forecast and made it more appropriate.
- In India, fungicide application is recommended when temperature is lower than normal and relative humidity is close to saturation point.

Apple scab forecasting

- In case of apple scab caused by an ascomycete Venturia inaequalis, the peudothecia present in the stromata embedded in the over-wintered infected leaf litter on the orchard floor start maturing in the spring.
- Ascospores are discharged from them continuously for 3-5 weeks and function as primary inoculum.
- Being air borne, ascospores land on young expanding apple leaves and start primary infections.

- Mills and La Plante (1954) developed a Mills' Chart according to which the primary infection could occur within a temperature range of 0 to 28oC and leaf wetness for 9 to 48 hours.
- A Mills' period was defined as a period of leaf wetness required at a particular temperature for causing light, medium or heavy scab infection on the leaves.
- By combining the temperature and duration of leaf wetness for varying periods, they suggested that combination of 30, 20, 14 and 10 hours of leaf wetness, respectively with a mean temperature of that period of 5.6, 7.2, 10 and 150C satisfied the requirements for successful infection among others.
- It also indicated the expected severity of disease along with incubation period. Later, Smith (1961) utilized a threshold level of 90% relative humidity following rain in place of leaf wetness record.
- Jones et al. (1981) and Ellis et al. (1984) devised computer based apple scab predictor, which utilized special purpose micro-processor for analyzing detailed information on various relevant components.
- Recommendations were made for need based timely fungicide application and even the type of fungicides to be used.

Apple scab forecasting in Himachal Pradesh

- In Himachal Pradesh, when the apple scab appeared in epidemic form in late 1970's onward, 5 Apple Scab Monitoring and Research Laboratories were established at Mashobra, Kotkhai, Thanedhar and Sarahan in Shimla, and Bajaura in Kullu district.
- Apple scab infection periods were predicted by using equipments, like de Wit 7 day leaf wetness recorder, Reuter Stokes apple scab predictor and SWG Biomat at different locations.
- When the conditions congenial for the development of apple scab were recorded, this information was given to the farmers by blowing sirens, radio broadcasts and personal contacts.
- Of all the systems, Reuter Stokes predictor was found to be the most efficient in predicting the infection periods at Kotkhai (Sharma and Gupta, 1995).
- Different fungicides could be effectively used depending on their after-infection (curative), pre-symptom or post-symptom (eradicative) activities. This is regarded as the best example of plant disease forecasting in India till date.

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LECTURE 25

MEASUREMENT OF PLANT DISEASES AND YIELD LOSS

MEASUREMENT OF PLANT DISEASES

Plant diseases are measured in terms of incidence and severity.

- Disease incidence is the number or proportions of plant units that are diseased (i.e. plants, leaves, flowers, fruits etc.) in relation to the total number of the units examined.

It is expressed in terms of percentage as follows:
The measurement of disease incidence is relatively quick and easy and is used to measure/assess a disease through a field, region or country.

- Disease severity
- In a few cases such as cereal smuts, neck blast, brown rot of stone fruits and vascular wilts of annuals where they cause total loss, disease incidence has a distinct relationship with the severity of the disease and yield loss.
- Disease severity is usually expressed as the proportion of plant area or fruit volume destroyed by the pathogen. It is expressed in the percentage as follows:
- In many diseases such as most leaf spots, root lesions and rusts in which plants are counted as diseased whether they are exhibiting a single lesion or hundred lesions, disease severity is of greater importance to the growers than the disease incidence.
- Disease severity is also referred to as disease intensity.
- For diseases, where the amount of disease varies greatly on different plants in the population, many arbitrary indices and ratings have been in practice.
- They are usually discouraged and are replaced by percentage scales and standard area diagrams of disease intensity.

Percentage Scales

- In this, usually the number of plants or organs falling into known percentage disease groups are recorded.
- The disease groups are the categories distinguished on the basis of per cent damage seen by human eye.
- A 12 grade scale was suggested by Horsfall and Baratt (1945) who took into the fact that the grades detected by the human eye are approximately equal divisions on a logarithmic scale and generally follow the Weber-Fechner law which states that visual activity depends on the logarithm of the intensity of the stimulus.
- In percentage disease assessment, the eye actually assesses the diseased area upto 50 per cent and the healthy area above 50 per cent.

In Horsfall and Baratt grading system, the categories were as follows: 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 %, 12 =100% disease.

- This is a logarithmic scale and is satisfactory not only for disease measurement, but also for epidemiological studies, because pathogens multiply at logarithmic rate and also for loss appraisal.
- A system using percentage scale was developed by British mycological Society (Anon, 1947) for measuring late blight of potato.

The percentage scales have many advantages such as:
i) The upper and lower limits of the scale are always well defined.
ii) The scale is flexible in that it can be divided and subdivided conveniently.
iii) It is universally known and can be used to record both the number of plants infected (incidence) and area damaged (severity) by a foliage or root pathogen.

Standard area Diagrams

- Nathan Cobb developed the first standard area diagram for leaf rust of wheat.
- It divided rust intensity into five grades representing 0, 5, 10, 20 and 50 per cent of leaf area occupied by the visible or sporulating rust pustules.
- The highest grade (50%) represented the maximum possible cover.
- A modified Cobb's scale was proposed by Melchers and Parleu (1922) for the estimation of stem rust of wheat.
- One of the most practical set of the area diagrams was prepared by James (1971). These diagrams represent the actual area of the leaves, stems, pods and tubers occupied by lesions in terms of per cent area covered.
- A common formula as follows is generally used to calculate the average infection or Infection Index, sometimes also known as Disease Index or Per cent Disease Index, which is calculated as follows:

Per cent Disease Index (PDI)	=	Sum of all disease ratings	X	100
		Total number of ratings	X	Maximum disease grade

- Severity estimates from fairly small areas can be combined to cover large areas,viz., village, district or state. This overall index can be obtained by using the formula:

Percent Disease Index (PDI)	=	Field rating class	X	Number of hectares in the class
		Total number of hectares		

Remote sensing

- Remote sensing provides a powerful tool for detection and measurement of diseases.
- It is used for survey of large crop areas by means of aerial photography.
- Diseased plants can be identified as distinct patches in an otherwise uniform picture.
- It is useful for measuring diseases such as late blight of potato where early disease foci can be located and their subsequent spread is followed.
- Remote sensing is also useful when sudden disaster strikes and a rapid appraisal of the situation is required.
- In wheat crop, aerial photography can be used to determine the extent of damage by take-all or eye spot disease. Similarly, the incidence of Heterobasidion can be monitored in pine plantation covering undulating, boggy or rocky land.

CROP LOSS ASSESSMENT

Crop loss can be summarized as the difference between the attainable yield from the healthy crop and that obtained from the diseased crop and is expressed as percentage mostly in terms of money.

- The most important purpose of disease appraisal is the assessment of crop loss.
- Various attempts have been made to utilize disease assessment data for estimation of loss.
- However, such conversion is not easy.
- There is no straight forward way to determine the amount of yield loss.
- While calculating the yield loss from a disease, its nature, extent of damage in terms of yield, quality and loss of market value are to be considered.
- Diseases like smuts, root rots, ergot etc. cause almost 100 per cent damage to the crop and the loss estimates are rather easy to make.
- However, those causing damage to the foliage and other debilitating diseases, thereby affecting the yield partially to different extents pose a great difficulty in assessing losses.
- Sometimes, the crop stage, when the crop is attacked becomes critical in this respect.

Estimation of yield loss

- For estimating yield loss due to diseases, comparisons between crops grown in different years or localities are not reliable as other factors are not the same.
- For valid comparisons, disease free plots are to be compared with those nearby with varying amount of disease.
- Disease free plots are mostly obtained by use of fungicides with little or no phytotoxicity.
- If the yield loss is to be estimated on a regional basis, data on disease incidence obtained from the disease surveys would be utilized using formulae based on fungicide trials.
- These data are usually employed in the ‘critical point’ models which are actually the regression equations.
- ‘Multiple point’ models in which the loss estimation is based on many diseases appear to be more reliable.
- These data are used to produce a multi-dimensional model whose dimensions include the date of disease onset, shape of the disease progress curve, the host cultivar and the yield loss as the dependent variables.

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LECTURE 26

PRINCIPLES OF PLANT DISEASE MANAGEMENT

PRINCIPLES OF PLANT DISEASE MANAGEMENT

Fundamental principles of disease management

i) Avoidance: Geographical area, selection of a proper field, planting time and disease escaping varieties, avoidance of insect vectors and wed hosts

ii) Exclusion: Quarantine, inspection & certification, seed treatment

iii) Eradication: Crop rotation, sanitation, rogouing, soil treatment, heat and chemical treatment to diseased plant material, use of antagonists

iv) Protection: Chemical treatment

v) Immunization: Resistant varieties, induced systemic resistance

vi) Therapy: Chemotherapy, thermotherapy

Avoidance

- It involves tactics that prevent contact between the host and the pathogen.
- The selection of geographic area, selection of a proper field, planting time and disease escaping varieties play an important role in avoiding the disease.
- For example bean anthracnose is common in wet areas. Similarly smut and ergot of pearl-millet are serious in areas where rainfall occurs for long durations during flowering of the crop.
- Successful cultivation of a crop depends to a great extent on the selection of a proper field especially in soil borne diseases, e.g., root knot nematode disease, wilt of pigeon-pea etc.
- In many diseases the incidence or disease severity depends upon the coincidence of susceptible stage of the host and favourable conditions for the pathogen.
- This can be achieved by alteration in the date of planting/sowing.
- Certain insects especially aphids, beetles and leafhoppers are known to transmit viruses and mollicutes from infected plants to healthy plants.
- Perennial weeds including pokeweed, milkweed, Johnson grass and horse nettle serve as over-wintering reservoirs of some viruses.
- Curly top in sugar-beet is a leaf hopper-transmissible viral disease and weeds play a significant role in its spread.
- Some of the important weeds involved in the spread of curly top disease are certain species of Chenopodium, Russian thistle, Amaranthus, deadly nigh shade, shepherd’s purse and knotweed.
- In some cases, aphids feed on some of the early-appearing weeds and then move to new crop plantings, thus introducing viruses which are then spread in secondary cycles within the planting.
- Bean yellow mosaic virus (BYMV) is a common problem in bean growing areas.
- Forage legumes (red clovers) are found to be the source of primary inoculum for aphids to carry BYMV into bean fields.

- For lettuce mosaic virus, only 10 to 15 seconds of feeding is needed by an aphid to acquire the virus and another 10 to 20 seconds on another plant suffices for the aphid to transmit the virus.

Exclusion

- It means preventing the entrance and establishment of pathogens in uninfected crops in a particular area.
- It can be achieved using certified seed or plants, sorting bulbs before planting, discarding any that are doubtful, possibly treating seeds, tubers or corms before they are planted and most importantly refusing obviously diseased specimens from dealers.
- In order to prevent the import and spread of plant pathogens into the country or individual states, certain federal and state laws regulate the conditions under which certain crops may be grown and distributed between states and countries.
- Such regulatory control is applied by means of quarantine, inspection of plants in the field or warehouse and occasionally by voluntary or compulsory eradication of certain host plants.
- Plant quarantines are carried out by experienced inspectors, stationed in all points of entry into the country, to stop persons or produce likely to introduce new pathogens.
- Similar quarantine regulations govern the interstate and even intrastate sale of nursery stock, tubers, bulbs, seeds and other propagative organs, especially of certain crops such as potatoes and fruit trees.
- For example, the outbreak of citrus canker in USA in 1910 through planting material imported from Southeast Asian countries.
- Due to heavy destruction, strict quarantine was imposed against entry of citrus planting material.
- However in 1981, 1984 and 1991, fresh outbreaks were reported due to illegal importation of citrus planting material. In India, interstate quarantine is in place for the movement of potato from Darjeeling area of West Bengal to prevent the spread of potato wart which is restricted to that area only.

Eradication

It involves elimination of a pathogen once it has become established on a plant or in a field. It can be accomplished by:

- Removal of diseased plants or parts as in roguing to control virus diseases or cutting off a cankered tree limb.
- Cultivating to keep down weed hosts and deep ploughing or spading to bury diseased plant debris.
- Rotation of susceptible with non-susceptible crops to starve out the pathogen.
- Disinfection usually by chemicals, sometimes by heat treatment.
- Spraying or dusting with sulphur to kill the mildew mycelium.
- Treating the soil with chloropicrin to kill nematodes and fungi.
- Soil treatment with various nematicides (Telone II, Temik 15G, Counter 15 and 20G) is useful to control sugar-beet nematodes.

Protection

- It is the use of some protective barrier between the susceptible part of the suscept or host and the pathogen.
- In most cases, a protective spray or dust applied to the plant in advance of the arrival of the fungus spores.
- Sometimes, it is achieved by killing insects or other inoculating agents.
- Sometimes it is achieved by erection of a wind-break or other mechanical barrier.
- Fungicidal sprays that act as protectants are used to control Cercospora leaf spot of sugar-beet, especially in those fields where inoculum has carried over from the previous year.
- The principle of protective fungicides is to disrupt the natural sequence of infection.
- These fungicides act on the leaf surface to kill the newly germinated spores.
- Sulphur is used as a protectant fungicide to control powdery mildew of sugar-beet.
- There is a long list of chemicals available in the literature that can be used in protective spraying and dusting, along with eradicant chemicals.
- The commercially sold chemicals are provided with instructions or notes on compatibility and possibilities of injury.
- Improvement of aeration under crop canopy reduces the humidity on aerial parts of the plant and thus checks the growth of fungi which flourish in humid atmosphere.

Immunization/Disease resistance

- Disease resistant and tolerant varieties are the cheapest, easiest and most efficient way to reduce disease losses.
- Varieties should be selected that possess resistance or tolerance to one or more disease organisms.
- For some diseases, such as the soil-borne vascular wilts and the viruses, the use of resistant varieties is the only means of ensuring control.
- Certified seed of resistant varieties is available and sold commercially.
- The use of varieties of plants resistant to particular diseases has proved to be very effective against stem rust of wheat, rust of dry bean and Rhizoctonia root rot of sugar beet.
- Most plant breeding is done for the development of varieties that produce greater yields of better quality.
- When such varieties become available, they are then tested for resistance against some of the most important pathogens present in the area where the variety is developed and where it is expected to be cultivated.
- If the variety is resistant to these pathogens for that area, it may be released to the growers for immediate production.
- There are degrees of resistance to certain diseases, some varieties being completely immune, others partially susceptible.
- Resistant varieties may become susceptible to new races of a pathogen, as happens with cereal rusts, powdery mildews, downy mildews and P. infestans.
- Modern DNA technology has made it possible to engineer transgenic plants that are transformed with genes for resistance against specific disease, for tolerance of adverse environmental factors or with nucleic acid sequence that lead to gene silencing of the pathogen.

- Use of microorganisms and chemicals to induce systemic acquired resistance and activations of plants' defense system could also be used for the management of plant diseases.

Therapy

- It is used on individual plants and can not be used on a large scale.
- It is achieved by inoculating or treating the plant with something that will inactivate the pathogen.
- Chemotherapy is the use of chemicals to inactivate the pathogen, whereas heat is sometimes used to inactivate or inhibit virus development in infected plant tissues so that newly developing tissue may be obtained which is free of pathogen.
- Thermotherapy involves the exposure of diseased plants or parts of them to hot water or high air temperature for different periods of time.
- Loose smut of wheat is controlled by treating the seeds with hot water, but growing resistant varieties iss a simpler method of control.
- Hot water treatment has been used to kill nematodes in bulbs, corms, tubers and fleshy roots while they are in a dormant condition.
- Dormant chrysanthemum stools can get rid of foliar nematodes by submerging in water at 112°F (44°C) for 30 minutes.

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LECTURE 27

PHYSICAL AND LEGISLATIVE METHODS OF PLANT DISEASE MANAGEMENT

PHYSICAL AGENTS USED FOR DISEASE CONTROL

The physical agents used most commonly in controlling plant diseases are:
i) Temperature (high or low)
ii) Dry air
iii) Unfavourable light wavelengths
iv) Various types of radiations
v) Cultivation in glass or plastic green houses
vi) Plastic or net covering

Soil sterilization by heat

- Soil sterilization is completed when the temperature in the coldest part of the soil has remained for at least 30 minutes at 82oC or above, at which temperature almost all plant pathogens in the soil are killed.
- Soil can be sterilized in greenhouses, and sometimes in seed beds and cold frames, by the heat carried in live or aerated steam or hot water.
- The soil is steam sterilized either in special containers (soil sterilizers), into which steam is supplied under pressure, or on the greenhouse benches, in which case steam is piped into and is allowed to diffuse through the soil.
- At about 50oC, nematodes, some oomycetes, and other water moulds are killed, whereas most plant pathogenic fungi and bacteria along with some worms, slugs, centipedes, are usually killed at temperatures between 60 and 72oC.
- Most weeds, rest of plant pathogenic bacteria, most plant viruses in plant debris, and most insects are killed at about 82oC.
- Heat tolerant weed seeds and some plant viruses, such as Tobacco mosaic virus (TMV) are killed at or near the boiling point that is between 95 and 100oC.
- Excessively high or prolonged high temperatures should be avoided during soil sterilization.
- High temperatures destroy all normal saprophytic microflora in the soil and result in release of toxic levels of some (e.g., Manganese) salts.
- High temperatures also result in the accumulation of toxic levels of ammonia (by killing the nitrifying bacteria before they kill the more heat resistant ammonifying bacteria), which may damage or kill plants planted afterward.

Soil solarization

- When clear polythene film is placed over moist soil during sunny summer days, the temperature at the top 5 cm of soil may reach as high as 52oC compared to a maximum of 37oC in unmulched soil.
- If sunny weather continues for several days or weeks, the increased soil temperature from solar heat, known as solarization inactivates (or kills) many soil borne pathogens, viz., fungi, nematodes, and bacteria near soil surface, thereby reducing the inoculum and its potential for causing disease.

Hot water treatment of propagating organs

- Hot water treatment of certain seeds, bulbs, and nursery stock is used to kill pathogens with which they are infected or which may be present in seed coats, bulbs, scales, and so on, or which may be present in external surfaces or wounds.
- Seed treatment with hot water was the only means of control in some diseases for many years, as in the loose smut of cereals, in which the fungus overwinters as mycelium inside the seed where it could not be reached by chemicals.
- Treatment of bulbs and nursery stock with hot water frees them from nematodes that may be present within them, such as Ditylenchus dipsaci in the bulbs of various ornamentals and Radopholus similis in citrus rootstocks.
- The effectiveness of this method is based on the fact that the dormant plant organs can withstand higher temperatures than those of their respective pathogens can do for a given time.
- The temperature of the hot water used and the duration of the treatment vary with the different host pathogen combinations.
- In case of loose smut of wheat, seed is kept in hot water at 50oC for 11 minutes, whereas bulbs treated for the control of Ditylenchus dipsaci are kept at 43oC for 3 hours.
- A short (15 seconds) treatment of melon fruit with hot (59 + 1oC) water rinse and brushes result in a significant reduction of fruit decay while maintaining fruit quality after prolonged storage.
- Treated fruit had less soil, dust, and fungal spores at its surface while many of its natural openings in the epidermis were partially or entirely sealed.

Hot air treatment of storage organs

- Treatment of certain storage organs with warm air (curing) removes excess moisture from their surfaces and hasten the healing of wounds, thus preventing their infection by certain weak pathogens.
- Keeping sweet potato at 28 to 320C for 2 weeks helps the wounds to heal and prevents the infection of Rhizopus and by soft rotting bacteria.
- Hot air curing of harvested ears of corn, tobacco leaves, and so on removes most moisture from them and protects them from attack by fungal and bacterial saprophytes.
- Dry heat treatment of barley seed at 72oC for 7 to 10 days eliminates the leaf streak and black chaff- causing bacterium Xanthomonas campestris pv. transluscens from the seed with negligible reduction of seed germination.

Control by eliminating certain light wavelengths

- Alternaria, Botrytis and Stemphylium are examples of plant pathogenic fungi that sporulate only when they receive light in the ultraviolet range (below 360 nm).
- Diseases can be controlled on greenhouse vegetables caused by several species of these fungi by covering or constructing the greenhouse with a special ultraviolet absorbing vinyl film that blocks the transmission of light wave lengths below 390 nm.

Drying stored grains and fruits

- All grains, legumes, and nuts carry with them a variety and number of fungi and bacteria that can cause decay of these organs in the presence of sufficient moisture.
- Such decay, however, can be avoided if seeds and nuts are harvested when properly mature and then are allowed to dry in the air or treated with heated air until the moisture content is reduced sufficiently (to about 12% moisture) before storage.
- Subsequently, they are stored under conditions of ventillation that do not allow build up of moisture to levels (about 12%) that would allow storage fungi to become activated.
- Fleshy fruits, such as peaches and strawberries, should be harvested later in the day, after dew is gone, to ensure that the fruit does not carry surface moisture with it during transit, which could result in decay of the fruit by fungi and bacteria.
- Many fruits can also be stored dry for a long time and can be kept free of disease if they are dried sufficiently before storage and if moisture is kept below a certain level during storage.
- Grapes, plums, dates and figs can be dried in the sun or through warm air treatment to produce raisins, prunes, and dried dates and figs, respectively, that are generally unaffected by bacteria and fungi as long as they are kept dry.
- Even slices of fleshy fruit such as apple, peaches, apricots can be protected from infection and decay by fungi and bacteria if they are dried sufficiently by exposure to the sun or to warm air currents.

Disease control by refrigeration

- Refrigeration is the most widely used and the most effective method of controlling post harvest diseases of fleshy plant products.
- Although low temperature at or slightly above the freezing point does not kill any of the pathogen that may be on or in the plant tissues, they do inhibit or greatly retard the growth and activities of all such pathogens, thereby reducing the spread of existing infection and the initiation of new ones.
- Most perishable fruits and vegetables should be refrigerated as soon as possible after harvest, transported in refrigerated vehicles, and kept refrigerated until used by the consumer.
- Regular refrigeration of especially succulent fruits and vegetables is sometimes preceded by quick hydrocooling or air cooling of these products, aimed at removing the excess heat carried in them from the field as quickly as possible to prevent the development of any new and latent infections.
- The magnitude of disease control through refrigeration and its value to growers and consumers is immense.

Disease control by irradiation

In this method, various electromagnetic radiations are used for controlling postharvest diseases of fruits and vegetables by killing the pathogens present on them, such as:

- UV light
- X-rays
- Gamma rays
- Particulate radiations, such as a-particles and ß-particles

LEGISLATIVE METHODS

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.

- Plant quarantine legislation has been placed on the statute book in most agriculturally advanced countries to restrict the movement of diseased plant material or of fungi, bacteria or viruses that can cause diseases in plants.

Quarantine measures are of three types:
i) domestic
ii) internal
iii) total embargoes

- The quarantine law was first enacted in USA in 1912, and was known as Federal Quarantine Act.
- In India, the Destructive Insect and Pest Act (DIPA) was passed in 1914 and subsequently supplemented by other provisions.
- Such quarantine laws were first enacted in France in 1660, and in Denmark in 1903.
- They aimed at the rapid destruction or eradication of barberry which has been known since early times to harbour black rust pathogen.

Some of the examples of disease which have been introduced into other countries are given below:
Examples of plant diseases introduced in India before and after the enforcement of quarantine are also below:Quarantine in India

- In India, there are 16 quarantine stations operating under the Directorate of Plant Protection, Quarantine and storage (DPPRS).
- Eight at sea ports, six at airports and two on land frontiers (Hussainiwala in Ferozepur in Punjab , and Sukhiapokri in Darjeeling district of West Bengal)

Destructive Insect and Pest Act

- The DIP Act in India was passed in 1914 and has been revised many times.
- A number of lacunae exit in the DIP Act; as a result, many serious plant pathogens have already been introduced in the country.
- 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, respectively.
- Timely action has prevented the spread of wart disease to other parts of India.
- *Synthytrium 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 in 1940. By 1943, it had spread to a few areas in Kottayyam and 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, quarantine measures exist for two insect pests and three diseases so far.

i) Fluted scale (*Icerva purcheri*)
ii) San Jose scale (*Quadraspiditus perniciosus*)
iii) Potato wart (*Synchytrium endobioticum*)
iv) Bunchy top (virus)
v) Mosaic (virus) of banana

International Plant Protection Convention

The problem of plant diseases is global. Hence, European Plant Protection Organization (EPPO) was formed prior to the treaty in Rome. In 1951 in Rome, an International Plant Protection Convention was drawn up which at present has about 50 signatory nations. Briefly, each contracting government agrees to make provisions for:

i) An official protection organization with the specific basis of inspecting, rowing crops and the produced derived from them and issuing phytosanitary certificates.

ii) The distribution of information regarding pests and diseases both within the country and to other countries through FAO, so that a world reporting service is established.

iii) Research and investigation in the field of plant protection on a cooperative basis for diseases which have international effects. Within the framework of the international cooperation, there are six regional groups.

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LECTURE 28

CULTURAL METHODS OF PLANT DISEASE MANAGEMENT

HOST ERADICATION

Host Eradication

When a plant pathogen enters into new area despite quarantine, a plant disease epidemic may occur. All the host plants infected by pathogen may have to be removed and burnt to prevent such epidemics. This eliminates the pathogen and prevents greater losses from the spread of pathogen to additional plants.

Eradication of the crop/main host

- This type of eradication of pathogen was done in Florida and other southern states for control of bacterial canker of citrus in 1915, where more than three million trees had to be destroyed.
- Another outbreak of citrus canker occurred in Florida in 1984, and by 1992; and the disease was apparently brought under control through painful destruction of nursery and orchard trees in the United States.
- Host eradication is also carried out routinely in many nurseries, greenhouses, and fields to prevent spread of numerous diseases by eliminating infected plants that provide a ready source of inoculum within this crop.
- However, attempts to eradicate certain diseases like fire blight of apple and pear caused by the bacterium Erwinia amylovora and plum pox virus of stone fruits in the United States, and coffee rust in several South American countries to eradicate them have not been successful.

Eradication of the wild/volunteer host plants

- Certain pathogens of annual crops, e.g., Cucumber mosaic virus overwinters only or mainly in perennial wild plants.
- Eradication of host in which the pathogen overwinters is sometimes enough to eliminate completely or to reduce drastically the amount of inoculum that can cause infection in the following season.
- In some crops like potatoes, the pathogens overwinter in the infected tubers.
- These tubers produce infected plants in the spring that allow pathogen to come on aboveground parts from where it can spread further by insects, rain and wind.
- Eradication of such volunteer plants of a crop helps greatly to reduce the inoculum of these pathogens.
- Eradication of alternate hosts
- Some pathogens require alternate hosts to complete their life cycle, e.g., Puccinia graminis tritici requires wheat and barberry, and Cronartium ribicola requires pine and currants.
- Eradication of wild or economically less important alternate host interrupts the life cycle of pathogen and leads to the control of the disease.

Crop Rotation

- Soil borne pathogens that infect plants of one or a few species or even families of plants can sometimes be reduced in the soil by planting non-host crops for 3 or 4 years.
- Crop rotation can reduce population of pathogens (e.g., Verticillium).

Fallowing

The field is tilled and left fallow for a year or part of year in some cases.

- During fallowing, pathogen debris and inoculum are destroyed by microorganism with little or no replacement.
- In areas with hot summer, fallowing allows greater heating and drying of the soil, which leads to a marked reduction of nematodes and some other pathogens.
- Other cropping systems utilize herbicides, reduced tillage and fallowing.
- In such systems, certain diseases, e.g. stalk rot of grain sorghum and corn, caused by Fusarium moniliforme have been reduced dramatically.
- In other diseases, such as Septoria leaf blotch of wheat and barley scab were increased.

Sanitation

Sanitation consists of all activities aimed at eliminating or reducing the amount of inoculum present in a plant, field or a warehouse and at preventing the spread of the pathogen to other healthy plants and plant products.

- Ploughing under infected plants after harvest, such as leftover infected fruit, tubers or leaves, helps cover the inoculum with soil and speed up its disintegration and concurrent destruction of most pathogens carried in or on them.
- Removing the infected leaves of house or garden plants helps remove or reduce the inoculum.
- Infected crop debris of grasses and rice crops is destroyed by burning in some parts of world, which reduces or eliminates the surface inoculum of several pathogens.
- By washing their hands before handling certain kinds of plants, such as tomatoes, workers who smoke may reduce the spread of Tobacco mosaic virus.
- Disinfecting the knives used to cut propagative stock, such as potato tuber and disinfecting pruning shears between trees reduce the spread of pathogen through such tools.
- Washing the soil of farm equipment before moving it from one field to another may also help in preventing the spread of pathogens present in the soil.

Practices for Creating Conditions Unfavourable to the Pathogen

- Stored product should be aerated properly to hasten the drying of their surface and inhibit germination and infection by any fungal or bacterial pathogens present on them.
- The appropriate choice of fertilizers or soil amendments may also lead to change in the soil pH, which may unfavourably influence the development of pathogen.

- In the production of many crops, particularly containerized stock, using decomposed tree bark in the planting medium has resulted in the successful control of diseases caused by several soil borne pathogens, e.g. Phytophthora, Pythium and Thielaviopsis causing root rots , Rhizoctonia causing damping off and crown rot, Fusarium causing wilt, and nematode diseases of several crops.

Polyethylene Traps and Mulches

- Many plant viruses, such as cucumber mosaic virus are brought into crops such as peppers, by airborne aphid vectors.
- When vertical, sticky, yellow polyethylene sheets are erected along edges of susceptible crop fields, a considerable number of aphids are attracted to and stick to them.
- If reflectant aluminum or black, whitish-grey or coloured polyethylene sheets are used as mulches between the plants or rows in the field, incoming aphids, thrips and possibly other insect vectors are repelled and misled away from the field.
- Reflectant mulches, however, cease to function as soon as the crop canopy covers them.

PRACTICES FOR EVADING OR AVOIDANCE OF THE PATHOGEN

For several plant diseases, control depends on attempts to evade pathogens.

- Bean anthracnose, caused by the fungus Colletotrichum lindemuthianum, and the bacterial blight of bean caused by bacteria Xanthomonas phaseoli and Pseudomonas phaseolicola are transmitted through the seed. Therefore, they can be successfully controlled by using disease free seed and seed treatments.
- In many cases, the susceptible crop is planted at a great enough distance from field containing infected plants so that the pathogen would not infect the crop.
- Crop isolation is practiced mostly with perennial plants, such as peach orchards isolated from choke cherry shrubs or trees infected with X disease phytoplasma.
- Various activities which evade the pathogens include:

i) Using vigorous seed

ii) Selecting proper dates and proper sites

iii) Maintaining proper distances between fields and between rows and plants

iv) Planting windbreaks or trap crops

v) Planting in well drained soil

vi) Using proper insect and weed control

Such practices increase the chances that the host will remain free of pathogen or at least that it will go through its most susceptible stage before the pathogen reaches the host.

Use of Pathogen Free Seed and Propagative Material

- Seed may carry internally one or a few fungi such as those causing anthracnose and smuts, certain bacteria causing bacterial wilts, spots and blights and certain viruses (Tobacco ring spot virus in soybean, Bean common mosaic virus, Lettuce mosaic virus, Barley stripe mosaic virus, Squash mosaic virus and Prunus nectroic ring virus). Such diseases cn be controlled effectively by producing and using disease free seed.
- True seed, however, is invaded by relatively few pathogens, although several may contaminate its surface.
- All types of pathogen can be carried in or on propagating material.
- When a pathogen is excluded from the propagating material of the host, it is often possible to grow the host free of that pathogen for the rest of its life, e.g., woody plants, generally affected by non-vectored viruses.

Production of pathogen free vegetative propagating material

- Vegetative propagating material free of pathogens that are distributed systemically throughout the plant is obtained from mother plant that had been tested and shown to be free of particular pathogen or pathogens.
- To ensure continuous production of pathogen free buds, grafts, cuttings, rootstocks and runners of trees, vines, and other perennials; the mother plant is indexed for the particular pathogen at regular intervals.

- For certain crops, such as potato, complex certification programmmes have been evolved to produce pathogen free seed potatoes.
- For the seed to be certified the plants must show disease level no higher than those allowed by particular state.
- Sometimes it is impossible to find even a single plant of variety that is free of particular pathogen, especially of viruses. In that case, one or few healthy plants are initially obtained by meristematic tissue culture which most viruses do not invade.

Practices for the Exclusion of Pathogens from Plant Surfaces by Epidermal Coatings

- The plants are sprayed with compounds that form a continuous film or membrane on the plant surface for controlling diseases of aboveground parts of plant and inhibit contact of pathogen with the host and penetration of host.
- Water emulsion of dodecyl alcohol forms a high quality of lipid membrane. The membrane allows diffusion of oxygen and carbon dioxide but not of water. The membrane is not easily washed by rain and remains intact for about 15 days.
- Kaolin based films have also proved effective in protecting apple shoot from becoming infected by the bacterial disease fire blight, and apple fruit from powdery mildew. It also protects grapevine from Pierce disease caused by Xylella fastidiosa by interfering with its transmission by the vector.

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LECTURE 29

BIOLOGICAL METHODS OF PLANT DISEASE MANAGEMENT

BIOLOGICAL CONTROL

Biological Control- Concept

- Biological control of plant pathogens refers to the total or partial destruction of pathogen population by other organisms.
- It occurs routinely in nature. For example, several diseases in which the pathogen can not develop in certain areas either because the soil, called suppressive soil, contains microorganisms antagonistic to the pathogen or because the plant that is attacked by a pathogen has also been inoculated naturally with antagonistic microorganisms before or after the pathogen attack.
- Sometimes, the antagonistic microorganisms may consist of avirulent strains of the same pathogen that destroy or inhibit the development of the pathogen, as happens in hypovirulence and cross protection.
- Agriculturalists have increased their efforts to take advantage of such biological antagonisms and to develop strategies by which biological control can be used effectively against several plant diseases.

Suppressive Soils

- Many soil borne pathogens, such as Fusarium oxysporum (causing vascular wilts), Gaeumannomyces graminis (causing take-all of wheat), Pythium spp. (causing damping-off) and Heterodera avenae (oat cyst nematode) develop well and cause severe diseases in some soils, known as conducive soils, whereas they develop much less and cause much milder diseases in other soils, known as suppressive soils.
- The mechanisms by which soils are suppressive to different pathogens may involve biotic and/or abiotic factors and may vary with the pathogen.
- They operate primarily by the presence in such soils of one or several microorganisms antagonistic to the pathogen.
- Many kinds of antagonistic microorganisms have been found to increase in suppressive soils; such as Trichoderma, Penicillium, and Sporidesmium, or bacteria Pseudomonas, Bacillus and Streptomyces.

Reducing Amount of Inoculum through Antagonistic Microorganisms
a) Control of soil borne pathogens

- Several non-plant pathogenic oomycetes and fungi including some chytridiomycetes and hyphomycetes, and some pseudomonad and actinomycetous bacteria infect the resting spores of several plant pathogenic fungi.
- Among the most common mycoparasitic fungi are Trichoderma sp., mainly T. viride and T. harzianum.
- It parasitizes mycelia of Rhizoctonia and Sclerotium and inhibits the growth of many oomycetes such as Pythium, Phythophthora, and other fungi, e.g., Fusarium and Heterobasidion (Fomes).

- Other common mycoparasitic fungi are Laetisaria arvalis (Corticium sp.), a mycoparasite and antagonist of Rhizoctonia and Pythium; Sporidesmium sclerotivorum, Gliocladium virens and Coniothyrium minitans.

b) Control of aerial pathogens

- Many fungi have been shown to antagonize and inhibit numerous fungal pathogens of aerial plant parts.
- *Chaetomium globosum* and *Athelia bombacina* suppress *Venturia inaequalis ascospore* and conidia production in the fallen and growing leaves, respectively.
- *Tuberculina maxima* parasitizes the white pine blister rust fungus *Cronartium ribicola*.
- *Darluca filum* and *Verticillium lecanii* parasitize several rusts.

Control through Trap Plants

- If a few rows of rye, corn, or other tall plants are planted around a field of beans, peppers, or squash, many of the incoming aphids carrying viruses that attack the beans, peppers, and squash will stop and feed on the peripheral taller rows of rye or corn.
- Trap plants are also used against nematodes which are sedentary endo- or ecto-parasites.
- Crotolaria plants trap the juveniles of root- knot nematodes.

Control through Antagonistic Plants

- Plants such as asparagus and marigold are antagonistic to nematodes
- They release substances in the soil that are toxic to several plant parasitic nematodes.

Use of Resistant Varieties

- Grow varieties that have both vertical (initial inoculum- limiting) and horizontal (rate limiting) resistance and most resistant varieties have both type of resistance.
- Many of them carry only one or a few genes of vertical resistance and an unspecified number of genes of horizontal resistance.
- Such varieties are resistant only to some of the races of pathogen and if the pathogen is air borne, a new race can be brought in easily as happens with cereal rusts, powdery mildews and Phytophthora infestans.
- The new race virulent to the resistant variety may appear and become wide spread in this way.

Use of transgenic biocontrol microorganisms

- Genetic engineering techniques have been used to add new genes or to enhance the genetic make up of the biocontrol organisms so that it may attack the pathogen better.
- Such genes may be of plant or microbe origin that code for toxins, enzymes, and other compounds affecting the pathogen adversely, or regulatory genes that over-express appropriate biocontrol genes already present in that organism.

Direct protection by biological control agents
The most commonly used microorganisms include:

- Gliocladium virens, for the control of seedling diseases of ornamental and bedding plants
- *Trichoderma harzianum*, for the control of several plant pathogenic fungi
- Trichoderma polysporum, for the control of wood decays
- Agrobacterium radiobacter K-84, for the control of crown gall
- Pseudomonas fluorescens, against Rhizoctonia and Pythium causing damping off and other diseases
- Bacillus subtilis, used as a seed treatment

Biological Control of Postharvest Diseases through Fungal and Bacterial Antagonists

- Post harvest rots of several fruits could be reduced by spraying the fruits with spores of antagonistic fungi and saprophytic yeasts at different stages of fruit development, or by dipping the harvested fruit in their inoculum.
- Yeast treatments reduced post harvest rotting of peach and apple.
- Botrytis rot of strawberries was reduced by several sprays of Trichoderma spores on strawberry blossoms and young fruits.
- Several antagonistic yeasts protected grapes and tomatoes from Botrytis, Penicillium, and Rhizoctonia rots.

Biological Control of Postharvest Diseases through Fungal and Bacterial Antagonists

- In bacterial antagonists, Pseudomonas protected lemons from Penicillium (green mould) and pear from various storage rots.
- Two Pseudomonas syringae strains control the post harvest decay in citrus, apple and pear under the trade name Bio-Save.
- Stone fruits such as peaches, nectarines, apricot and plums when treated with suspensions of the antagonistic bacterium Bacillus subtilis, they remain free from brown rot, caused by the fungus Monilinia fructicola for nine days.
- Bacillis subtilis also protected avocado from storage rots.
- Pseudomonas protected lemons from Penicillium (green mould) and pear from various storage rots.
- Two Pseudomonas syringae strains control the post harvest decay in citrus, apple and pear under the trade name Bio-Save.
- Stone fruits such as peaches, nectarines, apricot and plums when treated with suspensions of the antagonistic bacterium Bacillus subtilis remain free from brown rot, caused by the fungus Monilinia fructicola up to nine days.
- Bacillus subtilis also protects avocado from storage rots.

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LECTURE 30

CHEMICAL CONTROL OF PLANT DISEASES

PESTICIDES AND METHODS OF APPLICATION

Chemicals used for controlling insect pests, diseases and weeds are known as pesticides; and those used for controlling fungal diseases are called fungicides, those used against viruses are called viricides. Antibiotics are generally used for controlling bacterial diseases.

- One of the important or common mean of controlling the plant diseases is through chemical compounds which are toxic to the pathogens.
- These chemicals inhibit the germination, growth and multiplication of the pathogen or are lethal to the pathogen.

Classification of Chemical Pesticides

- Depending upon the pathogens they affect, they may be classified as fungicides, bactericides, nematicides, viricides etc.
- Out of these, some chemicals are broad-spectrum and they are toxic to all pathogens.
- Most of the chemicals used in plant protection are foliar and are used as aboveground parts of the plants.
- Some of them are soil disinfectants, and some are used as protectants for seed, tubers and culms etc. There are some of the chemicals which have been used prior to disease spread.
- A few chemicals are aimed to eradicate the general inoculum before it comes in contact with the plant hosts. They are called eradicants or chemotherapeutants.

Methods of Applications of Fungicides
Spraying and dusting

- Most important method of applying chemicals on the aerial plant parts which are exposed to different pathogens.
- Different chemicals particularly fungicides are sprayed either as protective, curative or post-symptom treatments.
- These chemicals provide a continuous covering on the vulnerable plant surfaces and do not allow the plant pathogens particularly fungi to invade them.
- They also eradicate the already established infections and reduce the secondary inoculum.
- Different equipments are available for high, low and ultra-low volume sprays in the field.
- Fungicides generally used as sprays are mancozeb, carbendazim, dodine, etc.
- Sulphur and copper fungicides can also be dusted on the crops for controlling some diseases under high humidity conditions.

Soil treatment

- Vegetables, ornamentals and trees are attacked by many pathogens which are present in the soil, like Fusarium and Verticillium and some bacteria.

- Different chemicals are used as soil drench, dust or granules inside the soil at the time of planting of the nursery or seedlings to control damping off, seedling blight, crown and root rot and many other soil borne diseases.
- These chemicals can also be applied with the irrigation water, wherever the irrigation is possible, especially with the drip irrigation system.
- Fungicides such as captan, metalaxyl, PCNB and chloroneb, etc. can be used as soil treatment to overcome above diseases.

Fumigation

- Most important method for controlling the nematodes and other soil borne diseases, and chemicals thus used are known as fumigants.
- Fumigants like formalin, chloropicrin, methyl-bromide, dazomet and metham sodium are now being used as fumigants in plant protection programmes.
- These chemicals are used as volatile or in gaseous form in the soil and are useful against various groups of organisms like nematodes, insects, fungi certain bacteria and weeds.

Disinfection of warehouses

- Stored products are the carrier of inoculum of many pathogens for the next season.
- These materials should be first treated with such chemicals before they are used for next planting season.
- The storage areas like rooms and the walls should also be bleached or treated with copper sulphate solution or some other sanitizing agents.

Seed treatment

- Seeds, tubers, bulbs and roots are usually treated with chemicals to prevent the pre- and post-emergence damping off of the young seedlings.
- These chemicals prevent the disease inoculum carried on the planting material.
- Since 1970's seed material is treated with the systemic fungicides to control and inactivate the pathogen in infected seed, e.g., carboxin for the control of loose smut of wheat, metalaxyl for the downy mildew of oats, etc.
- The fungicides used for seed treatment are chloroneb, captan, maneb, mancozeb, PCNB carboxin, benomyl, thiabendazole and triadimenol.
- Some are used for specific diseases and a few of them are used for controlling various type of diseases caused by fungi.
- They are applied directly on seed as dust or as thick water suspension mixed with the seed or tossed soaking with the chemical solution which is allowed to dry thereafter.

Tree wound treatment

- Fruit plants are often prone to cuts and wounds during the dormant period when they are pruned.
- The exposed portion of the plant is first sterilized by swabbing it with antiseptic solution of either sodium hypochlorite or ethyl alcohol.
- Finally, the entire wounded portion is painted with permanent tree wound dressing such as lanolin paste, Chaubattia paste or Bordeaux paste/ paint.

Post harvest treatment

- There are number of fungicides evolved for the control of post harvest diseases.
- Most of them are used as dilute solutions into which the fruits or vegetables are dipped before storage or as solution used for the washing of fruits and vegetables immediately after harvesting.
- Among the compounds used for commercial control of post harvest diseases of fruits are borax, biphenyl, sodium o-phenylphanate and widely used fungicides benomyl, thiabendazole and imazalil.

TYPES OF CHEMICAL COMPOUNDS USED FOR PLANT DISEASE CONTROL

A. Inorganic chemicals
i) Copper compounds
ii) Inorganic sulphur
iii) Carbonate compounds
iv) Phosphate and phosphonate compounds

Copper compounds

- The Bordeaux mixture (copper sulphate + calcium hydroxide), named after the region of Bordeaux of France, where it was developed against the downy mildew of grapes.
- It is still a widely used fungicide to control many diseases like bacterial leaf spot, blights, anthracnose, downy mildews and cankers throughout the world.
- Phytotoxicity of Bordeaux mixture can be reduced by increasing the ratio of hydrated lime to the copper sulphate.
- Copper oxychloride (Brand names: Blitox 50, Blue copper, Fytolan, etc.) is used to control diseases caused by oomycetes and cankers of fruit trees.

Inorganic sulphur

- Elemental sulphur is known as the oldest fungicide.
- It is used as a dust, wettable powder, paste or liquid formulation.
- It primarily controls powdery mildews, certain rusts, leaf blights and fruit rots.
- These are available in different trade names like Sulfex, Wettasul, Cosavet etc.

Carbonate compounds

- Sodium bicarbonate, as well as bicarbonate salts of ammonium, potassium and lithium are used as fungicides.
- These compounds plus 1 per cent superfine oil are inhibitory and fungicidal to the powdery mildew fungi on roses, grey mould and southern blight fungus.

Phosphate and phosphonate compounds

- Spraying cucurbits or grapevines with either of monopotassium or dipotassium phosphate gives satisfactory control of powdery mildew diseases of these two hosts.

B. Organic Chemicals
i) Organic sulphur compounds or dithiocarbamates
ii) Quinones
iii) Aromatic compounds
iv) Heterocyclic nitrogenous compounds

Organic sulphur compounds or dithiocarbamates

- Organic sulphur compounds form the most versatile and widely used group of modern fungicides.
- This group includes thiram, ziram, ferbam, nabam, maneb, mancozeb and zineb.
- They are the derivatives of dithiocarbamic acid which are toxic to fungi due to isothiocynate radicals and inactivate the sulphydral (SH) group in amino acids and enzymes within the fungus cells.

Quinones

- Quinones occur naturally in many plants and are used as fungicides.
- Only two quinone compounds chloranil and dichlone are used.

Aromatic compounds

- Many unrelated compounds that have benzene ring in centre are toxic to microorganisms, and several of them have been used as fungicide.
- Penta-chloro-nitro benzene (PCNB) sold as Brassicol is a long lasting soil fungicide which controls various soil borne diseases of vegetables and ornamentals and is applied as dip or furrow treatment.
- Another fungicide dichloran (DCNA) sold as Botran is widely used against diseases caused by Botrytis, Sclerotinia and Rhizopus.
- Chlorothalonil available as Bravo, Daconil and many other brand names is excellent broad-spectrum fungicide and is used against many leaf spots, blights, downy mildews, rusts, anthracnose, scab and fruit rots of fruits and vegetables.
- Biphenyl is used against various diseases caused by Penicillium, Diplodia, Botrytis and Phomopsis in case of citrus.

Heterocyclic nitrogenous compounds

- This group includes important fungicides, like captan, captafol and folpet.
- Captan is excellent fungicide for control of leaf spots, blights and rots of many fruits and vegetables and is used as seed treatment and foliar spray.

Systemic Fungicides
Acylalanines

- Most important fungicide in this group is metalaxyl which is effective against oomycetes, like Pythium, Phytophthora and downy mildews.

Benzimidazoles

- They include some of the systemic fungicides like benomyl, carbendazim, thiabandazole and thiophanate methyl.
- Benomyl (which is sold as Benlate) and carbendazim (sold as brand names, like Bavistin, etc.) control various types of diseases like leaf spots, blights, rots,

scab and seed borne diseases; but are not effective against oomycetes and dark colour spore forming fungi including Alternaria spp.
- They are effective against powdery mildews on many crops, apple scab and brown rot of stone fruits.
- Thiophanate methyl sold as Topsin M, is a broad-spectrum fungicide is also used against powdery mildew of various crops.

Oxanthiins

- They were the first to be discovered as having systemic fungicide activity.
- Carboxin is sold as Vitavax used against damping-off disease caused by Rhizoctonia and various smuts of grain crops.
- Oxycarboxin- marketed as Plantvax is effective against wheat rusts.

Organophosphates

- They include primarily fosetyl-Al, sold as Aliette which is very effective against many foliar, root and stem diseases caused by oomycetes such as Pythium, Phytophthora, and downy mildews in a variety of crops.
- Fosetyl-Al has been reported to stimulate defence reactions and the synthesis of phytoalexins against oomycetes.

Pyrimidines

- They include dimethirimol (Milcurb), ethirimol (Milstem) and bupirimate (Nimrod), all of which are effective against powdery mildew of various crops.
- Fenarimol (Rubigan) and Nuarimol (Trimidal) are effective against powdery mildew and also other scab, leaf spot, rust and smut diseases.

Triazoles

- Triazoles (-conazoles or imidazoles) include several excellent systemic fungicides such as triadimefon, bitertanol, difenoconazole, propiconazole, myclobutanil, cyprconazole and tebuconazole, etc.
- They show long protective and curative activity against broad-spectrum of foliar, root and seedling diseases like leaf spot, blights, powdery mildew and rusts causing fungi.
- They can be applied as foliar as well as seed and soil treatments.

Strobilurins

- These are the latest fungicides, also known as QoI fungicides.
- The most important strobilurin fungicides are azoxystrobin, trifloxystrobin and kresoxim methyl.
- These strobilurins can be used for controlling the diseases of grapevines, pome and stone fruits, cucurbits, sugar beet and rice.

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LECTURE 31

USE OF RESISTANT VARIETIES IN PLANT DISEASE MANAGEMENT

RESISTANCE

Introduction

- Use of resistant varieties for crop cultivation provides the most cost-effective, the easiest, and the safest of all the methods used for disease control.
- Both from economic point of view, and the possible health hazards involved in some of other methods of disease control, this can probably be termed as the "painless method". This approach costs little to the farmer and is, therefore, suitable for the developing countries like India.
- Cultivation of resistant varieties provides probably the only means of producing acceptable yields without using toxic compounds for many diseases like the vascular wilts, viral diseases, cereal rusts, powdery mildews, and root rots, etc.
- Several other kinds of fungal diseases and also many others caused by bacteria, nematodes, and viruses are best controlled by this approach.
- However, resistant varieties could be effectively used only in limited number of cases against the diseases of forest and fruit trees, e.g., blister rust of white pine (Cronartium ribicola), fusiform rust of pine (C. quercuum f. sp. fusiforme), and apple scab (Venturia inaequalis).
- It is always preferable to use resistant host varieties that have both vertical and horizontal resistance.
- Most resistant varieties have only one or few (2 - 3) genes for vertical resistance (mono- or oligogenic resistance, respectively) and an unspecified number of genes for horizontal resistance (polygenic resistance).
- Where inoculum production is rapid and its buildup high, and it is air borne, new races of the pathogen may appear quite often and soon become widespread. Such examples are cereal rusts, powdery and downy mildews and late blight of potato.

Resistance break down

- As the new race takes over, resistance of the old variety is no longer effective.
- Depending on the genetic plasticity of the pathogen and the particular gene or combination of genes involved in host resistance, resistant varieties with only vertical resistance, need to be replaced periodically.
- This means that breeding programmes for new resistant varieties has to continue so that some new varieties can be kept in readiness for the replacement of the old ones in case of any eventuality.
- It is hoped that genetic engineering techniques would come to the aid of such breeding programmes and make it possible for a quick transfer of individual genes or a combination of such genes to preferred susceptible host varieties in a much shorter time.

Maintenance of resistance

- Disease management strategies, such as sanitation, seed treatment or use of fungicide reduce the exposure of resistant variety to large pathogen population.
- For pathogens with low inoculum production and slow dispersal rate, resistance of the host variety usually lasts longer.
- The use of varietal mixtures has been widely used in a variety of crops as a possible measure in disease control in cereals, legumes and potatoes.
- A cultivar mixture is simply compounded by mixing seeds of cultivars on the basis of their predicted performance.
- Diversification of resistance naturally presents the pathogen with a difficult target than in the traditional monoculture.

Multiline varieties

- Jenson (1952) first proposed the idea of multiline varieties that is a composite of various isogenic lines sharing most agronomic characters, but carrying different genes for vertical resistance in one or a few of its constituents of the multiline variety.
- Use of multiline variety results in overall reduction of pathogen for a disease, which consequently reduces the rate of disease and also the inoculum presence on each of the component varieties.
- The most fully developed multiline programme involved wheat rusts and crown rust of oats.
- Multilines can delay the onset of disease and also reduce the rate of an epidemic.
- If a constituent variety loses its resistance to a new race of the pathogen, it can be replaced by a suitable alternative line.
- There are, however, certain limitations on the use of multiline varieties.
- The components must be distinct from each other, have different race-specific genes, and also ripen simultaneously.

Reliable resistance

- It is now accepted that crop resistance based on single or few vertical resistant genes is liable to become nonfunctional soon, mostly within 4 years.
- In the long run, the production of varieties with many additional genes for horizontal resistance may perhaps provide the only answer.

Breeding of resistant varieties

- Quite early in the twentieth century it became evident that breeding of resistant plant varieties was possible, and this provided the most desirable approach to plant disease control.
- The environment pollution in chemical control further highlighted the importance of such breeding.
- Plant breeding represents the most significant form of biological control of plant diseases.
- Genetic diversity can be regularly introduced into the plant genome through such breeding programme.

- Cultivated crop plants that we see today represent the results of natural selection or selection and breeding of different lines that evolved naturally in different regions over many thousands of years.
- It has been a very slow process.
- Many of them still exist as wild types at the place of their origin and have survived over such long periods in attack of various pathogens, because of many resistance genes they carried and also gradually acquired through natural crossing within the plant population.
- Weak and susceptible ones were eliminated in course of time.
- The survivors had sets of major and minor genes for resistance and much genetic diversity, adapted to the local health environment and suited to the needs of local population.
- Numerous varieties of each crop plant are cultivated throughout the world and they represent a non-uniform population.
- Widespread systematic efforts of plant breeders all over the world have further increased this diversity.
- Now, biotechnology has come in a big way with techniques aimed at further increasing this.
- The first step in breeding for disease resistance is mostly to decide on type and level of resistance required and whether the pathogen is seed-, soil- or air-borne.
- The decision will depend on the availability of a suitable source of resistance and whether or not it can be manipulated in a breeding programme.
- Many plant diseases cannot yet be properly controlled by host resistance, for example, powdery mildews of cereals, as this is complicated by pathogenic specialization and a complex resistance pattern.

Source of resistance

- Search for resistance is initially restricted to crop cultivars currently in use locally.
- Search has to be widened to include varieties grown in the adjacent regions, wild plant relatives, and species growing in the area where the disease is severe, or where the disease is originated.
- Plant breeders often take recourse to creation of new resistant genotypes for this purpose by inducing mutation or approach gene banks maintained in different countries.
- Larger public collections are maintained in different countries.
    - United States Department of Agriculture (USDA) for many crop species at Beltsville, Maryland, USA
    - CIMMYT at Londres, Mexico, for maize and wheat
    - International Rice Research Institute (IRRI) at Los Bagos, Phillippines, for rice
    - International Crop Research Institute for Semi Arid Tropics (ICRISAT) at Hyderabad, India, for legumes and small grain cereals.

METHODS OF DEVELOPING RESISTANCE

There are three common methods of developing resistance in the host.

i) Selection

ii) Hybridization

iii) Mutation

- Selection is an old practice of developing resistant varieties. When a large number of individuals grow under disease favourable environment, some individuals show some resistance to the disease which might be selected and tested again before recommendation as a resistant variety.
- Hybridization involves the crossing of two individuals (parents) with good commercial qualities lacking resistance to specific pathogens and another, a source of resistance lacking desired commercial traits.
- The source of resistance can be obtained by selection from variety or species much prevalent in the area.
- If such variety is not available in the area under cultivation in cultivated varieties or species, the desired individual can be obtained from some other species or related wild plants.
- Successful crossing of wild Lycopersicon pimpinellifolium with cultivated tomato Lycoperiscon esculentum has produced material for the development of varieties resistance to Fusarium wilt.
- The varieties developed by hybridization and selection for disease resistance in different vegetables are: Tomato (Hisar Anmol, Hisar Gaurav, H-86 and H-88 against Tomato leaf curl virus), brinjal (Pant Rituraj against bacterial wilt), pea (Palam Priya, JP 83 against powdery mildew), cowpea (Pusa Komal against bacterial blight), cauliflower (Pusa Shubhra against black rot and curd and inflorescence blight), cabbage (Pusa Mukta against black rot, Pusa Drumhead against black leg), watermelon (Arka Manik against anthracnose, powdery mildew and downy mildew), etc.
- Mutation is a sudden heritable change in the genetic makeup of the individual plant. In nature, chance mutations are possible, however, little success has been found for developing resistant varieties by this method in the field. The variety Pusa Parvati of French bean resistant to mosaic and powdery mildew diseases; and variety Punjab 8 (EMS 8) carrying field resistance to yellow vein mosaic have been developed by this method.
- Newly developed resistant plants have to be tested for resistance after artificial inoculation with the pathogen or natural infection under field conditions.
- Recently, molecular markers have been used in place of such inoculation for the selection of resistance, at least in the early stage of breeding.
- Resistance is not always stable and may also fail to function under certain conditions.
- To minimize such possibilities, precise standards have been set in respect of conditions for inoculation, environmental conditions in which inoculated plants are to be kept, and assessment of disease symptoms and incidence.

While searching for resistant genotypes, selection is done from existing crops in the following way:

i) Mass selection

ii) Pure line selection

iii) Pedigree selection

iv) Bulk hybrid method

v) Recurrent selection

vi) Other techniques

Mass selection

- Seeds are collected in mass from some selected, highly resistant plants surviving in a cropped field where natural infection occurs regularly, and seeds are composited after harvest for use in the next season.
- This method is no doubt simple, but plant improvement is slow. Further, in cross-pollinated plants there is no control over the source of pollen.
- In onion, the variety Arka Kalyan is resistant to purple blotch and is developed by mass selection only.

Pure line selection

- In pure line selection, seeds are collected only from individual highly resistant plants, and the progenies are grown separately.
- They are repeatedly inoculated with the target pathogen for disease resistance.
- This method is very effective for self-pollinated crops but not so with cross-pollinated ones.
- No new genotype is created by this method, which simply isolates the best genotype present in a mixed population.
- This, however, represents a more rapid method than allowing natural selection to take place and eliminates the more susceptible genotypes.
- Traditionally, mass or pure line selection methods are adopted for heterogenous plant populations. In chilli, the varieties G 4 is fairly tolerant to diseases, NP 46A is tolerant to virus, Arka Lohit is tolerant to powdery mildew and Musalwadi Selection is tolerant to powdery mildew and dieback diseases. These varieties were developed by pure line selection method.

Two more procedures of selection are commonly followed after hybridization to sort out desirable genotypes from the segregating progeny. These are pedigree selection and bulk hybrid methods.

Pedigree selection

- In this method of selection, plants with desired combination of characters are selected in the F2 generation after hybridization between two homozygous lines carrying different genes for resistance.
- Their progenies are propagated separately and inoculated, and the progenies of each selected plants are maintained in succeeding generations for resistance.

- These steps are continued up to F7 or F8 generation, when a high degree of homozygosity is achieved. This method takes advantage of the phenomenon of heterosis (hybrid vigour).
- The disease resistant varieties developed by pedigree selection in different vegetables are: Chilli (Pant C1 tolerant to chilli viruses; Punjab Lal resistant to TMV, CMV and leaf curl viruses; Jawahar 218 tolerant to leaf curl and fruit rot; Pusa Sadabahar, Jwalamukhi and Jwala Sakshi resistant to viruses), Okra (Parbhani Kranti, Arka Abhay, Arka Anamika and Punjab Padmini highly tolerant to YVMV), etc.

Bulk hybrid method

- This method is practiced following hybridization between two selected parents.
- Their seeds are bulked, grown out again, and the process is repeated.
- At each generation, plants are exposed to natural infection or artificial inoculation with the pathogen and reselected for resistance.

Recurrent selection

When it is desired to quickly introduce a single simply inherited, dominant, resistant character into an existing susceptible plant with desirable agronomic qualities, a back-cross or recurrent selection is adopted. This involves a succession of crossing of the 'donor' plants with the dominant resistant progeny with the existing cultivar, i.e. the recurrent parent, ultimately consolidates the resistant gene in the genetic background of the desirable susceptible variety. However, this method is time-consuming, and not equally effective in all cases, particularly for the self-pollinated plants.

Other techniques

Some other techniques are also occasionally used for introducing disease resistance in plants.

- Both natural and artificially induced mutants that exhibit improved resistance and a change in the chromosome number in plants or production of euploids (4N, 6N) or aneuploids (2N + 1 or 2 chromosomes) by the use of mutagenic chemicals like colchicine have also shown good effect in some cases.

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LECTURE 32

INTEGRATED PLANT DISEASE MANAGEMENT

INTRODUCTION

Integrated Disease Management
Integrated disease management (IDM) came under focus in 1960's when chemicals especially, fungicides and insecticides came under the attack from environmentalists due to the overuse of chemicals that created the problems of environmental pollution, chemical residues in food stuff, land, water and air, and the associated health hazards.

- It focused on the other methods of disease control.
- It involved cultural, biological, epidemiological and alternative means to achieve the disease control.
- Nowadays, there is an emphasis on disease "management" rather than on "Control".

Definition of IDM
"Disease management system that in the context of associated environment and population dynamics of microorganisms, utilizes all suitable techniques and methods in a manner as compatible as possible and maintains the disease below economic level".

- In general, it is the integration of all possible and suitable management techniques for the control of diseases.
- The practices which need to be avoided in IDM are indiscriminate use of fungicides, monoculture and growing of susceptible cultivars.
- Integrated disease management ensures the proper management of soil health, use of healthy seeds and planting material, application of fungicides when required, field sanitation, cultural practices which suppress the disease , use of bio-control agents and growing resistant plant genotypes .

Different Approaches of Integrated Disease Management System

1. The combined control approach: It is a combination of control methods like adjustment in sowing time, seed treatment, use of resistant variety, chemical spray schedule etc. This type of IDM is widely practiced as a package of practice where the occurrence of disease is certain and sure.
2. The surveillance based approach: It is an advanced IDM approach based on crop health monitoring and surveillance, and takes into account the economic threshold levels or economic damage levels.
3. Advanced integrated disease management system: It involves the high input technology like computer supported forecasting, remote sensing, scouting, multiple pathogen thresholds, information on life cycle of pathogens, epidemiology of diseases, environmental factor and knowledge based decision making.

MAIN COMPONENTS

Main components of integrated disease management (IDM)

1. Host resistance
2. Induced systemic resistance
3. Genetically improved plants
4. Cultural practices
5. Physical methods
6. Plant nutrition
7. Biological control
8. Use of pesticides of plant origin
9. Judicious use of chemicals

Host resistance

- Resistant varieties can be the simple, practical, effective and economical method of plant disease control.
- Apart from ensuring protection from diseases, they can also save time, money and energy spent on other methods of control and avoid environmental pollution with chemicals.
- They are the only practical method of controlling such diseases as wilts, rusts and others caused by viruses in which chemical control is very expensive and impractical.
- In low value crops, where other methods are often too expensive, development of varieties resistant to common and important diseases can be an acceptable recommendation for the farmers.
- Disease resistance in plants is also governed by their genetic constitution and can be monogenic, oligogenic or polygenic.

Advantages of host plant resistance

- No adverse effect on environment and man, rather the resistant cultivars put a constant and cumulative effect on pathogen.
- Host plant involves no extra cost to the farmers and does not require inputs and application skills.

Disadvantages of host plant resistance

- The development of pathogen resistant variety takes 5-10 years.
- Host plant resistance can put a selection pressure on pathogen to the extent that it may lead to the evolution of new biotypes of pathogen.
- Introduction of varieties with resistance to one pathogen leads to the emergence of new pathogen problem because of the absence of competition from the key pathogen.

Induction of host resistance

- Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability.
- Plants also respond to a variety of chemical stimuli produced by soil- and plant-associated microbes.
- Such stimuli can either induce or condition plant host defence through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens.
- Induction of host defence can be local and/or systemic in nature depending on the type, source, and amount of stimuli.
- The systemic acquired resistance (SAR) is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins.
- These PR proteins include a variety of enzymes, some of which may act directly to lyse the invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death.
- Whereas, the induced systemic resistance (ISR) is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some non-pathogenic rhizobacteria.
- Interestingly, the SA- and JA- dependent defense pathways can be mutually antagonistic, and some bacterial pathogens take advantage of this to overcome the SAR.
- Pathogenic strains of Pseudomonas syringae produce coronatine, which is similar to JA, to overcome the SA-mediated pathway.
- Because various host-resistance pathways can be activated to varying degrees by different microbes and insect feeding, it is plausible that multiple stimuli are constantly being received and processed by the plant.
- Thus, the magnitude and duration of host defence induction will likely vary over time.

Genetically improved plants

- Genes from plants, microbes and animals can be combined and introduced in to the living cells of other organisms, and the organisms that have genes from other species inserted into their genome are called transgenics.
- Production of disease resistant transgenic plants has been achieved by this method; certain genes are inserted in to plant genome that confer resistance to pathogens such as viruses, fungi and insects.
- These transgenic plants reduce the pesticide use and thereby provide environmental benefits while reducing farmers cost.
- Genetically modified plants are generally used to control the viral diseases, e.g., a transgenic papaya cultivar 'Rainbow' has been developed which is resistant to papaya ring spot virus in the US.

Integration of different cultural practices

- Different cultural practices like crop rotation, mulching, tillage, different soil amendments, soil solarization, soil sterilization, change in date of sowing, plant spacing etc. when applied alone are able to control diseases up to some extent; but when these cultural practices are combined with each other, they not only control the diseases but also increase the yield of crops.

- The inter-cropping of maize and sorghum with peppers serves as barriers against the aphid vectors of pepper veinal mottle virus and reduces the virus spread.
- Soil solarization for 40 days along with the addition of cabbage, cauliflower, broccoli and sarson leaf residues controlled the gladiolus wilt (Fusarium oxysporum f.sp. gladioli) by 74.6% whereas soil solarization (for 40 days) alone reduced the gladiolus wilt by 67.3% compared to the un-solarized control.

Physical methods of disease control

- Solar heat treatment of the water soaked wheat seed in May-June for 5-6 hours provides good control of loose smut of wheat.
- Most of the post harvest diseases can be avoided by irradiation, refrigeration, Controlled Atmosphere Storage etc.
- Soil solarization has been used to control soil borne diseaseS caused by otherwise difficult to control fungi, e.g., Rhizoctonia solani, Fusarium spp., Sclerotium etc .
- In this the soil beds are first irrigated and then covered with thin (20 µm) transparent mulch in the months of April, May and June.
- It raised the soil temperatures in some cases up to 500C, which is deleterious to many plant pathogens in the soil.
- It has been used in raising disease free nursery in tropical and subtropical climatic areas. It also provides excellent weed control.
- Hot water treatment of cabbage seed at 520C for 15-20 minutes controls black rot disease (caused by Xanthomonas campestris pv. campestris).

Plant nutrition

- The nutrition of crop plants has direct effect on the diseases, and is an important component of integrated disease management (IDM).
- Both deficient and over-nourished plants invite high incidence of diseases as well as loss in yield and quality of produce and products.
- The amount, proportion, time and method of application of fertilizers affect the metabolism of plants and thus occurrence and severity of diseases.
- Fertilization with both P and K significantly reduces the leaf rust damage and powdery mildew infection in wheat.
- The deficiency of macronutrients may also affect the incidence of many diseases.
- Potassium (K) plays an important role in survival of crop plants under environmental stress conditions.
- Potassium also affects the reaction of plants to pests or diseases by having direct effect on the pathogen number, development, multiplication, survival, vigour and length of life cycle.

Biological control

- Biocontrol agents are used as a core component of integrated disease management system.

- The science and art of using living organisms as biocontrol agents is an important component of environment friendly disease management procedures.
- These biocontrol agents are of enormous value in integrated diseases management for sustainable agriculture where they often replace the need of fungicides.
- The biocontrol agents either suppress the pathogen growth either by the antibiotic production, hyperparasitism or by competition.
- Various biocontrol agents used in control of various diseases are Bacillus subtilis, Pseudomonas fluorescens, Gliocladium spp., Trichoderma spp., Chaetomium globosum, Pseudomonas cepacia, Bacillus cereus, Agrobacterium radiobacter etc.
- Trichoderma viride is the most important and versatile biocontrol agent used for the control of a number of plant pathogens like Rhizoctonia solani and Sclerotium rolfsii which are otherwise difficult to control by other methods.
- Similarly, Fusarium lateriticum has been used to cover primary wounds of apricot for avoiding the canker disease caused by Eutypa armeniacae.
- Application of Peniophora gigantea oidia paste on pine stumps provided effective control of Heterobasidion annosus root rot disease which spreads through unprotected stumps left over after felling.
- Ampelomyces quisqualis and Darluca spp. hyperparasitize powdery mildew and rust fungi, respectively, and therefore exploited for their biological control.
- Agrobacterium radiobacter K-84 strain has been used against crown gall disease world over.

Use of pesticides of plant origin

- Pesticides of plant origin are derived from plant parts and their genes are also used to transform crops to express resistance to insect, fungal and viral attack.
- The plant parts and their extracts with antifungal properties play an important role in plant disease management.
- Plants with pest killing properties have been recorded as early as Rig Veda in India.
- Garlic (Allium sativum) has a long history of reputed value and actual use for its medicinal, antimicrobial and pesticidal properties.
- The growth of Rhizoctonia solani can be reduced with ethanolic extracts of Eucalyptus sp., Chenopodium ambrosioides, Lippia alba, Aegle marmelos and Cestrum diurnum leaves.
- The seed extract of Piper nigrum was found to be effective against R. bataticola.

Judicious use of fungicides

- Chemicals have been used successfully to combat the ravages of these diseases for many years.
- Fungicides with different modes of action like protective (broad spectrum fungicides), post infection activity (EBI), pre- symptom and post symptom (benzimidazoles and triazoles) may be used for controlling a wide array of plant diseases ravaging various crops.
- The over-use of these chemicals resulted in water pollution, residues on food and fruit crops, effect on non- target organisms and development of resistance in pathogens against the chemicals have drawn the attention toward the rational use of fungicides by including monitored control strategies and cultural practices.

Types of Integrated Disease Management

i) Integration of cultural and chemical control

- The integration of chemicals and cultural practices (including improved cultivars) has resulted in a continuous supply of fresh watermelons, reduced diseases caused by *Colletotrichum lagenarium*, *Pseudomonas syringae* pv. *lachrymans* and *Pseudoperonospora cubensis*.
- The covering the tomato nursery seedlings with nylon net for 25-30 days plus 4 sprays of monocrotophos at 10-days intervals after transplanting, delayed the spread of Tomato leaf curl virus for 3-5 weeks and increased tomato yields.

ii) Integration of chemical and biological control

Bio-control agents such as *Pseudomonas fluorescens*, *Trichoderma viride*, *T. harzianum*, *Bacillus subtilis*, *Pseudomonas putida*, *P. cepacia*, *Talaromyces flavus,* and *Agrobacterium radiobacter* strain K 84 etc. can be used with integration of chemicals for the effective control of certain diseases.

iii) Integration of resistance, cultural, biological and chemical control

The integration of cultural practices (crop rotation, good farm hygiene procedures, quarantine), fertilizers, soil fumigation and solarization, pesticides (fungicide transplant dips, soil drench, soil incorporations, seed treatments, trace elements and surfactants), resistant cultivars and biocontrol agents are used for the control of club root (*Plasmodiophora brassicae*) of vegetables.

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PRACTICAL EXERCISES

EXERCISE 1

General Plant Pathological Laboratory Equipments

Objective: To familiarize the students with general plant pathological equipments like compound microscope, autoclave, laminar air flow, incubators and hot air oven

1. Compound Microscope

It is used for microscopic examination of the samples in the laboratory.

i) Construction: A compound microscope consists of 3 major parts i.e. body, lens system and illumination system

a) The body of a microscope consists of the base, arm, body tube, stage, iris diaphragm and fine/coarse adjustment knobs etc. The base provides firm support for the rest of the microscope. Arm is used for carrying the microscope and supporting the optical/lens system. Coarse adjustment knob moves the body tube and objectives to bring the specimen roughly into focus. The fine adjustment knob brings the specimen into exact focus. Upper body tube encloses light path between objective and ocular. Lower body tube provides movable section for focusing objectives. Revolving nosepiece holds the objectives, which can be rotated into light path. The stage provides a platform with an opening in the centre on which a slide is placed which contains the specimen to be observed. The clamps or a movable slide holder is used to hold the slide in place. Condenser collects the light rays and focuses them on the specimen. Iris diaphragm is an assembly of thin metal leaves controllable by a lever to produce variable sized openings.

Fig. 1.1 Compound Microscope

b) The lens system consists of eye piece, objectives and condenser

Eyepiece or the ocular of the compound microscope is composed of 2 or more lenses; the upper compartment or the eye lens is the magnifier and the lower compartment is called the field lens. The commonly used eyepieces are available with magnifications like 1X, 2X, 5X and 10X etc.

Objectives are considered to be the most important part of a compound microscope since they affect the quality of image formation. Most compound microscopes are equipped with 3 objectives having different magnifying powers. They are low power (10X), high power (40X) and oil-immersion (100X) objectives. Low power objective is the shortest and oil-immersion is the longest.
The primary function of a condenser is to supply sufficient cone of light to fill the objective aperture for getting maximum resolving power. Generally, condensers also incorporate iris diaphragm and filter holder. Iris diaphragm is used to control the light intensity.

c) Proper illumination is essential for the efficient utilization of the magnification and resolution of a microscope. The readily available source of illumination is ordinary daylight. As the intensity of daylight varies greatly, artificial light sources (generally a tungsten lamp) are more often used. The most precise of such light sources control the intensity, colour and size of the light beam. The size of the light cone differs with each objective. As the magnification of objective lens increases, the working distance decreases, and the angle of aperture of the objective increases. Therefore, with increasing magnification a larger cone of light must enter the objective.
ii) **Principle of working:** A compound microscope works on the basic principle of magnification, resolving power and illumination.
Magnification is obtained by a series of 2 lens system, the lens system nearest the specimen called objective, magnifies the specimen and produces a real image. The ocular or eye lens system magnifies the real image, yielding a virtual image that is seen by the eye. The total magnification is equal to product of the ocular magnification and the objective magnification.
Resolving power of a lens is its ability to show two closely adjacent points on the object as distinct and separate. This characteristic of a microscope is a function of the wave length of the light used and a characteristic of the lens system known as its numerical aperture:
Wave length
Resolving power = Diameter of the smallest structure visible =

Numerical aperture

The above relationship between the wavelength of light used and numerical aperture in determining resolving power holds good only for parallel light rays. When the specimen is illuminated with oblique rays in addition to direct light rays, the relationship becomes:
Wavelength
Resolving power =

2 x Numerical aperture

Use of compound microscope

- Place a slide on the stage with specimen side up, and centre the section to be examined as accurately as possible over the hole in the centre of the stage.
- Adjust the light source until it passes the maximum amount of light through the specimen. With low power objective in position, lower the body tube by means of the coarse adjustment until the objective is about 5-6 mm from the slide.
- Look through the eyepiece and slowly raise the objective with the coarse adjustment until the specimen is in approximate focus. Never focus downward while looking through the eyepiece. Bring the specimen into sharp focus with the fine adjustment.

Adjust the iris diaphragm and sub-stage condenser until the light intensity is optimum, being neither glaring nor dull.

- After examining the specimen with the low-power objective, shift to the high power dry objective by rotating the nosepiece until the objective clicks into place, first making certain that the portion of the specimen you wish to view is exactly centered in the field of the low power objective.
- Look through the eyepiece and slowly raise the body tube with the coarse adjustment until the specimen comes into approximate focus. Then bring the image into final accurate focus by using the fine adjustment.
- Focusing of the oil-immersion objective requires more care than that of the other objectives, but the procedure is essentially the same. First use the low power objective to locate the portion of the specimen to be examined. Raise the body tube and then rotate the nosepiece until the immersion oil such as clove oil or cedar oil on the portion of the slide directly under the objective. Watching the objective form the side, carefully lower it into the oil. Do not allow the objective to touch the slide. Look through the ocular and slowly focus upward with the fine adjustment until the image appears.

Precautions

- Never touch the lenses. If the lenses become dirty, wipe them gently with lens paper.
- Never leave a slide on the microscope when it is not in use.
- Always remove oil from the oil immersion objective after its use. If by accident oil gets on either of the lower power objectives, wipe it off immediately with lens paper.
- Keep the stage of the microscope clean and dry.
- Do not tilt the microscope when working with the oil-immersion system.
- When the microscope is not in use, keep it covered and in the microscope compartment.
- Never allow an objective lens to touch the cover glass or the slide.
- Never force the microscope. All adjustments should work freely and easily.

2. Autoclave

An autoclave is used for sterilization of media.

Principle: The principle of autoclave is that the water boils at about 100oC, depending upon the vapour pressure of the atmosphere. If the vapour pressure is increased, the temperature will be increased.

Construction: An autoclave is basically a double walled metallic vessel made of thick stainless steel or copper, one end of which has an opening fitted with a tightly closed lid. The lid is provided with pressure gauze to measure the steam pressure and a safety valve. There is also an exhaust valve below to let the steam escape from the bottom of the inner chamber. Autoclave may be jacketed or non-jacketed types. In Jacketed types, the duration of heating is less than in non-jacketed types, however, in non-jacketed ones, water does not condense on objects and the steam is dry, i.e. it does not contain particulate water.

Fig.1.2 Autoclave

Working: For most purposes, sterilization in autoclave is done for 15 minutes at 121oC temperature which is achieved at 1.05 kg/cm2 pressure.

Precautions

- Autoclave should not be overloaded.
- All the air must be removed from within the autoclave before closing the exhaust valve by keeping the outlet valve open until a jet of continuous air comes out of it.
- Sterilization time must be counted not from the time it is switched on but from the time the required pressure is built up.
- Ensure that there is sufficient water in the autoclave before switching on.
- At the end of the sterilization period, allow the steam pressure to drop to zero and only then open the lid.

3. Laminar Air Flow

The laminar air flow system is used for reducing the chances of contamination of cultures while isolation and purification of microorganisms.

Principle: The laminar air flow system works on the principle of application of fibrous filters in air filtration. In this system, air of a closed cabinet is made to pass through high efficiency particulate air filters (HEPA) which filter the air and do not allow any suspended particle above 0.3 mm dimension to go out and as such the air is free of all suspended particles. The air is blown out at uniform velocity and in parallel flow line. Under operational conditions, it provides class 100 air cleanliness levels (3.5 particles of 0.5 µm or larger size per litre) in the work area.

Fig.1.3 Laminar Air Flow

Working

- Just prior to using the working station, the working surface should be wiped clean with isopropyl alcohol or spirit.
- UV lamp should be turned on at least 15 minutes before work is performed.
- Air supply must be turned on while working on the station.

Precautions

- UV light should be switched off before starting work on the station.
- HEPA filters should be checked after every six months and should be changed when a filter is ruptured or so much loaded with contaminants that it will not produce the proper velocity across the working surface.

4. Hot Air Oven

Hot air oven is a sterilizer using dry heat and is primarily used for sterilization of glassware.

Principle: The hot air oven is used for sterilizing glassware which are not burnt or damaged by high temperature. The action of dry heat is an oxidation process resulting from heat conduction from the contaminated object. Thus the entire object must be heated to a temperature for a sufficient length of time to destroy contaminants.

Construction: Hot air oven consists of an insulated double walled cabinet heated by electricity and can withstand high temperature. The walls of the oven are made up of stainless steel or aluminum and are designed to prevent heat conduction from inside the chamber. There is a motor and fan fitted either at the bottom or back to circulate hot air inside the chamber. This hot air increases the temperature inside the chamber thus sterilizing it. A thermostat regulates the temperature at the desired level and a thermometer is fitted for recording the temperature. The shelves within the hot air oven are perforated to allow proper air circulation.

Fig.1.4 Hot Air Oven

Working: Generally, the oven is operated at a temperature of 160-180oC for 1 to 1½ hour. The time required for sterilization is inversely correlated to temperature. Commonly used time for different temperature regimes is 1 hour at 180oC, 2 hours at 170oC, 4 hours at 140oC, 12 to 16 hours at 120oC.

Precautions

- Exposure time is counted from the time when objects to be sterilized have reached the desired temperature in the oven.
- Calibrated glass should not be sterilized in hot air oven since the expansion and contraction can cause changes in the graduations.
- Rubber goods and culture media should not be sterilized in the hot air oven.
- After the sterilization process, the oven and its contents should be allowed to reach the ambient temperature before opening the door to prevent breakage and recontamination by cool air rushing into the chamber.

5. Incubators

Incubators are important equipments required for providing an optimum temperature condition for growing micro-organisms including plant pathogens. They are of many types.
i) Bottom heated incubator: A temperature from ambient to 60o C can be maintained in such incubator. Heating elements are provided at the bottom of the equipment, which is fitted with a thermostat, temperature regulatory knob and power on and off indicators.

ii) B.O.D. incubator: Also called cooling incubator, it is designed for the control of incubation/storage temperatures. Mostly, it consists of a cast iron wheel mounted cabinet which is double walled with inner chamber made of stainless steel and outer body made of mild steel duly powder coated. The chamber is provided with two/three removable shelves, made of stainless steel for complete flexibility in use. At the back of the chamber a special compartment is formed which is fitted with a refrigeration evaporator, heater and two powerful centrifugal air circulators for creating a positive air flow throughout the inner chamber for temperature uniformity. A temperature ranging from 5 to 60oC can be maintained by a thermostat.

Fig.1.5 BOD Incubator

iii) Orbital shaking incubator: It is especially designed to combined advantage of a closely controlled incubator/ storage temperatures with orbital shaking system necessary for many laboratory procedures. Such a set up is more useful for growing cultures of microorganisms and tissue cells under controlled conditions of temperature and agitation. The variable speed platform containing samples is set into the circular motion in a horizontal plane, which ensures uniform mixing, minimizes accretion of material on the sides of flasks and provides good transfer of gas to the culture, and avoids undue buffeting of the organisms and cells. It consists of the same cabinet as of BOD incubator and can maintain a temperature ranging from 5 to 60oC.

Examination and record

1. Draw and label the various parts of these equipments.
2. Understand their principle and working, and record the same.

EXERCISE 2

Plant Pathological Field Equipments

Objective: To familiarize the students with some plant pathological field equipments like disease predictors, spore traps, leaf wetness recorder, etc. and their functioning

1. Disease predictors

The predictor is a unique outdoor monitoring system designed to take the guess work out of the spray programmes. Just by pushing the button, we can know the current and past environmental conditions, current disease status and history of infection periods or the probability of disease occurrence. It enables us to time the spraying operations for optimum disease control.

Reuter stokes predictor: It is an electronic device used to monitor temperature, leaf wetness and relative humidity in apple orchards and was basically programmed to predict apple scab infection periods. A leaf wetness sensor is placed on the tree about 1m above ground and two temperature sensors (linear thermistors) enclosed in a standard weather shelter are connected to the instrument. One thermistor measures dry bulb or ambient temperature, the other, presented in a wet wick measures wet bulb temperature. The other end of the wick is put in the water reservoir through the hole on the top of the sensor shelter. Tipping bucket rain guage, hand held printer, temperature probe and sensor extension cable are optional accessories available with the instrument. Predictor is mounted on 4 cm diameter pipe in the orchard and the reservoir is filled with distilled water. Sensors are connected to the processor. A 6-volt dry battery is used and date and time are keyed in. The instrument measures and compares current environmental conditions with formula developed. It then calculates the onset of specific plant disease for timely application of preventive chemicals. The information is gathered, processed and stored automatically. We can retrieve the information by pushing buttons, which is displayed on Liquid Crystal Display (LCD). It gives current weather data, back data up to 16 days, apple scab history up to last 16 infection periods recorded. Current data on the environment are processed and related to the apple scab status as none, low, medium, and heavy; and fungicide spray options depending upon kick-back action. A degree day accumulator accurately calculates the number of degree days which have transpired for various base temperatures between 0 and 24oC.

Fig.2.1 Reuter Stokes Predictor installed in an apple orchard

. Spore traps

Spore traps are used to capture and quantify a broad spectrum of fungal spores (both culturable and non-culturable) present in the air.

i) Burkard's volumetric spore sampler: A compact unit with built-in vacuum pump, designed to sample airborne particles such as fungus spores and pollens, continuously for periods of up to seven days without attention. Particles are impacted on adhesive coated transparent plastic tape supported on a clock-wise driven drum. Performance of the standard model is similar to the trap described by Hirst in 1952, but interchangeable orifices can be supplied to special order to improve the trapping efficiency for particles in the range 1 to 10 µm diameter. The pore trap is supplied with one roll of 'Melinex' tape and one roll of double sided tape, together with the laboratory stand and Perspex cutting block.

Fig.2.2 Burkard's Spore Sampler

ii) Rotorod sampler: Rotorod sampler is a volumetric rotating arm impaction device capable of obtaining quantitative data on air-borne particulate in the size of 1 to 100 µm at sampling rates up to 120 litres/min. In Plant Pathology, it is used to monitor the amount of inoculum in the form of spores present in the atmosphere in order to assess the disease risk in advance and thus forewarn the managers to get ready for timely spray treatments for effective control of the disease. Its basic components are a constant speed motor and aerodynamically designed collector rods, which are rotated by sampler motor. The type H, V and I rods form the basic collector rod geometry.

Sampling for spores in an orchard should be done at a height consistent with level of concern near the fruit, leaves or new woody growth. The collector rods are smeared with silicon grease adhesive before they are put in the place on the fixed compatible sampling heads on the samplers. Mount the coated collector rod on sampler by sliding the centre clip portion of the rod into the slotted hub of the Rotorod motor. Connect the sampler leads to 12 volt battery. The rods will be rotated at the calibrated RPM as long as the DC voltage is in between 9 to 15 volts in a clock-wise direction. The sampler should be operated according to the purpose. For example, in apple scab forecasting, the sampler should be operated immediately after rain for a period of 3-4 hours to trap discharged ascospores in the orchard. After switching the sampler off, remove the rods and mount them in specially designed grooved stage adapter. The spore deposit is evaluated by counting under light microscope. Nowadays, counting reticules are also available for facilitating the process.

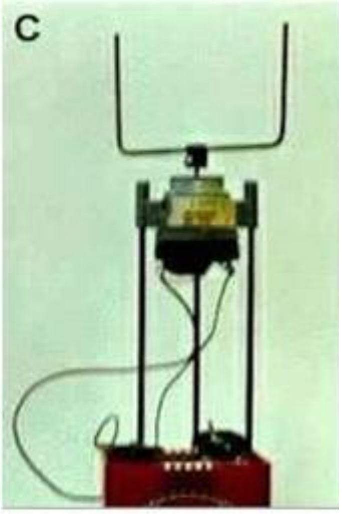


Fig.2.3 Rotorod sampler

iii) METOS automatic weather station

The heart of METOS system is a central processing computer powered by rechargeable batteries. All full function systems are equipped with a built in Epson graphics plotter, washable numeric keypad with 9 functional keys and an 80-character LCD screen. This recording unit is housed indoors or in a weatherproof shelter and includes the main power switch and ports for battery charger, optimal external power supply and RS-232 computer interface. The sensor unit is contained in an all weather, rustproof ventilated housing with standard air temperature, relative humidity and solar radiation sensors and is connected by 15 (5m) cable to a rain guage with 0.2 mm sensitivity and by 36 (10m) cable to two leaf wetness sensors. The recording and sensor units are joined by a 36 (10m) quick connect cable with optional extensions to 500 (150m).

The latest addition to the METOS line is the METOS-DAT, a compact data recorder with multiple channels. This compact version includes many standard and optional sensor inputs, without keyboard, printer or display. Output is channeled to the PC by direct cable or optional cellular or standard phone modem. The METOS system automatically scans all sensors every 12 minutes and stores these data for up to 4 weeks (extendable to 12 months) in internal ROM. In between scans, the METOS remains in standby mode drawing very little power. Each fully functional METOS model contains specific software to convert weather data into easily understandable information on disease conditions, accessed immediately at any time on the LCD by pressing function keys, or automatically at one hour intervals on the graphics plotter. The latest environmental data can be transferred to PC or compatible computer via direct cable or modem.

MET9(R)

Fig.2.4 METOS Automatic Weather Station

MET9(R) is a comprehensive software package for IBMTM and compatible personal computers for the calculation of disease management decisions. MET9(R) operated with an easy to use windows TM like format under DOS 3.0 or higher and need the use of the MET8TM database. Temperature, relative humidity rainfall and leaf wetness etc. largely determine the presence of plant diseases. This connection has been established for various harmful organisms. The MET9 TM disease and pest warning programme indicates favourable conditions and gives instant on line warnings for scab of apples, powdery mildew, black rot, downy mildew of grapes, late and early blight of potatoes and Pythium blight, brown patch and dollar spot of turf grasses.

3. de Wit leaf wetness recorder:
Leaf wetness recorder is built on hygrothermograph principle with the addition of wetness sensing element and pen arm. de Wit leaf wetness recorder records only leaf wetness. In this, the chart revolves in a clockwise direction. If the weather is dry, the pen makes recording on the margin of the chart. The beginning of a rainy period is indicated by a clear kink in the line. As the moistened sensor shrinks, the pen moves towards the centre of the chart and keeps recording there for the duration of the rain. When the rain stops, the sensor dries again and the pen returns to its former position.

Generally, the sensor dries sooner than the leaves and a correction should be made by applying correction of half to one hour. The best spot to mount the instrument is where the leaves remain wet the longest time. The instrument in the orchard is fitted at eye level (approximately 1.5 m high) in a vertical position on a pole with cross beam with the sensor in a horizontal position. The sensor should not be touched with the fingers during installation or afterwards. Leaf wetness recorders are efficiently used to predict the infection periods in case of apple scab.

Examination and record
1. Draw and label the parts of these instruments.
2. Understand the principle and working of these instruments.

EXERCISE 3

Diseases Caused by Plasmodiophoromycota, Chytridiomycota and Oomycota

Objective: To study the symptoms, signs and host-parasite relationship of diseases caused by Plasmodiophorocota, Chytridiomycota and Oomycota,

i). Club root of Cabbage

Causal organism: *Plasmodiophora brassicae*
Classification: Kingdom: Protozoa, Division: Plasmodiophoromycota, Class: no class assigned, Order: Plasmodiophorales: family: Plasmodiophoraceae
Symptoms: The hypertrophied club roots develop before symptoms appear on the above ground parts.The roots swell 10-12 times then original size and develop into numerous spindle- shaped knobby or club-shaped galls.The leaves show chlorosis and wilting.If the infection occur early,the young plants are killed within a few days.
Disease Cycle: The pathogen survives in the soil as resting spores(+resting sporangia),which remain viable for 7-10 years or even more. They germinate under moist conditions, each releasing a single biflagellate zoospore, with unequal, whiplash flagella. These penetrate the root hairs of the seedling and form the thallus, a haploid, primary plasmodium.

Fig.3.1 Club root of cabbage

ii). Wart Disease of Potato
Causal organism: *Synchytrium endobioticum*
Classification: Kingdom: Fungi, Division: Chytridiomycota, Class: Chytridiomycetes, Order: Chytridiales Family: Chytridiaceae
Symptoms: Dark-brown to black, cauliflower-like outgrowths (warts) appear on the tubers.Buds on tubers and stolons are the main sites of infection and origin of the warts. Roots are, however, not infected. The cells adjoining the infected site undergo hyperplasia and hypertrophy and form the tumors. The infected plants, some times, show symptoms on the above ground parts, like formation of warts at the base of the stem near the soil, and stunted growth. The crop produced is unmarketable and the soil is rendered unfit for potato cultivation for several years due to the long survival of the fungus in the soil.

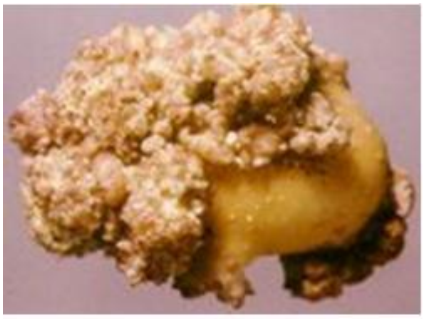


Fig.3.2 Potato Wart

iii). Damping-off of vegetable crops

Causal organism: *Pythium debaryanum, Pythium* spp.

Classification: Kingdom: Chromista, Division: Oomycota, Class: Oomycetes, Order: Pythiales, Family: Pythiaceae

Symptoms: When seeds of susceptible crops are planted in infested soils and are attacked by the damping-off fungi, they fail to germinate, become soft and mushy and then turn brown, shrivel, and finally disintegrate. Young seedlings can be attacked before emergence at any point, from which the infection spreads rapidly, the invaded cells collapse, and the seedling is overrun by the oomycete and is killed even before emergence from the soil (thereby called pre-emergence damping off).

Seedlings that have already emerged are usually attacked at the roots and sometimes in the stems at or below the soil line. The invaded areas become water soaked and discolored and they soon collapse. The basal part of the seedling stem becomes softer and much thinner than the uninvaded parts above it and as a result, the seedlings fall-over on the ground. The fungus continues to invade the fallen seedlings, which quickly wither and die out. It is also called post-emergence damping-off.

Disease cycle: Spore germ tubes or saprophytic mycelium of Pythium coming in contact with seeds or seedling tissues of host plants enter by direct penetration and grows between and through the cells. Infected seeds and seedlings are killed and turn into a rotten mass consisting primarily of the oomycete.

Microscopic observations: Pythium produces a rapidly growing mycelium which is coenocytic. The mycelium gives rise to sporangia, which germinate directly by producing one to several germ tubes or by producing a short hypha at the end of which forms a balloon like secondary sporangium called a vesicle. Sometimes oospores are produced which are thick walled structures and are resistant to adverse temperature and moisture conditions and serve as the survival and resting stage of the fungus.

Fig.3.3 Damping off of tomato seedlings

EXERCISE 4

Diseases Caused by Oomycota and Zygomycota

Objective: To study the symptoms, signs and host-parasite relationship of diseases caused by Oomycota and Zygomycota

i). Late blight of potato

Causal organism: *Phytophthora infestans*
Classification: Kingdom: Chromista, Division: Oomycota, Class: Oomycetes, Order: Pythiales, Family: Pythiaceae
Symptoms: Symptoms appear at first as water soaked spots usually on the edges of the lower leaves. In moist weather, the spots enlarge rapidly and form brown, blighted areas with indefinite borders. A zone of white, downy growth 3 to 5 mm wide appears at the border of the lesions on the undersides of the leaves. Soon entire leaves are infected, die and become infected. Under continuously wet conditions, all tender, aboveground parts of the plants blight and rot away. In dry weather, the activities of the pathogen are slowed or stopped. Existing lesions stop enlarging, turn black, curl and wither, and no growth appears on the underside of the leaves. When the weather becomes moist again, the fungus resumes its activities; and the disease once again develops rapidly.
Disease cycle: The fungus overwinters as oospores or as mycelium only in the infected tubers. The mycelium from infected tubers or from germinating oospores and zoospores spreads into shoots produced from infected or healthy tubers, causing discoloration and collapse of the cells. When the mycelium reaches the aerial parts of plants, it produces sporangiospores, which emerge through the stomata of the stems and leaves and produce sporangia. The sporangia when ripe, become detached and are carried off by the wind or are dispersed by rain and if they land on wet potato leaves or stems, they germinate and cause new infections. The germ tube penetrates directly or enters through a stoma, and the mycelium grows profusely between the cells, sending long, curled haustoria into the cells. Older infected cells die while the mycelium continues to spread into fresh tissue. In cool, moist weather, new sporangia may form within four days from infection, thus a large number of asexual generations and new infections may be produced in one growing season. As the disease develops, established lesions enlarge and new ones develop, often killing the foliage and reducing potato tuber yield.
Microscopic observations: The aseptate, coenocytic mycelium produces branched indeterminate sporangiophores that in turn produce lemon-shaped sporangia. At the places where sporangia are produced, sporangiophores form swellings that are characteristic for this fungus.

ii). White rust of crucifers
Causal organism: *Albugo candida*
Classification: Kingdom: Chromista, Division: Oomycota, Class: Oomycetes, Order: Pythiales, Family: Albuginaceae

Symptoms: The white rust, sometimes also called white blisters, is easily recognized by the chalk-white, cheesy, raised spore masses (sori) which occur mostly on the underleaf surfaces. The floral parts of radish, cabbage, and cauliflower seed plants are grossly deformed and sterile, and called 'stag heads'. Occasionally, swollen galls form in the petioles and stems of some plants and even the roots of radish, horseradish, and a few other plants.
Disease cycle: The white rust pathogen of crucifers infects only cruciferous plants.The pathogen survives in infected plant debris in the soil. If free moisture and cool temperature are present, the sporangia germinate by producing several smaller motile spores (zoospores) that swim and enter susceptible young tissues. Because A. candida is dependent on cool, wet conditions, the disease is consistently more severe during winter and early spring months. In addition to sporangia, A. candida also produces oospores that can resist drying conditions and enable the fungus to survive in a dormant state in soil or crop residue. The white rust pathogen exists in the form of distinct races.
Microscopic observations: The mycelium of white rust fungus is intercellular in host tissues except for one to several, very small spherical haustoria in each host cell which serve to supply the white rust fungus with nourishment. The colourless, nearly spherical to rectangular sporangia are borne on short, club-shaped stalks (sporangiophores), each of which produces a chain of spores with distinct thickening between the sporangia.

iii). Downy mildews
Causal organisms : Some of the most common or most serious downy mildew oomycetes and the diseases they cause are:

Bremia lactucae, causing downy mildew of lettuce
Hyaloperonospora parasitica, causing downy mildew of crucifers
Peronospora destructor, causing downy mildew of onion, P. effusa, causing downy mildew of spinach, P. tabacina, causing blue mold of tobacco
Plasmopara viticala, causing downy mildew of grapes
Peudoperonospora cubensis, causing downy mildew of cucrbits
Sclerophthora sp. causing downy mildew of cereals
Sclerospora graminicola, causing downy mildew or green ear of grasses and millets

Classification: Kingdom: Chromista, Division: Oomycota, Class: Oomycetes, Order: Peronosporales, Family: Peronosporaceae
Symptoms: At first, small, pale yellow, irregular spots appear on the upper surface of the leaves and a white downy growth of the sporangiospores of the oomycete appears on the underside of the spots. Later, the infected leaf areas are killed and turn brown, while the sporangiospores of the oomycete turn grey. The spots often enlarge, coalesce to form large dead areas on the leaf, and frequently result in premature defoliation.

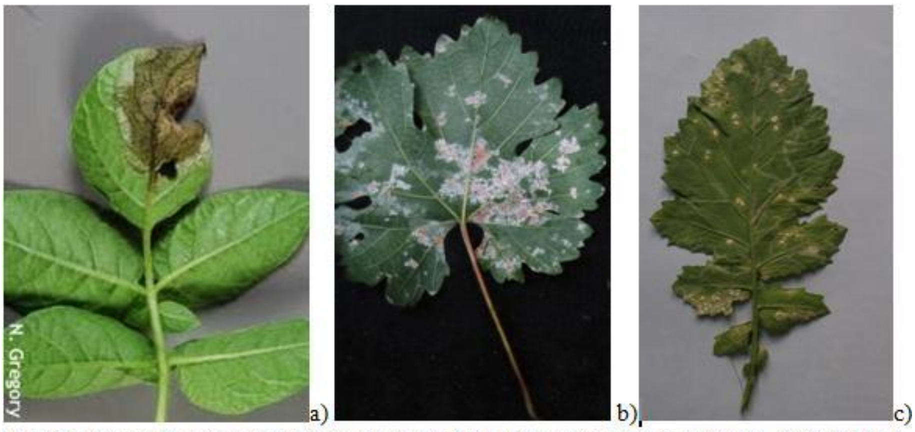


Fig.4.1: Oomycota diseases; a-Potato late blight, b- Grapevine downy mildew, c) White rust

Disease cycle: The fungus overwinters in or on plant parts as mycelium or oospores (thick-walled, gumball-like structures that form the resting stage of the pathogen). Temperature and humidity play a key role in the pathogen development. During cool (50-75ºF), wet conditions with high relative humidity (85% or higher), downy mildew outbreaks develop when germinating oospores form sporangiophores, which resemble a bunch of grapes emerging from the plant stomata. Each "grape" is a sporangium. And each sporangium is filled with dozens of zoospores that swim to susceptible plants and infect them even when a film of free water is available.

Prolonged periods of leaf wetness promote spore germination and the diseases spread. Thus increasing air circulation around the plant by thinning and pruning it, reduces humidity and minimizes infection. The disease cycle, from the initial infection to the production of additional spores and secondary infection, is usually seven to ten days, but can be as short as four days under warm and humid conditions.

Microscopic observations: The characteristic structures of the sporangiophores of different downy mildew genera are observed under the microscope as follows:

Basidiophora- The sporangiophore is club shaped with a swollen head over which the sporangia are borne on minute sterigmata.

Sclerospora- The sporangiophore is a long, stout hyphae, with many upright branches near the end, bearing sporangia at the tips.

Plasmopara – The branches and their subdivisions occur typically at right angles and are irregularly spaced.

Peronospora and Pseudoperonospora- The sporangiophores are dichotomously branched at acute angles, and taper to gracefully curved pointed tips on which sporangia are borne and do not produce zoospores.

Bremia – is similar to Peronospora except that the tips of the branches are expanded into cup shaped apophyses with four sterigmata each, bearing the sporangia along their margins and do not produce sporangia.

Bremiella is again similar to Bremia except in having the tips of its branches inflated into bulbous apophyses on which sterigmata bearing sporangia are produced.

Examination and record:

1. Draw and label the life cycle of oomycetous fungi.
2. Observe the pathogen structures under microscope.

iv). Rhizopus soft rot of fruits and vegetables

Symptoms

Infected organs of fleshy organs appear water soaked at first and are very soft. Fungal hyphae grow outward through the wounds and cover the affected portions by producing tufts of whisker-like grey sporangiophores and sporangia. The bushy growth of the fungus often spreads over the surface of the healthy portions of affected fruit and even to the surface of the containers.

Causal organism: *Rhizopus* spp.

Disease cycle

Rhizopus spores are ubiquitous and continually present in the air around us. The fungus can survive in crop debris in the soil, in fruits and vegetables, and to some extent on contaminated equipment. *Rhizopus* spp. require wounds and necrotic tissue for infection. When either airborne spores or infested soil comes into contact with a wound, the spores germinate, producing hyphae that enter the root. Pectolytic and other enzymes produced by Rhizopus quickly cause host discoloration and liquefy host tissues. The pathogen usually reproduces asexually without the overwintering sexual stage seen in temperate zones. It is an efficient saprophyte, surviving on the dead tissues of its plant hosts or on rotting fruits in fields, in packinghouses, in field soils, and on partly decomposed, stored products such as old bread.

Microscopic observations

The genus Rhizopus is characterized by the presence of stolons and pigmented rhizoids, the formation of sporangiophores singly or in groups from nodes directly above the rhizoids, and apophysate, columellate, multi-spored, generally globose sporangia. After spore release the apophyses and columella often collapse to form an umbrella-like structure. Sporangiospores are globose to ovoid, one-celled, hyaline to brown and striate in many species.

Examination and Record

1. Note the symptoms of the disease and make a detailed study of the life cycle of the pathogens.
2. Observe the morphological differences between Rhizopus and Mucor under the compound microscope.

EXERCISE 5

Diseases Caused by Ascomycota - Powdery mildews

Objective: To study the symptoms, signs and host-parasite relationship of diseases caused by Ascomycota - Powdery mildews

i) Powdery mildew diseases

Causal organisms

Blumeria graminis, causing powdery mildew on cereals and grasses
Erysiphe cichoracearum, causing powdery mildew of cucurbits
E. polygoni, causing powdery mildew of pea
Leveillula taurica, causing powdery mildew of tomato
Phyllactinia spp., causing powdery mildew of shade and ornamental plants
Podosphaera leucotricha, causing powdery mildew of apple and pear
P. oxycanthae, causing powdery mildew of apricot, cherry, plum and peach
Sphaerotheca macularis, causing powdery mildew of strawberry
S. pannosa, causing powdery mildew of peach and rose
Uncinula necator, causing powdery mildew of grapes

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Not assigned, Order: Erysiphales, Family: Erysiphaceae

Symptoms

Powdery mildew appears as spots or patches of a white to greyish, powdery growth on young plant tissues, sometimes entire leaves and other organs are completely covered by the white powdery mass. Tiny, pinhead sized, spherical at first white, later becoming yellowish brown and finally black ascocarps may be present singly or in groups on the white to greyish mildew growth in the older areas of infection. Powdery mildew is most common on the upper side of leaves, but it also affects the underside of leaves, young shoots and stems, buds, flowers and young fruit.

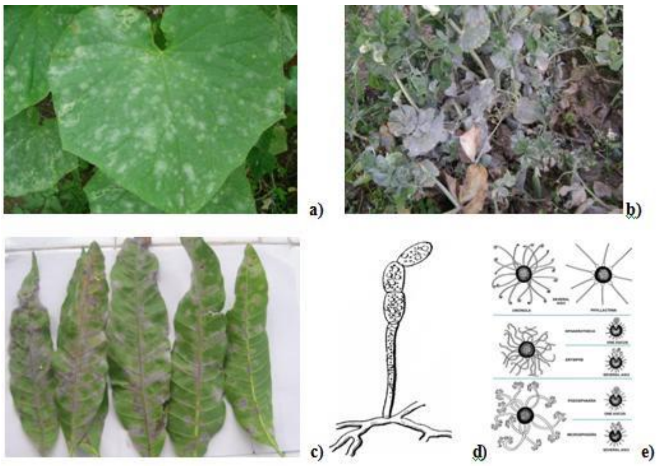


Fig.5.1 Powdery mildew; a- on cucumber , b- on pea , c- mango , d- conidia on conidiophore, e- perithecia of different powdery mildew genera

Disease cycle

Fungi causing powdery mildews are obligate parasites. They produce mycelium that grows only on the surface of plant tissues but do not invade the tissues. They obtain nutrients from the plant by sending haustoria into the epidermal cells of the plant organs. The mycelium produces short conidiophores on the plant surface. Each conidiophore produces chains of ovoid, or round conidia that are carried by air currents. When environmental or nutritional conditions become unfavourable, the fungus may produce ascocarps containing one or a few asci containing ascospores.

Microscopic observations

Blumeria graminis – Ascocarps with myceloid appendages resembling somatic hyphae in being flaccid and indefinite

Erysiphe cichoracearum- Ascocarps with myceloid appendages resembling somatic hyphae in being flaccid and indefinite

Leveillula taurica- Ascocarps with myceloid appendages resembling somatic hyphae in being flaccid and indefinite

Phyllactinia spp.- Ascocarps have rigid, spear like appendages with a bulbous base and pointed tips.

Podosphaera leucotricha- Ascocarps have rigid, dichotomously branched appendages at tips.

Sphaerotheca macularis- Ascocarps having rigid, myceloid appendages.

Uncinula necator- Ascocarps have appendages that are rigid with curled tips.

Examination and Record

1. Note the symptoms of the diseases and make a detailed study of the life cycle of the pathogens.
2. Observe the host-parasite relationship under the compound microscope. Differentiate between the morphological features of various powdery mildew genera.

EXERCISE 6

Diseases Caused by Ascomycota - Wilts and root rots

Objective: To study the symptoms, signs and host-parasite relationship of diseases caused by Ascomycota - Wilts and root rots

i) Vascular wilts

a) Fusarium wilts
Causal organisms

Fusarium oxysporum f. sp. *lycopersici*, infecting tomato
Fusarium oxysporum f. sp. *conglutinans*, infecting cabbage
Fusarium oxysporum f. sp. c*ubense,* infecting banana
Fusarium oxysporum f. sp. *vasinfectum*, infecting cotton
Fusarium oxysporum f. sp. *dianthii,* infecting carnation

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Deuteromycetes, Order: Moniliales, Family: Tuberculariaceae

Symptoms

The first symptoms appear as slight vein clearing on the outer, younger leaflets. Subsequently, the older leaves show epinasty caused by drooping of the petioles. Plants infected at the seedling stage usually wilt and die soon after appearance of the first symptoms. Older plants may show vein clearing and leaf epinasty followed by stunting of the plants, yellowing of the older leaves occasional formation of the adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of the remaining leaves and finally death of the plant.

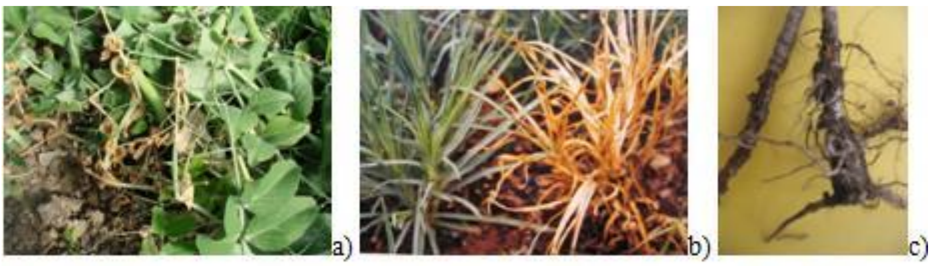


Fig.6.1 Wilts and root rots; a- Fusarium wilt of pea, b- Corm rot and wilt of carmation, c- Root rot of apple |

Disease cycle

This pathogen is soil-borne and survives in infected plant debris and in soil as mycelium and in all its spore forms most commonly as chlamydospores. It spreads short distances by means of water and contaminated farm equipment and over long distances in infected plant material. When healthy plants grow in contaminated soil, the germ tube of spores or the mycelium penetrates root tips directly or enters the roots through wounds or at the point of formation of lateral roots. The mycelium advances through the root cortex intercellularly and when it reaches xylem cells, it enters them through the pits. While in the vessels, the mycelium branches and produces microconidia, which are detached and carried upward in the sap stream. A combination of vessel clogging by mycelium, spores, gels, gums and tyloses and crushing of the vessels by proliferating adjacent parenchyma cells is responsible for the breakdown of the water supply in the plant, ultimately leading to wilting of the plants.

Microscopic observations

Mycelium is extensive and cottony in culture, often with some tinge of pink, purple or yellow, in the host or medium; conidiophores variable, slender and simple, or stout, short, branched irregularly or bearing a whorl of phialides, single or grouped into sporodochia; condia hyaline, variable, principally of two kinds often held in a mass of gelatinous material; macroconidia several-celled slightly curved or bent at the pointed ends, typically canoe-shaped; microconidia 1-celled, ovoid or oblong, borne singly or in chains; some conidia intermediate, 2- or 3- celled, oblong or slightly curved; parasitic on higher plants or saprophytic on decaying plant material. Chlamydospores are one- or two-celled, thick walled, round spores produced within or terminally on older mycelium.

b) Verticillium wilts

Causal organism: *Verticillium albo-atrum* and *V. dahliae*, cause Verticillium wilts in most plants.

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Deuteromycetes, Order: Moniliales, Family: Tuberculariaceae

Symptoms

The symptoms of Verticillium wilt are almost identical to those of Fusarium wilts. However, Verticillium spp. induce wilt at lower temperature than the Fusarium spp. Moreover, the symptoms develop slowly. Verticillium infection may result in defoliation, gradual wilting and death of successive branches, or abrupt collapse and death of the entire plant.

Disease cycle

Verticillium overwinters as mycelium within perennial hosts, in propagative organs or in plant debris.

Microscopic observations

Conidiophores slender, branched, at least some of the branches verticillate (in whorls); conidia ovoid to ellipsoid, hyaline, 1-celled, borne singly or in small clusters apically; vascular parasites causing wilts of higher plants, parasitic on other fungi, or growing saprophytically.

ii) Root rots

a) White root rot of apple

Causal organism: *Rosellinia necatrix* (*Dematophora necatrix*)

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Pyrenomycetes, Order: Xylariales, Family: Xylariaceae

Symptoms

Trees show symptoms of the disease first by yellowing of foliage and poor growth of the branches. This is followed by defoliation and tree death. Below ground, the fibrous roots are rotted away and a white fluffy mycelium may remain in the soil around the rotted roots. Under the cortex of the affected roots is found a whitish weft to floccose layer of mycelium. In excessive soil moisture conditions, the infected roots become covered with white fluffy mycelium.

Disease cycle:

Mycelium of the fungus remains viable in the soil for several years even under most unfavourable conditions. Infection occurs entirely by penetration of mycelium into susceptible roots. The fungus is spread by growth of the mycelium throughout the soil.

Spread of the fungus over long distances occurs through the transportation of the affected nursery stock. The fungus develops more rapidly in comparatively cool, moist soils.

Microscopic observations

Mycelium is white initially and partially pigmented later. Hyphae are hyaline or pigmented, less than 5µm in width, mostly with pear shaped swellings at the septa.

Examination and record

1. Note the symptoms of the various wilt and root rot diseases and make a detailed study of the life cycle of the pathogens.
2. Observe the host-parasite relationship under the compound microscope.

EXERCISE 7

Diseases Caused by Ascomycota –Stem, leaf and fruit diseases

Objective: To study the symptoms, signs and host--parasite relationship of diseases caused by Ascomycota –stem, leaf and fruit diseases

i. Stem and twig cankers

Causal organism:

Botryosphaeria dothidea, causing canker on apple, peach, pecan etc.

Ceratocystis fimbriata, causing canker diseases on cacao, coffee, stone fruits etc.

Dibotryon morbosum, causing black knot of plum and cherry

Eutypa armeniacae, causing die back in grape and apricot

Nectria galligena, causing canker of apple, pear and many forest trees

Leucostoma sp. causing canker of peach and many fruit trees

Kingdom: Fungi, Division: Ascomycota, Class: Pyrenomycetes

Symptoms

Cankers are localized wounds or dead areas in the bark of the stem or twigs of woody plants that are often sunken beneath the surface of the bark. In some cankers, the healthy tissues immediately next to the canker may increase in thickness and appear higher than the normal surface of the stem. Cankers generally begin at a wound or at a dead stub. From that point they expand in all directions but much faster along the main axis of the stem, branch or twig. Under some environmental conditions, the host may survive the disease by producing callus tissue around the dead areas and thus limiting the canker.

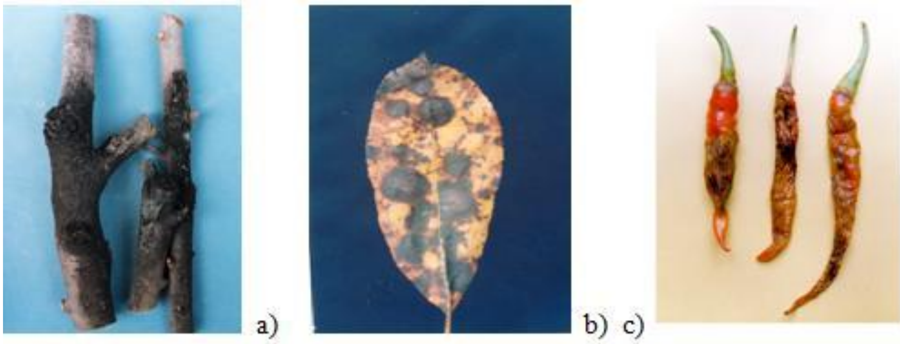


Fig7.1 Stem, leaf and fruit diseases; a- Apple stem black, b- Apple leaf spot, c- Anthracnose and ripe rot of chilli

Disease cycle

Although most canker causing fungi are ascomycetes, only a few of them produce their sexual stage regularly. The other canker causing fungi produce primarily conidia, usually in pycnidia embedded in the bark and only occasionally do they produce perithecia.

Microscopic observations

Botryosphaeria dothidea- Ascomata initially immersed, either separate or grouped in complex multilocular stromata, becoming erumpent through the epidermis and opening through a well-developed ostiole, wall composed of dark, thick-walled textura angularis, becoming paler and thin-walled towards the interior, contents conspicuously white when dry, opening through a periphysate ostiole. Asci bitunicate, clavate, stipitate. Ascospores ellipsoid to broadly fusoid, widest in the middle or upper third, hyaline, smooth, thin-walled, unicellular, tapering to the obtuse base and apex, multiguttulate, eight spores in each ascus, irregularly biseriate. Pseudoparaphyses hyaline, septate, branched.

Ceratocystis fimbriata- Perithecia are long necked, brown to black with globose base, necks almost 800-900 µm long with ostiolar hyphae, asci are produced from ascogenous cells that line the inner wall of the perithecium, ascospores are elliptical, hyaline, non-septate, hat-shaped in appearance. Conidiophores are hyaline, septate upto 150 µm long. Conidia cylindrical, sometimes in chains, truncate at the ends.

Dibotryon morbosum- Stromata are black, hard, produced in branches. Ascostromata black, globose, sometimes turbinate, with a slightly flattened apex and central ostiole. Asci are clavate, sessile, bitunicate, 8-spored. Ascospores clavate, apex obtusely rounded, tapered towards the base, 1-septate near the base, smooth-walled, olivaceous. Conidiophores erect, pale brown, flexuous, geniculate with thickened scars on small denticles. Conidia pale brown, 0—1 septate, smooth, singly or in short chains.

Eutypa armeniacae- The asci are club shaped to cylindrical, having an apical ring and long, tapering, persistent stalks forming a definite hymenial layer in the perithecium. Asci are sometimes polysporous. Paraphyses develop but may gelatinize by the time the perithecium is mature. The lightly pigmented to brown, one-celled ascospores are sausage shaped.

Nectria galligena- Nectria produces its perithecia on the surface of a cushion shaped stroma and the ascocarps are brightly coloured shades of orange and red. Asci may or may not be formed from crosiers, and micro-conidia function as spermatia. The ascospores are one- or two-celled, hyaline, and often boat shaped.

Leucostoma cincta (Anamorph: Cytospora rubscens).- Pycnidia formation on PDA is usually observed 15-20 days after isolation, but sometimes after more than 20 days, and two months after inoculation on twigs and branches. Pycnidia are rounded, dark, 0.2-2.0 mm in diameter. The pycnidia formation varies from sporadic to abundant. The pycnidiospores are extruded in the form of orange to red droplets/globules. They are one-celled, smooth, slightly curved with rounded ends, hyaline. There are many locules variable in size and shape seen on vertical and horizontal sections of pycnidia. Individual locules are fused into a single central cavity and one ostiole.

1. Foliar diseases

Causal organisms:

i) Diplocarpon rosae, causing black of rose

Kingdom: Fungi, Division: Ascomycota, Class: Discomycetes, Order: Helotiales, Family: Dermatiaceae

Mycosphaerella, causing Sigatoka disease of banana (M. musicola), leaf spot of strawberry (M. fragariae), citrus greasy spot (M. citri).

Kingdom: Fungi, Division: Ascomycota, Class: Pyrenomycetes

Venturia inaequalis, causing apple scab

Kingdom: Fungi, Division: Ascomycota, Class: Loculoascomycetes, Order: Pleosporales, Family: Venturiaceae.

Foliar diseases caused by anamorphic/mitosporic fungi

i) Alternaria solani, causing leaf spots and blights on various crops

ii) Ascochyta pisi, causing leaf spot and blight of pea

iii) Cercospora zinniae, causing leaf spot of zinnia

iv) Cladosporium fulvum, causing leaf mold of tomato
v) Marssonina coronaria, causing leaf spot or blotch of apple
vi) Phyllosticta minima, causing leaf spot of apple and red maple
vii) Septoria lycopersici causing Septoria leaf spot of tomato
viii) Stemphylium vesicarium causing Stemphylium leaf blight and stem rot of onion and garlic
Kingdom: Fungi, Division: Ascomycota, Class: Deuteromycetes, Order and Family: Varied

Symptoms
Symptoms of foliar diseases include small, oval, circular, oblong spots produced on leaves initially. These spots may be white to grey, light brown to dark brown with dark green to brown or dark brown borders surrounded by a yellow halo. The lesions may enlarge, coalesce and kill entire leaves.

Disease cycle
Most foliar ascomycetes reproduce by means of conidia during the growing season and by their perfect stage at the end of the season and overwinter. Some produce ascocarps and ascospores along with conidia throughout the growing season. The primary inoculum of these fungi, therefore, may be either ascospores or conidia and usually originates from infected fallen or hanging leaves of the previous year.
Mitosporic fungi attack primarily the foliage of plants by means of conidia. On the infected areas, numerous conidia are produced that spread to other plants by wind, wind blown rain, water and insects, and cause more infections. In most cases, these fungi overwinter primarily as conidia or mycelium in fallen leaves or other plant debris. Some, however, overwinter as conidia or mycelium in or on seed of infected plants or as conidia in the soil.

Microscopic observations
Diplocarpon rosae- Its conidial state is Marssonina rosae. This fungal pathogen grows sub-cuticularly below the epidermis, and produces saucer shaped acervuli bearing typical Marssonina-type conidia on short conidiophores. The conidia are hyaline, bicelled, guttullate, somewhat curved, and pointed at on end.It also produces well developed erumpent apothecia, and more or less crescent shaped ascospores in asci.
Mycosphaerella- The pseudothecia of this genus are small, separate and immersed in host tissue, usually in dead or overwintered leaves and stems. The ascospores are hyaline or light brown and have one central septum. Conidial stages are varied, mostly Cercospora in present cases. Hyaline, multi-celled, filiform conidia are formed on the host leaf surfaces as well as cultures.
Alternaria – Conidiophores dark, simple, rather short or elongate, typically bearing a simple or branched chain of conidia; conidia dark, typically with both cross and longitudinal septa, obclavate to elliptical or ovoid, frequently borne acropetally in long chains, less often borne singly and having an apical simple or branched appendage.
Ascochyta- Pycnidia dark, globose, separate, immersed in host tissue, ostiolate; conidia hyaline, 2-celled, ovoid to oblong; parasitic, principally causing leaf spots.
Cercospora- Conidiophores dark, simple, arising in clusters and bursting out of leaf tissue, bearing conidia successively on new growing tips, conidia hyaline or dark, filliform, several-celled, parasitic on higher plants, commonly causing leaf spots.
Cladosporium- Conidiophores dark, branched variously near the apex or middle portion, clustered or single; conidia dark, 1-or -2 celled, variable in shape and size, ovoid to cylindrical and irregular, some typically lemon-shaped; parasitic on higher plants or saprophytic.

Phyllosticta- Pycnidia dark, ostiolate, lenticular to globose, immersed in host tissue, erumpent or with a short beak piercing the epidermis; conidiophores short or obsolete; conidia small, 1-celled, hyaline, ovoid to elongate; parasitic, producing spots, principally on leaves.
Septoria- Pycnidia dark, separate, globose, ostiolate, produced in spots, erumpent; conidiophores short; short hyaline, narrowly elongate to filliform, several septate; parasitic, typically causing leaf spots.
Stemphylium- Conidiophores dark, mostly simple, short to long, bearing a single, terminal conidium, or successive conidia on new growing tips, conidiophore proliferating through old conidial scar; conidia dark with cross and longitudinal septa, variable in shape, frequently globose to broadly ellipsoid, or ovoid, smooth, verrucose or echinulate; parasitic or saprophytic.

2. Fruit diseases

i) Anthracnose diseases
Causal organisms
Elsinoe, (conidial stage: Sphaceloma), causing anthracnose of grape (E. ampelina), raspberry (E. veneta), and scab of citrus (E. fawcetii) and avocado (E. perseae)
Kingdom: Fungi, Division: Ascomycota, Class: Pyrenomycetes
Glomerella (conidial stage: Colletotrichum or Geotrichum), causing anthracnose of many annual and perennial plants, bitter rot of apple and ripe rot of grape and other fruits.
Kingdom: Fungi, Division: Ascomycota, Class: Pyrenomycetes
Gnomonia, causing anthracnose of walnut and many forest plants
Kingdom: Fungi, Division: Ascomycota, Class: Pyrenomycetes
Diplocarpon, causing apple blotch, (D. mali)
Kingdom: Fungi, Division: Ascomycota, Class: Pyrenomycetes
Symptoms
Anthracnose diseases are diseases of foliage, stem or fruits that typically appear as dark-coloured spots or sunken lesions with a slightly raised rim. Some cause twig or branch die back. In fruit infections, the spots are raised and have corky surfaces. And often result in fruit drop and fruit rot.
Disease cycle
Anthracnoses are caused by fungi that produce conidia within black acervuli. They are found in nature mostly in their conidial stage and can overwinter as mycelium or conidia.
Microscopic observations
Elsinoe- The ascospores are four celled, hyaline produced within the globose asci that are borne singly within cavities scattered in the ascostroma.
Glomerella – it produces dark perithecia either in groups or separately. Perithecial ostioles ae periphysates and well developed. Ascospores are hyaline, unicellular, oblong, ellipsoid or curved with pointed tips.
Gnomonia- Perithecia are buried in the substrate and their prominent necks protrude from the surface and each ascus has a small refractive ring at the apex.

ii) Fruit spot diseases
Causal organisms
Diaporthe vexans, causing fruit rot of eggplant
Venturia inaequalis, causing apple scab
Monilinia fructicola, causing brown rot of stone fruits

Symptoms

These fungi cause most of their damage by their effect on the fruit, but they may affect other parts as well.

Disease cycle

Most of these fungi produce ascospores in perithecia and conidia on free hyphae, but they differ from one another in life cycles and the diseases they cause.

Microscopic observations

Diaporthe vexans- It produces perithecial ascocarps in stromata of fungal and substrate tissues or directly from somatic hyphae on the substrate. The asci have a pore and refractive apical ring and ascospores are hyaline and two-celled. Its anamorphic stage is Phomopsis vexans which produces hyaline, ovoid, one-celled conidia on conidiophores in the pycnidia .

Venturia inaequalis- They produce globose pseudothecia or ascostromata with several locules. Ascocarps are firm and immersed in the substrate. Setae may surround the ostiole of the short neck. The ascospores are two celled, yellowish green, with the upper cell shorter and somewhat wider than the lower. Its anamorphic stage is Spilocaea pomi. It produces pale coloured, usually one-celled, flame shaped conidia on characteristic annelied (ringed) conidiophores.

Monilinia fructicola- the apothecia are generally brown, and most often borne on stalks. Ascospores are generally hyaline, one celled, ovate or somewhat elongated. Its anamorphic stage is Monilia. It produces hyaline, one-celled, conidia in chains in sporodochia.

Examination and record

1. Note the symptoms of different fruit spot diseases and make a detailed study of the life cycle of the pathogens.
2. Observe the host-parasite relationship under the compound microscope.

EXERCISE 8

Post Harvest Diseases of Fruits and Vegetables

Objective: To study the symptoms, signs and host-parasite relationship of post harvest diseases of fruits and vegetables

i). Penicillium rots (blue mould and green mould rots)

Causal organisms:
i. Penicillium expansum, causing soft or blue mould rot of apple
ii. Penicillium italicum, causing blue mould rot of citrus fruits
iii. Penicillium digitatum, causing green mould rot of citrus fruits
(Teleomorph: Talaromyces spp.)

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Plectomycetes, Order: Eurotiales, Family: Eurotiaceae
Symptoms: Symptoms at first appear as soft, watery, slightly discoloured spots of varying size and on any side of the fruit. The spots are shallow at first but quickly become deeper. Soon a white mould begins to grow on the surface of the fruit, near the centre of the spot and starts producing spores. The sporulating area has a blue, bluish green or olive green colour and is usually surrounded by white mycelium, and a band of water-soaked tissue.

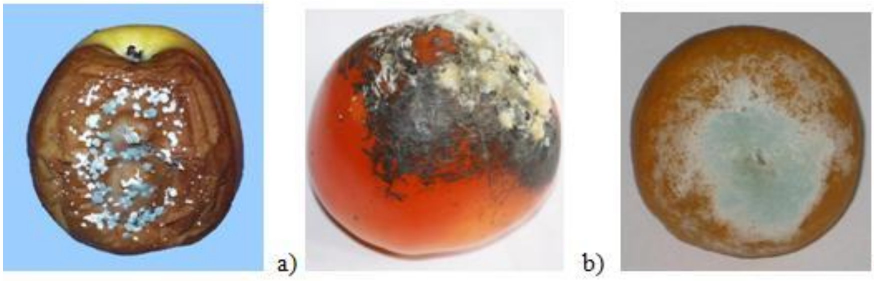

a) b) c)

Fig.8.1 Post harvest diseases of fruits; a- Apple blue mould rot, b- Perssimon grey mould rot, c- Citrus green mould rot

Disease cycle: Penicillium spp. overwinter in the mummified fruits and enter tissue through wounds. However, it can spread from infected fruit in contact with healthy ones through the uninjured skin also.

Microscopic observations: The mycelium produces simple, long, erect conidiophores that branch about two thirds up to the tip and make broomlike structures, called 'penicillus'. The multiple branching of the conidiophores ends in a group of phialides that bear the long conidial chains. Conidia are globose to ovoid and resemble glass beads.

ii). Aspergillus rot (black mould rot)
Causal organism: Aspergillus niger (Teleomorph: Eurotium sp.) , causing black mould rot of pome and stone fruits, and black mould of onion

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Plectomycetes, Order: Eurotiales, Family: Eurotiaceae

Symptoms: On plants such as onion or peanut, Aspergillus niger can cause black mould to develop on bruised tissue, such as the roots or the neck where the vegetable meets the stalk. Often black mould will appear as a black discolouration after the vegetables have been pulled from the ground and stored for some time in a dark location with little ventilation.

Disease cycle: The pathogen is soil-borne. It occurs widely in field soils, and as a saprophyte on dead planting material. During harvesting operations, the spores on infected foliage are dispersed and they spread the disease in the storage. Relatively high temperature and humidity favour the spore germination. Free water is required for fresh infection.

Microscopic observations: The pathogen is dark brown to black in culture. The fungus produces readily recognizable conidiophores that consist of an erect unbranched stalk, a single swollen spherical vesicle, prophialides and phialides that line the surface of the vesicle and densely packed chains of spherical, black spiny conidia (2-5 μm in diameter). The conidia are dry and are easily dispersed by wind.

iii). Brown rot
Causal organism: Monilinia fructigena, M. fructicola, M. laxa (Anamorph: Monilia sp.)

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Discomycetes, Order: Helotiales, Family: Sclerotiniaceae

Symptoms: Brown rot is a major disease of pome and stone fruits in certain countries while minor in others. The initial symptoms are the development of small circular brown spots. Infection may occur around the stem, calyx, injuries and insect punctures. Under humid conditions as the decay develops, tufts of white mould break through the skin often forming concentric circles around the point of infection site. In the presence of light abundant grey brown conidia are produced on the lesions. In absence of light, the fruits turn shiny black in storage, and there may be no spore formation.

Disease cycle: Fruits infected in the early stages of development undergo rotting on the tree and turn into mummies which may remain attached or fall to the ground. These fungi overwinter on mummified fruits and blighted twigs in the orchard. In the sexual stage pathogen produces apothecia giving rise to ascospores and in asexual stage conidia are produced. The initial release of ascospores in the spring coincides with the emergence of blossom and young shoots. Occurrence of sexual stage is common in M. fructicola and less common in the other two species. Older fruits are infected through wounds by the conidia disseminated by air, rain splashes or insects.

Microscopic observations: The conidia of Monilinia spp. are produced in sporodochia in monilioid chains without disjunctures. The apothecia, although rare except M. fructicola are light brown, about 3 mm across at maturity, cupulate or stipitate. Asci are eight-spored, hyaline and narrow ellipsoidal to ovoid.

iv). Grey mould rot
Causal organism: Botryotinia fuckeliana (Anamorph: Botrytis cinerea)

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Discomycetes, Order: Helotiales, Family: Sclerotiniaceae

Symptoms: Grey mould rot can cause heavy losses especially in cherry and pear, perssimon, etc. The grey mould symptoms vary with the variety of fruit infected, the stage of ripeness and the temperature at which the rot develops. In calyx-end rot, the first symptom is a slight reddening of the skin surrounding the eye of the fruit. This lesion develops into a soft brown rot affecting the entire fruit. In the due course, the rotted portion is covered with a dirty white mouldy growth on the fruits.

Disease cycle: The pathogen survives as sclerotia on plant debris and in the soil. Under favouable conditions conidia are produced which infect drying blossom and late invade the fruit. Grey mould rot that develops in storage from incipient infections at harvest occur principally at the stem or calyx end. Direct infections may occur through wounds.

Microscopic observations: Conidia are one-celled, hyaline and round to ovoid. They are produced in clusters on the ends of branched conidiophores. Irregularly shaped sclerotia are also formed on the infected fruits fallen on the ground.

v). Alternaria rot
Causal organism : Alternaria alternata

Classification: Kingdom: Fungi, Division: Ascomycota, Class: Deuteromycetes, Order: Moniliales, Family: Dematiaceae

Symptoms: Alternaria rot has been recorded on pome and stone fruits from all producing areas. Typical Alternaria rot symptom is the dark grey mould which colonized the core cavity and proceed to rot the surrounding flesh. Occasionally the stem undergoes a black decay. On firm ripe fruits, Alternaria rot spots are dry, firm and shallow. Injured tissues are often invaded resulting in skin blemishes, small black corky lesions or yellow dark coloured rot.

Disease cycle: A. mali can survive in soil as microsclerotia and chlamydospores. Under suitable conditions conidia are produced on plant debris and spread by rain and wind. These fungi are weak pathogens and unable to infect uninjured fruit. However, A. kikuchiana can invade healthy young fruits through stomata and lenticels causing numerous small black spots.

Microscopic observations: The simple or branched conidiophores bear conidia singly or in chains. The conidia have up to eight transverse septa; and are obclavate , ovoid or ellipsoidal; and often have short beak.

Examination and record
1. Note the symptoms of different post harvest diseases of fruits and vegetables and make a detailed study of the life cycle of the pathogens.
2. Observe their host-parasite relationship under the compound microscope.

EXERCISE 9

Diseases Caused by Basidiomycota- Rusts

Objective: To Study the symptoms, signs and host-parasite relationship of diseases caused by Basidiomycota- Rusts

The rust fungi mostly attack leaves and stems of various crop plants. Rust infections usually appear as numerous rusty, orange, yellow or even white coloured pustules that rupture the epidermis. Some cause swellings and even galls. Most rust infections are strictly local spots but some may spread internally to some extent.

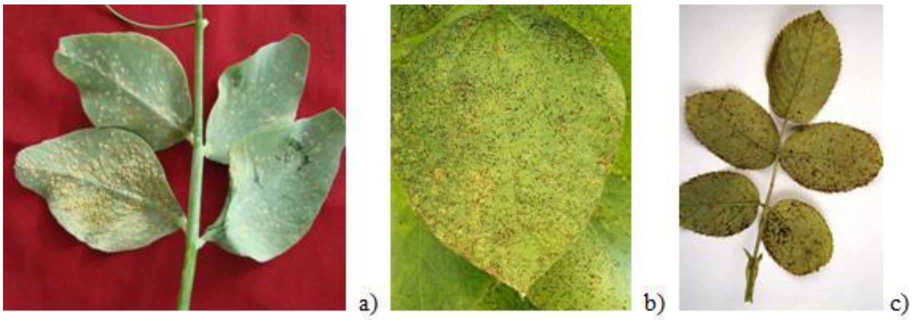


Fig.9.1 Rusts diseases; a- Pea rust, b- Bean rust, c- Rose rust

Causal organisms
Puccinia graminis tritici, causing stem rust of wheat
P. dianthi, causing rust of carnation
Gymnosporangium juniperi-virginianae, causing cedar apple rust
Phragmidium mucronatum, causing rose rust
Uromyces appendiculatus, causing rust of legumes
M. ciliata, causing poplar rust
Tranzschelia discolor, causing rust of almond, peach and other stone fruits
Classification:
Kingdom: Fungi, Division: Basidiomycota, Class: Urediniomycetes, Order: Uredinales, Family: Pucciniaceae

i) Stem rust of wheat (*P. graminis tritici*)
Symptoms: The symptoms appear as elliptical blisters or pustules, known as uredia, that develop parallel with the long axis of the stem, leaf or leaf sheath. The epidermis covering the pustules is later ruptured irregularly and pushed back, revealing a powdery mass of brick red coloured uredospores. Later in season, the rusty colour of the pustules turns black and the fungus produces teliospores instead of uredospores and uredia are transformed into black coloured telia.
Disease cycle: Puccinia graminis is a macrocyclic, heteroecious rust fungus producing spermogonia and aecia on barberry and uredia and telia on wheat and other cereals and grasses. In cooler regions, the fungus overwinters as teliospores on infected wheat straw debris. The stem rust pathogen is an obligate parasite and produces five distinct fruiting structures with five different spore forms that appear in a definite sequence. Basidiospores, aeciospores and uredospores can attack and infect host plants. The teliospores represent the only sexual, overwintering stage, which on germination produce the basidia. The basidium,

following meiosis, produces four haploid basidiospores. The basidiospores, on infection, produce haploid mycelium that forms spermogonia, containing haploid spermatia and receptive hyphae on the barberry leaves. Spermatia act as male gametes and are unable to infect plants. Their function is the fertilization of receptive hyphae of the compatible mating type and subsequent production of dikaryotic mycelium and dikaryotic spores. This mycelium forms aecia producing aeciospores on barberry leaves itself, which on infection of wheat further form more dikaryotic mycelium and finally uredia containing uredospores. Uredospores also infect and produce either more uredia and uredospores or near host maturity, telia and teliospores. The rust cycle is thus completed. However, in India, there is no role of barberry in the survival and yearly recurrence of this disease. The black rust pathogen is believed to survive on the 'off season' wheat, volunteer and stubbles plants in the hills in the form of uredospore, from where the fresh infections start and travel to the plains in the North and South India.
Microscopic observations: The teliospores are brown in colour, bicelled and stalked. The uredospores are oval, yellowish, spiny and pedicellate.

ii). Bean rust (*Uromyces appendiculatus*)
Symptoms: The fungus produces characteristic rust pustules on the hosts. The pustules are mostly found on the leaf blade, though often they are found on the petiole and stem of some hosts. The sori are small, round, open, powdery and brown coloured. They appear in groups, several sori coalescing to cover a large area of the leaf blade. In severe infections, the leaves wither away, resulting in considerable damage to the crop.
Disease Cycle: Since the fungus is an obligate pathogen and autoecious in nature, it completes its life cycle on beans. The uredial stage repeats several times and it is often noticed on such hosts as species of Dolichus and Vigna. The urediospores build up in masses and are spread by wind to cause secondary infection. The fungus can perpetuate through the uredial stage as well as through the telial stage. The teliospores germinate to produce basidiospores, which after infection, produce pycnia, aecia and uredia. Pycnia appear in yellowish spots on the upper surface of leaves. Orange coloured aecia are formed on the lower surface of the leaf around the pycnia on the opposite side.
Microscopic observations: The uredospores are echinulate, oval, and yellowish brown in colour, and measure 20-33 × 16-23 μ. The dark brown teliospores are elliptical to ovate, pedicellate, smooth walled, single-celled, with warty papillae at the top, and measure 24-33 × 20-26 μ in size.

Examination and record
1. Study and record the symptoms of rust on different hosts.
2. Observe the teliospores and uredospores under compound microscope.
3. Draw diagrams of different stages of the rust fungi.

EXERCISE 10

Diseases Caused by Basidiomycota-Smuts

Objective: To study the symptoms, signs and host parasite relationship of diseases caused by Basidiomycota- Smuts

Causal organisms:

Ustilago, causing corn smut (U. maydis), loose smut of cereals (U. avenae, U. nuda and U. iritici)
Urocystis, causing onion smut (U. cepulae)

Classification: Kingdom: Fungi, Division: Basidiomycota, Class: Ustilaginomycetes, Order: Ustilaginales, Family: Ustilaginaceae

i). Loose smut of wheat
Causal organism: *Ustilago tritici*
Symptoms: In an infected plant usually all the heads and all the spikelets and kernels of each head are smutted and are transformed into a smut mass consisting of olive-green spores. Smutted kernels are at first covered by a delicate greyish membrane, which soon bursts and sets the powdery spores free.
Disease cycle: The pathogen overwinters as dormant mycelium in the cotyledon of infected kernels. When planted, infected kernels begin to germinate and the mycelium resumes its activity and grows intercellularly through the tissues of the young seedling until it reaches the growing point of the plant. When the plant forms the head, the mycelium invades all the young spikelets, where it grows intracellularly and destroys most of the tissues of the spike, except the rachis. The mycelium in the infected kernels is soon transformed into teliospores, covered only by a delicate membrane. The membrane bursts open soon and spores are released.
Microscopic observations: The mycelium is hyaline during its growth through the plant and it is hyaline changing to brown near maturity. The mycelial cells are transformed into brown, spherical teliospores, which germinate readily and produce a basidium consisting of one to four cells. The basidium produces no basidiospores, but its cells germinate and produce short, uninucleate hyphae that fuse in pairs and produce dikaryotic mycelium.

ii). Corn smut
Causal organism: *Ustilago maydis*
Symptoms: When young corn seedlings are infected, minute galls form on the leaves and stems and the seedlings may remain stunted or may be killed. On older plants, infections occur on the young, actively growing tissues of axillary buds, individual flowers of the ear and tassel, leaves and stalks. Galls are fist covered with a greenish white membrane. Later, as the galls mature, they darken and turn into a mass of powdery, dark, olive-brown spores. The silvery grey membrane then ruptures and exposes the millions of sooty teleutospores, which are released into air.

Fig.10.1 Onion smut

Disease cycle: The fungus overwinters as teleutospores in crop debris and in the soil, where it can remain viable for several years. In the spring and summer, the teleutospores germinate and produce basidiospores, which are carried by air currents or are splashed by water to young developing tissues of corn plants. The basidiospores germinate and produce a fine hypha, which can enter epidermal cells directly. Later the hypha dies or fuses with a haploid hypha of compatible mating type. The resulting hypha becomes dikaryotic, enlarges in diameter and grows into the plant tissues mostly intercellularly and the cells surrounding it produce galls. The galls consist primarily of dikaryotic mycelium and plant cell remains, and the dikaryotic mycelium develops into teleutospores.
Microscopic observations: The fungus produces dikaryotic mycelium, the cells of which are transformed into black, spherical, or ellipsoidal teleutospores. They germinate by producing a four-celled basidium (promycelium), from each cell of which a basidiospore (sporidium) develops.

iii). Onion smut
Causal Organism: *U. cepulae*.
Symptoms: The fungus attacks the cotyledons of young plants soon after their emergence, causing dark, elongated, eruptive spots. On the older leaves the lesions may extend from the base to the tip, and appear as blisters. The severely affected plants may be killed in about a month after they emerge. On the bulb the outer scales are attacked similarly, resulting in blisters. The blisters rupture to expose masses of black powdery spores.
Examination and record:
1. Study the symptoms of various smuts of crop plants.
2. Observe the teleutospores of different smut fungi under microscope and point out salient differences.
3. Draw and label the life cycle of various smut fungi.

iv). Rhizoctonia Diseases:
Rhizoctonia diseases occur throughout the world. They cause losses on almost all vegetables and flowers, several field crops, turf grasses, and even perennial ornamentals, shrubs, and trees.
Causal organism: Rhizoctonia solani (Thanatephorus cucumeris) causing root rot and web blight of many plants including beans, and black scurf of potato.
Classification: Kingdom: Fungi, Division: Basidiomycota, Class: Basidiomycetes, Order: Ceratobasidiales, Family: Ceratobasidiaceae
Symptoms: Symptoms may vary some-what on the different crops, with the stage of growth at which the plant becomes infected, and with the prevailing environment conditions. The

most common symptoms on most plants are damping-off of seedling and root rot, stem rot, or stem canker of growing and grown plants. On some hosts, however, Rhizoctonia also causes rotting of storage organs and foliage blights or spots especially of foliage near the ground.
Microscopic observations: Rhizoctonia spp. exist primarily as sterile mycelium and, sometimes, as small sclerotia that show no internal tissue differentiation. Mycelial cells of the most important species, R solani, contain several nuclei (multinucleate Rhizoctonia), whereas mycelial cells of several other species contain two nuclei (binucleate Rhizoctonia). The mycelium, which is colourless when young but turns yellowish or light brown with age, consists of long cells and produces branches that grow at approximately right angles to the main hypha, are slightly constricted at the junction , and have a cross wall near the junction. The perfect stage of the multinucleate R.solani is Thanatephorus cucumeris, whereas that of binucleate Rhizoctonia is Ceratobasidium. A few multinucleate Rhizoctonia spp. (R.zeae and R.oryzae) have Waitea as their perfect basidiomycetous stage.
Disease cycle: The pathogen overwinters usually as mycelium or sclerotia in the soil and in on infected perennial plants or propagative material such as potato tubers. In some hosts the fungus may even be carried in the seed. Its inoculum is present in most soils, and, once established in a field, remains there indefinitely. The fungus spreads with rain, irrigation, and flood water; with tools and anything else that carries contaminated soil; and with infected or contaminated propagative materials.
Things to do:
1. Study the symptoms of various Rhizoctonia diseases of crop plants.
2. Observe different fungal structures under microscope and record salient features.

v). Sclerotium Diseases:
Causal organism: Sclerotium rolfsii (Teleomorph: Aethalium rolfsii)
Classification: Kingdom: Fungi, Division: Basidiomycota, Class: Basidiomycetes, Order: Ceratobasidiales, Family: Ceratobasidiaceae
Symptoms: Sclerotium diseases occur primarily in warm climates. They cause damping off of seedlings, stem canker, crown blight, root, crown bulb and tuber rot , and fruit rots. Sclerotium frequently causes severe losses of fleshy fruits and vegetables during shipment and storage. In the United States, they are often called southern wilts or southern blights and affect a wide variety of plants, including most vegetables, flowers, legumes, cereals, forage plants, and weeds.
Disease cycle: The fungus overwinters mainly as sclerotia. It is spread by moving water, infested soil, contaminated tools, infected transplant seedlings, infected vegetables and fruits, and, in some hosts, as sclerotia mixed with seed. The fungus attacks tissues directly. However, the mass of mycelium it produces secrets oxalic acid and also pectinolytic, cellulolytic and other enzymes, and it kills and disintegrates tissues before it actually penetrates the host. Once established in the plants, the fungus advances and produces mycelium and sclerotia quite rapidly, especially at high moisture and high temperatue (between 30 and 35ºC).
Microscopic observations: *Sclerotium rolfsii* produces abundant white, fluffy, branched mycelium that forms numerous sclerotia but is usually sterile, i.e., does not produce spores. *Sclerotium rolfsii*, which causes the symptoms described earlier on most of the hosts, occasionally produces basidiospores at the margins of lesions under humid conditions.
Things to do:
1. Study the symptoms of various Sclerotium diseases of crop plants.
2. Observe different fungal structures under microscope and record salient features.

vi). Wood rots and decays caused by Basidiomycetes:

Huge losses of timber trees in the forest and in harvested wood are caused every by the wood-rotting Basidiomycetes.

Causal organisms:

Heterobasidion, causing root and butt rot of living trees

Polyporus, a few species causing heart rot of living trees and rot of dead trees or logs

Phellinus, causing root rots on most conifers and many hardwood trees, and brown cubical rot in buildings and in stored lumber

Ganoderma lucidum, causing root and basal rots in conifers and hardwoods, in palms, and in other tropical plantation trees

Chondrostereum purpureum, causing 'silver leaf' of fruit trees as a result of decay of the interior of the tree trunk and branches, and heart rots of other trees

Peniophora gigantia, causing decay in coniferous logs and pulpwood

Symptoms: Depending on the tree part attacked, wood rots may be called root rots, root and butt rots, or stem rots. Fungi that cause tree or wood product decays grow inside the wood cells and utilize the cell wall components for food and energy. Some of them, the brown-rot fungi, attack preferably softwoods and break down and utilize primarily the cell wall polysaccharides (cellulose and hemicellulose), leaving the lignin more or less unaffected. Other wood rotters, the white-rot fungi, either decompose lignin and hemicellulose first and cellulose the last or decompose all wood components simultaneously, in either case reducing the woodpeckers or streaks separated by thin areas of firm wood. White-rot fungi are able to or preferably attack hardwoods normally resistant to brown-rot fungi.

Things to do:

1. Collect and study various sporophores of root and wood rotting fungi affecting fruit and forest trees.
2. Obrserve the basidia and basidiospores under microscope.

EXERCISE 11

Bacterial Plant Diseases

Objective: To study the symptoms, signs and host-parasite relationship of bacterial plant diseases

Bacteria are very prokaryotic unicellular organisms. About 200 species of bacteria cause diseases in plants.

i). Morphology

Most plant pathogenic bacteria are rod shaped except Streptomyces, which is filamentous. The cell walls of bacteria of most species are enveloped by a viscous, gummy material, which if thin and diffused, is called a slime layer; but if thick, forming a definitive mass around the cell, is called a capsule. Most plant pathogenic bacteria have delicate, threadlike flagella, considerably longer than the cells on which they are produced. In some bacterial species, each bacterium has only one flagellum (monotrichous) and others have a tuft of flagella at one end of the cell (lophotrichous), and still others have flagella, which are distributed over the entire surface of the cell (peritrichous).

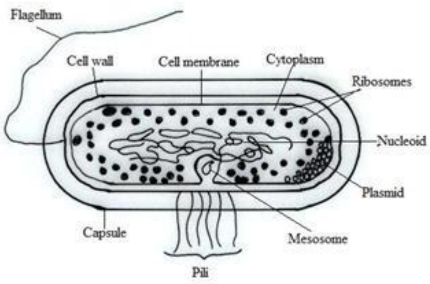


Fig.11.1 A typical bacterial cell

Observations and record

1. Draw a well labeled diagram of a typical bacterial cell.
2. Study the shape of different bacteria, and their flagellation.

ii). Gram staining reaction

Gram staining reaction differentiates bacteria into gram positive and gram negative types. In this reaction, bacteria fixed on a glass slide are treated with a crystal violet solution for 30 seconds, rinsed gently, treated with iodine solution and rinsed again with water and then alcohol. Gram positive bacteria retain the violet-iodine stain combination because it forms a complex with certain components of their cell walls and cytoplasm. Gram negative bacteria have no affinity for the stain combination which is therefore, removed by the alcohol rinse and bacteria remain as nearly invisible as before. Of the rod shaped phytopathogenic bacteria, only the genera Clavibacter and Curtobacterium, and also Bacillus and Rhodococcus are gram positive. Agrobacterium, Erwinia, Pseudomonas, Xanthomonas and Xylella are gram negative.

Procedure

1. Make thin smears of bacterial culture on separate glass slides.
2. After air drying, heat fix the smears.

3. Cover the smears with crystal violet for 30 seconds.
4. Wash the slide with distilled water for a few seconds using wash bottle.
5. Cover smear with iodine solution of 60 seconds.
6. Wash off the iodine solution with 95 per cent ethyl alcohol. Add ethyl alcohol drop by drop, until no more colour flows from the smear.
7. Wash the slides with distilled water and drain.
8. Apply safranin to smear for 30 seconds.
9. Wash with distilled water and dry the slides with blotting paper.

Observations and record

1. Note the Gram's reaction under microscope for pink or violet colour of the bacterial cells.

iii). Bacterial Diseases

Symptoms

Plant pathogenic bacteria cause a variety of disease symptoms in plants, viz. leaf spots and blights, soft rots of fruits, roots, and storage organs, wilts, overgrowths, scabs and cankers. Any given type of symptom can be caused by bacterial pathogens belonging to several genera, and each genus may contain pathogens capable of causing different types of diseases.

a. Bacterial spots and blights

Causal organisms:

Pseudomonas syringae pathovars cause diseases like wildfire of tobacco (P. syringae pv. tabacci), angular leaf spot of cucumber (P. syringae pv. lachrymans), halo blight of bean (P. syringae pv. phaseolicola), lilac blight (P. syringae pv. syringae) and bacterial speck of tomato (P. syringae pv. tomato).

Xanthomonas campestris pathovars cause common blight of beans (X. campestris pv. phaseoli), bacterial spots of stone fruits (X. arboricola pv. pruni), and of tomato and pepper (X. campestris pv. vesicatoria).

b. Bacterial vascular wilts

Clavibacter (Corynebacterium) causes ring rot of potato (C. michiganense subsp. sepedonicum) and bacterial wilt and canker of tomato (C. michiganense subsp. michiganense).

Curtobacterium (Corynebacterium) flaccumfaciens causes bacterial wilt of bean.

Erwinia causes bacterial wilt of cucurbits (E. tracheiphila), Stewart's wilt of corn (E. stewartii) and fire blight of pome fruits (E. amylovora)

Ralstonia (Pseudomonas), causes the southern bacterial wilt of solanaceous crops and the Moko disease of banana (R. solanacearum).

Xanthomonas, causes black rot and black vein of crucifers (X. campestris pv. campestris).

Fig.11.2 Bacterial wilt of capsicum

c. Bacterial soft rots

Erwinia "carotovora" or "soft rot" group, causes soft rot of numerous fleshy fruits, vegetables, and ornamentals (E. carotovora pv. carotovora), and blackleg of potato (E. carotovora pv. atroseptica).

Pseudomonas causes soft rot of fleshy fruits and vegetables (P. fluorescens), such as the pink eye disease of potato, the slippery skin disease and the sour skin of onion.

Bacillus, causes rotting of potatoes and tobacco leaves in storage, of tomato seedlings, and those of soybean.

Clostridium causes rotting of potatoes, and tobacco leaves in storage and the wet wood syndrome of poplar and elm.

Fig.11.3 Soft rot of bell pepper

d. Bacterial galls

Agrobacterium causes crown gall of many woody plants, primarily stone and pome fruits, willow, and grapes (A. tumefaciens), hairy root of apple (A. rhizogenes), and cane gall of raspberries and blackberries (A. rubi). The kind of symptoms produced is actually determined not by the species of Agrobacterium, but by the kind of plasmid they carry: bacteria carrying a tumor inducing (Ti) plasmid induce crown gall, whereas bacteria carrying a root inducing (Ri) plasmid produce hairy root symptoms. Another species, A. radiobacter carries neither of the plasmids, and thus causes no disease.

Pseudomonas causes the olive knot disease and the bacterial gall or canker of oleander (P. syringae subsp. savastanoi).

Fig.11.4 Crown gall of stone fruits

e. Bacterial cankers

Pseudomonas causes the bacterial canker of stone and pome fruit trees (P. syringae pv. syringae and P. syringae pv. morsprunorum).

Xanthomonas causes the bacterial canker of citrus (X. axonopodis, formerly X. campestris pv. citri).

Fig.11.5 Citrus canker

f. Bacterial scabs
Bacterial scabs are caused by species of Streptomyces. The most important ones are the common scab of potato and of other belowground crops (S. scabies) and the soil rot or pox of sweet potato (S. ipomoeae).

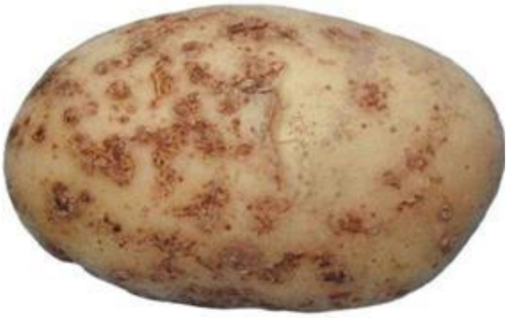


Fig.11.6 Common scab of potato

Fastidious vascular bacteria
The fastidious vascular bacteria that cause plant diseases cannot be grown on simple culture media in the absence of host cells.

a. Fastidious xylem limited bacteria
Fastidious xylem-inhabiting bacteria are generally rod shaped and are bounded by a cell membrane and a cell wall. They have no flagella. The cell is usually undulating or rippled. Nearly, all fastidious xylem limited bacteria are gram negative. Several such xylem-limited bacteria have been placed in the genus Xylella. All fastidious xylem bacteria are unable to grow on conventional bacteriological media. Gram negative xylem-inhabiting fastidious bacteria are transmitted by xylem-feeding insects, such as sharp shooter, leaf hoppers and spittle bugs.

Symptoms: The symptoms of diseases caused by fastidious xylem-inhabiting bacteria often consist of marginal necrosis of leaves, stunting, and general decline and reduced yields. Such symptoms are probably caused by plugging of the xylem by bacterial cells and by a matrix material of bacterial and of plant origin. In some diseases, however, such as phony peach, no marginal leaf necrosis occurs, and in others, such as sugarcane ratoon stunting, the only diagnostic symptom is stunting and an internal discoloration of the stalk.

Among the most important plant diseases caused by fastidious xylem-limited, gram-negative bacteria are Pierce's disease of grapevine, citrus variegation chlorosis, phony peach disease, almond leaf scorch, and plum leaf scald.

b. Fastidious phloem limited bacteria

Fastidious phloem-limited vascular bacteria are generally rod-shaped cells 0.2 to 0.5 µm in diameter by 1 to 4 µm in length. They are bounded by a cell membrane and a cell wall, although in some phloem inhabiting bacteria the cell wall appears more as a second membrane rather than a cell wall. They have no flagella. The cell is usually undulating or rippled. Nearly all fastidious vascular bacteria are gram negative.

Symptoms: The symptoms of diseases caused by fastidious phloem-inhabiting bacteria often consist of leaf stunting and clubbing; in some cases they may appear as shoot proliferation and witches' broom, and as greening of floral parts. In some of these diseases, symptoms are often mild and sometimes are followed by spontaneous recovery. Phloem-limited bacteria are so far known to cause very important diseases like citrus greening, yellow vine disease of watermelon and other cucurbits, the bunchy top disease of papaya, and some minor diseases of clover (club leaf) and periwinkle.

Phytoplasma

Phytoplasma are wall-less, pleomorphic, unicellular, prokaryotic organisms; bound by aunit memberane, and have cytoplasm, ribosomes and strands of nuclear material. They were earlier called MLO's and were found to be associated with several yellows and witches' broom diseases after their discovery by Doi et al. in 1967. They are different from mycoplasma in the sense that they can not be cultured on synthetic media. The change in terminology from MLO's to phytoplasma occurred since the studies of DNA homology in the highly conserved genes encoding ribosomal RNA and ribosomal protein. It showed that the phytoplasma comprise a coherent group distinct from other prokaryotes. Their closest relatives are in the genus Acholeplasma. As they have not been cultured on artificial medium in vitro and characterized apart from their host, they are referred to Candidatus status. They are associated with about 200 plant diseases including aster yellows, apple proliferation, peanut witches' broom, peach-x-disease and elm yellows. They are phloem inhabiting organisms and are graft transmissible in nature, and can also be transmitted by leaf hoppers.

Fig.11.7 Witches' broom on weeping willow

Observations and record

1. Study the symptoms of bacterial and phytoplasma diseases of various horticultural crops.
2. Examine cultures of various types of bacteria and describe their characteristics in terms of nature of growth, margin, topography and colour.
3. Examine specimens of various bacterial diseases.
4. Perform Gram's reaction.

EXERCISE 12

Viral Diseases of Horticultural Plants

Objective: To study the symptoms, signs and host-parasite relationship of diseases caused by viruses and virus-like plant pathogens in horticultural crop plants

A virus is a nucleoprotein that multiplies only in living cells and has the ability to cause disease. It is too small to be seen individually with a light microscope.

Characteristics of plant viruses

Plant viruses differ greatly from all other plant pathogens not only in size and shape, but also in the simplicity of their chemical constitution and physical structure, methods of infection, multiplication, translocation within the host, dissemination, and the symptoms they produce on the host. Because of their small size and the fact that they are transparent, viruses generally cannot be viewed and detected by the methods used for other plant pathogens. Cell inclusions consisting of virus particles, however, are visible by light microscopy. Viruses are not cells nor do they consist of cells.

Morphology

Plant viruses are of different shapes and sizes. Nearly half of them are elongate (rigid rods or flexuous threads), and almost as many are spherical (isometric or polyhedral), with the remaining being cylindrical bacillus-like rods. Some elongated viruses are rigid rods about 15 x 300 nm, but most appear as long, thin, flexible threads that are usually 10 to 13 nanometers wide and range in length from 480 to 2,000 nm. Rhabdoviruses are short, bacillus like, cylindrical rods, approximately three to five times as long as they are wide (52–75 x 300–380nm). Most spherical viruses are actually polyhedral, ranging in diameter from about 17 nanometers (tobacco necrosis satellite virus) to 60 nanometers (wound tumor virus). Tomato spotted wilt virus is surrounded by a membrane and has a flexible, spherical shape about 100 nanometers in diameter. Many plant viruses have split genomes, i.e., they consist of two or more distinct nucleic acid strands encapsidated in different-sized particles made of the same protein subunits. Thus, some, like tobacco rattle virus, consist of two rods, a long one (195 x 25nm) and a shorter one (43 x 25 nm), whereas others, like alfalfa mosaic virus, consist of four components of different sizes. Also, many isometric viruses have two or three different components of the same size but containing nucleic acid strands of different lengths.

Symptoms of viral diseases

Almost all viral diseases seem to cause some degree of dwarfing or stunting of the entire plant and reduction in total yield. The most obvious symptoms of virus-infected plants are usually those appearing on the leaves, but some viruses may cause striking symptoms on the stem, fruit, and roots; while they may or may not cause any symptom development on the leaves. The most common types of plant symptoms produced by systemic virus infections are mosaics and ring spots.

Mosaics are characterized by light-green, yellow, or white areas intermingled with the normal green of the leaves or fruit or of lighter–colored areas intermingled with areas of the normal color of flowers or fruit. Depending on the intensity or pattern of discolourations, mosaic-type symptoms may be described as mottling, streak, ring pattern, line pattern, vein clearing, vein banding, or chlorotic spotting.

Ring spots are characterized by the appearance of chlorotic or necrotic rings on the leaves and sometimes also on the fruit and stem. In many ring spot diseases, the symptoms but not the virus, tend to disappear later on.

A large number of other, less common virus symptoms have been described and include stunt (e.g., tomato bushy stunt), dwarf (barley yellow dwarf), leaf roll (potato leaf roll), yellows (beet yellows), streak (tobacco streak), pox (plum pox), enation (pea enation mosaic), tumours (wound tumor), pitting of stem (apple stem pitting), pitting of fruit (pear stony pit), and flattening and distortion of stem (apple flat limb). These symptoms may be accompanied by other symptoms on other parts of the same plant.

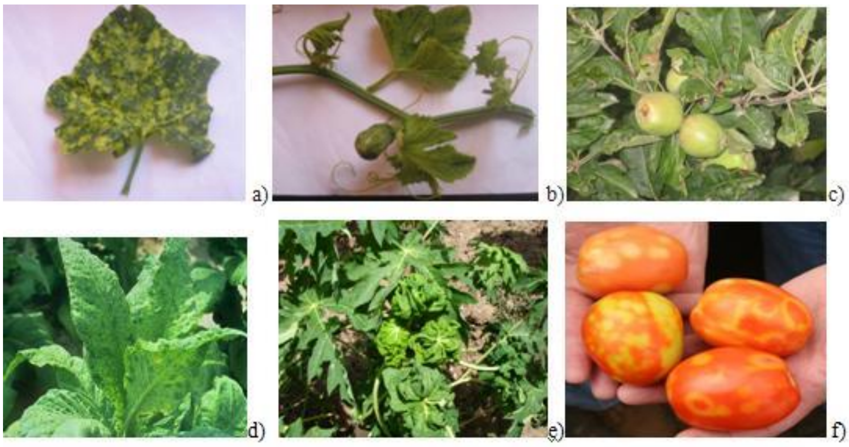


Fig.12.1 Virus diseases; a. Cucumber mosaic, b. Zucchini mosaic, c. Apple chlorotic leaf spot, d. Tobacco mosaic, e. Papaya leaf curl, f. Tomato ring spot

Observations and Record

1. Study the symptoms produced by viruses and viroids in the horticultural crops.
2. Study the morphology of common virus particles infecting horticultural plants of your area under electron microscope.

EXERCISE 13

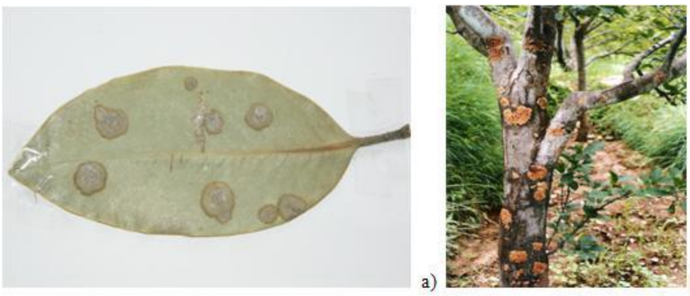
Parasitic Algae and Flowering Plants

Objective: To study the characteristics of parasitic algae and flowering plants and their relationship with their hosts

Algae

Parasitic green algae occur on cultivated plants but are generally overlooked because of their green colour. Cephaleuros is the best known genus. It is a plant parasite living under leaf cuticle. It was first reported from India in19th century, causing damage to tea and coffee plantations. Now, over 400 hosts of Cephaleuros are recorded all over the world infecting hibiscus, orchids, euphorbias, citrus and forest trees. Ninety per cent of the hosts are dicots. Cephaleuros infections were earlier confused with fungal infection. There are 13 species of Cephaleuros, out of which C. parasiticus and C. virescens are most common and cause maximum damage. C. virescens causes red rust of tea and mango.

Symptoms: Fluffy, bright orange red spots occur on leaves and stems that look very much like rust fungi. In fact, C. virescens has the misleading common name ‘red rust’. Species of Cephaleuros have fungus-like filaments, sterile hairs and produce sporangiophores and zoosporangia on the lower surface of leaves that look like downy mildew fungi. Necrosis may be limited to the epidermis or spread into the deeper tissues of the leaves. Severe damage usually occurs on older leaves leading to defoliation.



a) b)

Fig.13.1 Algal parasites; a- Algal spot of magnolia, b- Lichen on apple tree trunk

Fungi parasitize Cephaleuros to form lichens. The lichenized state of C. virescens is identified as Strigula elegans. Early literature suggests that the fungus portion of the lichen (mycobiont) was responsible for plant damage. But, more recent findings show that the fungus parasitizes the alga, not the plant. Plant injury is caused by the alga much before a fungus colonizes it.

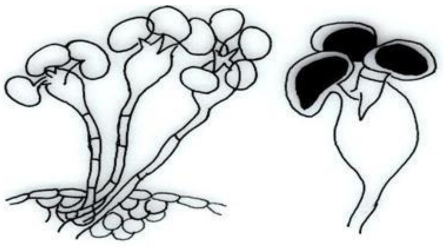


Fig.13.2 Zoosporangia and zoosporangiophores of *C. virescens*

Flowering Parasitic Plants

Root parasites

Striga: Striga (witchweed) is a root hemiparasite, and the seedlings above ground do form chlorophyll. Striga has made greater impact than any other parasitic angiosperm. It attacks important crops like maize, sorghum, pearl millet, rice, sugarcane and legumes (cowpea, groundnut etc.). Two species, S. asiatica and S. hermonthica cause maximum damage to crops. Its English name witchweed is due to its effects on the host, prior to the appearance of the parasite above ground.

Fig.13.3 Striga

Striga has a complex life cycle. It produces thousands of 'dust' seeds that are disseminated by wind and rain. The seeds after a dormant 'ripening' period of several months, respond to chemical signals exuded by the host. The chemical signals enable the Striga seeds to detect the type of host and its distance from the host. Seed germination of Striga, as in all obligate root parasites, is cryptocotyler i.e. the cotyledons remain within the seed when the radical comes out. The radical produces root hair like structures that glue it to the host. If the host is suitable, a haustorium is formed that penetrates and forms a link with the host vascular system. Once the parasite is established, the distinctive seedling of Striga is formed underground, which lacks chlorophyll, possesses scale leaves, and produces abundant adventitious roots that form additional haustoria, establishing more connections with the host. The parasitic seedlings exert great influence on the growth-regulating metabolism of the host, stimulating root production. Significant damage to the host occurs at this stage. The next stage is emergence of the seedlings above ground. Chlorophyll develops, and in due course, flower and seeds are formed. The life cycle is ready for a repeat.
A major problem in control is persistence of the tiny seeds in the soil. Ethylene gas is introduced into the soil to induce seed germination, which becomes suicidal in absence of the

host. Equipments and application methodologies have been developed to introduce the gas into the soil. Up to 90% seeds germinate by this method, and die in absence of the host.

Orobanche (broomrape): This is an obligate root holoparasite, infecting legumes, solanaceous crops, carrot, cabbage, cauliflower, lettuce and sunflower. Total crop failure may occur in heavily infested soils. The parasite appears as whitish, yellowish or brownish stems, about 30 cm high that arise from the roots of infected host, and bear beautiful flowers, besides bracket-like leaves lacking, chlorophyll. In general, Orobanche is a parasite of colder climate and need 10-20oC of temperature for seed germination. This is the reason why it attacks tobacco during winter in India, but fails to infect sunflower during summers in the same field.

Fig.13.4 Orobanche (broomrape)

Seed germination requirements of Orobanche are different from those of Striga. It needs low temperature (10-20oC); the germinated seeds are geotropically neutral i.e. they do not grow downward in the soil and ethylene has no stimulatory effect. Its control is difficult due to the high longevity (more than 5 decades) of the seeds in the soil, their extremely small size (less than the thickness of human a hair), their production in extremely large number, and subterranean infection.

Stem Parasites

Cuscuta (dodder)

It is obligate stem holoparasite and is among the best-known of all parasitic plants. Its slender, twining, orange-yellow, leaf less stems form conspicuous tangled mass on the host. The host range is large, though monocots are less preferred. Dodders are most important parasites of legumes. C. campestris is the most widely distributed among the ten species that attack crops. It causes considerable damage to alfalfa, flax, sugar beet, onion and other crops. It also transmit viruses.

Fig.13.5 Cuscuta

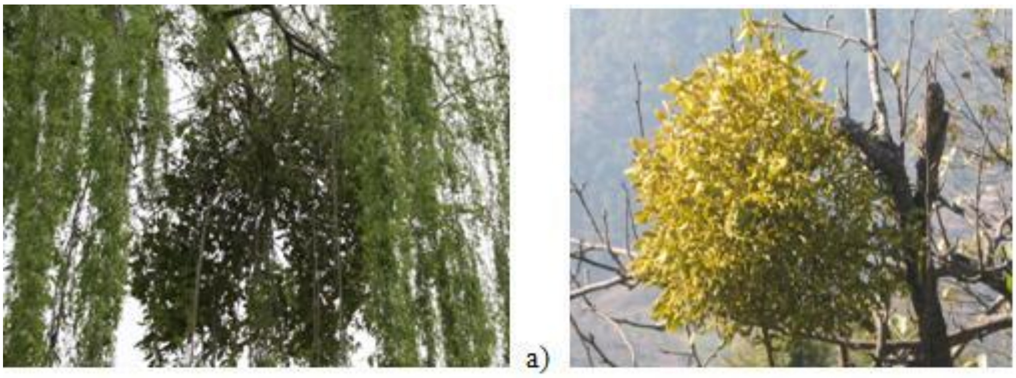
The most effective means of control is seed sanitation and mechanical removal from the infested plants.. Several herbicides are effective on newly-germinated seeds.

Mistletoes

Mistletoes are stem hemiparasites occurring in three families of the order Santalales as follows:

Family Loranthaceae: Showy mistletoes
Family Santalaceae: sandalwood
Family Viscaceae: Viscum album (leafy mistletoe)

The showy "mistletoes" produce large and beautiful flowers that are pollinated by birds. The co-evolution of these parasites and the birds is also suggested by the seed dispersal mechanism operating in the birds. Santalaceae, the sandalwood family, have a few members (Pyrularia etc.) that cause negative impact on their hosts. Family Viscaceae is called "Christmas mistletoe family", because their shoots with the white berries are used as door festoons during Christmas in temperate countries. Numerous folklores are associated with mistletoes.



a) b)

Fig.13.6 Mistletoes; a- *Loranthus*, b- *Viscum*

Examination and record

1. Study the disease symptoms caused by algae.
2. Draw and label the life cycle of parasitic flowering plants.

EXERCISE 14

Culture Media and Sterilization

Objective: Preparation of culture media and sterilization

Culture media

Microorganisms can be cultured in the laboratory on artificial substrates called culture media which contain a variety of substances. Most microorganisms require about one per cent of a carbon source in the form of sugar, less than 0.5 per cent of a nitrogen source as salt or yeast extract and small quantities of phosphate, sulphur, potassium, magnesium and traces of calcium, iron, zinc, manganese and molybdenum. These nutrients are supplied to the organisms in different inorganic forms, depending upon their capacity to utilize them.
The culture media commonly used in the laboratory are classified into non-synthetic (natural) and synthetic or organic and inorganic media. Non-synthetic media include organic substances with complex or simple molecules, while synthetic media consist of chemicals of known molecular structure and composition. Such media may be in liquid, solid or semi-solid form. In order to solidify the liquid medium containing the various ingredients one to two per cent agar agar or 10-20 per cent gelatin is added. In a Plant Pathology Laboratory, different synthetic media are prepared, e.g. Richard's, Czapeck-Dox etc. along with some specific media required for specific purposes. However, a non-synthetic potato-dextrose-agar (PDA) medium is routinely prepared and used.

Preparation of synthetic media
Dissolve weighed quantities of chemicals in measured quantities of water. Adjust the pH to the required level by adding N/10 NaOH or N/10 HCl. Filter the contents through absorbent cotton wool or a double layer of cheese cloth. Dispense filtrate into test-tubes or flasks. Plug them with cotton, and sterilize the medium in the test tubes and flasks in an autoclave at the

right temperature and steam pressure. For preparing solid agar medium, half the quantity of water is used for dissolving the chemicals and the remaining half for dissolving the agar agar powder. The agar is dissolved in water by slow heating with constant stirring. The melted agar is added to the other half of the medium containing the chemicals. Usually the pH is adjusted before mixing with agar. The mixture is then dispensed into tubes or flasks as desired, the mouth plugged with non-absorbent cotton and sterilized in an autoclave.

Preparation of potato-dextrose-agar medium
Composition

Peeled potatoes – 250 g
Dextrose (D-glucose) – 20 g
Agar agar – 20 g
Distilled water- 1000 ml

Procedure
Boil the peeled and sliced potatoes (250 g) in water for 30 minutes and then strain the broth through double-layered cheesecloth. Make the volume to one litre by adding distilled water. Add dextrose (20g) and agar agar powder (20 g) and sterilize the media in an autoclave.

Sterilization
Sterilization is the process of freeing a substance from living organisms. Several sterilization processes can be adopted in the laboratory, depending upon the material to be sterilized. Sterilization can be accomplished by direct heating over a flame, exposure to dry heat in a hot air oven or steam heat under pressure in an autoclave, filtration through suitable microbiological filters, chemical treatments and exposure to UV light, X-ray and radioactivity as follows:

i. The inoculating needle, scalpel, scissors and other metallic instruments used in handling microorganisms and the host tissues in the laboratory are usually surface sterilized by rectified spirit, and by heating over a flame for a few seconds.

ii. Glassware such as Petri dishes and pipettes are sterilized by heating at about 100oC for 2 hours in a hot air oven.

iii. Since moist heat is efficient in penetrating materials, it is used for sterilizing laboratory media. Usually test tubes or flasks containing media are autoclaved at 10 p.s.i. pressure (115.5oC) for 30 minutes, 15 p.s.i. pressure (121.6oC) for 15 minutes or 20 p.s.i. pressure (126.6oC) for 10 minutes, depending upon the substance contained in the medium.

iv. In case high temperature is likely to spoil the chemical composition of the medium, pasteurization can be adopted. It is a process by which the flasks or tubes containing the medium are steam heated at 60oC for one hour, on three successive days.

v. Materials which are destroyed even by minimum heating are sterilized either by treating with chemicals or by exposure to UV or X-ray.

vi. Sterilization through a sintered glass filter or Seitz filter or Millipore filter helps to preserve the qualities of the media which are likely to be destroyed by heat or exposure to different light rays.

Fig.14.1 Culture media

Things to do:
1. Prepare the Richard's and potato dextrose agar media and make slants and keep in 250 ml Erlenmeyer flasks, sterilize at 15 p.s.i. pressure in the autoclave and store in a refrigerator for further use. Record the procedure in the note book.

EXERCISE 15

Isolation of Fungal and Bacterial Plant Pathogens

Objective: To isolate the fungal and bacterial plant pathogens from soil, seed and affected plant parts

Preparing for isolation

1. Procure already sterilized glassware, such as petri dishes, test tubes, and pipettes
2. Prepare solutions for treating the surface of the infected or infested tissue to eliminate or markedly reduce surface contaminants that could interfere with the isolation of the pathogen. The most commonly used surface sterilants are 0.5 per cent sodium hypochlorite solution, used both for wiping infected tissues or dipping sections of such tissues in it and for wiping down table or bench surfaces before making isolations; and 70 per cent ethyl alcohol, which is used for leaf dips for three seconds or more. The tissues must be blotted dry with a sterile paper towel.
3. Prepare culture media on which the isolated fungal or bacterial pathogens will be grown. The most commonly used medium is potato dextrose agar (PDA), which is good for most, but not all fungi; water agar or glucose agar (1–3% glucose in water agar) for separating some oomycetes (Pythium) and fungi (Fusarium) from bacteria; V-8 and other less rich media, which encourage fungal sporulation; and nutrient agar, which contains beef extract and peptone and is good for isolating bacterial plant pathogens. Fungi can also be separated in culture from bacteria by adding 1 or 2 drops of a 25 per cent solution of lactic acid to 10 milliliters of the medium before pouring it in the plate, which inhibits the growth of bacteria.

Pouring the plates

Properly sterilized culture media are melted (if stored as in the previous exercise), allowed to cool somewhat, and are subsequently poured from the flasks into sterilized petri dishes, test tubes, or other appropriate containers. If agar was added, the medium will soon solidify and is then ready to be used for growth of the fungus or bacterium. Pouring of the culture medium into petri dishes, tubes, and so on is carried out as aseptically as possible either in a separate culture room or in a clean room free from drift and dust. In either case, the work table should be wiped with ethyl alcohol, hands should be clean, and tools such as scalpels, forceps, and needles should be dipped in alcohol and flamed to prevent introduction of contaminating microorganisms. Working in a laminar flow hood greatly helps to grow the desired fungus free of airborne contaminants.

Although most fungi and most bacteria can be cultured on nutrient media with ease, some of them have specific and exacting requirements and will not grow on most commonly used nutrient media. Some groups of fungi, namely Erysiphales causing the powdery mildew diseases, and the oomycetes (Peronosporaceae) causing downy mildews, are considered strictly obligate parasites and cannot be grown on culture media but can be grown on leaf-containing dishes. Another group of fungi, Uredinales, which cause the rust diseases of plants, were, until the late 1960s, also thought to be strictly obligate parasites. Since then, however, it has become possible to grow some stages of some rust fungi in culture by adding certain components to the media so rust fungi are no longer impossible to grow in culture, although they are, of course, obligate parasites in nature and, therefore, are aptly termed as biotrophs. Fastidious phloem- and xylem-limited bacteria also either are impossible to grow in culture so far or must be grown on special complex nutrient media. Of the other pathogens, only spiroplasmas have been grown in culture. None of the phytoplasmas and none of the viruses, nematodes, or protozoa have been grown on nutrient culture media so far.

Methods of isolation

Methods to be adopted for isolation of plant pathogens depend on the nature of the latter particularly in relation to its presence in the host cells and the form in which it is present. Methods that are recommended for different cases are stated below:

S No.	Method	Pathogens involved
1.	Induction of sporulation prior to isolation	Leaf spotting fungi which sporulate comparatively easily
2.	Induction of mycelial growth	For deep seated infections and poorly sporulating fungi
3.	Host inoculation	Badly contaminated and slow growing pathogens
4.	Dilution technique	For bacteria and micro-organisms with large number of reproductive bodies
5.	Direct isolation	Organisms that are present in pure colonies

Isolating the Pathogen

From Leaves
i) Fungi

1. Cut across several small sections of 5 to 10 mm square size from the margin of the infected lesion so that they contain both diseased and healthy looking tissue.
2. Place them in one of the surface sterilant solutions, making sure that the whole surface gets wet. After about 15 to 30 seconds, the sections are taken out aseptically one by one and at regular intervals (e.g. every 10–15 seconds) so that each of them has been surface sterilized for different times.
3. The sections are then washed in three changes of sterile water; blotted dry on clean sterile paper towels, and are finally placed on the nutrient medium, usually three to five bits per petri dish aseptically.
4. Incubate the inoculated petri dishes at 25OC for 3-5 days.
5. If fruiting structures (pycnidia, perithecia) are present on the leaf, it is sometimes possible to pick them out, drop them in the surface sterilant for a few seconds, and then plate them on the nutrient medium.

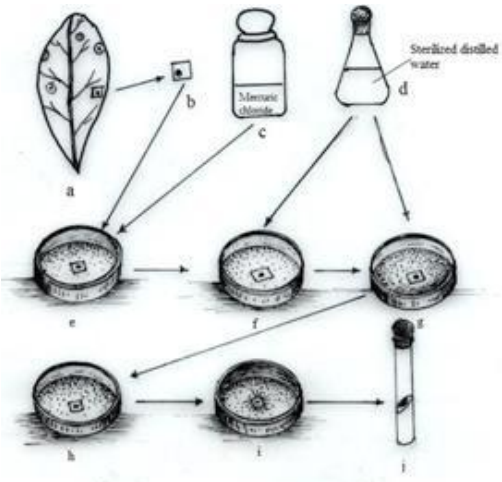


Fig.15.1 Isolation procedure

ii) Bacteria
The serial dilution method is often used to isolate pathogenic bacteria from diseased tissues contaminated with other bacteria. After surface sterilization of sections of diseased tissues from the margin of the infection, the sections are ground aseptically but thoroughly in a small volume of sterile water and then part of the homogenate is diluted serially in equal volumes or 10 times the volume of the initial water. Finally, plates containing nutrient agar are streaked with a needle or loop dipped in each of the different serial dilutions, and single colonies of the pathogenic bacterium are obtained from the higher dilutions that still contain bacteria.

From stems, fruits, seeds, and other aerial plant parts
Almost all the methods described for isolating fungal and bacterial pathogens from leaves can also be used to isolate these plant pathogens from superficial infections of stems, fruits, seeds, and other aerial plant parts. Entire seeds can be plated. In addition to these methods, however, plant pathogens can often be isolated easily from infected stems and fruits in which the they have penetrated fairly deeply. This is accomplished by splitting the stem or breaking

the fruit from the healthy side first and then tearing it apart toward and past the infected margin, thus exposing tissues not previously exposed to contaminants and not touched by hand or knife and therefore not contaminated. Small sections of tissue can be cut from the freshly exposed area of the advancing margin of the infection with a flamed scalpel and can be plated directly on the culture medium.

From roots, tubers, fleshy roots, vegetables and fruits in contact with soil

Isolating pathogens from any diseased plant tissue in contact with soil present the additional problems of numerous saprophytic organisms invading the plant tissue after it has been killed by the pathogen. For this reason, the first step in isolating the pathogen is repeated thorough washing of such diseased tissues to remove all soil and most of the loose, decayed plant tissue in which most of the saprophytes are present. If the diseased root is small, once it is washed thoroughly, pathogens can be isolated from it by following one of the methods described for isolating pathogens from leaves. If isolation is attempted from fleshy roots or other fleshy tissues penetrated only slightly by the pathogen and showing only surface lesions, the tissue is washed free from adhering soil, and several bits of tissue from the margin of the lesions are placed in Clorox solution. The tissue sections are picked from the solution one by one, washed in sterile water, blotted, and placed on agar in petri dishes. If the pathogen has penetrated deeply into the fleshy tissue, the method described earlier for stems and fruits can be used most effectively, namely breaking the specimens from the healthy side first and then tearing toward the infected area and plating bits taken from the previously unexposed margin of the rot.

Proving Koch's postulates

German microbiologist, Robert Koch, identified a set of four conditions which had to be satisfied to establish that a particular organism is the causative agent of a particular disease. These conditions are known as Koch's postulates.

1. Isolate the associated microorganism from the infected tissues using the standard isolation method.
2. Purify the culture, if contaminated, following the purification techniques like hyphal tip method in case of fungi.
3. Wash a healthy leaf or fruit or a twig.
4. Surface sterilize it with ethyl alcohol or other disinfectant and rinse with 2-3 changes of sterile water.
5. Transfer these sterilized parts into Petri plates or trays lined with moistened sterile filter paper.
6. Inoculate them at the centre with a mycelial disk cut from the margins of the growing pathogen culture keeping one uninoculated plant part as control.
7. Incubate the plates at an optimum temperature (say 25oC) for 3-5 days or until the disease appears.
8. Re-isolate the microorganism from the diseased plant part in the potato dextrose agar plates.
9. Incubate the plates at 25oC for 5-7 days.
10. If the symptoms both on the naturally and artificially inoculated parts are similar and the pathogen is the same in both the isolations, the Koch's isolates are proved.

Things to do:

1. Isolate fungal and bacterial plant pathogens one each; and prove Koch's postulates for them.

EXERCISE 16

Fungicidal Solutions, Slurries and Pastes, and their Applications

Objective: To prepare the fungicidal solutions, slurries and pastes and their applications along with precautions in their handling

Fungicides are the chemicals used against fungi to control diseases caused by the latter. Due consideration should be given to their efficacy, economy and safety.

i) Fungicide formulations

Fungicides are manufactured as technical material which are further processed into formulations to get even distribution of the active ingredients over the application area. Each formulation has its own special characteristics. The most commonly used formulations are as under:

a) Solid

- • Dusts are free flowing powders containing technical material in the range of 2 to 10 per cent and inert carrier.
- • Granule products contain technical material in the range of 3 to 10 per cent and granule base.
- • Water dispersible powders or Wettable Powders (WP) are free flowing powders containing technical material mostly in the range of 25 to 75 per cent, and contain wetting and dispersing agents and carriers.

b) Liquid

- Water soluble liquids (SL) are liquid formulations based on technical material which are insoluble in water and contain 36 to 85 per cent technical material and solvent.

- Emulsifiable concentrates (EC) are liquid formulations based on technical material which are not soluble in water and contain 25 to 80 per cent technical material, solvent and emulsifier.
- Fumigant formulations used for indoor application for storage of grains.

c) Other formulations

- Water soluble powders are similar to water dispersible powders but active ingredient is insoluble in water.
- Flowable concentrates are slurry like formulations mixable in water.
- Aerosols are liquids under pressure filled in cans which on release give a misty spray.
- ULV formulations are liquid formulations suitable for ultra low volume applications.

Fig.16.1 Commonly available fungicide formulations

ii) Significance of symbols on the pesticide label

Toxicity levels of different pesticides are based on their LD50 values and are evident from the warning (triangle) symbols present on the package as below:

Toxicity category	**Acute oral toxicity LD_{50} (mg/Kg)**	**Colour of the triangle**	**Signal word required on the label**	**Warning symbols on the label**
Extremely toxic	0-50	Red	POISON (With skull & bone mark	

			above it)	
Moderately toxic	501-5000	Blue	DANGER	
Slightly toxic	More than 5000	Green	CAUTION	

Preparation of spray solution

To ascertain the quantity of fungicide needed for required gallonage of spray solution, following formula is used:
Quantity of the fungicide required (in g/ml)=
Percentage of solution desired x Quantity of spray solution required (in ml)
____
Given strength of fungicide formulation

Example: If 100 litres of 0.25 per cent mancozeb is to be prepared from Dithane M-45 (75 % W.P.), the required quantity of fungicide can be calculated as:
Quantity of the fungicide required=
0.25 x 100 x 1000 = 25 x 100 x 1000 = 0.333 x 1000 = 333g
75 100 x 75

However, in case of fungicides, the strength of the formulated product is generally not considered and the given strength is always taken as 100 per cent. Because mostly, the spray recommendations are made on the basis of the finished products/formulations, and the given strength is, thus, taken as 100 percent, until and unless desired and spray recommendation is made on the basis of on the basis of active ingredient (a.i.).

Therefore, mancozeb required for 100 L of water will routinely be calculated as follows:

Quantity of the fungicide required (in g/ml)= 0.25 x 100 x 1000
100

= 25 x 100 x1000 = 250g
100 x 100

Procedure: Mix the measured quantity of the fungicide in a small quantity of water, say 5-10 litres water in a separate bucket. Put this concentrated mixture in the required quantity of water in a bigger container as per the requirement. Then, add the required quantity of the sticker etc. if needed as in the rainy weather. Keep stirring the contents with a wooden rod frequently, so that the fungicidal suspension does not settle down, thereby varying its concentration in different fractions.

iv) Application of fungicides
Fungicides are applied by different appliances or methods, based on their formulations and nature of pathogen attack.

Pesticide application equipments are available in a variety of sizes ranging from small to big keeping in view the application capacity and the source of energy. These application equipment may be either manually or power operated. They can be further classified into movable and portable appliances. Movable appliances are those which can be moved around on wheels or lifted by two or more persons. Portable equipments can be carried by one person. In the field, knapsack, foot and power sprayers, and rotary dusters are commonly used.

Things to do:
1. Acquaint yourself with different fungicide formulations. Note down their common names, brand names, contents, use and application rates.
2. Prepare the fungicidal spray solutions, paints and pastes.
3. Spray and apply them in the field, and observe and note down all the precautions while their handling.