

**B.Sc. Ag  
II Sem**

**Fundamentals of Plant  
Pathology**

**Credit - 4(3+1)**

**As per ICAR  
5<sup>TH</sup> Dean Syllabus**

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**PRINCIPLES OF PLANT PATHOLOGY**

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## LECTURE 1

### INTRODUCTION TO PLANT PATHOLOGY

#### **Why Plant Pathology?**

Plants are essential for maintenance of life. Plants not only sustain the man and animals, they are also the source of food for multitudes of micro-organisms living in the ecosystem. Thus, while man has been able to subjugate plants and animals for his own use, the competing micro-organisms still defy his efforts and claim a major share of resources which man would like to use for himself. It is in this context that the need for fighting the competing micro-organisms and other agencies that lack loss of productivity has been felt. The attack on plants by these micro-organisms changed the appearance and productivity of the crop and this observed change was called a disease. Plant diseases have been considered as stubborn barriers to the rapid progress of food production.

We call a plant healthy only so long as it continues to perform all its normal physiological activities and give the expected yield according to its genetic potentiality.

#### **Physiological activities of a healthy plant**

1. Normal cell division, differentiation and development.
2. Uptake of water and nutrients from the soil.
3. Synthesis of food from sunlight by photosynthesis.
4. Translocation of water and food to the sites of necessity through xylem and phloem.
5. Metabolism of synthesized material
6. Reproduction

A diseased plant fails to perform one or more of these functions. The effect of a disease on functioning of an organ depends on which cells or tissues were first attacked by the pathogen.

For example, rotting of root tissues will affect the absorption of water and minerals from soil and if vascular tissues have been affected, the translocation of water and photosynthetates will be stopped or reduced. If leaf tissues are attacked by a pathogen, photosynthesis is affected and plant suffers from deficiency of carbohydrates essential for supplying energy for other activities. Thus, disease can be defined as malfunctioning process that is caused by continuous irritation by a pathogen (Dimond, 1959).

#### **Definitions:**

1. **Disease** is a malfunctioning process that is caused by continuous irritation which results in some suffering producing symptoms (American Phytopathological society & British Mycological society).
2. **Disease** is an alteration in one or more of the ordered sequential series of physiological processes culminating in a loss of coordination of energy utilization in a plant as a result of continuous irritation from the presence or absence of some agent or factor.
3. **Disease:** Any malfunctioning of host cells and tissues that result from continuous irritation by a pathogenic agent or environmental factor and leads to development of symptoms (G.N.Agrios, 1997).

Pathogens bring about these irritating processes through different but inter-related pathways

1. by utilizing the host cell contents,
2. by causing death of cells or by interfering with their metabolic activities through their enzymes, toxins and growth regulators,
3. by weakening of tissues due to continuous loss of nutrients, and
4. by interfering with translocation of food, minerals and water.

#### **OBJECTIVES OF PLANT PATHOLOGY:**

The science of plant pathology has four main objectives:

1. to study the living, non-living and environmental causes of plant diseases,
2. to study the mechanisms of disease development by pathogens,
3. to study the interactions between the plants and the pathogen, and

4. to develop the methods of controlling the diseases and reducing the losses caused by them.

## **HISTORY OF PLANT PATHOLOGY:**

### **Ancient period:**

A literature of European and vedic eras will give us some information on the plant diseases and their control measures. Greek philosopher **Theophrastus** recorded some observations on the plant diseases in his book **enquiry into plants**. His experiences were mostly based on imagination and observation but not on experimentation. He had mentioned that plants of different groups have different diseases which were autonomous or spontaneous, i.e., no external cause was associated.

In India, the information on plant diseases is available in ancient literature such as Rigveda, Atharveda (1500-500BC), Arthashastra of Kautilya (321-186 BC), Sushruta Samhita (200-500AD), Vishnu Purana (500AD), Agni Purana (500-700AD), Vishnu Dharmottara (500-700AD), etc. In Rigveda, not only the classification of plant diseases has been given but the germ theory of disease was also advocated.

**Vriksha ayurveda** by **Surpal** in ancient India is the first book in which lot of information on plant diseases is available. In this book, plant diseases were categorized into two groups, internal (probably physiological diseases) and external (probably infectious diseases). External diseases were supposed to be due to attack of microorganisms and insects. In this book, a mention of treatments for different diseases caused by different agencies was prescribed which were based on superstition as well as scientific observation. Hygiene, tree surgery, protective covering with pastes and special culture of plants are practices which are still recommended. In chemical treatments, use of honey, ghee, milk, barley flour, pastes made from herbs, plant extracts, etc., were recommended. For the control of root diseases, oilcakes of mahua, mustard, sesame, castor, etc., were used.

Symptoms of plant diseases such as rust, downy mildew, powdery mildew and blight are often mentioned in the Bible, Shakespeare's poems and dramas of other Christian literature. In Jataka of Buddhism, Raghuvansh of Kalidasa there was also a mention about different symptoms of plant diseases.

In Europe and other western countries, after the time of Theophrastus (about 286 BC) no definite opinion could be formed about plant disease for the next 2000 years. In ancient period, the plant diseases were attributed to many causes which include divine power, religious belief, superstition, effect of stars and moon, bad wind and wrath of god, etc.

## **PRE-MODERN PERIOD**

### **1) PIER ANTONIO MICHELLI (Italian):**

- He was an Italian botanist.
- He was the *founder and father of Mycology*.
- He was the first person who observed fungal spores for the first time and conducted many spore germination studies (by growing fungus organisms on freshly cut pieces of melons and pears).
- He was the first person who observed Cystidia on the lamellar edge or hymenial layer of Agaricales.
- In 1729 he published a book "**Nova Plantarum Genera**" in which he gave descriptions about 1900 species in Latin out of which 900 were fungi. The important genera are *Aspergillus niger*, *Botrytis sps.*, *Polyporus sps.* etc.

### **2) TILLET (French)**

- In 1755, he published a paper on **bunt or stinking smut of wheat**
- By well planned experiments he proved that wheat seeds that contained black powder on their surface produced more diseased plants than clean seeds.
- He emphasized that bunt was an infectious disease and it was closely related with fungus. However, he believed that the disease was caused by some toxin produced by the black powder. He did not know that the black powder contained the spore mass of the fungus.
- He reported that the chemical treatment of seeds with common salt and lime inhibited the contagious activity.

## MODERN PERIOD

### 1) BENEDICT PREVOST (French)

- He proved that diseases are caused by micro-organisms
- He studied wheat bunt disease for about 10 years and in 1807, he published his findings in the paper “memoir on the immediate cause of bunt or smut of wheat and of several other diseases of plants and on preventives of bunt”
- He proved that the bunt of wheat was caused by the fungus *Tilletia caries*
- Studied and observed the germination of bunt species. He confirmed the findings of Tillet by mixing the spores of fungus with clean seeds.
- Discovered the **life cycle of bunt fungus**
- He showed that the solution containing copper sulphate prevented the germination of bunt spores and can be used for control of bunt diseases.
- He mentioned the fungicidal and fungistatic properties of chemical treatments

### 2) CHRISTIAN HENDRICK PERSOON (1761-1831):

- Persoon first published observations Mycologicae.
- In 1801, he published “**Synopsis methodica fungorum**” for nomenclature of Ustilaginales, Uredinales and Gasteromycetes.
- He also published Mycologica Europica in 1822.
- He gave the name to rust pathogen of wheat as *Puccinia graminis*.

### 3) ELIAS MAGNUS FRIES (1821):

- He published three volumes of “*Systema Mycologium*” for nomenclature of hymenomycetes.
- Person and fries first time introduced binomial system of nomenclature to classify the fungal organisms.

During 1830-1845, when late blight of potato was fast spreading in England, Ireland and continental Europe, there was no one opinion among the scientists about the disease-fungus relationship.

### 1) ANTON De BARY (Germany):

- He was the father and founder of modern Mycology.
- He was the founder of modern experimental plant pathology
- In 1863, he studied the epidemics of late blight and renamed the casual organism as *Phytophthora infestans*.
- He discovered **heteroecious nature of rust fungi** (1865).
- He gave detailed account on life cycles of downy mildew genera.
- He studied about vegetable rotting fungi and damping off fungi.
- He wrote a book named “*Morphology and Physiology of fungi, lichens and Myxomycetes*” (1866).
- He reported the role of enzymes and toxins in tissue disintegration caused by *Sclerotinia sclerotiorum*

### Students of De Bary:

1. Marshal Ward (UK) - Studied coffee rusts and its epidemics
2. M.S. Woronin (USSR) - Studied about life cycle of club root fungi, i.e, *Plasmodiophora brassica*
3. Farlow - Fungi and bibliography. He established Farlow cryptogamic herbarium. **Farlow**, first introduced independent course of plant pathology at Harvard University.
4. Millardet - Discovered Bordeaux mixture for the control of downy mildew of grapevine

**Oscar Brefeld**, a colleague of De Bary (Germany) -Pioneer in pure culture techniques

### 2) E. J. Butler (Edwin John Butler):

- He was the **father of modern plant pathology and father of Indian Mycology**.
- He worked at IARI for 20 years from 1901 to 1920.

- He was the founder and first director of imperial Mycological institute, Kew, England (1920-35).
- Monograph: *Pythiaceae and allied fungi*.
- Books: a) Fungi and Disease in Plants (1918)  
b) Fungi in India (with B.R.Bisby) and  
c) Plant Pathology (with S.G.Jones).

### 3) E.C. STAKMAN

- He studies the variability in rust fungus. Contributed valuable information on physiological races of pathogen
- He concluded that due to continuous evolution of races and biotypes in the species of the rust fungus its pathogenic capability goes on changing and as a result the resistant capability of the host also changes.

4) **T. J. BURRILL (USA):** He proved for the first time that fire blight of apple and pear was caused by a bacterium (now known as *Erwinia amylovora*)

### 5) E.F.SMITH (U.S.A)

- He gave the final proof of the fact that bacteria could be incitants of plant diseases.
- He also worked on the bacterial wilt of cucurbits and crown gall disease. He is also called as "**Father of Phyto bacteriology**".
- In 1981, he demonstrated for the first time that budding or grafting could be another method of transmission of plant viruses.
- He showed the contagious nature of peach yellows.

### 6) DOI AND ISHIE (JAPANESE)

- They found that mycoplasma like organisms (MLO) could be responsible for the disease of the yellows type.
- Doi observed that MLO's are constantly present in phloem while Ishie observed MLO's temporarily disappeared when the plants are treated with tetracycline antibodies.

### 7) BEIJERINCK (Dutch)

- **Founder of virology**
- He proved that the virus inciting tobacco mosaic is not a living microorganism.
- He believed it to be *contagium vivum fluidum* (infectious living fluid)

### 8) W.H.STANLEY

- In 1935, he proved that **viruses can be crystallised**. He got Nobel Prize.
- He treated the sap from diseased leaves of tobacco with ammonium sulphate and obtained a crystalline protein which, when placed on healthy tobacco leaves, could reproduce the disease.
- He finally proved that viruses are not living micro-organisms because no living form can be chemically treated and crystallized and still remain viable.

9) **BAWDEN F.E. and PIRIE (Britain):** They found that the crystalline nature of the virus contains nucleic acid and protein.

10) **DIENER and RAYMER** discovered the potato spindle tuber was caused by small naked ssRNA which he called **viroid**.

## INDIAN SCIENTISTS

### 1) B.B MUNDKUR:

- He worked on the control of **cotton wilt diseases**.
- He is responsible for the identification and classification of large number of **Indian smut fungi**
- He started Indian Phytopathological Society in 1948 and published a journal Indian Phytopathology.
- His book – **Fungi and Plant diseases**.

2) **J.F.DASTUR:**

- First Indian plant pathologist who was credited for his detailed studies on fungi and plant diseases.
- He studied the characters of Phytophthora and Phytophthora diseases of potato and castor.
- He established *Phytophthora parasitica* from castor.

3) **K.C. MEHTA** – Life cycle of cereal rusts in India

4) **T.S. SADASIVAN**

- Started the studies on bio-chemistry of host-parasite relationship at University of Madras
- Contributed to the concept of vivotoxins
- Studied on mechanism of wilting in cotton by *Fusarium vasinfectum*. The production of fusaric acid by this fungus outside the host was demonstrated.

## LECTURE 2

### TERMS AND CONCEPTS USED IN PLANT PATHOLOGY

**Disease:** Any malfunctioning of host cells and tissues that result from continuous irritation by a pathogenic agent or environmental factor and leads to development of symptoms (G.N.Agrios, 1997).

**Disorder:** Non-infectious plant diseases due to abiotic causes such as adverse soil and environmental conditions are termed disorders. The common characteristic of non-infectious diseases of plants is that they are caused by the lack or excess of something (temperature, soil moisture, soil nutrients, light, air and soil pollutants, air humidity, soil structure and pH) that supports life. Non-infectious plant diseases occur in the absence of pathogens, and cannot, therefore, be transmitted from diseased to healthy plants.

**Pathogen:** An entity, usually a micro-organism that can incite disease. In a literal sense a pathogen is any agent that causes *pathos* (ailment, suffering) or damage. However, the term is generally used to denote living organisms (Fungi, bacteria, MLO's, nematodes etc.) and viruses but not nutritional deficiencies.

**Parasite:** Organisms which derive the materials they need for growth from living plants (*host or suscept*) are called parasites.

**Pathogenicity** is the ability of the pathogen to cause disease

**Pathogenesis** is the chain of events that lead to development of disease in the host (or) sequence of progress in disease development from the initial contact between the pathogen and its host to the completion of the syndrome

**Sign:** The pathogen or its parts or products seen on a host plant.

**Symptom:** The external or internal reactions or alterations of a plant as a result of a disease.

**Syndrome:** The set of varying symptoms characterizing a disease are collectively called a syndrome.

**Biotroph:** An organism that can live and multiply only on another living organism. They always obtain their food from living tissues on which they complete their life cycle.  
Ex: Rust, smut and powdery mildew fungi.

**Hemibiotroph (Facultative Saprophyte):** The parasites which attack living tissues in the same way as biotrophs but will continue to grow and reproduce after the tissue is dead called as *facultative saprophytes*.

**Perthotrophs or perthophytes (Necrotroph):** A parasite is a *necrotroph* when it kills the host tissues in advance of penetration and then lives saprophytically  
Ex: *Sclerotium rolfsii*.

**Inoculum:** It is the part of the pathogen which on contact with susceptible host plant causes infection (or) the infective propagules which on coming in contact with the host plant causes an infection are known as inoculum

**Inoculum potential:** The energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected (or) The resultant of the action of environment, the vigour of the pathogen to establish an infection, the susceptibility of the host and the amount of inoculum present

**Incubation period:** The period of time (or time lapse) between penetration of a host by a pathogen and the first appearance of symptoms on the host. It varies with pathogens, hosts and environmental conditions.



**Predisposition:** It is the action of set of environments, prior to penetration and infection, which makes the plant vulnerable to attack by the pathogen. It is related to the effect of environments on the host, not on the pathogen, just before actual penetration occurs

**Hypersensitivity:** Excessive sensitivity of plant tissues to certain pathogens. Affected cells are killed quickly, blocking the advance of obligate parasites.

**Infection** is the establishment of parasitic relationship between two organisms, following entry or penetration (or) the establishment of a parasite within a host plant.

**Systemic infection:** The growth of pathogen from the point of entry to varying extents without showing adverse effect on tissues through which it passes.

**Epidemic or Epiphytotic disease:** A disease usually occurs widely but periodically in a destructive form is referred as epidemic or Epiphytotic disease.

Ex: Late blight of potato – Irish famine (1845)

**Endemic:** Constantly present in a moderate to severe form and is confined to a particular country or district.

Ex: Club root of cabbage in Nilgiris

Black wart of potato – *Synchytrium endobioticum*

Onion smut – *Urocystis cepulae*

**Sporadic disease:** Occur at very irregular intervals and locations and in relatively fewer instances. Ex: Udbatta disease of rice, Angular leaf spot of cucumber – *Pseudomonas lachrymans*

## LECTURE 3

### SURVIVAL OF PLANT PATHOGENS

The means of survival are the **first link** in infection chain or disease cycle. The initial infection that occurs from the sources of pathogen survival (Infected host as a reservoir of inoculum, saprophytic survival outside the host or dormant spores and other structures in or on the host or outside the host) in the crop is *primary infection* and the propagules that cause this infection are called *primary inoculum*. After initiation of the disease in the crop, the spores or other structures of the pathogen are sources of *secondary inoculum* and cause *secondary infection*, thereby spreading the disease in the field.

Ex: The fungus (*Phytophthora infestans*) causing late blight of potato survives in seed tubers or in soil. **Infected tubers** bring the **primary infection** in the field while primary inoculum (PI) present in soil causes primary infection of the crop from healthy seed. The PI may also be brought by wind from neighboring fields or long distances. Then the fungus produces spores on leaves. These spores are dispersed by wind and water and reach healthy plant surfaces to cause new infections. This is **secondary infection**. **The primary infection initiates the disease and secondary infection spreads the disease.**

#### SOURCES OF SURVIVAL OF PATHOGENS:

- 1) Infected host as reservoir of inoculum (or) survival in vital association with living plants.
- 2) Survival as saprophytes outside the host.
- 3) Survival by means of specialized resting structures in or on the host or outside the host.
- 4) Survival in association with insects, nematodes and fungi.

#### **1) Infected host as reservoir of inoculum:**

The infected host serving as reservoir of active inoculum is grouped into

- a) **Seed:** Seed may be externally or internally infected by plant pathogens during the course of development and maturation in fruit or pod. Most seed borne pathogens survive as long as seed remains viable.  
**Ex 1:** The pathogen of loose smut of wheat, *Ustilago nuda tritici*, enters the stigma and style and infects the young seed, in which it survives as mycelium.  
**Ex 2:** *Pseudomonas syringae pv. tomato* has been shown to survive in dried tomato seed for 20 years.
- b) **Collateral hosts / Alternative hosts (wild hosts of same families):** Collateral hosts are those which are susceptible to the plant pathogens of crop plants and provide adequate facilities for their growth and reproduction of these pathogens during off-season. Weeds which survive and live during non-cropping season provide for the continuous growth and multiplication of the pathogen. Thus the weed hosts help to bridge the gap between two crop seasons.  
**Ex:** The fungal pathogen for blast disease of rice, *Pyricularia grisea* (Teleomorph: *Magnaporthe grisea*) can infect the grass weeds like *Brachiaria mutica*, *Dinebra retroflexa*, *Leersia hexandra*, *Panicum repens* etc., and survive during off-season of rice crop. As soon as a fresh rice crop is raised, the conidia (inoculum) liberated from the weed host disseminated by wind infects the fresh rice crop.
- c) **Alternate hosts (Wild hosts of other families):** The role of alternate hosts is not as important as of collateral hosts. However, when a pathogen has very wide host range (as *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium moniliforme* etc.) and is tolerant to wide range of weather conditions the alternate hosts become very important source of survival of the pathogen. These alternate hosts are very important for the completion of the life cycle of heteroecious rust pathogens.  
For example in temperate regions the alternate host of *Puccinia graminis tritici* (black or stem rust pathogen of wheat), the barberry bush (*Berberis vulgaris*) grows side by side with the cultivated host. In such areas this wild host belonging to a different family is important for survival of the fungus.

- d) **Self sown crops:** Self sown crops, voluntary crops and early sown crops are reservoirs of many plant pathogens. Ex: Self sown rice plants harbour the pathogen (*Rice tungro virus*) as well as vector (*Nephotettix virescens*).
- e) **Ratoon crops:** Sometimes ratoon crops also harbour the plant pathogens.  
Ex: Sugarcane mosaic.
- f) **Survival by latent infection:** Latent infection refers to the conditions in which the plant pathogens may survive for a long time in plant tissue without development of visual symptoms. Ex: *Xylella fastidiosa*, the causal agent of pierce's disease of grapevine infect different weeds without developing visible symptoms.

## 2) Saprophytic survival outside the host:

The ability to live saprophytically enables many plant pathogens to survive in the absence of growing susceptible plants. Saprophytic survival usually occurs in or on the soil. Waksman (1971) distinguished between soil inhabitants and soil invaders; the former comprise the basic fungal flora of the soil, whereas the later are short lived exotics.

In the absence of the cultivated host plant, fungi are capable of surviving as saprophytes and can be studied under three categories:

- 1) **Soil inhabitants:** Those organisms which survive indefinitely in the soil as saprophytes in the absence of the host plant. Ex: Species of *Pythium*, *Rhizoctonia* and *Sclerotium*
- 2) **Root inhabitants:** These are more specialized parasites that survive in soils in close association with their hosts. The active saprophytic phase remains as long as the host tissue in which they are living as parasites is not completely decomposed. Ex: Species of *Fusarium*, *Verticillium* (vascular wilt causing fungi) and root rot of cotton (*Phymatotrichum omnivorum*)
- 3) **Rhizosphere colonizers:** Those organisms which colonize the dead substrates in the root region and continue to live like that for a longer period which are more tolerant to soil antagonism. Ex: Leaf mold in tomato: *Cladosporium fulvum*

### Differentiate Soil inhabitants and soil invaders:

#### Soil inhabitants

1. These are unspecialized parasites with a wide host range that are able to survive indefinitely in the soil as saprophytes.
2. Soil inhabitants include obligate saprophytes and facultative parasites they are exo-pathogens
3. Soil and plant debris serve as media for their saprophytic survival.
4. They have high competitive saprophytic survival ability.
5. Species of *Pythium*, *Rhizoctonia*, *Sclerotium*, etc., survive as soil inhabitants for considerable length of time in absence of the host.

#### Soil invaders / Root inhabiting fungi

1. These are more specialized parasites that survive in soils in close association with their hosts.
2. Soil invaders include facultative saprophytes which are endo-pathogens (root infecting fungi).
3. The active saprophytic phase remains as long as the host tissue in which they were living as parasites is not completely decomposed.
4. They have low competitive saprophytic survival ability.
5. Most plant pathogenic fungi and bacteria are soil invaders. Many vascular wilt causing species of *Fusarium*, *Verticillium*, etc., are soil invaders.

## 3) Survival as dormant spores or specialized resting structures:

**Plant viruses** have no resting stage and are transmitted through a continuous infection chain.

**Phytopathogenic bacteria:** The plant **bacteria** also do not produce resting spores or similar structures. They continuously live in their active parasitic stage in the living host or as active saprophytes on dead plant debris.

**Nematodes:** They survive in the form of active parasitic phase on a living host and also survive through dormant structures, i.e., eggs, cysts, galls, formed in host tissues. These structures may be present in soil or in seed lots

**Phanerogamic parasites:** They survive in dormant state for many years through seeds. Ex; Seeds of Orobanchae survive in soil for more than 7 years.

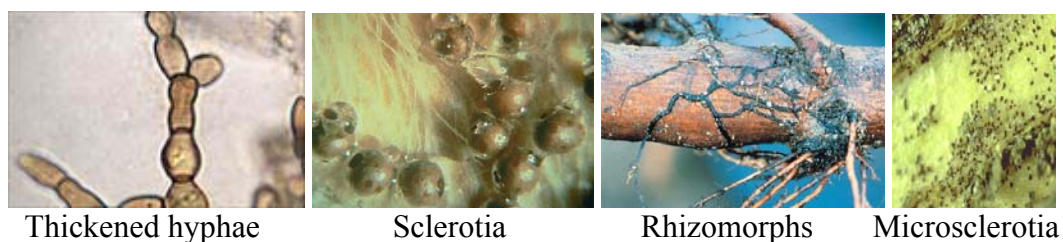
Among plant pathogens, fungi are the only organisms that produce spores, analogous to eggs of nematodes, and other resting structures for their inactive survival. These dormant structures of survival can be classified in the following categories.

1) **Soil borne fungi:**

- a) Dormant spores {Conidia (Peach leaf curl pathogen, *Taphrina deformans*), Chlamyospores (Wilt pathogen, *Fusarium* sp.), oospores (Downy mildew fungi), perithecia (Apple scab pathogen, *Venturia inaequalis*) etc.}.



- b) Other dormant structures such as thickened hypha, sclerotia (Cottony rot fungus, *Sclerotinia sclerotiorum*), microsclerotia (*Verticillium*), Rhizomorphs (*Armillaria mellea*), etc.



- c) Factors affecting the survival of pathogen in the soil are **a)** physical factors (high temperature, irradiation, dessication and anaerobiosis), **b)** chemical factors (antibiotics, antagonistic chemicals produced by other microbes) and **c)** biotic factors (parasitism, predation by microflora and microfauna).

2) **Seed borne fungi:**

- a) **Externally seed borne:** Dormant spores on seed coat Ex: Covered smut of barley, grain smut of jowar, bunt of wheat, etc.  
b) **Internally seed borne:** Dormant mycelium under the seed coat or in the embryo Ex: Loose smut of wheat (*Ustilago nuda tritici*)  
c) Factors affecting the survival of the pathogen on/in the seed are temperature and moisture.

- 3) **Dormant fungal structures on dormant or active host** Ex: In downy mildew of grapevine, powdery mildew of grapevine, apple etc., The fungus mycelium may be present in dormant state in the affected twigs or its oospores or perithecia may be embedded in the tissues of the affected organs.

Parasitic phanerogams survive in the form of seeds, and in plant parasitic nematodes eggs, cysts and larvae serve as over seasoning structures.

4) **Survival in association with insects, nematodes and fungi**

Several important plant pathogens may survive within the insect body and over winter therein. The corn flea beetle, *Ceatoconema pulicaria* carries inside its body, the corn wilt pathogen, *Xanthomonas stewartii* and thus helps in over wintering.

Plant viruses like wheat mosaic, tobacco necrosis, tobacco rattle and tobacco ringspot viruses survive with nematodes or fungi found in the soil between crop seasons. Tobacco ringspot is associated with the nematode *Xiphinema americana*. The fungi, *Polymyxa graminis* (Wheat soil borne mosaic & Barley yellow mosaic) and *Spongospora subterranea* (Potato mop top virus) carry the viruses internally and transmit them through the resting spore.

## LECTURE 4

### DISPERSAL OF PLANT PATHOGENS

The **second link** in infection chain is the dissemination of plant pathogens. Transport of spores or infectious bodies, acting as inoculum, from one host to another host at various distances resulting in the spread of the disease, is called **dispersal, dissemination or transmission** of plant pathogens. The dispersal of the pathogen or disease is important not only for spread of plant diseases but also for continuity of the life cycle and evolution of the pathogen. The knowledge of these methods of dispersal is essential for effective control of plant diseases because possibilities of preventing dispersal and thereby breaking the infection chain exist.

In **fungi**, productions of asexual and sexual spores follow the active vegetative growth of the fungus in or on the host tissues and are dispersed mechanically in time and space by various means. In **bacterial diseases**, the bacterial cells come out on the host surface as ooze or the tissues may be disintegrated so that the bacterial mass is exposed and then dispersed by various physical and biological agencies. **Viral diseases** which have no such organs are transmitted by insects, mites, phanerogamic parasites, nematodes and human beings.

The two links in the infection chain of an animate pathogen, *Viz.*, survival through dormant structures and the dispersal of the pathogen are very closely bound with each other. Actually the dormant structures provide means of *dispersal in time*, i.e., the pathogen is retained viable over a period of time enabling it to be transported through physical agencies without being harmed.

The dispersal of infectious plant pathogens in *space* occurs through two ways:

1. Autonomous or direct or active dispersal.
2. Indirect or passive dispersal.

#### **1) Autonomous or direct or active dispersal:**

In this method the dispersal of plant pathogens takes place through soil, seed and planting material during normal agronomic operations. There is no major role of external agencies like insects, wind, water, etc. in this type of dispersal.

##### **1) Seed as the source of autonomous dispersal:**

Since most of the cultivated crops are raised from seed the transmission of diseases and transport of pathogens has much importance. The dormant structures of the pathogen (Ex: seeds of *Cuscuta*, *Sclerotia* of ergot fungus, smut sori, etc.) are found mixed with seed lots and they are dispersed as **seed contaminants**. The bacterial cells or spores of fungi present on the seed coat (such as in smuts of barley, sorghum, etc.) are transported to long distances. Dormant mycelium of many fungi present in the seed is transmitted to long distances. There are three types of dispersal by seed, *viz.*, contamination of the seed, externally seed borne and internally seed borne.

a) *Contamination of the seed:* Seed borne pathogens move in seed lot as **separate contaminants** without being in intimate contact with the viable crop seeds. The seeds of the pathogen or parasite and the host are mixed during harvest of the crop. In many cases, the identity of the seeds of the two entities (host and the pathogens) is difficult to separate. Ex: Smut of pearl millet and ergot of rye. Smut sori and ergots mix easily with the seed lots during harvest and threshing.

b) *Externally seed borne:* **Close contact between structure of the pathogen and seeds** is established where the pathogen gets lodged in the form of dormant spores or bacteria on the seed coat during growth of the crop or at the time of harvest and threshing. Ex: Short smut of sorghum, bacterial blight of cotton, loose smut of barley etc.

In many pathogens the externally seed borne structures such as smut spores can persist for many years due to their inherent capacity for long survival. Ex: The spores of *Tilletia caries* (Stinking smut of wheat) remain viable even after 18 years and those of *Ustilago avenae* (Oat smut) for 13 years.

c) *Internally seed borne*: The pathogen may penetrate into the ovary and cause **infection of the embryo** while it is developing. They become internally seed borne. Ex: **Loose smut of wheat**.

### **Differentiate Seed infection and infestation**

**Seed infection**: The seed is infected only when the pathogen has grown in or on it for sometime and established its relationship with the seed tissues. Ex: Loose smut of wheat, where the fungus grows in the embryonic tissues and becomes dormant when the seed enters dormancy.

**Seed infestation**: When the fungus or the pathogen is present on the seed coat and in the seed lot, it is only transport of the pathogen and the seed is infested.

**2) Soil as a means of autonomous dispersal**: Soil borne facultative saprophytes or facultative parasites may survive through soil. The dispersal may be by movement of pathogen in the soil or by its growth in soil or by movement of the soil containing the pathogen. The former is known as *dispersal in soil* while the latter is called *dispersal by soil*.

**a) Dispersal in soil**: The following are the three stages of dispersal in soil

**i) Contamination of soil**: Contamination of the soil takes place by gradual spread of the pathogen from an infested area to a new area.

**ii) Growth and spread of a pathogen in soil**: Once the pathogen has reached the soil it can grow and spread based on its ability to multiply and spread. Among characters of the pathogen its adaptability to soil environment including its **saprophytic survival ability** are most important. The survival ability of the pathogen is governed by high growth rate, rapid spore germination, better enzymatic activity, capability to produce antibiotics and tolerance to antibiotics produced by other soil-microorganisms.

On the basis of this competitive saprophytic ability the pathogens in soil can be of three types. **Specialized facultative parasites** (Saprophytes) can pass their life in soil in the absence of host plants, but they depend more on the residues of the host plant (ex: *Armillariella mellea*, *Ophiobolous graminis* etc.). **Unspecialized facultative parasites** can pass their entire life in the soil (*Pythium* sp., and *Phytophthora* sp.). The soil borne **obligate parasites** such as *Plasmodiophora brassicae*, *Synchytrium endobioticum* require the presence of active host.

**iii) Persistence of the pathogen in soil**: The pathogens persist in the soil as dormant structures like **oospores** (*Pythium*, *Phytophthora*, *Sclerospora* etc.), **Chlamydospores** (*Fusarium*), smut spores (*Ustilago*) and **sclerotia** (*Rhizoctonia*, *Sclerotium*).

**b) Dispersal by the soil**: The pathogen is dispersed by the soil during cultural operations through the agricultural implements, irrigation water, workers feet etc. Propagules of fungi and the plant debris containing the fungal and bacterial pathogens thus spread through out the field. The transfer of soil from one place to another along with propagating materials is the most important method of dispersal of pathogen. For example transfer of papaya seedlings from a nursery infested with *Pythium aphanidermatum* (causal agent of stem or foot rot of papaya) can introduce the pathogen in new pits for transplanting the seedlings. Similarly grafts of fruit trees transported with soil around their roots can transmit pathogens present in the nursery to the orchards.

### **3) The plant and the plant organs as a means of autonomous dispersal**

The plants, plant parts **other than seed** that are used for vegetative propagation, raw field produce and plant debris that accumulates during the course of cropping constitute the third method of autonomous dispersal. Ex: Late blight of potato was introduced in North America and in Europe through seed tubers brought from the native source of the in South America. Citrus canker was introduced into California from Asia. The climatic conditions favoured its epidemic in California.

## II) Passive or Indirect dispersal:

Passive dispersal of plant pathogens happens through animate and inanimate agents.

### 1) Animate agents:

a) **Insects:** Insects carry plant pathogens either externally (**epizoic**) or internally (**endozoic**). They can disseminate bacteria, fungi, viruses, mycoplasmas, spiroplasmas, rickettsia, etc.

**Fungal diseases:** The external transmission is of special interest in those fungi which produce **conidia, oidia and spermatia in honey secretions** having attractive odours. Ex: Sugary disease of sorghum. The spermatial oozings at the mouth of spermatogonia in the ascomycetes attract various type of insects, flies, pollinating bees and wasps which play a dual role, viz., pollination and transmission of plant pathogens. Dutch elm disease (*Ceratostomella ulmi*) is transmitted internally by elm bark beetles.

**Bacterial diseases:** The fire blight organism (*Erwinia amylovora*) and citrus canker bacterium (*Xanthomonas axonopodis pv. citri*) are transmitted by flies (bees) and ants and the later by leaf miner respectively. The cucumber wilt bacterium, *Erwinia tracheiphila* is spread by the striped cucumber beetles (*Acalymma vittata*) and the spotted cucumber beetle (*Diabrotica undecimipunctata*). When the beetles are feeding on the diseased plant, the bacterium contaminates the mouth parts and passes into the gut of the beetle and over winters inside the beetle during the winter season. Thus the beetle helps the bacteria in two ways, i.e., in their transmission and survival.

**Viral diseases:** More than 80 per cent of the viral and phytoplasmal diseases are spread by different types of insects. The insect which acts as specific carriers in disseminating the diseases are called *insect vectors*. Both **Aphids** (Aphididae) and **leaf hoppers** (Cicadellidae or Jassidae) in the order **Homoptera** contain largest number and the most important insect vectors of plant viruses. Certain species of mealy bugs and scale insects (Coccoidae), whiteflies (Aleurodidae) and tree hoppers (Membracidae) in Homoptera also transmit virus diseases. Insect vectors of plant viruses are few in true bugs (Hemiptera), thrips (Thysanoptera), beetles (Coleoptera) and grasshoppers (Orthoptera).

S.No.	Vector	Virus
1.	<b>Aphid transmitted viruses</b>	
	<i>Myzus persicae</i>	Beet mosaic, Lettuce mosaic, Potato virus Y, Turnip mosaic, Beet yellows
	<i>Acyrtosiphon pisum</i>	Bean common mosaic, Bean yellow mosaic, Soybean mosaic, Pea enation mosaic
	<i>Toxoptera citricidus</i>	Citrus tristeza
2.	<b>Leaf hopper transmitted viruses</b>	
	<i>Nephotettix impicticeps</i> , <i>N. nigropictus</i> , <i>N. virescens</i>	Rice tungro virus
	<i>Nephotettix cincticeps</i> , <i>N. nigropictus</i>	Rice dwarf virus
	<i>Circulifer tenellus</i>	Beet curly top
	<i>Agallia constricta</i>	Potato yellow dwarf
	<i>Graminella nigrifrons</i>	Maize chlorotic dwarf
3.	<b>Tree hopper transmitted viruses</b>	
	<i>Micrutalis malleifera</i>	Tomato-pseudo curly top
4.	<b>Plant hopper transmitted viruses</b>	
	<i>Perigrinus maidis</i>	Maize mosaic
	<i>Sogatodes oryzicola</i>	Rice hoja blanca
5.	<b>Whitefly transmitted viruses</b>	
	<i>Bemesia tabaci</i>	Bhendi yellow vein mosaic, Bhendi leaf curl, Chilli leaf curl, Cotton leaf curl, Papaya leaf curl, Mungbean yellow mosaic
6.	<b>Thrips transmitted viruses</b>	



	<i>Thrips tabaci</i> , <i>Frankliniella schultzei</i> , <i>Scirtothrips dorsalis</i>	Tomato spotted wilt virus
7.	<b>Mealy bugs transmitted viruses</b>	
	<i>Planococcoides njalensis</i>	Cocoa swollen shoot
	<i>Pseudococcus saccharifolii</i>	Sugarcane spike (Phytoplasma)
8.	<b>Grass hoppers transmitted viruses</b>	
	<i>Melanophus differentialis</i>	Potato virus X, Tobacco mosaic virus (Mechanical transmission)
9.	<b>Lace bugs transmitted viruses</b>	
	<i>Piesma quadratum</i>	Beet leaf curl virus
	<i>Stephanites typicus</i>	Root (wilt) disease of coconut (Phytoplasma)
10.	<b>Beetle transmitted viruses</b>	
	<i>Ceratoma trifurcata</i>	Cowpea mosaic
	<i>Acalymma trivittata</i>	Squash mosaic
	<i>Diabrotica longicornis</i>	Brome mosaic

**Mycoplasma diseases:** Plant MLO's are phloem inhabitants and those insects which are feeding on phloem of plants transfer the MLO's. Mycoplasmal diseases are mostly transmitted by leaf hoppers. Ex: Sesamum phyllody (*Orosious albicinctus*) and little leaf of brinjal (*Hishimonas phycitis*)

**b) Mites:** Mites belonging to the families Eryophyiidae (eryophyiid mite) and Tetranychidae (spider mite) of class Arachnida transmit plant viruses. The genera *Abacarus*, *Aceria*, *Eriophyes* and *Brevipalpus* are important.

Ex: *Aceria cajani* transmits Pigeonpea sterility mosaic virus

*Aceria tulipae* transmits wheat streak mosaic

**c) Fungi:** Some soil borne fungal plant pathogens carry plant viruses in or on their resting spores and zoospores, and transmit them to susceptible hosts during the infection process. *Tobacco necrosis virus* and *Cucumber mosaic virus* are carried outside the fungi, while lettuce big vein virus is carried inside the zoospores. Many soil borne viruses are transmitted by the members of Chytridiales and Plasmodiophorales.

#### Fungal transmitted viruses

S.No.	Fungal vector	Disease
1.	<i>Olpidium brassicae</i>	<b>Tobacco necrosis</b> , Tobacco stunt, Lettuce big vein
2.	<i>Olpidium cucurbitacearum</i>	Cucumber necrosis
3.	<i>Polymyxa graminis</i>	Barley yellow dwarf mosaic, Wheat soil borne mosaic, Peanut clump
4.	<i>Polymyxa betae</i>	Beet necrotic yellow vein
5.	<i>Spongospora subterranea</i>	<b>Potato mop top</b>
6.	<i>Synchytrium endobioticum</i>	<b>Potato virus X</b>

**d) Nematodes:** Several nematodes act as vectors for transmission of fungi, bacteria and viruses.

**Bacterial diseases:** The bacterium which causes **yellow ear rot** of wheat (*Corynebacterium tritici* or *Clavibacter tritici*) is disseminated by **ear cockle nematode**, *Anguina tritici*. If these two diseases appear together, a complex disease called **tundu** of wheat occurs. *Corynebacterium tritici* is not capable of dispersal and infection unless it is carried by *Anguina tritici*.

**Fungal diseases:** Similarly, root rot and wilt pathogens such as *Phytophthora*, *Fusarium*, *Rhizoctonia*, *Verticillium*, etc., are disseminated by nematodes.

**Viral diseases:** Plant nematodes play a vital role in transmitting certain virus diseases. Many soil borne viruses are known to be transmitted by the nematodes. *Xiphenema*, *Longidorus*, *Trichodorus* and *Paratrichodorus* are the nematode genera belonging to Dorylaimoidea which are known to transmit plant viruses. The nematode transmitted viruses are divided into two groups on the basis of shape of their particles: nematode

transmitted polyhedral viruses (NEPO) and nematode transmitted tubular (NETU) viruses.

**NEPO viruses:** These are nematode transmitted viruses with **polyhedral particles**. These are generally transmitted by species of *Xiphenema* and *Longidorus*. Ex: Tobacco ringspot virus, Tomato ringspot virus, Tomato black ring virus, Arabis mosaic virus

**NETU viruses:** These are nematode transmitted viruses with **tubular particles**. NETU viruses are transmitted by *Trichodorus* and *Paratrichodoros*. Ex; Pea early browning virus (*Trichodorus* sp.), Tobacco rattle virus (*Trichodorus pachydermis*)

**Nematode transmitted viruses:**

S.No.	Nematode vector	Virus	Virus group
1.	<i>Paratrichodorus</i> sp. & <i>Trichodorus</i> sp.	Pea early browning, <b>Tobacco rattle</b>	NETU group
2.	<i>Xiphenema index</i>	<b>Grapevine fan leaf</b>	NEPO virus
3.	<i>Xiphenema americanum</i>	Tobacco ringspot, Tomato ringspot	NEPO virus
4.	<i>Longidorous elongatus</i>	Raspberry ringspot	NEPO virus

e) **Human beings:** Human beings role in dissemination of plant pathogens is more direct than indirect. The ways and means in which human beings help in dispersal are as follows.

- **Transportation of seeds (seed trade):** The import and export of contaminated seeds without proper precautions lead to movement of pathogens from one country to another or from one continent to another. The diseases which are amenable to such transmission are mainly those that are carried in or on the propagative parts and seed. Ex: Late blight of potato, Downy mildew of grapevine, Citrus canker, *Fusarium* wilt of banana, etc.
  - **Planting diseased seed materials:** Planting diseased bulbs, bulbils, corms, tubers, rhizomes, cuttings, etc., of **vegetatively propagated** plants such as potato, sweet potato, cassava, sugarcane, banana, many ornamentals and fruit trees etc., help in dispersal of pathogens from field to field, orchard to orchard, locality to locality or from one country to another.
  - **During adoption of normal farming practices:** Human beings engaged in preparatory cultivation, planting, irrigation, weeding, pruning etc., help in dispersal of plant pathogens. Spores and other external structures of fungi can be carried by workers clothing's, shoes, and hands etc., from plant to plant and from field to field.
  - **By use of contaminated implements:** Pathogens are transferred from one area to another through implements used in various cultural operations (weeding, thinning, hoeing etc.) in the field. Ex: Soil borne diseases such as root rot, wilt etc. Cutting knives and pruning knives also help in dispersal from one plant to another. Ex: Bunchy top of banana.
  - **By use of diseased grafting and budding material:** Grafting and budding between healthy and diseased plants is the most effective method of distribution of pathogens of horticultural crops.
- f) **Dispersal by phanerogamic parasites:** Phanerogamic parasites transmit the viruses by acting as a bridge between the diseased and healthy plants. Ex: **Dodder** (*Cuscuta California*, *C. campestris*, *C. subinclusa* etc.)
- Cuscuta subinclusa* – Cucumber mosaic virus
  - Cuscuta californica* – Tobacco mosaic virus  
Tobacco rattle virus  
Tomato spotted wilt virus
  - Cuscuta campestris* - Tomato bushy stunt virus

- g) **Dispersal by birds:** This mode of dispersal is important in dissemination of seeds of flowering parasites and certain fungi. In tropics, crows feeding on the fleshy, sticky and gelatinous berries of gaint mistletoe (*Dendrophthoe* sp.) deposit the seeds on the other trees with excreta. Seeds of *Loranthus* are disseminated by birds by sticking on their beaks and also through excreta. Stem segments of dodder are carried by birds for preparing their nests and thus get transported to new areas. Moreover, spores of chestnut blight fungus, *Endothea parasitica* are disseminated by more than 18 species of birds. Cleistothecia of many powdery mildew fungi are carried by feathers of birds.
- h) **Farm and wild animals:** Farm animals (cattle) while feeding on diseased fodder ingest the viable fungal propagules (spores or oospores or sclerotia) and pass out as such in the dung. This dung when used as manure spread in the field and act as source of inoculum. Further, soil inhabiting fungi especially sclerotia adhere to the hoofs and legs of animals and get transported to other places.

## 2) Inanimate agents:

a) **Wind:** The dispersal of pathogens by wind is known as **anemochory**. Wind transmission involves the upward air currents, velocity and the downward movements of the wind. Wind acts as a potent carrier of propagules of fungi, bacteria and viruses.

**Fungi:** Usually the fungal pathogens are light in weight and are well adapted to wind dispersal. The **adaptations for wind dispersal** in fungal pathogens include production of numerous spores and conidia, discharge of spores with sufficient force, production of very small and light spores so that they can move to long distances. Ex: Powdery mildew, downy mildew, rusts, smuts etc.

Both short and long distance dissemination is possible by means of wind.

i) Spores adapted for **short distance** dissemination- sporangia of downy mildew fungi, conidia of powdery mildew fungi and basidiospores of rust fungi. In the plains of northern India the annual recurrence of cereal rusts is solely due to uredospores brought by wind from the source of survival in the hills in the far north (Himalayas) and south (Nilgiris).

ii) Spores adapted to **long distance** dispersal – uredospores of rust fungi, Chlamydospores of smut fungi and conidia of *Alternaria*, *Helminthosporium* and *Pyricularia*

Uredial stages of the rust fungi travel long distances through air currents and thus are responsible for destructive epidemics over wide areas. Ex: The uredospores of *Puccinia graminis* var. *tritici* have been detected as high as **14000** feet above infected wheat fields (Stackman and Christensen). Similarly, *Alternaria* spores at 8000 feet, *Puccinia recondita* and *Cronartium ribicola* spores at 12500 feet were reported.

**Dispersal distance:** In USA, uredospores of this fungus are blown from the far south (Mexico) into Dakota and Minnesota (far north) travelling more than 1000 miles in about two days without losing their viability. If the uredospores reach an altitude of 5000 feet, their distance dispersal in a 30 mile per hour wind could be about **1100 miles**, without losing viability.

**Nematodes:** In addition to fungi, it also helps in the dissemination of the cysts of nematodes and also the seeds of phanerogamic parasites. Ex: Cysts of the nematode *Heterodera major*, which causes **molya disease of wheat and barley**, are carried by dust storms from **Rajasthan** to **Haryana**

**Bacteria:** Some pathogenic bacteria are carried along with the infected material to short distances by wind. Ex: *Erwinia amylovora*, the causal agent of fire blight of apple and pear, produces fine strands of dried bacterial exudates which may be broken off and are transmitted by wind.

**Viruses and phytoplasmas** are not directly transmitted by wind, but the insect and mite vectors that carry the viruses move to different directions and distances based on the direction and speed of the air.

b) **Water**: Transmission of plant pathogens by water is called as **hydrochory**. Water is less important than air in long distance transport of pathogens, but it is more efficient as the pathogens land on the wet surface and can germinate immediately. Water dissemination occurs mainly through **surface running water** and **rain splash**.

The **surface flow of water** after heavy rains or during irrigation from canals and wells carries the pathogens to short distances. Ex: The mycelial fragments, spores or sclerotia of fungi, *Colletotrichum falcatum* (**red rot of sugarcane**), *Fusarium*, *Ganoderma*, *Macrophomina*, *Pythium*, *Phytophthora*, *Sclerotium*, etc., are transmitted through rain or irrigation water. Long distance dispersal is also possible by water only when the floods cover larger areas or when the water flows from the sources of survival of pathogens to longer distances.

Dissemination by rain splash is also called as **splash dispersal**. It is one of the efficient methods of dispersal of **bacterial plant pathogens**. Rain drops falling with force on sori, pustules, cankers or even soil surface may splash the propagules in small droplets and enable them to land on neighbouring healthy susceptible surfaces or the water droplets may be carried to long distances by air. Ex: Bacterial leaf spot of rice (*Xanthomonas campestris* pv. *oryzae*), Bacterial leaf streak of rice (*Xanthomonas campestris* pv. *oryzicola*), Green ear of bajra (*Sclerospora graminicola*).

Fungal spores and bacteria present in the air or plant surface are washed downward by rain splash or drops from overhead irrigation and are deposited on susceptible healthy plants. Water not only plays an important role in the dissemination of plant pathogens, but also helps in the growth and spore discharge of many fungi. It also helps in the spore germination and infection process.

## LECTURE 5 & 6

### PHENOMENON OF INFECTION/ INFECTION PROCESS

It is the **third link** in the infection chain after survival and dispersal of inoculum. Infection process means establishment of pathogen in the host plant. Entry and colonization of pathogen in the host tissues is known as establishment and the infective propagules coming in contact with the host are known as **inoculum**.

**Inoculum potential:** It is the inoculum needed for successful infection. It is a function of **inoculum density** and their **capacity**.

**Def:** It is defined as the resultant of the action of environment, the vigour of pathogen to establish an infection, susceptibility of the host and amount of inoculum present (Dimond and Horsfall, 1960)

*Or*

It is defined as the energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected (Garret, 1960)

In case of specialized pathogens as rusts and powdery mildews, very few or even one spore is capable of causing infection successfully. In case of non-specialized pathogens such as *Pythium*, *Phytophthora*, *Rhizoctonia* and *Sclerotium* require high density of inoculum on the surface of susceptible host for successful infection.

The success of process of infection depends on

#### 1. **Host factors**

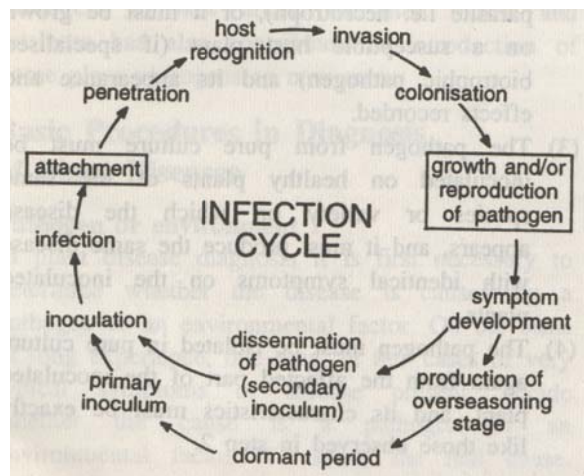
- *Susceptibility of host:* It is genetically controlled by DNA and it is an inheritable character which is transmitted from parents to off springs.
- *Disease proneness of the host:* It is decided by the external factors such as host nutrition, i.e., more nitrogen application makes the host more susceptible and more potash application leads to less susceptibility.

#### 2. **Pathogen factors**

- *Virulence / aggressiveness of the pathogen:* It is determined by genetic material which is inheritable.
- *High multiplication rate of the pathogen:* Chances of infection increases with high rate of multiplication. High birth rate and low death rate is highly essential for successful infection.
- *Proper inoculum potential:* In case of specialized pathogens very few or even one spore is capable of causing infection successfully, whereas, non-specialized pathogens require high density of inoculum on the surface of susceptible host for successful infection.

3. **Environmental factors:** Environmental conditions such as temperature, relative humidity, moisture, etc., are very important for survival, dissemination and infection process.

Process of infection can be grouped into three stages, *i.e.*, pre-penetration, penetration and post-penetration.



**Stages in the development of infection or disease cycle**

**1. PRE-PENETRATION:** Depending upon the plant pathogen activity, the plant pathogens are classified in to 2 categories

1. Active invaders and 2. Passive invaders

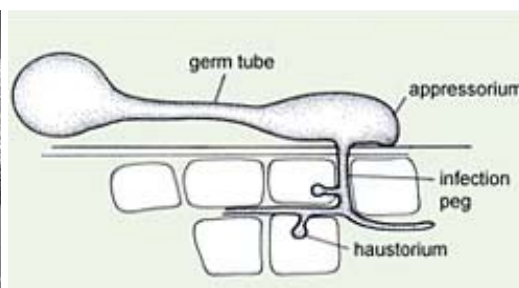
Active Invaders	Passive Invaders
1. Pathogens which make an aggressive effort to gain entry into intact host cells. 2. They do not require help of any external agency to gain entry into host cells. 3. Eg. Phyto-pathogenic fungi Phanerogamic parasites	1. No aggressive effort 2. Require help of external agencies like insect vectors or wounds caused by agricultural implements. 3. Eg. Plant viruses Phyto-pathogenic bacteria

Plant viruses are particulate in nature and they do not have any capacity to enter the host cell so they do not make any aggressive effort for entry, but depend on different insect vectors for their entry into host cell. Bacteria have no dormant structures; hence no pre-penetration activity except for multiplication in infection drops on the natural openings. However, nematodes show some orientation towards root surface before actual penetration.

In fungal pathogens, pre-penetration includes spore germination and growth of the resulting germ tube on the surface of the host plant. Germination is essentially the change from low metabolic rate to a high metabolic rate and involves a change from near dormancy to intense activity; for this an energy source is needed such as a carbohydrate or fat reserve in the propagule. Fungal invasion is chiefly by germ tubes or structures derived from them. In some fungi like *Rhizoctonia solani* and *Armillariella mellea*, the hypha act in a concerted way to achieve the penetration. In *Rhizoctonia solani*, the fungus on coming in contact with root surface, first forms infection cushions and **appressoria** and from these multiple infections takes place by means of infection pegs. In *Armillariella mellea*, the fungus hyphae form the **rhizomorphs** (aggregation of hyphae into rope like strands) and only these can cause infection.



Rhizomorphs



Appressorium

**2. PENETRATION:** Pathogens penetrate plant surfaces by direct penetration or indirectly through wounds or natural openings. Bacteria enter plants mostly through wounds and less frequently through natural openings. Viruses, viroids, mollicutes, fastidious bacteria enter through wounds made by vectors. Fungi, nematodes and parasitic higher plants enter through direct penetration and less frequently through natural openings and wounds.

## A. Indirect Penetration

1. **Wounds:** Wounds caused by farm operations, hail storms, or insect punctures, etc., will help in the entry of different plant pathogens into the host cells. Organisms which cause storage diseases and ripe rots will enter through the wounds caused by farm operations.

Ex. *Rhizopus*, *Gloeosporium*, *Aspergillus*, *Penicilium*, *Colletotrichum*, *Diplodia*, etc.

Weak parasites enter through the wounds caused by hail storms and freezing

Ex. *Macrophomina phaseolina*

Pathogen causing brown rot of fruits (*Sclerotinia fructicola*) enters through the wounds caused by insect punctures. Similarly, causal organism of Dutch elm disease (*Ceratostomella ulmi*) enters through the wounds caused by elm bark beetle.

## 2. Natural openings

a) **Stomata:** There is variation in the behaviour of germ tube at the time of penetration through the stomata. In *Puccinia graminis tritici*, the uredospore germinates and forms a germ tube which on approaching stoma swells at the tip to form an appressorium in the stomatal aperture. From the appressorium a blade like wedge grows through the stomatal slits and swells inside to form a sub-stomatal vesicle from which the haustoria penetrating the cells are produced.

In *Peronospora destructor* infecting onion leaves, the germ tube continues to grow after the formation of first appressorium. In *Pseudoperonospora cubensis*, the hyphae penetrate the stomatal aperture and swell to form a sub-stomatal vesicle from which in turn other hyphae grow to form haustoria in the adjacent cells of the leaves. *Mycosphaerella musicola* forms a small structure called **stomatopodium** over the pore of the stoma after growing for few days on the surface of the leaf. A hypha then arises from it which grows into the sub-stomatal chamber and swells to form a vesicle, which in turn gives rise to hyphae which invade palaside tissues.

Other examples: *Xanthomonas campestris* pv. *malvacearum* (Black arm of cotton), *Xanthomonas phaseoli* (Bacterial leaf spot of green gram), *Phytophthora infestans* (Late blight of potato), *Albugo candida* (White rust of crucifers) and uredospores of *Puccinia graminis tritici* (Black stem rust of wheat).

b) **Lenticels:** *Sclerotinia fructicola* (Brown rot of fruits), *Streptomyces scabies* (Scab of potato), *Phytophthora arecae* (Mahali disease of arecanut)

c) **Hydathodes:** *Xanthomonas campestris* pv. *campestris* (Black rot of crucifers)

**B) Direct penetration:** Most fungi, nematodes and parasitic higher plants are capable of penetrating the host surface directly. However, the plants are provided with different mechanisms of defense which include structural features of the host, presence of chemical coverings on the cell walls, and anti-infection biochemical nature of the protoplasm. Hence, the pathogen should have mechanisms to overcome these barriers for direct penetration.

a) *Breakdown of physical barriers.* Viruses have no physical force or enzyme system of their own to overcome structural or chemical barriers of the host and therefore come in contact with the host protoplasm only through wounds. Bacteria are mostly weak parasites and cannot employ force to effect penetration. Fungi and nematodes are the only group of plant pathogens that employ force for direct penetration of the host. Fungi penetrate host plants directly through a fine hypha produced directly by the spore or mycelium or through a penetration peg produced by an appressorium. These structures exert pressure on the surface which results in stretching of the epidermis which becomes thin. Then the infection peg punctures it and effects its entry.

b) *Breakdown of chemical barriers:* the host is provided with defense mechanisms against invasion which include i) presence of cuticular layer on the epidermis, ii) lack of suitable nutrients for the pathogen in the host cells, iii) presence of inhibitory or toxic substances in the host cells, iv) exudation of substances toxic to pathogen or stimulatory

to antagonists of the pathogen. Ex: The glands in leaf hairs of begalgram contain maleic acid which is antifungal and provide resistance to infection by the rust fungus (*Uromyces ciceris arietini*). Similarly, protocatecheuic acid and catechol in the red scales of onion provide resistance to onion smudge pathogen, *Colletotrichum circinans*. To overcome these physical and chemical barriers, the fungi produce various enzymes, toxins organic acids and growth regulators.

**Through non-cutinized surfaces:**

**a) Seedlings:** Grain smut of jowar (*Sphacelotheca sorghi*), Loose smut of jowar (*Sphacelotheca cruenta*), Downy mildew of jowar and bajra (*Sclerospora graminicola*), Wheat bunt disease (*Tilletia caries*, *Tilletia foetida*)

**b) Root hairs:** Wilt causing fungi (*Fusarium* sp.), Club root of cabbage (*Plasmodiophora brassicae*), Root rot of cotton (*Phymatotrichum omnivorum*)

**c) Buds:** Pea rust fungi (*Uromyces pisi*), Witches broom of cherries (*Taphrina cerasi*)

**d) Flowers:** Loose smut of wheat (*Ustilago nuda tritici*), Long smut of jowar (*Tolyposporium ehrenbergi*), Bunt of rice (*Neovossia horrida*), **Ergot of rye** (*Claviceps purpurea*)

**e) Leaves:** Basidiospores of white pine blister rust fungus (*Cronartium ribicola*) germinate and grow down into branches and leaves, where aecia are produced.

**d) Nectaries: Fire blight of apple** (*Erwinia amylovora*)

**e) Stalk ends:** *Penicillium italicum*, *Theilaviopsis paradoxa* (Post harvest disease fungi)

**Through cutinized surfaces:**

**a) Cuticle:** Leaf spot of spinach (*Cercospora beticola*), early blight of solanaceous plants (*Alternaria solani*), Tikka disease of groundnut (*Cercospora personata*)

### 3. POST PENETRATION

**Invasion and colonization:** Infection is the process by which pathogens establish contact with the susceptible cells or tissues of the host and derive nutrients from them. A parasitic relationship is formed between host cytoplasm and parasite cytoplasm. During infection, pathogens grow and multiply within the plant tissues. **Invasion** of plant tissues by the pathogen, and growth and reproduction of the pathogen (**colonization**) are two concurrent stages of disease development.

Fungi spread into all parts of host organs, either by growing directly through the cells as an intracellular mycelium or by growing between the cells as an intercellular mycelium. During establishment, pathogen produces different substances which include enzymes, toxins, growth hormones and polysaccharides which will help in colonization of the host.

In **ectoparasites** the main body of the pathogen lies on the surface of the host with only feeding organs (haustoria) penetrating the tissues Ex: Most of the powdery mildew fungi. Some fungal parasites develop both external and internal mycelium Ex: *Rhizoctonia solani*. The endophytic parasites or **endoparasites** grow subcuticularly (*Diplocarpon rosae*, black spot of rose), in parenchyma tissues (most fungal and bacterial pathogens as well as many nematodes) or in vascular tissues (vascular wilt parasites). Some pathogens are **endobiotic**, *i.e.*, mycelium is not produced and the thallus is entirely present within a host cell Ex: *Synchytrium endobioticum*.

Bacteria invade tissues intercellularly, but also grow intracellularly when parts of the cell walls dissolve. Viruses, viroids, mollicutes and fastidious bacteria invade tissues by moving from cell to cell intracellularly.



Infection caused by microbes may be local (involve single cells or few cells or small area) or **systemic** (pathogen spreads and invades most or all susceptible cells and tissues throughout the plant Ex: *Sclerospora graminicola*). The time interval between inoculation and appearance of disease symptoms is called the **incubation period**.

#### **Exit of the pathogen**

After invasion and colonization of the host, the pathogens come out of the host to maintain the continuity of the infection chain or disease cycle and escape death due to overcrowding. Once the pathogens exit from the host, they survive and are disseminated to other hosts and continue the infection cycle.

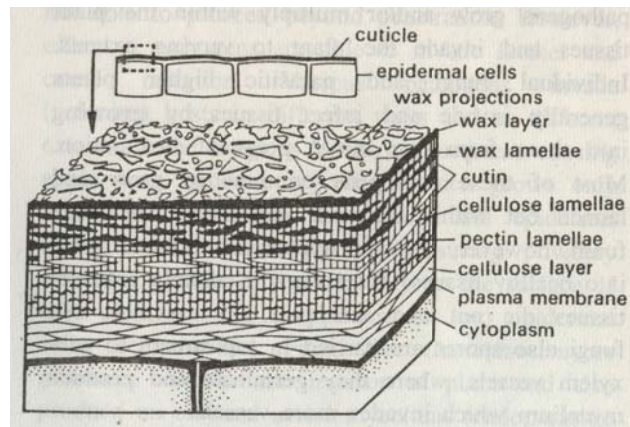
**Viruses** can exist only with the living protoplasm and hence disseminated through their animate vectors like insects, fungi, nematodes, etc. The **bacteria** ooze out in the form of slime on the host surface from where they can be disseminated through water and insects. However, the **fungi** have the most elaborate system of exit. Most plant pathogenic fungi grow out on the host surface and produce repeating spores (secondary inoculum), usually asexually, under favourable conditions. The spores thus formed are disseminated through wind, water, soil, seed, vegetative propagating material, agricultural implements, etc.

## LECTURE 7

### ROLE OF ENZYMES IN PATHOGENESIS

Enzymes are large protein molecules which catalyze all inter-related reactions in the living cell. Most pathogens derive energy principally from enzymatic break down of food materials from host tissue.

**Composition of the cell wall:** Functionally cell wall is divided into 3 regions, viz., middle lamella (made of pectins), primary wall (cellulose, pectic substances) and secondary cell wall (entirely cellulose).



Middle lamella acts as intercellular cement which binds the cells together in tissue system. Pectin or pectic substances are major chemical constituents of wall layers and entire middle lamella, where as in other layers, cellulose is found in good amounts.

Besides these two major components, other components such as hemicelluloses, lignin and some amount of protein is also present. Main components of cell wall are pectic substances, cellulose, hemicelluloses, lignin and small quantity of protein.

The epidermis of plants is covered by cuticle, whose major chemical substance is cutin in addition to cuticular wax.

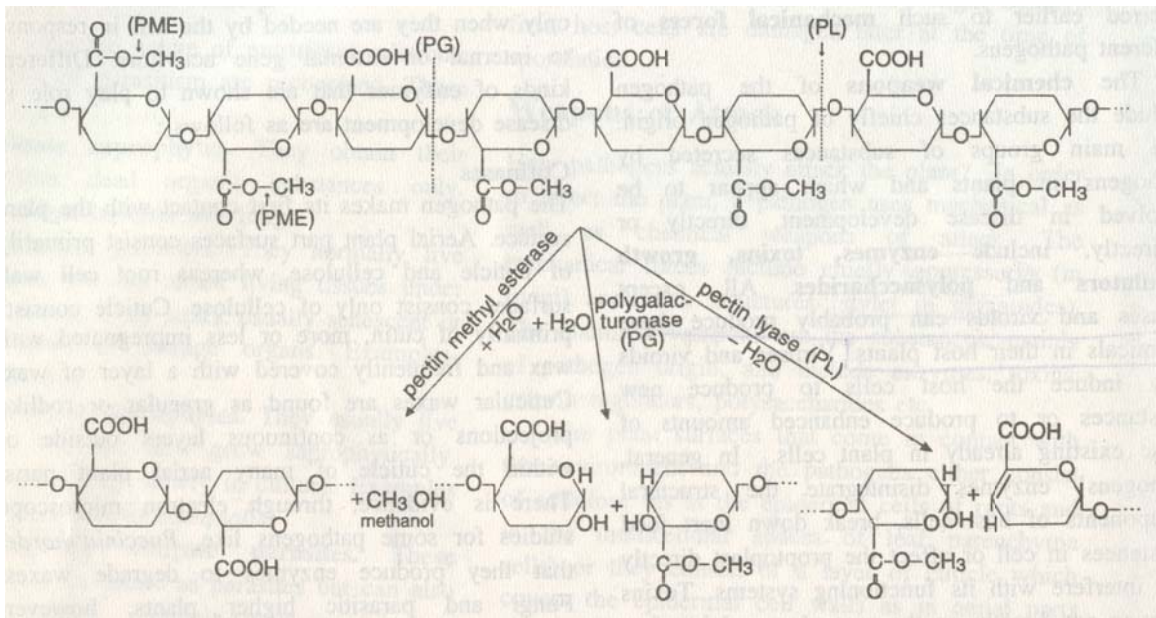
**Cuticular wax:** Plant waxes are found as granular or rod like projections or as a continuous layer outside / within the cuticle. Wax formation is a continuous process and it is not a terminal phase in the development of leaf. Cuticular waxes are made up of long chain molecules of paraffin, hydrocarbons, alcohols, ketones and acids. Most of the fungi and parasitic higher plants penetrate wax layers by means of mechanical force alone.

**Cutin:** It is an insoluble polyester of unbranched derivatives of **C<sub>16</sub> and C<sub>18</sub> hydroxy fatty acids**. Cutin is admixed with waxes on upper side and with pectin and cellulose on the lower side. **Cutinases** break cutin molecules and release monomers as well as oligomers from insoluble cutin polymer. Cutinases reaches its highest concentration at penetrating point of the germ tube and at infection peg of appressorium forming fungi

Ex: *Colletotrichum gloeosporioides*, *Sphaerotheca pannosa*, *Venturia inaequalis*, *Helminthosporium victoriae*.

**Pectic substances:** These are major components of middle lamella (intercellular cement that holds in place the cells of plant tissues). They also make up a large portion of primary cell wall in which they form an amorphous gel filling the spaces between cellulose microfibrils. Pectic substances are polysaccharides consisting mostly of **d-galactouronic acid** units with  **$\alpha$ -1,4-glycosidic bonds**. These chains are esterified with **methyl** groups or linked with other carboxyl groups in calcium and magnesium salt bridges.

Pectic substances are of three types, namely, **pectic acid** (non methylated units), **pectinic acid** (<75% methylated galacturonan units) and **pectin** (>75% methylated units). Term **protopectin** is used to denote substances which are soluble in water and upon restricted hydrolysis yields pectinic acid.



The enzymes that degrade pectic substances are known as **pectinases** or **pectolytic** enzymes. Pectinases and pectolytic enzymes are pectin methyl esterases (PME's), polygalactouronases (PG's) and pectin lyases (PL's).

1. **Pectin methyl esterases:** Breaks ester bonds and removes methyl groups from pectin leading to the formation of **pectic acid** and **methanol** (CH<sub>3</sub>OH).
2. **Polygalacturonases:** Split pectin chain by adding a molecule of water and breaks the linkage between two galacturonan units. These enzymes catalyze reactions that break  $\alpha$ -1,4-glycosidic bonds.
3. **Pectin lyases:** Split pectin chain by removing a molecule of water from the linkage, thereby breaking it and releasing products with unsaturated double bonds.

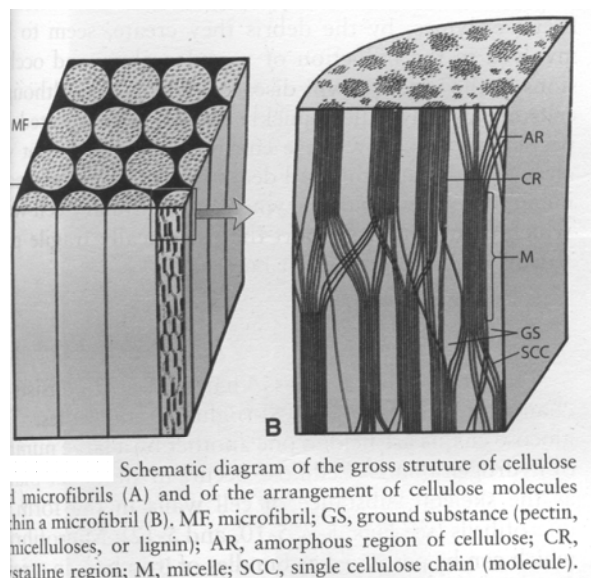
These pectin enzymes can be **exopectinases** (break only terminal linkage) or **endopectinases** (break pectin chain to random sites). Pectin degradation results in liquefaction of the pectic substances and weakening of cell walls, leading to tissue maceration

Ex: Soft rot bacterium, *Erwinia caratovora* subsp. *caratovora* and other fungi like *Botrytis cinerea*, *Sclerotium rolfii*, etc.

**Cellulose:** Cellulose is a polysaccharide, made of chains of  **$\beta$ -D-glucopyranose** units (where C<sub>1</sub> is linked to C<sub>4</sub>). Glucose chains are held by **hydrogen** bonds. Cellulose occurs in all higher plants as the skeletal substance of cell walls in the form of microfibrils. Primary and secondary wall consists of a matrix in which a large number of microfibrils are embedded. These microfibrils are like bundles of iron bars in a reinforced concrete building. In some parts of microfibrils the chains are arranged in an orderly fashion attaining crystalline form, when arranged in less orderly fashion, it attains amorphous form. If the proportion of crystalline portion is more, the resistance of the host to pathogen is more. The space between microfibrils and between micelles or cellulose chains is filled with pectins, hemicelluloses and also lignin at maturity.

Cellulose is insoluble in **crystalline** form (native form), and soluble in **amorphous** form (modified cellulose). The enzymatic breakdown of cellulose results in final production of glucose molecules.

Cellulose is degraded by **cellulases**. Cellulase one (C<sub>1</sub>) attacks native cellulose by cleaving cross-linkages between chains. A second cellulase (C<sub>2</sub>) also attacks native cellulose and breaks into shorter chains. These shorter chains are then attacked by C<sub>x</sub> enzyme, which degrade them into disaccharide, **cellobiose**. Finally cellobiose is degraded by the enzyme,  **$\beta$ -glucosidase** into glucose.



Cellulase degrading enzymes play a role in softening and degradation of cell wall material and facilitate easy penetration and spread of pathogen in the host.

Ex: Basidiomycetes fungi

**Hemicellulose:** These are the major constituents of **primary cell wall** and also seen in middle lamella and secondary cell wall. The hemicellulose polymers include primarily **xyloglucan** but also glucomannans, galactomannans, arabinogalactans, etc. Hemicelluloses link the ends of pectic polysaccharides and various points of the cellulose microfibrils.

Hemicellulases degrade hemicelluloses and depending on the monomer released from polymer on which they act, they are termed as xylanase, galactanase, glucanase, arabinase, mannanase, and so on. Ex: *Sclerotinia sclerotiorum*, *Sclerotinia fructigena*.

**Lignin:** Lignin is found in the **middle lamella**, as well as in the secondary cell wall of xylem vessels and the fibres that strengthen plants. It is an amorphous, three-dimensional polymer made up of basic structural unit, **phenylpropanoid**. Lignin forms by oxidative condensation (C-C and C-O bond formation) between phenylpropanoid units or substituted **cinnamyl alcohols** (p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol). **White rot fungi** (Basidiomycetes) secrete one or more ligninases which enable them to utilize lignin. Ex: *Xylaria*, *Chaetomium*, *Alternaria*, *Cephalosporium*, etc.

**Cell wall proteins:** Cell wall proteins are similar to other proteins, except that they are rich in amino acid, **hydroxy proline**. Five classes of structural proteins are found in cell walls: extensins, proline-rich proteins (PRP's), glycine-rich proteins (GRP's), Solanaceous lectins and arabinogalactan proteins (AGP's). Proteins are degraded by means of enzymes, **proteases** or **proteinases** or **peptidases**.

**Lipids:** Various types of lipids occur in all plant cells. The most important ones are **phospholipids** and **glycolipids**. These lipids contain fatty acids, which may be saturated or unsaturated. Lipolytic enzymes, called **lipases** (phospholipases, glycolipases) hydrolyze lipids and release fatty acids.

**Starch:** Starch is the main reserve polysaccharide found in plant cells. It is a glucose polymer and exists in two forms: **amylose**, a linear molecule, and **amylopectin**, a highly branched molecule. Starch is degraded by enzyme, amylases.

## LECTURE 8

### ROLE OF TOXINS IN PLANT PATHOGENESIS

**Def:** Toxin can be defined as a microbial metabolite excreted (**exotoxin**) or released by lysed cells (**endotoxin**) which in very low concentration is directly toxic to the cells of the susceptible (host).

The term toxin is used for a product of the pathogen, its host, or pathogen host interaction which even at very low concentration directly acts on living host protoplasm to influence disease development or symptom expression.

Toxins are different from enzymes in that they do not attack structural integrity of host tissues but affect the metabolism of the host because the toxins will act on protoplast of the cell.

#### **Toxin hypothesis (Luke and Wheeler, 1955):**

1. A toxin should produce all symptoms characteristic of the disease
  2. Sensitivity to toxin will be correlated with susceptibility to pathogen
  3. Toxin production by the pathogen will be directly related to its ability to cause disease.
- Except, **victorin**, the toxic metabolite of *Cochliobolus victoriae*, the vast majority of toxins associated with plant diseases fail to exhibit all the above characters.

#### **Classification of toxins (Wheeler and Luke, 1963)**

According to the **source of origin**, toxins are divided into 3 broad classes namely, *pathotoxins*, *vivotoxins* and *phyto toxins*.

1. **Pathotoxins:** These are the toxins which play a major role in disease production and produce all or most of the symptoms characteristic of the disease in susceptible plants. Most of these toxins are produced by pathogens during pathogenesis.

Ex: **Victorin:** *Cochliobolus victoriae* (*Helminthosporium victoriae*), the causal agent of Victoria blight of oats. This is a host specific toxin.

Other examples:

a) *Selective*

T- toxin: *Helminthosporium maydis* race T

HC-toxin: *Helminthosporium carbonum*

HS- toxin: *Helminthosporium sacchari*

Phyto-alternarin: *Alternaria kikuchiana*

PC- toxin: *Periconia circinata*

b) *Non-selective*

Tentoxin: *Alternaria tenuis*

Tabtoxin or wild fire toxin: *Pseudomonas tabaci*

Phaseolotoxin: *Pseudomonas syringae* pv. *phaseolicola*

c) Produced by *plant or plant X pathogen interaction*

Amylovorin: *Erwinia amylovora* (Fire blight of apple and pears)

2) **Phyto toxins:** These are the substances produced in the host plant due to host-pathogen interactions for which a causal role in disease is merely suspected rather than established. These are the products of parasites which induce few or none of the symptoms caused by the living pathogen. They are non-specific and there is no relationship between toxin production and pathogenicity of disease causing agent.

Ex: Alternaric acid – *Alternaria solani*

3) **Vivotoxins:** These are the substances produced in the infected host by the pathogen and / or its host which functions in the production of the disease, but is not itself the initial inciting agent of the disease.

Fusaric acid – Wilt causing *Fusarium* sp.

Lycomarasmin – *Fusarium oxysporum f.sp. lycopersici*

Piricularin – *Pyricularia oryzae*

### Classification based on specificity of toxins

1. **Host specific / Host selective toxins:** These are the metabolic products of the pathogens which are selectively toxic only to the susceptible host of the pathogen

Ex: Victorin, T-toxin, Phyto-alternarin, Amylovorin

### 2. Non-specific / Non-selective toxins

These are the metabolic products of the pathogen, but do not have host specificity and affect the protoplasm of many unrelated plant species that are normally not infected by the pathogen

Ex: Ten-toxin, Tab-toxin, Fusaric acid, Piricularin, Lycomarasmin and Alternaric acid

### Differentiate host – specific and non-host specific toxins

#### Host specific

1. Selectively toxic only to susceptible host of the pathogen

2. Primary determinants of disease

3. Produce all the essential symptoms of the disease

4. Ex: Victorin, T- toxin

#### Non-host specific

1. No host specificity and can also affect the physiology of those plants that are normally not infected by the pathogen

2. Secondary determinants of disease

3. Produce few or none of the symptoms of the disease

4. Ex: Tentoxin, Tabtoxin

### Effect of toxins on host tissues

A) *Changes in cell permeability:* Toxins kill plant cells by altering the permeability of plasma membrane, thus permitting loss of water and electrolytes and also unrestricted entry of substances including toxins. Cellular transport system, especially,  $H^+ / K^+$  exchange at the cell membrane is affected.

B) *Disruption of normal metabolic processes*

- Increase in respiration due to disturbed salt balance
- Malfunctioning of enzyme system Ex: Piricularin inhibits polyphenol oxidase
- Uncoupling of oxidative phosphorylation

C) *Interfere with the growth regulatory system* of host plant Ex: Restricted development of roots induced by *Fusarium moniliforme*

## ROLE OF GROWTH REGULATORS IN PLANT PATHOGENESIS

### Growth regulators

Growth regulators are of two types

1. Growth promoting substances and 2. Growth inhibiting substances

Auxins, gibberellins and cytokinins are growth promoting substances, whereas, dormin, ethylene and abscissic acid are growth inhibiting substances. The imbalance in growth promoting and growth inhibiting substances causes **hypertrophy** (excessive increase in cell size) and **atrophy** (decrease in cell size). Symptoms may appear as tumors, galls, knots, witches broom, stunting, excessive root branching, defoliation and suppression of bud growth.

#### 1. Growth promoting substances:

a) **Auxins:** *Indole-3-acetic acid (IAA)* is the naturally occurring auxin. It is continuously produced in young meristematic tissue and moves rapidly to older tissues. If auxin concentration is more, its concentration is reduced by the enzyme, **IAA oxidase**.

*Functions:* IAA regulates cell elongation and differentiation, also affects permeability of the membrane, increases respiration, and promotes synthesis of mRNA.

### How disease is induced?

Increased IAA results in **hypertrophy** and decreased IAA results in **atrophy**. Increased IAA may be due to inhibition of IAA oxidase.

Ex: *Ralstonia solanacearum* (*Pseudomonas solanacearum*), the causal agent of wilt of Solanaceous plants, induces a 100 fold increase in IAA level in diseased plants. Increased plasticity of cell walls as a result of high IAA levels renders the pectin, cellulose and protein components of the cell wall more accessible to pathogen degradation. Increase in IAA levels may also inhibit lignifications of tissues.

Increased IAA levels have been reported in plants infected with the following pathogens *Phytophthora infestans* (Late blight of potato), *Ustilago maydis* (Maize smut), *Plasmiodiophora brassicae* (Club root of crucifers), *Sclerospora graminicola* (Downy mildew of sorghum), *Agrobacterium tumefaciens* (Crown gall of apple), and *Meloidogyne* (Root knot nematode).

**b) Gibberellins:** First isolated from *Gibberella fujikuroi* (Conidial stage: *Fusarium moniliforme*), the causal agent of bakanae or foolish seedling disease of rice. Infected seedlings show abnormal elongation due to excessive elongation of internodes. Best known gibberellin is **Gibberellic acid**.

*Functions:* Cell elongation, stem and root elongation, promote flowering and growth of fruits. It also induces IAA synthesis. IAA and GA act synergistically. Ex: *Sclerospora sacchari*, the causal agent of downy mildew of sugarcane induces GA production.

**c) Cytokinins:** **Kinetin** was the first compound isolated from herring sperm DNA and does not occur naturally in plants. Cytokinins, such as **zeatin** and **isopentenyl adenosine** (IPA) have been isolated from plants

*Functions:* Cytokinins are necessary for cell growth and differentiation. It inhibits breakdown of proteins and aminoacids and thereby inhibit senescence and they have the capacity to direct the flow of aminoacids and other nutrients towards high cytokinin concentration. Cytokinin activity increases in club root, in crown galls and in rust infected bean leaves. Ex: Green islands are formed around infection in bean (*Phaseolus vulgaris*) leaves infected by *Uromyces phaseoli*.

## 2. Growth inhibiting substances

**a) Ethylene (CH<sub>2</sub>=CH<sub>2</sub>):** Ethylene exerts a variety of effects on plants, viz., chlorosis, leaf abscission, epinasty, stimulation of adventitious roots, fruit ripening and increased permeability of cell membranes.

Ex: Ethylene is involved in premature ripening of fingers in banana infected by *Pseudomonas solanacearum*, the causal agent of moko disease of banana. Ethylene was also detected in leaf epinasty symptom of the vascular wilt syndrome. Ex: *Fusarium oxysporum f.sp. lycopersici* (Wilt in tomato).

**b) Abscissic acid:** It exerts dormancy in seeds, closure of stomata, inhibition of seed germination and growth and stimulated germination of fungal spores. It is one of the factors involved in stunting of plants.

**c) Dormin / Abscissin II:** Dormin induces dormancy by converting developing leaf primordia of a bud into bud scales. It acts as an antagonist of gibberellins and masks the effect of IAA. However, the exact role of dormin is not known.

## ROLE OF POLYSACCHARIDES IN PATHOGENESIS

**Polysaccharides:** Fungi, bacteria and nematodes release varying amounts of mucilaginous substances that coat their bodies and provide interface between the outer surface of the micro-organism and its environment. The role of slimy polysaccharides is of utmost importance in wilt diseases. In the vascular wilts, large polysaccharide molecules released by the pathogen in the xylem causes mechanical blockage of vascular bundles and initiate wilting.

Ex: *Ralstonia solanacearum* (Bacterial wilt of Solanaceous plants)



## LECTURE 9, 10 & 11

### DEFENSE MECHANISM IN PLANTS

In general plants defend themselves against pathogens by two ways: structural or morphological characteristics that act as physical barriers and biochemical reactions that take place in cells and tissues that are either toxic to the pathogen or create conditions that inhibit the growth of the pathogen in the plant.

**I. Structural defense mechanisms:** These may be pre-existing, which exist in the plant even before the pathogen comes in contact with the plant or induced, *i.e.*, even after the pathogen has penetrated the preformed defense structures, one or more type of structures are formed to protect the plant from further pathogen invasion.

#### **A) Pre-existing structural defense structures**

These include the amount and quality of wax and cuticle that cover the epidermal cells and the size, location and shapes of natural openings (stomata and lenticels) and presence of thick walled cells in the tissues of the plant that hinder the advance of the pathogen.

**i) Waxes:** Waxes on leaf and fruit surfaces form a hydrophobic or water repellent surface preventing the germination of fungi and multiplication of bacteria.

**ii) Cuticle and epidermal cells:** A thick cuticle and tough outer wall of epidermal cells may increase resistance to infection in diseases in which the pathogen enters its host only through direct penetration. Ex: Disease resistance in Barbery species infected with *Puccinia graminis tritici* has been attributed to the tough outer epidermal cells with a thick cuticle. In linseed, cuticle acts as a barrier against *Melampsora lini*.

The silicification and lignifications of epidermal cells offers protection against *Pyricularia oryzae* and *Streptomyces scabies* in paddy and potato, respectively.

**iii) Sclerenchyma cells:** The sclerenchyma cells in stems and leaf veins effectively blocks the spread of some fungal and bacterial pathogens that cause angular leaf spots.

#### **iv) Structure of natural openings:**

**a) Stomata:** Most of the pathogens enter plants through natural openings. Some pathogens like stem rust of wheat can enter its host only when the stomata are open. The wheat varieties (Cultivar, **Hope**) in which stomata open late in the day are resistant as the germ tubes of the spores germinating in the night dew desiccate owing to evaporation of the dew before stomata begin to open. This can also be called as functional resistance. The structure of stomata provides resistance to penetration by certain plant pathogenic bacteria.

Ex: The citrus variety, **szinkum**, is resistant to citrus canker because it posses a broad cuticular ridge projecting over the stomata and a narrow slit leading to the stomatal cavity thus preventing the entry of bacterial and fungal spores into the interior of the leaf.

**b) Lenticels:** The shape and internal structure of lenticels can increase or decrease the incidence of fruit diseases. Small and suberised lenticels will offer resistance to potato scab pathogen, *Streptomyces scabies*.

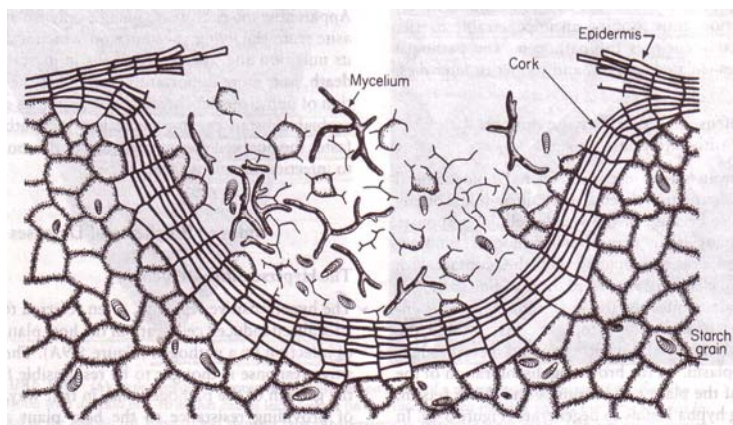
**B) Post-infectious structural defense mechanisms/Induced structural barriers:** These may be regarded as histological defense barriers (cork layer, abscission layers and tyloses) and cellular defense structures (hyphal sheathing).

#### **i) Histological defense structures**

**a) Cork layer:** Infection by fungi, bacteria, some viruses and nematodes induce plants to form several layers of cork cells beyond the point of infection and inhibits the further invasion by the pathogen beyond the initial lesion and also blocks the spread of toxin substances secreted by the pathogen. Furthermore, cork layers stop the flow of nutrients and water from the healthy to the infected area and deprive the pathogen of nourishment.

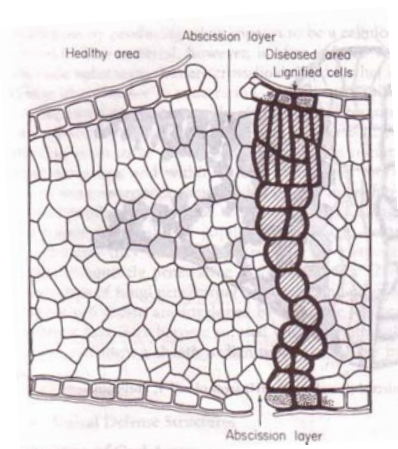
Ex: Potato tubers infected by *Rhizoctonia*; *Prunus domestica* leaves attacked by *Coccomyces pruniphorae*.





### b) Abscission layers

An abscission layer consists of **a gap formed between infected and healthy cells** of a leaf surrounding the locus of infection due to the disintegration of the middle lamella of parenchymatous tissue.



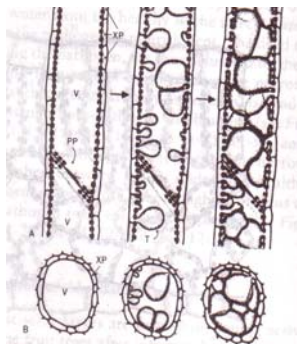
Gradually, infected area shrivels, dies, and sloughs off, carrying with it the pathogen. Abscission layers are formed on young active leaves of stone fruits infected by fungi, bacteria or viruses.

Ex: *Xanthomonas pruni*, and *Closterosporium carpophyllum* on peach leaves

### c) Tyloses

Tyloses are the overgrowths of **the protoplast of adjacent living parenchymatous cells, which protrude into xylem vessels through pits**. Tyloses have cellulosic walls and are formed quickly ahead of the pathogen and may clog the xylem vessels completely blocking the further advance of the pathogen in resistant varieties. In susceptible varieties, few or no tyloses are formed ahead of pathogen invasion.

Ex: Tyloses form in xylem vessels of most plants under invasion by most of the **vascular wilt** pathogens.



### ii) Cellular defense structures:

**Hyphal sheathing:** The hyphae penetrating the cell wall and growing into the cell lumen are enveloped by a cellulosic sheath (callose) formed by extension of cell wall, which become infused with phenolic substances and prevents further spread of the pathogen.

Ex: Hyphal sheathing is observed in flax infected with *Fusarium oxysporum f.sp. lini*.

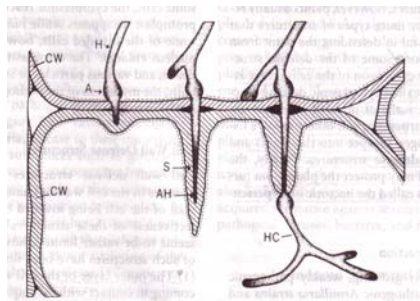


FIGURE 5-3 Formation of a sheath around a hypha (H) penetrating a cell wall (CW). A, Appressorium; AH, advancing hypha still enclosed in sheath; HC, hyphae in cytoplasm; S, sheath.

**II) Biochemical defense mechanisms:** These can be classified as pre-existing and induced biochemical defenses.

### 1) Pre-existing chemical defenses:

#### a) *Inhibitors released by the plant in its environment:*

Plants exude a variety of leaf and root exudates which contain aminoacids, sugars, glycosides, organic acids, enzymes, alkaloids, flavones, toxic materials, inorganic ions and also certain growth factors. The inhibitory substances directly affect micro-organisms or encourage certain groups to dominate the environment which may act as antagonists to pathogen.

- Ex 1: Tomato leaves secrete exudates which are inhibitory to *Botrytis cinerea*
- Ex 2: Red scales of red onion contain the phenolic compounds, **protocatechuic acid** and **catechol**, which diffuse out to the surface and inhibits the conidial germination of **onion smudge** fungus, *Colletotrichum circinans*. However, these fungitoxic phenolic compounds are missing in white scaled onions.
- Ex 3: Resistant varieties of apple secrete waxes on the leaf surface which prevents the germination of *Podosphaera leucotricha* (powdery mildew of apples).
- Ex 4: In *Cicer arietinum* (chickpea), the *Ascochyta* blight resistant varieties have more glandular hairs which have **maleic acid** which inhibit spore germination.
- Ex 5: Resistant varieties of linseed secrete HCN in roots which are inhibitory to linseed wilt pathogen, *Fusarium oxysporum f.sp. lini*.
- Ex 6: Root exudates of marigold contain  $\alpha$ -terthinyll which is inhibitory to nematodes.
- Ex 7: **Chlorogenic acid** present in sweet potato, potato and carrot inhibits *Ceratocystis fimbriata*. Similarly **caffeic acid** and **phloretin** are present in sweet potato and apple, respectively.

#### b) *Inhibitors present in plant cells before infection:*

- Antimicrobial substances pre-existing in plant cells include unsaturated lactones, cyanogenic glycosides, Sulphur containing compounds, phenols, phenolic glycosides and saponins
- Several phenolic compounds, **tannins**, and some fatty acid like compounds such as **dienes**, which are present in high concentrations in cells of young fruits, leaves or seeds are responsible for the resistance of young tissues to *Botrytis*. These compounds are potent inhibitors of many hydrolytic enzymes.  
Ex: Chlorogenic acid in potato inhibits common scab bacteria, *Streptomyces scabies*, and to wilt pathogen, *Verticillium alboatrum*
- **Saponins** have antifungal membranolytic activity which excludes fungal pathogens that lack saponinases. Ex: **Tomatine** in tomato and **Avenacin** in oats
- Similarly, **lectins**, which are proteins that bind specifically to certain sugars and occur in large concentrations in many types of seeds, cause lysis and growth inhibition of many fungi.
- Plant surface cells also contain variable amounts of **hydrolytic enzymes** such as **glucanases** and **chitinases** which may cause breakdown of pathogen cell wall.

### 2) Post inflectional or induced defense mechanisms:

#### a) *Phytoalexins* (Phyton = plant; alexin = to ward off)

- **Muller** and **Borger** (1940) first used the term phytoalexins for fungistatic compounds produced by plants in response to injury (mechanical or chemical) or infection.
- Phytoalexins are toxic antimicrobial substances produced in appreciable amounts in plants only after stimulation by phytopathogenic micro-organisms or by chemical or mechanical injury.

- Phytoalexins are not produced by uninfected healthy plants, but produced by healthy cells adjacent to localized damaged or necrotic cells in response to materials diffusing from the infected cells. These are not produced during compatible biotrophic infections.
- Phytoalexins accumulate around both resistant and susceptible necrotic tissues. However, resistance occurs when one or more phytoalexins reach a concentration sufficient to restrict pathogen development.

### Characteristics of phytoalexins

1. Fungitoxic and bacteriostatic at low concentrations.
2. Produced in host plants in response to stimulus (elicitors) and metabolic products.
3. Absent in healthy plants
4. Remain close to the site of infection.
5. Produced in quantities proportionate to the size of inoculum.
6. Produced in response to the weak or non-pathogens than pathogens
7. Produced within 12-14 hours reaching peak around 24 hours after inoculation.
8. Host specific rather than pathogen specific.

Synthesis and accumulation of phytoalexins are shown in diversified families, viz., Leguminosae, Solanaceae, Malvaceae, Chenopodiaceae, Convolvulaceae, Compositae and Graminaceae.

S.No.	Phytoalexin	Host	Pathogen
1	Pisatin	Pea	<i>Monilinia fructicola</i>
2	Phaseolin	French bean	<i>Sclerotinia fructigena</i>
3	Rishitin	Potato	<i>Phytophthora infestans</i>
4	Gossypol	Cotton	<i>Verticillium albo-atrum</i>
5	Cicerin	Bengalgram	<i>Ascochyta rabiei</i>
6	Ipomeamarone	Sweet potato	<i>Ceratocystis fimbriata</i>
7	Capsidol	Pepper	<i>Colletotrichum capsici</i>

### b) Hypersensitive response (HR)

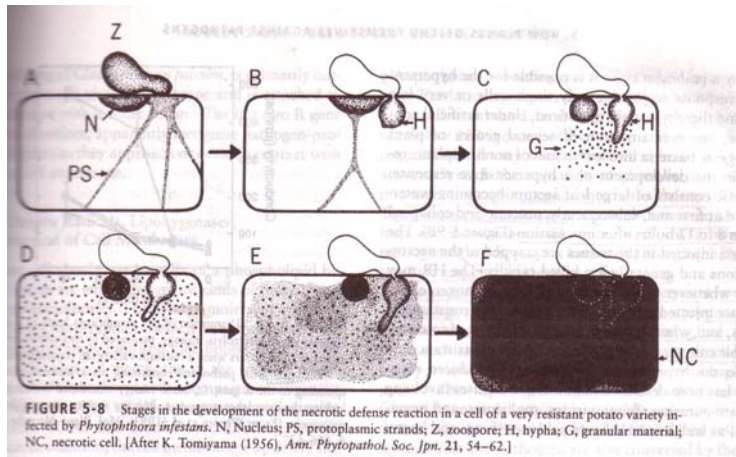
- The term hypersensitivity was first used by **Stakman** (1915) in wheat infected by rust fungus, *Puccinia graminis*.
- The hypersensitive response is a **localized induced cell death** in the host plant at the site of infection by a pathogen, thus limiting the growth of pathogen. In the infected plant part, HR is seen as water soaked large sectors which subsequently become necrotic and collapsed.
- HR occurs only in **incompatible** host-pathogen combinations. HR may occur whenever virulent strains or races of pathogens are injected into non-host plants or into resistant varieties, and when avirulent strains or races of pathogens are injected into susceptible cultivars.
- HR is initiated by the recognition of specific pathogen-produced signal molecules, known as **elicitors**. Recognition of the elicitors by the host results in altered cell functions leading to the production of defense related compounds.

The most common **new cell functions** and compounds include:

- A rapid burst of oxidative reactions
- Increased ion movement, especially of K<sup>+</sup> and H<sup>+</sup> through cell membrane
- Disruption of membranes and loss of cell compartmentalization
- Cross-linking of phenolics with cell wall components and strengthening of plant cell wall
- Production of antimicrobial substances such as phytoalexins and pathogenesis-related proteins (such as chitinases)

### Cellular responses during HR

- In many host-pathogen combinations, as soon as the pathogen establishes contact with the cell, the nucleus moves toward the invading pathogen and soon disintegrates.
- Brown resin like granules form in the cytoplasm, first around the point of penetration of pathogen and then throughout the cytoplasm
- As the browning discolouration of the cytoplasm continues and death sets in, the invading hypha begins to degenerate and further invasion is stopped.



**c) Plantibodies:** Transgenic plants have been produced which are genetically engineered to incorporate into their genome, and to express foreign genes, such as mouse genes that produce antibodies against certain plant pathogens. Such **antibodies, encoded by animal genes, but produced in and by the plant**, are called plantibodies. Ex: Transgenic plants producing plantibodies against coat protein of viruses, such as, **artichoke mottle crinkle virus** have been produced.

## LECTURE 12

### PLANT DISEASE EPIDEMIOLOGY

**Epiphytology or Epidemiology** of plant diseases is essentially a study of the rate of multiplication of a pathogen and spread of the disease caused by it in a plant population. Epidemiology deals with outbreaks and spread of diseases in a population.

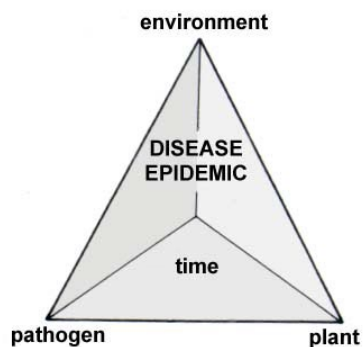
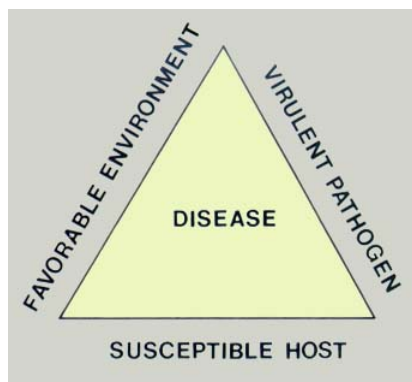
**Importance of epidemiology:**

Knowledge of epidemiology is useful in forecasting of a disease and also for the management of a disease

Terms compound interest and simple interest diseases were given by **Vanderplank** (1963) in his book “Plant Disease Epidemics and control”

S.No.	Compound interest disease/ polycyclic	Simple interest disease/Monocyclic
1	Rate of increase of disease is mathematically analogous to compound interest in money (Interest is added periodically to the capital; interest gets interest)	Rate of increase of disease is mathematically analogous to simple interest in money (Interest is added only at the end; interest does not get interest)
2	Pathogen produces spores at rapid rate	Pathogen produces spores at very slow rate
3	Propagules disseminate by air	Propagules disseminate by soil or seed
4	Incubation period and sporulation period is short	Incubation period and sporulation period is long
5	There are several generations of the pathogen in the life of a crop	There is only one generation of the pathogen in the life of a crop
6	Ex: Rusts of cereals	Ex: Smuts of wheat, barley & sorghum

**Disease Triangle:** The interactions of three components of disease, i.e., the host, pathogen and environment, can be visualized as a disease triangle. The length of each side is proportional to the sum total of the characteristics of each component that favour disease.



The interaction of susceptible host plant, virulent pathogen and favourable environmental conditions leads to the development of the disease.

**Disease Pyramid:** The disease triangle can be expanded to include two more components, time and humans. The amount of each of the three components of disease and their interaction in the development of the disease are affected by fourth component, time. Thus addition of time component to the disease triangle results into a **tetrahedron** or **disease pyramid**. The effect of time on disease development becomes apparent when we consider the importance of time of year, the duration and frequency of favourable temperature and rain, the time of appearance of the vector, the duration of the cycle of a particular disease. If the four components of disease pyramid could be quantified, its volume would be proportional to the amount of disease on a plant or in plant population. Humans affect disease development in various ways. They affect the type of plants grown in an area, their level of resistance, time of planting, density of planting, etc.



### Essential components/conditions for an Epiphytotic:

1. Host factors
2. Pathogen factors
3. Environmental factors

#### **1. Host factors**

*i) Distance of susceptible plants from the source of primary inoculum:* Longer the distance from the source of survival of the pathogen, longer will be the time required for the buildup of an Epiphytotic in a susceptible crop.

*ii) Abundance and distribution of susceptible hosts:* Continuous cultivation of a susceptible variety over a large contiguous area helps in the buildup of the inoculum and improves the chances of epiphytotics.

*iii) Disease proneness in the host due to environment:* Susceptibility is genetically controlled but the disease proneness in the plant to get infected can be induced by environment and other factors (Host nutrition, excessive application of nitrogenous fertilizers, etc).

*iv) Presence of suitable alternate or collateral hosts:* These host plants help in the survival of inoculum of different pathogens in off season. Presence of Barbery which is an alternate host to *Puccinia graminis tritici* helps in the heterogenous infection chain. Presence of grass hosts helps in the survival of *Pyricularia oryzae* in the off-season.

#### **2. Pathogen factors:**

*i) Presence of virulent/aggressive isolate of a pathogen:* For any epiphytotic, rapid cycle of infection is essential, and successful infection can be caused only by virulent isolates of the pathogen.

*ii) High birth rate:* The fungi that assume epiphytotic form invariably have the capacity to produce enormous quantity of spores that are adapted to long distance dissemination in a short time.

*iii) Low death rate of the pathogen:* Epiphytotics is attributed to low death rate of the pathogens in those in which the causal agent is systemic and protected by the plant tissues.

*iv) Easy and rapid dispersal of the pathogen:* The ability of a pathogen to cause epiphytotics is much more dependent on its dispersal rate. The units of propagation need to be dispersed by external agencies, if epiphytotics are to develop.

Ex: Fungal spores disseminated by wind, water, etc

Viruses disseminated by insect vectors

Bacteria dispersed through rain splashes and water

*v) Adaptability of the pathogen:* Most of the pathogens causing epiphytotics adapt themselves to various adverse conditions.

**3. Weather factors:** Assuming that a particular fungus meets all the above requirements for causing an epidemic, the infection, invasion and development of epidemic may not occur if weather is not favourable for the germination of spores. Congenial environmental conditions, viz., optimum weather conditions for sporulation, dispersal, infection and survival of pathogen, are very important.

Weather conditions such as, optimum temperature, moisture, light, etc., are very essential for the development of an epidemics.

Science which deals with the relationship between weather and epiphytotics is called **metereopathology**.

### REMOTE SENSING

Remote sensing is estimating an object/phenomenon without being in physical contact with it. Remote sensing is a science/art that permits us to obtain information about an object/a phenomenon through analysis of data obtained through sensory devices without being in physical contact with that object.

### **Objectives of remote sensing in plant Pathology**

1. Assessment of disease over a vast area
2. To know the relationship of diseases and environment
3. To know the origin and development of epidemics
4. Quantitative assessment of the disease

### **Remote sensing techniques of importance to Plant Pathology**

1. Aerial photography and 2. Satellite remote sensing

**1. Aerial photography:** Aerial photography can detect objects on land over a larger area. **Colwell** (1956) first used remote sensing technique for monitoring stem rust of wheat. He showed that panchromatic colour and especially infrared aerial photography could be used to detect rusts and viral diseases of small grains and certain diseases of citrus. Later, infrared photography was used in England for late blight of potato.

The key to distinguish diseased and healthy parts of a crop is to use appropriate film or filter combinations. The main film types used are panchromatic, infrared, normal colour and colour infrared. The **infrared** films are preferred because of their superior sensitivity to visible light and to near infrared wavelengths of radiation (700-900 m $\mu$ ). The colour infrared or Ektachrome Aero Infrared (Camouflage Detection Film) is superior as it can show the difference between diseased and healthy patches of plants in colour. The healthy foliage is highly reflective to the infrared wavelengths and appears red on this film whereas blighted or diseased foliage has low infrared reflectance and does not appear red in the photograph.

### **2. Satellite Imaging**

#### ***Weather satellites***

Often cyclones create heavy clouds with rains and an anti-cyclone creates a cloudless sky. All these can be effectively monitored by weather satellites. Sequential pictures show the movement of these systems before they arrive in an area. Therefore by monitoring epidemic favouring systems using a satellite, the disease occurrence on the field can be monitored. Ex: The spread and deposition of stem rust pathogen of wheat is influenced by definite synoptic weather conditions called **Indian stem rust rules**.

#### ***Earth resources technology satellites*** (LANDSAT, 1972, USA)

LANDSAT covers the entire globe every 18 days scanning the same area at a fixed time. The scanned data is compared for any major differences happened within 18 days. Nagarajan utilized LANDSAT infrared spectral bands 6 (0.7-0.8 $\mu$ m) and 7 (0.8-1.1 $\mu$ m) to differentiate healthy wheat crop of India and severe yellow rust affected crop of Pakistan.

Examples: Coconut root rot and wilt, black stem rust of wheat, citrus canker

### **Advantages of Remote sensing**

1. Reveals pattern of disease incidence, intensity and development over large area
2. Data generated by remote sensing is amenable to multidisciplinary approach
3. Gives synoptic view of large areas
4. Data generated is on a permanent scale and is unbiased
5. Data acquisition is fast compared to traditional methods and data analyzed is effectively utilized
6. Satellite data (ERTS) obtains information of an area periodically so that the information can be updated.
7. It frequently poses questions for ground investigators which cannot be generated by ground parties

## LECTURE 13

### PRINCIPLES OF PLANT DISEASE MANAGEMENT

**Management:** It conveys a concept of continuous process which is based not only on the principle of eradication of the pathogen but mainly on the principle of minimizing the damage or loss below economic injury level.

**Importance:** Plant diseases are important because of the losses (qualitative and quantitative) they cause. Loss may occur at any time between sowing of the crop and consumption of the produce. Measures taken to prevent the incidence of the disease, reduce the amount of inoculum that initiates and spreads the disease and finally minimize the loss caused by the disease are called as management practices.

#### Essential considerations in plant disease Management:

1. Benefit-cost ratio
2. Procedures for disease control should fit into general schedule of operations of crop production
3. Control measures should be adopted on a **co-operative basis** over large adjoining areas. This reduces frequency of applications, cost of control and increases chances of success of control measures
4. Knowledge aspects of disease development is essential for effective economical control. Information is needed on the following aspects
  - a. Cause of a disease
  - b. Mode of survival and dissemination of the pathogen
  - c. Host parasite relationship
  - d. Effect of environment on pathogenesis in the plant or spread in plant population
5. Prevention of disease depends on management of primary inoculum
6. Integration of different approaches of disease management is always recommended

#### General principles of plant disease management

1. **Avoidance:** Avoiding disease by planting at times when, or in areas where, inoculum is ineffective due to environmental conditions, or is rare or absent
2. **Exclusion of inoculum:** Preventing the inoculum from entering or establishing in the field or area where it does not exist
3. **Eradication:** Reducing, inactivating, eliminating or destroying inoculum at the source, either from a region or from an individual plant in which it is already established
4. **Protection:** Preventing infection by creating a chemical toxic barrier between the plant surface and the pathogen
5. **Disease resistance (Immunization):** Preventing infection or reducing effect of infection by managing the host through improvement of resistance in it by genetic manipulation or by chemical therapy.

**I. Avoidance of the pathogen:** These methods aim at avoiding the contact between the pathogen and susceptible stage of the crop. This is achieved by

- a. Proper selection of geographical area
- b. Proper selection of the field
- c. Adjusting time of sowing
- d. Disease escaping varieties
- e. Proper selection of seed and planting material

**a) Proper selection of geographical area:** Many fungal and bacterial diseases are more severe in wet areas than in dry areas. Cultivation of bajra in wet areas is not profitable due to the diseases, smut (*Tolyposporium penicillariae*) and ergot (*Claviceps microcephala*).

**b) Proper selection of the field:** Proper selection of field will help in the management of many diseases, especially the soil borne diseases. Raising of a particular crop year after year in the same field makes the soil sick, where disease incidence and severity may be more.

Ex: Wilt of redgram, late blight of potato (*Phytophthora infestans*), green ear of bajra (*Sclerospora graminicola*), etc.



**c) Time of sowing:** Generally pathogens are able to infect the susceptible plants under certain environmental conditions. Alteration of date of sowing can help in avoidance of favourable conditions for pathogen.

Ex: *Rhizoctonia* root rot of redgram is more severe in the crop sown immediately after the rains. Delayed sowing will help in reducing the incidence of disease.

Ex: Infection of black stem rust of wheat (*Puccinia graminis tritici*) is more in late sowing, hence, early sowing helps in reduction of stem rust incidence.

**d) Disease escaping varieties:** Certain varieties of crops escape the disease damage because of their growth characteristics. Ex: Early maturing varieties of wheat or pea escape the damage due to *Puccinia graminis tritici* and *Erysiphe polygoni*, respectively.

**e) Proper selection of seed and planting material:** Selection of seed and seedling material from healthy sources will effectively manage the diseases such as loose smut of wheat (*Ustilago nuda tritici*), bunchy top of banana (*Banana virus-1*), Panama wilt of banana (*Fusarium oxysporum f.sp. cubense*) and whip smut of sugarcane (*Ustilago scitaminae*). Potato seed certification or tuber indexing is followed for obtaining virus free seed tubers. Citrus bud wood certification programme will help in obtaining virus free planting material.

**II. Exclusion of the pathogen:** These measures aim at preventing the inoculum from entering or establishing in the field or area where it does not exist. Different methods of exclusion are seed treatment, seed inspection & certification, and plant quarantine regulation.

**a) Seed inspection and certification:** Crops grown for seed purpose are inspected periodically for the presence of diseases that are disseminated by seed. Necessary precautions are to be taken to remove the diseased plants in early stages, and then the crop is certified as disease free. This practice will help in the prevention of inter and intra regional spread of seed borne diseases.

**b) Plant quarantine regulation:** Plant quarantine is defined as “ a legal restriction on the movement of agricultural commodities for the purpose of exclusion, prevention or delaying the spread of the plant pests and diseases in uninfected areas”.

Plant quarantine laws were first enacted in **France** (1660), followed by Denmark (1903) and USA (1912). These rules were aimed at the rapid destruction or eradication of barberry bush which is an alternate host of *Puccinia graminis tritici*.

In India, plant quarantine rules and regulations were issued under **Destructive Insects and Pests Act (DIPA)** in 1914. In India, 16 plant quarantine stations are in operation by the “Directorate of plant protection and quarantine” under the ministry of food and agriculture, government of India.

Plant quarantine measures are of 3 types.

**1. Domestic quarantine:** Rules and regulations issued prohibiting the movement of insects and diseases and their hosts from one state to another state in India is called domestic quarantine. Domestic quarantine in India exists for two pests (Rooted scale and Sanjose scale) and three diseases (Bunchy top of banana, banana mosaic and wart of potato).

**Bunchy top of banana:** It is present in Kerala, Assam, Bihar, West Bengal and Orissa. Transport of any part of *Musa* species excluding the fruit is prohibited from these states to other states in India.

**Banana mosaic:** It is present in Maharashtra and Gujarat. Transport of any part of *Musa* species excluding the fruit is prohibited from these states to other states in India.

**Wart of potato:** It is endemic in Darjeeling area of West Bengal, therefore seed tubers are not to be imported from West Bengal to other states.

**2. Foreign quarantine:** Rules and regulations issued prohibiting the import of plants, plant materials, insects and fungi into India from foreign countries by air, sea and land. Foreign quarantine rules may be general or specific. General rules aim at prevention of introduction of pests and diseases into a country, whereas the specific rules aim at

specific diseases and insect pests. The plant materials are to be imported only through the prescribed ports of entry.

1. **Airports:** Bombay (Santacruz), Calcutta (Dum Dum), Madras (Meenambakam), New delhi (Palam, Safdarjung) and Tiruchurapally.

2. **Sea ports:** Bombay, Calcutta, Vishakapatnam, Trivandrum, Madras, Tuticorin, Cochin and Dhanushkoti.

3. **Land frontiers:** Hussainiwala (Ferozpur district of Punjab), Kharla (Amritsar district of Punjab) and Sukhiapokri (Darjeeling district of West Bengal)

3. **Total embargoes:** Total restriction on import and export of agricultural commodities.

**Phytosanitary certificate:** It is an official certificate from the country of origin, which should accompany the consignment without which the material may be refused from entry.

Plant diseases introduced into India before/after enforcement of plant quarantine laws:

S.No.	Disease	Year	Introduced into	From
1	Late blight of potato	1883	India	Europe
2	Coffee rust	1879	India	Srilanka
3	Flag smut of wheat	1906	India	Australia
4	Downy mildew of grapes	1910	India	Europe
5	Bacterial blight of rice	1964	India	Phillippines
6	Rice blast	1918	India (Madras)	South East Asia
7	Downy mildew of maize	1912	India (Madras)	Java
8	Ergot of bajra	1957	India (Bombay)	Africa
9	Panama wilt of banana	1920	India	Panama canal
10	Bunchy top of banana	1940	India	Srilanka
11	Wart of potato	1953	India	Netherlands
12	Golden cyst nematode of potato	1961	India	Europe

**Diseases not entered into India:** Swollen shoot of cocoa, leaf blight of rubber and many viral diseases.

**III. Eradication:** These methods aim at breaking the infection chain by removing the foci of infection and starvation of the pathogen (i.e., elimination of the pathogen from the area by destruction of sources of primary and secondary inoculum). It is achieved by

**a) Rouging:** Removal of diseased plants or their affected organs from field, which prevent the dissemination of plant pathogens.

Ex: Loose smut of wheat and barley, whip smut of sugarcane, red rot of sugarcane, ergot of bajra, yellow vein mosaic of bhendi, khatte disease of cardamom, etc. During 1927-1935, to eradicate citrus canker bacterium in USA, 3 million trees were cut down and burnt.

**b) Eradication of alternate and collateral hosts:** Eradication of alternate hosts will help in management of many plant diseases.

Ex: Barbery eradication programme in France and USA reduced the severity of black stem rust of wheat

Ex: Eradication of *Thalictrum* species in USA to manage leaf rust of wheat caused by *Puccinia recondita*.

Eradication of collateral hosts, such as *Panicum repens*, *Digitaria marginata* will help in the management of rice blast disease (*Pyricularia oryzae*)

**c) Crop rotation:** Continuous cultivation of the same crop in the same field helps in the perpetuation of the pathogen in the soil. Soils which are saturated by the pathogen are often referred as **sick soils**. To reduce the incidence and severity of many soil borne diseases, crop rotation is adopted. Crop rotation is applicable to only root inhabitants and facultative saprophytes, and may not work with soil inhabitants.

Ex: Panama wilt of banana (long crop rotation), wheat soil borne mosaic (6 yrs) and club root of cabbage (6-10 yrs), etc.

**d) Crop sanitation:** Collection and destruction of plant debris from soil will help in the management of soil borne facultative saprophytes as most of these survive in plant debris. Collection and destruction of plant debris is an important method to reduce the primary inoculum.

**e) Manures and fertilizers:** The deficiency or excess of a nutrient may predispose a plant to some diseases. Excessive nitrogen application aggravates diseases like stem rot, bacterial leaf blight and blast of rice. Nitrate form of nitrogen increases many diseases, whereas, phosphorous and potash application increases the resistance of the host. Addition of farm yard manure or organic manures such as green manure, 60-100 t/ha, helps to manage the diseases like cotton wilt, Ganoderma root rot of citrus, coconut, etc.

**f) Mixed cropping:** Root rot of cotton (*Phymatotrichum omnivorum*) is reduced when cotton is grown along with sorghum. Intercropping sorghum in cluster bean reduces the incidence of root rot and wilt (*Rhizoctonia solani*)

**g) Summer ploughing:** Ploughing the soil during summer months expose soil to hot weather which will eradicate heat sensitive soil borne pathogens.

**h) Soil amendments:** Application of organic amendments like saw dust, straw, oil cake, etc., will effectively manage the diseases caused by *Pythium*, *Phytophthora*, *Verticillium*, *Macrophomina*, *Phymatotrichum* and *Aphanomyces*. Beneficial micro-organisms increases in soil and helps in suppression of pathogenic microbes.

Ex: Application of lime (2500 Kg/ha) reduces the club root of cabbage by increasing soil pH to 8.5

Ex: Application of Sulphur (900 Kg/ha) to soil brings the soil pH to 5.2 and reduces the incidence of common scab of potato (*Streptomyces scabies*).

**ij) Changing time of sowing:** Pathogens are able to infect susceptible plants under certain environmental conditions. Alternation in date of sowing can help avoidance of favourable conditions for the pathogens.

Ex: Rice blast can be managed by changing planting season from June to September/October.

**j) Seed rate and plant density:** Close spacing raises atmospheric humidity and favours sporulation by many pathogenic fungi. A spacing of 8'X8' instead of 7'X7' reduces sigatoka disease of banana due to better ventilation and reduced humidity. High density planting in chillies leads to high incidence of damping off in nurseries.

**k) Irrigation and drainage:** The amount, frequency and method of irrigation may affect the dissemination of certain plant pathogens. Many pathogens, including, *Pseudomonas solanacearum*, *X. campestris pv. oryzae* and *Colletotrichum falcatum* are readily disseminated through irrigation water. High soil moisture favours root knot and other nematodes and the root rots caused by species of *Sclerotium*, *Rhizoctonia*, *Pythium*, *Phytophthora*, *Phymatotrichum*, etc.

## LECTURE 14

**PHYSICAL METHODS:** Physical methods include soil solarization and hot water treatments.

**i. Soil solarization:** Soil solarization or slow soil pasteurization is the hydro/thermal soil heating accomplished by covering moist soil with polyethylene sheets as soil mulch during summer months for 4-6 weeks. Soil solarization was developed for the first time in Israel (Egley and Katan) for the management of plant pathogenic pests, diseases and weeds.

**ii. Soil sterilization:** Soil can be sterilized in green houses and sometimes in seed beds by aerated steam or hot water. At about 50°C, nematodes, some oomycetous fungi and other water molds are killed. At about 60 and 72°C, most of the plant pathogenic fungi and bacteria are killed. At about 82°C, most weeds, plant pathogenic bacteria and insects are killed. Heat tolerant weed seeds and some plant viruses, such as TMV are killed at or near the boiling point (95-100°C).

**iii. Hot water or Hot air treatment:** Hot water treatment or hot air treatment will prevent the seed borne and sett borne infectious diseases. Hot water treatment of certain seeds, bulbs and nursery stock is done to kill many pathogens present in or on the seed and other propagating materials. Hot water treatment is used for controlling sett borne diseases of sugarcane [whip smut, grassy shoot and red rot of sugarcane (52°C for 30 min)] and loose smut of wheat (52°C for 10 min).

### **Biological methods:**

**Def:** Biological control of plant disease is a condition or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except human beings), with the result that there is reduction in incidence of the disease caused by the pathogen (Garett, 1965).

**Def:** Biological control is the reduction of inoculum density or disease producing activity of a pathogen or a parasite in its active or dormant state by one or more organisms accomplished naturally or through manipulation of the environment of host or antagonist by mass introduction of one or more antagonists (Baker and Cook, 1974)

### **Mechanisms of biological control**

**1. Competition:** Most of the biocontrol agents are fast growing and they compete with plant pathogens for space, organic nutrients and minerals. Most aerobic and facultative anaerobic micro-organisms respond to low iron stress by producing extracellular, low molecular weight (500-1000 daltons) iron transport agents, designated as **Siderophores**, which selectively make complex with iron ( $Fe^{3+}$ ) with very high affinity. Siderophore producing strains are able to utilize  $Fe^{3+}$  - Siderophore complex and restrict the growth of deleterious micro-organisms mostly at the plant roots. Iron starvation prevents the germination of spores of fungal pathogens in rhizosphere as well as rhizoplane. Siderophores produced by

*Pseudomonas fluorescens* (known as **pseudobactins** or **pyoverdins**) helps in the control of soft rot bacterium, *Erwinia caratovora*.

**2. Antibiosis:** Antagonism mediated by specific or non-specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances is known as antibiosis.

**a. Antibiotics:** Antibiotics are generally considered to be organic compounds of low molecular weight produced by microbes. At low concentrations, antibiotics are deleterious to the growth or metabolic activities of other micro-organisms.

Ex: *Gliocladium virens* produces **gliotoxin** that was responsible for the death of *Rhizoctonia solani* on potato tubers.

Ex: Colonization of pea seeds by *Trichoderma viride* resulted in the accumulation of significant amount of the antibiotic **viridin** in the seeds, thus controlling *Pythium ultimum*.

Ex: Some strains of *Pseudomonas fluorescens* produce a range of compounds, viz., 2,4-diacetyl phloroglucinol (DAPG), phenazines, pyocyanin, which have broad spectrum activity against many plant pathogenic bacteria and fungi

b. **Bacteriocins:** These are antibiotic like compounds with bactericidal specificity closely related to the bacteriocin producer. Ex: The control of crown gall (caused by *Agrobacterium tumefaciens*) by the related *Agrobacterium radiobacter* strain K 84 is by the production of bacteriocin, **Agrocin K84**.

c. **Volatile compounds:** Antibiosis mediated by volatile compounds has been observed in the management of soil borne pathogens, viz., *Pythium ultimum*, *Rhizoctonia solani* and *Verticillium dahlia*, by *Enterobacter cloacae*. The volatile fraction responsible for inhibition was identified as ammonia.

3. **Hyperparasitism:** Direct parasitism or lysis and death of the pathogen by another micro-organism when the pathogen is in parasitic phase is known as hyperparasitism.

Ex: *T. harzianum* parasitize and lyse the mycelia of *Rhizoctonia* and *Sclerotium*.

### **Biocontrol agents for the management of plant pathogens**

<b>Biocontrol agent</b>	<b>Pathogen/disease</b>
1. <i>Ampelomyces quisqualis</i>	Powdery mildew fungi
2. <i>Darluca filum</i> , <i>Verticillium lecanii</i>	Rust fungi
3. <i>Pichia gulliermondii</i>	<i>Botrytis</i> , <i>Penicillium</i>

<b>Biocontrol agent</b>	<b>Nematode</b>
1. <i>Pasteuria penetrans</i> (Bacteria)	Juvenile parasite of root knot nematode
2. <i>Paecilomyces lilacinus</i> (Fungus)	Egg parasite of <i>Meloidogyne incognita</i>

### **Important fungal biocontrol agents:**

Most of the species of Trichoderma, viz., *T. harzianum*, *T. viride*, *T. virens* (*Gliocladium virens*) are used as biocontrol agents against soil borne diseases, such as, root rots, seedling rots, collar rots, damping off and wilts caused by the species of Pythium, Fusarium, Rhizoctonia, Macrophomina, Sclerotium, Verticillium, etc.

Formulations of biocontrol agents available: *T. viride* (**Ecofit**, **Bioderma** in India), *G. virens* (**GlioGard** in USA), *T. harzianum* (**F-Stop** in USA) and *T. polysporum* (**BINAB-T**)

### **Important bacterial biocontrol agents:**

1. *Pseudomonas fluorescens* (**Dagger-G** against damping off of cotton seedlings in USA)
2. *Bacillus subtilis* (**Kodiak** against damping off and soft rot in USA)
3. *Agrobacterium radiobacter* K-84 (**Gallex** or **Galltrol** against crown gall of stone fruits caused by *Agrobacterium tumefaciens*)

### **Plant growth promoting Rhizobacteria (PGPR):**

Rhizosphere bacteria that favourably affect plant growth and yield of commercially important crops are designated as plant growth promoting rhizobacteria. The growth promoting ability of PGPR is due to their ability to produce phytohormones, Siderophores, Hydrogen cyanide (HCN), chitinases, volatile compounds or antibiotics which will reduce infection of host through phyto-pathogenic micro-organisms.

Many bacterial species, viz., *Bacillus subtilis*, *Pseudomonas fluorescens*, etc., are usually used for the management of plant pathogenic microbes. *Bacillus* has ecological advantages as it produces endospores that are tolerant to extreme environmental conditions. *Pseudomonas fluorescens* have been extensively used to manage soil borne plant pathogenic fungi due to their ability to use many carbon sources that exude from the roots and to compete with microflora by the production of antibiotics, HCN and Siderophores that suppress plant root pathogens.

## LECTURE 15

**PROTECTION:** Use of chemicals for the control of plant diseases is generally referred to as protection or therapy.

**Protection:** The prevention of the pathogen from entering the host or checking the further development in already infected plants by the application of chemicals is called protection and the chemicals used are called **protectants**.

**Therapy** means cure of a disease, in which fungicide is applied after the pathogen is in contact with the host. Chemicals used are called **therapeutants**.

**Fungicide:** Any agent (chemical) that kills the fungus

**Fungistat:** Some chemicals which do not kill fungi, but simply inhibit the fungus growth temporarily.

**Antisporulant:** The chemical which inhibits spore production without affecting vegetative growth of the fungus.

Fungicides are classified into three categories: Protectants, eradicants and therapeutants.

**1. Protectants:** These are the chemicals which are effective only when used before infection (prophylactic in behavior). Contact fungicides which kill the pathogen present on the host surface when it comes in contact with the host are called protectants. These are applied to seeds, plant surfaces or soil. These are non-systemic in action (i.e, they cannot penetrate plant tissues). Ex: Zineb, sulphur, captan, Thiram, etc.

**2. Eradicants:** Those chemicals which eradicate the dormant or active pathogen from the host. They can remain on/in the host for some time. Ex: Lime sulphur, Dodine.

**3. Therapeutants:** These are the agents that inhibit the development of a disease syndrome in a plant when applied after infection by a pathogen. Therapy can be by physical means (solar and hot water treatment) and chemical means (by use of systemic fungicides, i.e., chemotherapy).

### **CLASSIFICATION OF FUNGICIDES BASED ON CHEMICAL NATURE**

Many fungicides have been developed for purpose of managing crop diseases which may be used as sprays, dusts, paints, pastes, fumigants, etc. The discovery of Bordeaux mixture in 1882 by Professor Millardet, University of Bordeaux, France led to the development of fungicides. Major group of fungicides used include salts of toxic metals and organic acids, organic compounds of sulphur and mercury, quinones and heterocyclic nitrogenous compounds. Copper, mercury, zinc, tin and nickel are some of the metals used as base for inorganic and organic fungicides. The non metal substances include, sulphur, chlorine, phosphorous etc. The fungicides have been classified based on their chemical nature as follows

**COPPER FUNGICIDES:** Copper fungicides can be classified as preparatory and proprietary copper compounds.

#### **PREPARATORY COPPER FUNGICIDES**

Common name	Chemical composition	Diseases managed
1. Bordeaux mixture	It is prepared by suspending 5 Kg of copper sulphate and 5 Kg of lime in 500 liters of water (1%)	Downy mildew of grapes, Coffee rust, Tikka leaf spot of groundnut, citrus canker, citrus scab, etc.
2. Bordeaux paste	It is prepared by mixing 1 Kg of copper sulphate and 1 Kg of lime in 10 liters of water	It is a wound dressing fungicide and can be applied to the pruned parts of the host plants such as fruit crops and ornamentals. Ex: Citrus gummosis, Stem bleeding of coconut, Bud rot of coconut, etc.

3. Burgundy mixture	Sodium carbonate is used in place of lime. It is prepared by mixing 1 Kg of copper sulphate and 1 Kg of sodium carbonate in 100 liters of water	Downy mildew of grapes, Coffee rust, Tikka leaf spot of groundnut, citrus canker, citrus scab
4. Cheshunt compound	It is a compound prepared by mixing 2 parts of copper sulphate and 11 parts of ammonium carbonate	It is used for soil drenching only. Sclerotial wilt diseases of chilli, tomato and groundnut. Fusarial wilt diseases. Damping-off diseases of solanaceous crops.
5. Chaubattia paste	It is a compound prepared by mixing 800g of copper sulphate and 800g of red lead in 1 liter of lanolin or linseed oil	Pink disease of citrus, stem canker and collar rot of apple and pears

**Proprietary copper fungicides or Fixed or insoluble copper fungicides:** In the fixed or insoluble copper compounds, the copper ion is less soluble than in Bordeaux mixture. So, these are less phytotoxic than Bordeaux mixture but are effective as fungicides.

Common name	Trade name	Dosage	Disease managed
1. Copper oxy chloride	Blitox-50, Blue copper-50, Cupramar-50	0.3 to 0.5% for foliar application, 25 to 35 Kg/ha for dusting	Anthracoise of grapevine, Tikka leaf spot of groundnut, Sigatoka leaf spot of banana, citrus canker, black arm of cotton
2. Cuprous oxide	Fungimar and Perenox	0.3% for foliar spray	Anthracoise of grapevine, Tikka leaf spot of groundnut, Sigatoka leaf spot of banana, citrus canker, black arm of cotton
3. Copper hydroxide	Kocide	0.3% for foliar spray	Blister blight of tea, False smut of rice, Tikka leaf spot of groundnut

### SULPHUR FUNGICIDES

Sulphur is probably the oldest chemical used in plant disease management for the control of powdery mildews and can be classified as inorganic sulphur and organic sulphur. Inorganic sulphur fungicides include lime sulphur and elemental sulphur fungicides. Organic sulphur fungicides, also called as carbamate fungicides, are the derivatives of dithiocarbamic acid.

### INORGANIC SULPHUR FUNGICIDES

Common name	Trade name	Dosage	Disease managed
Preparatory sulphur compounds			
1. Lime sulphur	It is prepared by mixing 20 Kg of rock lime and 15 Kg of sulphur in 500 liters of water	10-15 liters in 500 liters of water	Powdery mildew of apple, Apple scab, bean rust
2. Sulphur dust	Kolo dust, Mico-999	4-5g/Kg seed for ST, 10-30 Kg/ha for dusting on crops, 100 Kg /ha for soil application in tobacco, 500 Kg/ha for furrow application in potato	Common scab of potato, Grain smut of jowar, Powdery mildew of tobacco, chilli, rose, mango, grapes, etc.
3. Wettable sulphur	Sulfex, Thiovit, Cosan	0.2-0.4 % for foliar spray	Powdery mildews of various crops

## ORGANIC SULPHUR COMPOUNDS

Organic sulphur compounds are derived from dithiocarbamic acid and are widely used as spray fungicides. In 1931, Tisdale and Williams were the first to describe the fungicidal nature of Dithiocarbamates. Dithiocarbamates can be categorized into two groups, viz., dialkyl dithiocarbamates (ziram, ferbam and thiram) and monoalkyl dithiocarbamates (nabam, zineb, vapam and maneb).

Common name	Trade name	Dosage	Diseases managed
<b>Dialkyl Dithiocarbamates</b>			
1. Ziram	Ziride, Hexazir, Milbam, Zerlate	0.15 to 0.25% for foliar spray	Anthracoise of pulses, tomato, beans, tobacco, etc., bean rust
2. Ferbam	Coromet, Ferbam, Fermate, Fermocide, Hexaferb, Karbam Black	0.15 to 0.25% for foliar spray	Fungal pathogens of fruits and vegetables, leaf curl of peaches, apple scab, downy mildew of tobacco
3. Thiram	Arasan, Hexathir, Tersan, Thiram, Thiride	0.15 to 0.2% as foliar spray, 0.2-0.3% as dry seed treatment, 15-25Kg/ha as soil application	Soil borne diseases caused by <i>Pythium</i> , <i>Rhizoctonia solani</i> , <i>Fusarium</i> , etc. Rust of ornamental crops, Scab on pears and <i>Botrytis</i> spp. on lettuce
<b>Monoalkyl dithiocarbamates</b>			
1. Nabam	Chembam, Dithane D-14, Dithane A-40 and Parzate liquid	0.2% as foliar spray	Used as foliar spray against leaf spot diseases of fruits and vegetables. Also used against soil borne pathogens, <i>Fusarium</i> , <i>Pythium</i> and <i>Phytophthora</i>
2. Zineb	Dithane Z-78, Hexathana, Lanocol and Parzate	0.1 to 0.3% for foliar application	Chilli die-back and fruit rot, Apple scab, Maize leaf blight, early blight of potato
3. Vapam or Metham sodium	Chem-vape, vapam, vitafume, VPM	1.5 to 2.5 liters per 10 m <sup>2</sup> area	Fungicide with fungicidal, nematicidal and insecticidal properties. Soil fungal pathogens like <i>Fusarium</i> , <i>Puthium</i> , <i>Sclerotium</i> and <i>Rhizoctonia</i> .
4. Maneb	Dithane M22, Manzate and MEB. Mancozeb (78% Maneb + 2% zinc ion): Dithane M 45, Indofil M 45	0.2% to 0.3% as foliar application	Early and late blight of potato and tomato, rust diseases of field and fruit crops

## HETROCYCLIC NITROGENOUS COMPOUNDS

The group of heterogeneous fungicides includes some of the best fungicides like captan, folpet, captafol, vinclozoline and Iprodione. Captan, folpet and captafol belong to dicarboximides and are known as phthalamide fungicides. The new members of dicarboximide group are Iprodione, vinclozolin, etc.



Common name	Trade name	Dosage	Diseases managed
1. Captan (Kittleson's killer)	Captan 50W, Captan 75 W, Ezzo fungicide, Orthocide 406, Hexacap, Vancide 89	0.2 to 0.3% for dry seed treatment, 0.2 to 0.3% for foliar spray, 25 to 30 Kg/ha for furrow application	Onion smut, Chilli die-back and fruit rot, Damping off of beans, chilli and tomato, seed rots and seedling blights of maize
2. Folpet	Phaltan	0.1 to 0.2% for spraying	Apple scab, tobacco brown spot, rose black spot
3. Captafol	Difosan, Difolaton, Sanspor, Foltaf	0.15 to 0.2% for spraying, 0.25% for seed treatment, 0.15% for soil drenching	Sorghum anthracnose, cotton seedling diseases, seed rot and seedling diseases of rice, downy mildew of crucifers, apple scab
4. Iprodione	Rovral, Glycophene	0.1 to 0.2% for foliar application	Diseases caused by <i>Botrytis</i> , <i>Monilinia</i> , <i>Alternaria</i> , <i>Sclerotinia</i> , <i>Helminthosporium</i> and <i>Rhizoctonia</i>
5. Vinclozolin	Ornalin, Ronilan, Vorlan	0.1 to 0.2% for foliar application	Effective against sclerotia forming fungi like <i>Botrytis</i> , <i>Monilinia</i> and <i>Sclerotinia</i>

#### MISCELLANEOUS FUNGICIDES

Common name	Trade name	Dosage	Diseases managed
1. Chlorothalonil	Bravo, Daconil, Kavach, Thermil, Exotherm, Safeguard	0.2 to 0.3% for foliar application	A broad spectrum contact fungicide often used in greenhouses for control of <i>Botrytis</i> on ornamentals and for several molds and blights of tomato. Also used for the control of sigatoka leaf spot of banana, onion purple blotch, tikka leaf spot and rust of groundnut
2. Dinocap	Karathane, Arathane, Capryl, Mildex, Mildont and crotothane	0.1 to 0.2% for spraying	It is a good acaricide and contact fungicide and it controls powdery mildews of fruits and ornamentals effectively. This can be safely used on sulphur sensitive crops like cucurbits and apple varieties for control of powdery mildews

3. Dodine	Cyprex, Melprex, Guanidol and Syllit	0.075% for spraying	Apple scab, black spot of roses and cherry leaf spot
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### SYSTEMIC FUNGICIDES

The systemic fungicides were first introduced by Von Schelming and Marshall Kulka in 1966. The discovery of Oxathiin fungicides was soon followed by confirmation of systemic activity of pyrimidines and benzimidazoles. A systemic fungicide is capable of managing a pathogen remote from the point of application. On the basis of chemical nature these fungicides are classified as follows

Common name	Trade name	Dosage	Diseases managed
<b>ACYLALANINES</b>			
1. Metalaxyl	Ridomil 25% WP, Apron 35 SD, Subdue, Ridomil MZ-72WP	3-6 g/Kg seed for seed treatment, 1 to 1.5 Kg a.i/ha for soil application, 0.1 to 0.2% for foliar spray	It is highly effective against <i>Pythium</i> , <i>Phytophthora</i> and many downy mildew fungi
2. Benalaxyl	Galben 25% WP and 5% G	0.1 to 0.2% for foliar spray, 1 to 1.5 Kg a.i/ha for soil application	Blue mold of tobacco, late blight of potato and tomato, downy mildew of grapevine
<b>AROMATIC HYDROCARBONS</b>			
1. Chloroneb	Demosan	0.2% for seed treatment	Seedling diseases of cotton, peanut, peas and cucurbits caused by species of <i>Pythium</i> , <i>Phytophthora</i> , <i>Rhizoctonia</i> and <i>Sclerotium</i>
<b>BENZIMIDAZOLES</b>			
1. Carbendazim	Bavistin 50WP, MBC, Derosol 60WP, Agrozim, Zoom	0.1% for foliar spray, 0.1% for soil drench, 0.25% for ST, 500-1000ppm for post-harvest dip of fruits	Effectively controls anthracnose, powdery mildews and rusts caused by various fungi. It is also used as a soil drench against wilt diseases and for post harvest treatment of fruits
2. Benomyl	Benlate 50WP	0.1 to 0.2% for ST, 50-60g/100 L for foliar spray, 50-200ppm for soil drenching, 12-45 Kg a.i/ha for soil broadcast, 100-500 ppm for post harvest fruit dip	Effective against powdery mildews of cucurbits, cereals and legumes. It is highly effective against diseases caused by the species of <i>Rhizoctonia</i> , <i>Theilaviopsis</i> and <i>Cephalosporium</i> . Benomyl has no effect against Oomycetes and some dark coloured fungi such as <i>Alternaria</i> and <i>Helminthosporium</i>
3. Thiabendazole	Mertect 60WP, Mycozol, Arbotect, Tecto and Storite	0.2 to 0.3% for spraying, 1000 ppm for fruit dip	Blue and green molds of citrus, loose smut of wheat, Tikka leaf spot of groundnut

<b>ALIPHATICS</b>			
1. Prothiocarb	Previcur, Dynone	5.6 Kg a.i/ha for soil application	Highly active against soil borne Oomycetes like <i>Pythium</i> and <i>Phytophthora</i>
2. Propamocarb	Previcur-N, Dynone-N, Prevex, Benol	3.4 and 4.8 Kg a.i/ha for soil application	Effective against soil borne Oomycetes like <i>Pythium</i> and <i>Phytophthora</i>
<b>OXATHINS or CARBOXIMIDES</b>			
1. Carboxin	Vitavax 75WP, Vitaflow	0.15 to 0.2% for seed treatment, 0.5% for spraying	Highly effective against smut diseases. Commonly used for the control of loose smut of wheat, onion smut, grain smut of sorghum. As a soil drench it is used for the control of diseases caused by <i>Rhizoctonia solani</i> and <i>Macrophomina phaseolina</i> .
2. Oxycarboxin	Plantavax 75 WP, Plantavax 20EC, Plantavax 5% liquid	0.1 to 0.2% for foliar spray, 0.2 to 0.5% for ST	Highly effective against rust diseases. Commonly used for the control of rusts of wheat, sorghum, safflower, legumes, etc.
<b>IMIDAZOLES</b>			
1. Imazalil	Fungaflor, Bromazil and Nuzone	0.1 % as post harvest dip	Blue and green molds of citrus
2. Fanapanil	Sistane 25 EC	0.05% foliar spray	Spot blotch of barley, loose and covered smut of barley
<b>MORPHOLINES</b>			
1. Tridemorph	Calixin 75EC, Bardew, Beacon	0.1% for foliar spray	Powdery mildew of cereals, vegetables and ornamentals. Rusts of pulses, groundnut and coffee, Sigatoka leaf spot of banana, pink disease of rubber, Ganoderma root rot & wilt
<b>ORGANOPHOSPHATES</b>			
1. Iprobenphos	Kitazin 48EC, Kitazin 17G, Kitazin 2% D	30-45 Kg of granules/ha, 1 to 1.5 liters of 48% EC in 1000 ml of water for foliar spray	Fungicide with insecticidal properties. Highly specific against rice blast, stem rot and sheath blight of rice
2. Ediphenphos	Hinosan 30 and 50% EC, Hinosan 2%D	400 to 500 ppm for spraying, 30 to 40 Kg/ha	Highly specific against rice blast, stem rot and sheath blight of rice
<b>ALKYL PHOSPHONATES</b>			
1. Fosetyl-Al or Aluminium Tris	Aliette 80WP	0.15% for foliar spray, 0.2% for soil drench	Ambimobile fungicide. Specific against Oomycetes fungi

<b>PYRIMIDINES</b>			
1. Fenarimol	Rubigan 50% WP, 12%EC	2g/Kg seed as ST, 20 to 40 ml/100 liters of water for spraying	Powdery mildew of cucurbits, apple, mango, roses, grapes and ornamental crops
<b>THIOPHANATES</b>			
1. Thiophanate	Topsin 50WP, Cercobin 50WP	0.1 to 0.2% for spraying	Powdery mildew of cucurbits and apple, club root of crucifers, rice blast
2. Thiophanate methyl	Topsin M 70WP, Cercobin M 70WP	0.1% for spraying	Blast and sheath blight of rice, sigatoka leaf spot of banana, powdery mildew of beans, chilli, peas and cucurbits
<b>TRIAZOLES</b>			
1. Triadimefon	Bayleton, Amiral	0.1 to 0.2% for spraying, 0.1% for seed treatment	Highly effective against powdery mildews and rusts of several crops. Effective against diseases caused by species of <i>Erysiphe</i> , <i>Sphaerotheca</i> , <i>Puccinia</i> , <i>Uromyces</i> , <i>Phakopsora</i> , <i>Hemileia</i> and <i>Gymnosporangium</i>
2. Tricyclazole	Beam 75WP, Baan 75WP, Trooper 75WP	2g/Kg seed for ST, 0.06% for spraying	Highly effective against blast of rice
3. Bitertanol	Baycor and Sibutol	0.05 to 0.1% for foliar spray	Powdery mildews and rusts of various crops, apple scab, <i>Monilinia</i> on fruit crops, late leaf spot of groundnut and sigatoka leaf spot of banana
4. Hexaconazole	Contaf 5%EC, Anvil	0.2% for spraying	Sheath blight of rice, powdery mildew and rust of apple, rust and tikka leaf spot of groundnut
5. Propiconazole	Tilt, 25% EC, Desmel	0.1% for foliar application	Sheath blight of rice, Sigatoka leaf spot of banana, brown rust of wheat
6. Myclobutanil	Systhane 10WP	0.1 to 0.2% for spraying	Apple scab, cedar apple rust and powdery mildew of apple
<b>STROBILURINS</b>			
1. Azoxystrobin	Amistar, Quadris	0.1% for spraying	Broad spectrum fungicide
2. Kresoxim methyl	Ergon, Discus, Stroby	0.1% for spraying	Commonly used for control of ornamental diseases

**CLASSIFICATION OF FUNGICIDES BASED ON METHOD OF APPLICATION**  
The fungicides can also be classified based on the nature of their use in managing the diseases.

1. Seed protectants: Ex. Captan, thiram, carbendazim, carboxin etc.
2. Soil fungicides (preplant): Ex. Bordeaux mixture, copper oxy chloride, Chloropicrin, Formaldehyde, Vapam, etc.
3. Soil fungicides: Ex. Bordeaux mixture, copper oxy chloride, Captan, PCNB, thiram etc.
4. Foliage and blossom: Ex. Captan, ferbam, zineb, mancozeb, chlorothalonil etc.
5. Fruit protectants: Eg. Captan, maneb, carbendazim, mancozeb etc.
6. Eradicants: EX. Lime sulphur
7. Tree wound dressers: Ex. Boreaux paste, chaubattia paste, etc.
8. General purpose sprays and dust formulations.

### **HOST PLANT RESISTANCE (IMMUNIZATION)**

**Disease resistance:** It is the ability of a plant to overcome completely or in some degree the effect of a pathogen or damaging factor.

**Susceptibility:** The inability of a plant to resist the effect of a pathogen or other damaging factor.

#### **Advantages of resistant varieties:**

1. Resistant varieties can be the most simple, practical, effective and economical method of plant disease management.
2. They not only ensure protection against plant diseases but also save the time, energy and money spent on other measures of control
3. Resistant varieties, if evolved can be the only practical method of control of diseases such as wilts, viral diseases, rusts, etc.
4. They are non-toxic to human beings, animals and wild life and do not pollute the environment
5. They are effective only against the target organisms, whereas, chemical methods are not only effective against target organisms but also effective against non-target organisms.
6. The resistance gene, once introduced, is inherited and therefore permanent at no extra cost.

#### **Disadvantages:**

1. Breeding of resistant varieties is a slow and expensive process
2. Resistance of the cultivar may be broken down with the evolution of the pathogen

#### **Types of resistance:**

1. **Vertical resistance:** When a variety is more resistant to some races of the pathogen than others, the resistance is called vertical resistance (race-specific resistance, qualitative resistance, discriminatory resistance). Vertical resistance is usually governed by single gene and is unstable.

2. **Horizontal resistance:** When the resistance is uniformly spread against all the races of a pathogen, then it is called horizontal/generalized/non-specific/field/qualitative resistance. Horizontal resistance is usually governed by several genes and is more stable.

3. **Monogenic resistance:** When the defense mechanism is controlled by a **single gene pair**, it is called monogenic resistance.

4. **Oligogenic resistance:** when the defense mechanism is governed by a **few gene pairs**, it is called oligogenic resistance.

5. **Polygenic resistance:** When the defense mechanism is controlled by **many genes** or more groups of supplementary genes, it is called polygenic resistance.

**Cross protection:** The phenomenon in which plant tissues infected with mild strain of a virus are protected from infection by other severe strains of the same virus. This strategy is used in the management of severe strains of *Citrus Tristeza virus*

## **INTEGRATED PLANT DISEASE MANAGEMENT (IPDM)**

IPDM involve management systems which utilize compatible combinations of all the available techniques to keep the pathogen population below the economic threshold level (ETL) which would not result in economically unacceptable damage to the crop. IPDM is based on five principles of plant disease management and integrates multidisciplinary approaches for the management of plant diseases.

### **Main components of IPDM:**

1. Cultural practices
2. Regulatory measures (quarantine)
3. Chemical methods
4. Biological methods
5. Physical methods
6. Genetic engineering

### **Main strategies of IPDM:**

1. Need based application of pesticides
2. Encouragement and enhancement of biocontrol agents
3. Use of resistant or tolerant cultivars of plants
4. Modification of cultural practices
5. Use of any other strategies that interrupts host-pathogen interactions

### **Advantages of IPDM:**

1. Avoids chemical pollution of soil, water, air and food products
  2. Avoids development of resistance in the plant pathogens against fungicides
  3. It is an eco-friendly strategy for management of plant diseases
  4. It is an economically feasible approach
  5. It is a multipronged strategy for efficient management of plant diseases
- Therefore, IPDM utilizes all suitable strategies in a compatible manner to reduce and maintain pathogen populations at levels below those causing economic losses.

### **Rice diseases and IPDM:**

Fungal diseases

1. Blast: Foliar disease and the pathogen survives on collateral hosts
2. Brown spot of rice – Seed borne and a foliar disease
3. Sheath rot, sheath blight, foot rot and stem rot – Soil borne diseases
4. False smut – seed borne disease

Bacterial diseases: Bacterial leaf blight and bacterial leaf streak – Seed borne and survives on collateral hosts and weeds

Viral or Phytoplasmal diseases – Rice tungro virus, Rice yellow dwarf – Survives on weeds and dissemination is by insect vectors

### **IPDM strategy in rice:**

1. Selection of healthy seed
2. Selection of resistant cultivars
3. Removal and destruction of collateral hosts
4. Balanced fertilization
5. Rouging of diseased plants
6. Seed treatment with carbendazim or tricyclazole at 2g/Kg seed
7. Need based foliar application of [carbendazim@0.1%](#) or [Tricyclazole@0.06%](#) for the management of blast.
8. Need based foliar application of validamycin for the management of sheath blight and sheath rot.
9. Soil application of carbofuran granules or foliar spray of any systemic fungicide is followed to manage insect vectors, thereby decreasing the spread of viral diseases.

### **Sugarcane diseases and IPDM**

1. Red rot – sett borne disease which spreads through irrigation water
2. Whip smut - sett borne and disseminate through wind borne sporidia

3. Pine apple disease, sett rot – Sett borne disease
4. Grassy shoot – Vector borne Phytoplasmal disease
5. Ratoon stunting – Sett borne (*Clavibacter xyli*)
6. Sugarcane mosaic – Survives on weeds and disseminated by insect vectors

**IPDM in sugarcane:**

1. Collection and destruction of infected crop debris
2. Hot water treatment of setts (52<sup>0</sup>C for 30 min)
3. Hot air treatment of setts (54<sup>0</sup>C for 2-3 hrs)
4. Balanced irrigation and fertilization
5. Avoid selection of seed material from Ratoon crop
6. Need based spray of systemic insecticides to minimize the spread of viral and Phytoplasmal diseases
7. Selection of disease resistant or tolerant cultivars

## LECTURE 16

**Biotechnology:** It is defined as genetic modification and manipulation of living organisms through the novel technologies such as tissue culture and genetic engineering resulting in production of improved or new organisms that can be used in variety of ways.

### APPLICATION OF BIOTECHNOLOGY IN PLANT DISEASE MANAGEMENT:

#### **1. Diagnosis of plant diseases**

- a) Diagnostic kits helps in identification of plant diseases, viz., bacterial canker of tomato, soybean root rot, viral diseases of potato, etc., at an early stage of development and helps in devising suitable management practices.
- b) Polymerase Chain Reaction (PCR): Detection of very small amount of pathogen in a sample by amplifying the pathogen sequences to a detectable level. PCR is especially used in plant quarantine.

#### **2. Strain improvement of biocontrol agents:** It has the following advantages

- a) Expanding the range of target species
- b) Restricting the range of non-target species
- c) To improve the survival ability or rhizosphere competence
- d) Expanding the bio-agents environmental range beyond its congenial habitat
- e) Development of fungicide tolerant strains

#### **3. Transgenics for plant disease management**

- a) Coat protein mediated resistance for papaya ring spot virus in Hawaii islands
- b) Cloning of resistance genes, viz., *Xa 21*, bacterial blight resistance gene isolated from African rice, *Oryza longistaminata* was introduced into cultivable rice, *Oryza sativa*

**4. Determination of biochemical nature** and the signals involved in plants reaction to pathogen invasion and disease development. Ex: Host-pathogen interaction has been studied in rice blast disease incited by *Magnaporthe grisea*.

**5. Manipulation of resistance of host** by expression of PR-proteins, antifungal peptides, etc. Ex: Expression of multiple PR-proteins (Chitinases and  $\beta$ -1,3 glucanases) in rice enhanced disease resistance to rice sheath blight pathogen, *Rhizoctonia solani*.

**PLANT TISSUE CULTURE:** *In vitro* culture of plant cells, tissues as well as organs.

**Totipotency** is the ability of a plant cell to perform all the functions of development which are characteristic of zygote, i.e., its ability to develop in to a complete plant.

### IMPORTANT TISSUE CULTURE TECHNIQUES OF IMPORTANCE TO PLANT PATHOLOGY:

1. Meristem tip culture
2. Protoplast culture

#### **A. Production of virus free plants through plant tissue culture:**

**Meristem tip culture:** Cultivation of axillary or apical meristems, particularly of shoot apical meristem, is known as meristem culture.

**1. Explant:** the explant must consist of the meristematic dome of cells together with atleast one leaf primordial. Meristem tips varying in size from 0.1 to 2.0 mm in diameter (usually 0.3-1.5 mm) can be used for meristem tip culture. The infected parent plant or organ of the plant from which explant is excised is generally subjected to thermotherapy in a temperature controlled cabinet at 30<sup>0</sup>C to 40<sup>0</sup>C for six to twelve weeks to inactivate the virus.

**2. Culture initiation on suitable medium:** In general Murashige and Skoog medium has been found satisfactory for most plant species. But for some species, a much lower salt concentration may be adequate or even necessary since the high salt concentration of MS medium may be deleterious or even toxic. Culture initiation consists of surface sterilization of explants and establishing them *in vitro* on culture medium. Culture



initiation often involves anti-metabolite chemicals such as ribavirin (virazole) in the tissue culture medium.

**3. Shoot multiplication:** After 2-3 weeks, the cultures are transferred to a shoot multiplication medium designed to promote axillary branching. This medium generally contains cytokinins, either alone or in combination with an auxin. Higher concentration of cytokinins induces adventitious buds. During culture initiation and shoot multiplication phases, the cultures are generally kept at 25<sup>0</sup>C.

**4. Rooting of shoots:** In general, the rooting medium has low salt (1/2 or even 1/4 salts of MS medium) and reduced sugar levels. But in most species, 0.1-1 mg/l Naphthalene Acetic Acid (NAA) or Indole-3-Butyric acid (IBA) is required for rooting. Rooting takes about 10-15 days depending on species.

**5. Transfer of plantlets to soil:** Rooted shoots are removed from the medium, agar sticking to roots is washed with tap water, and they are transplanted into plastic cups containing a suitable potting mix. Plants are kept in high (>90%) humidity and initially low light intensities. The humidity is generally decreased to the ambient level after about 7-15 days, and the light intensity is increased. The plants are finally exposed to greenhouse conditions (**hardening**).

**6. Indexing, clone selection and stock maintenance:** Virus indexing is done several times during first year and the virus free plantlet is used as a nuclear stock material for commercial multiplication. Virus indexing is generally made by Enzyme Linked Immuno-Sorbent Assay (ELISA) or Immuno Sorbent Electron Microscopy (ISEM).

**B. Protoplast culture:** Fungal protoplasts are important tools in physiological and genetic research. Interspecific, intraspecific and intragenetic hybridization could be done by this technique for strain improvement of biocontrol agents to enhance the biocontrol potential for the management of pathogenic fungi. Isolation and self-fusion of protoplasts were achieved in *Trichoderma harzianum* and *T. viride*.

#### **Steps in protoplast fusion:**

1. Isolation of protoplasts is achieved by treating cells with a suitable mixture of cell wall degrading enzymes.
2. The pH of enzyme solution is adjusted between 4.7 and 6.0 and temperature is kept around 25-30<sup>0</sup>C. The osmotic concentration of enzyme mixture and of subsequent media is elevated to stabilize the protoplasts and to prevent them from bursting. Usually, 50-100 m mol/l CaCl<sub>2</sub> is added to the osmoticum as it improves plasma membrane stability.
3. The protoplasts of different strains are treated with 28-50% **Poly Ethylene Glycol (fusogen)** for 15-30 min followed by gradual washing of the protoplasts to remove PEG. The washing medium may be alkaline and contain high calcium ion concentration (50 m mol/l). Protoplast fusion occurs during washing step.
4. Selection of hybrid cells and culturing on suitable medium.

#### **Gene cloning/ Recombinant DNA technology / Genetic engineering:**

Integration of specific fragment of foreign DNA into a cell through a suitable vector in such a way that the inserted DNA replicate independently and transferred to progenies as a result of cell division.

Recombinant DNA molecule is a vector into which the desired DNA fragment has been inserted to enable its cloning in an appropriate host. Recombinant DNA molecule is produced by joining together two or more DNA segments usually originated from different organisms.

#### **Steps in gene cloning:**

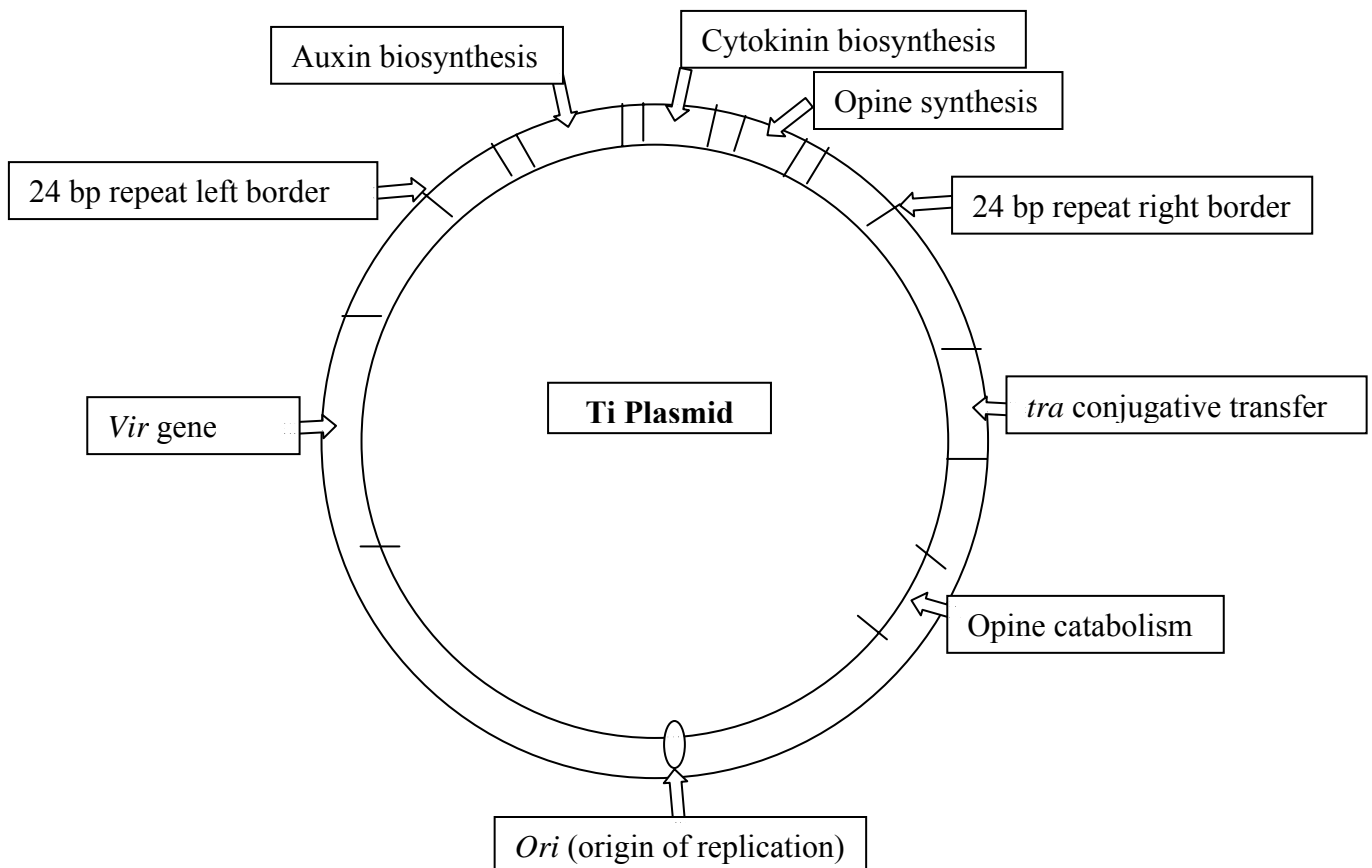
1. Identification and isolation of the desired gene or DNA fragment to be cloned (Restriction digestion and electrophoresis)
2. Insertion of the isolated gene in a suitable vector (ligation)
3. Introduction of this vector into a suitable organism or cell called host (transformation)
4. Selection of transformed host cells (selectable markers)
5. Multiplication / integration followed by expression of the introduced gene in the host

**Enzymes involved:** Restriction endonucleases, DNA ligases, DNA polymerases, RNA polymerases and reverse transcriptases.

**Vectors used in gene cloning:** A vector is a DNA molecule that has the ability to replicate in an appropriate host cell, and into which the DNA fragment to be cloned (called DNA insert) is integrated for cloning. Ex: Tumor inducing (Ti) plasmid of *Agrobacterium tumefaciens*, pBR322, Bacteriophages, cosmid vectors (derived from phage  $\lambda$ ).

**Ti plasmid of *Agrobacterium tumefaciens*:**

- Ti plasmid is a large conjugative plasmid or megaplasmid of about 200 kb.
- Ti plasmid has a T-DNA region (15-24 kb) which is bounded by a pair of 24 bp repeats. T-DNA carries genes for **auxin**, **cytokinins** and **opine** synthesis which are responsible for tumor formation (tumorigenesis).
- Transfer of T-DNA depends on 35 kb **virulence (vir) region** of the Ti plasmid. This region has 7 operons ranging from *vir A* to *vir H* (*vir A*, *vir B*, *vir C*, *vir D*, *vir E*, *vir G* and *vir H*). The protein products of these genes respond to phenolics to generate a copy of T-DNA and mediate its transfer into the cell.
- The T-DNA when transferred from the *Agrobacterium* to the plant cell integrates with the chromosome, and the plant cells which are affected begin to synthesize opines, auxins and cytokinins.



- Opines are tumor specific compounds formed by the condensation of amino acid, keto acid and sugar. The **opines (octopine, nopaline, succinamopine or leucinopine)** can be metabolized only by *Agrobacterium*.
- The IAA (auxin) and Isopentenyl-AMP (cytokinins) are phytohormones which cause the proliferation of plant cells and induction of the gall.
- Plant wound exudates contain phenolics, which attract *Agrobacterium* and induce *vir* genes. The strong *vir* gene inducers are syringic acid, ferulic acid, **acetosyringone** and sinapinic acid. Only *Agrobacterium* with Ti plasmid are attracted by these compounds.
- The exogenous DNA is inserted into the T-DNA region of the Ti plasmid by homologous recombination using an intermediate vector system or directly using binary vectors.

**DEVELOPMENT OF DISEASE RESISTANT TRANSGENIC PLANTS THROUGH TI PLASMID MEDIATED GENE TRANSFER:**

1. The appropriate gene construct is inserted within the T-DNA of a disarmed Ti plasmid; either a co-integrate or binary vector is used. The recombinant vector is placed in *Agrobacterium*, which is co-cultured with the plant cells or tissues to be transformed for about 2 days.
2. In case of many plant species, small (a few mm diameter) leaf discs are excised from surface sterilized leaves and used for co-cultivation. In general the transgene construct involves a selectable reporter gene (Bacterial *neo* gene), the presence of which confers resistance to kanamycin.
3. During the leaf disc-*Agrobacterium* co-culture, **acetosyringone** released by plant cells induces the *vir* genes which bring about the transfer of recombinant T-DNA into many of the plant cells. The **T-DNA** would become integrated into the plant genome, and the transgene would be expressed. As a result, the transformed plant cells would become resistant to kanamycin.
4. After 2 days, the leaf discs are transferred onto a regeneration medium containing appropriate concentrations of kanamycin and carbenicillin. **Kanamycin** allows only transformed plant cells to divide and regenerate shoots in about 3-4 weeks, while **carbenicillin** kills *Agrobacterium* cells. The shoots are separated, rooted and finally transferred into soil.

**B.Sc. Ag  
II Sem**

**Fundamentals of Plant  
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**Credit - 4(3+1)**

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