RAMA UNIVERSITY

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FACULTY OF AGRICULTURAL SCIENCES & ALLIED INDUSTRIES

Lecture 6

CHEMICAL WEAPONS OF PATHOGENS

Enzymes, Toxins and Growth Hormones in Plant Disease

Plant cells consist of cell wall, cell membranes, and cytoplasm, which contains the nucleus and various organelles (Fig. 1). The cytoplasm and the organelles it contains are separated from each other by membranes that carry various types of proteins embedded in them (Fig. 2). The intact, healthy plant is a community of cells built in a fortress-like fashion. Plant cells consist of cell wall, cell membranes, and cytoplasm, which contains the nucleus and various organelles and all the substances for which the pathogens attack them. The cytoplasm and the organelles it contains are separated from each other by membranes that carry various types of proteins embedded in them. The plant surfaces that come in contact with the environment either consist of cellulose, as in the epidermal cells of roots and in the intercellular spaces of leaf parenchyma cells, or consist of a cuticle that covers the epidermal cell walls, as is the case in the aerial parts of plants. Often an additional layer, consisting of waxes, is deposited outside the cuticle, especially on younger parts of plants (Fig. 3).

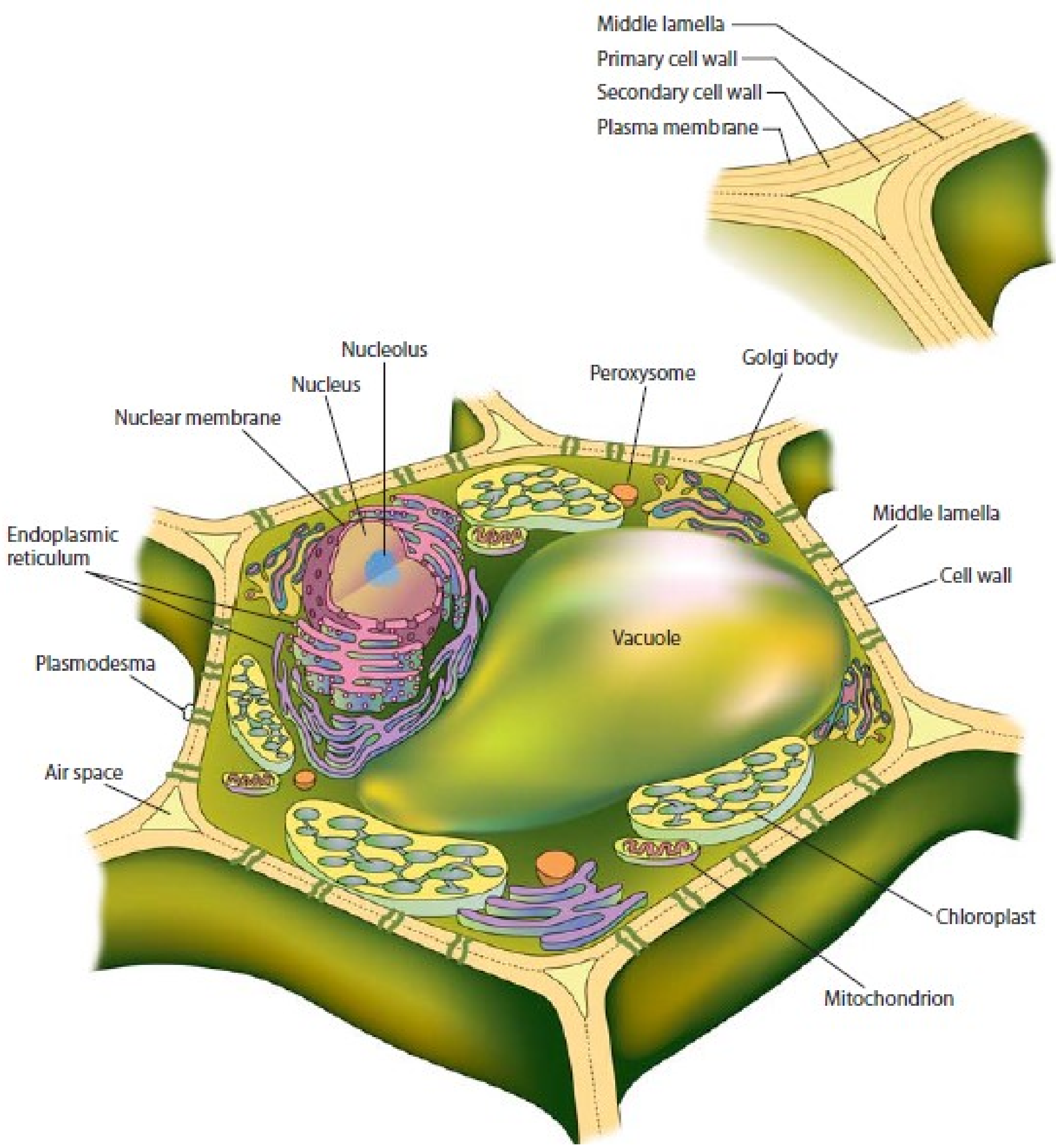


Fig.1 Schematic representation of a plant cell and its main components

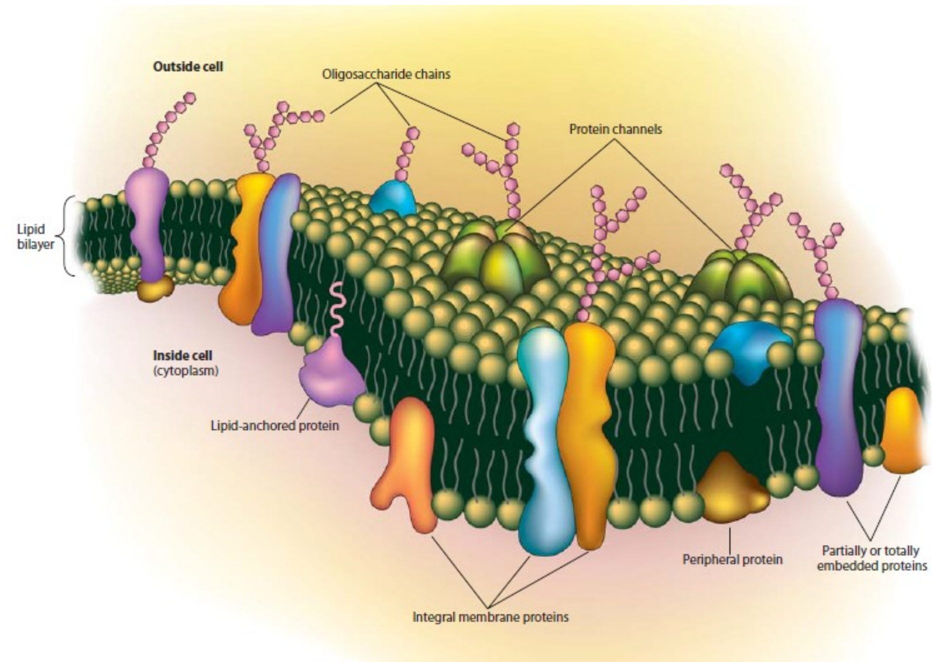


Fig. 2 Schematic representation of a portion of a cell membrane and of the arrangement of protein molecules in relation to the membrane

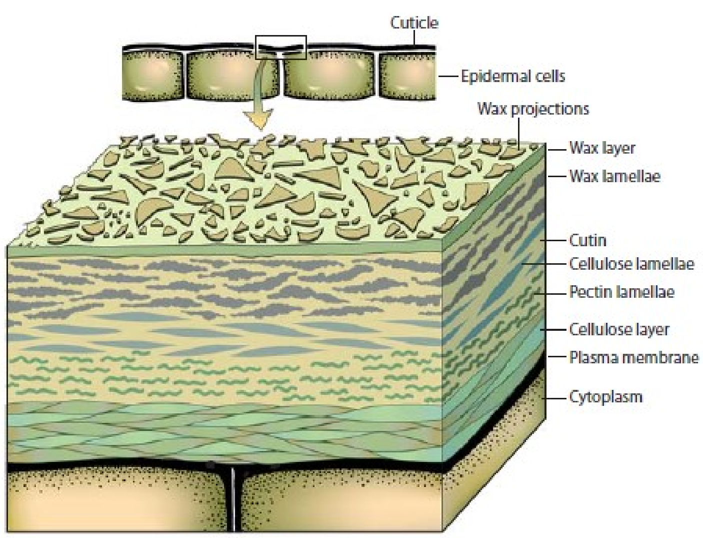
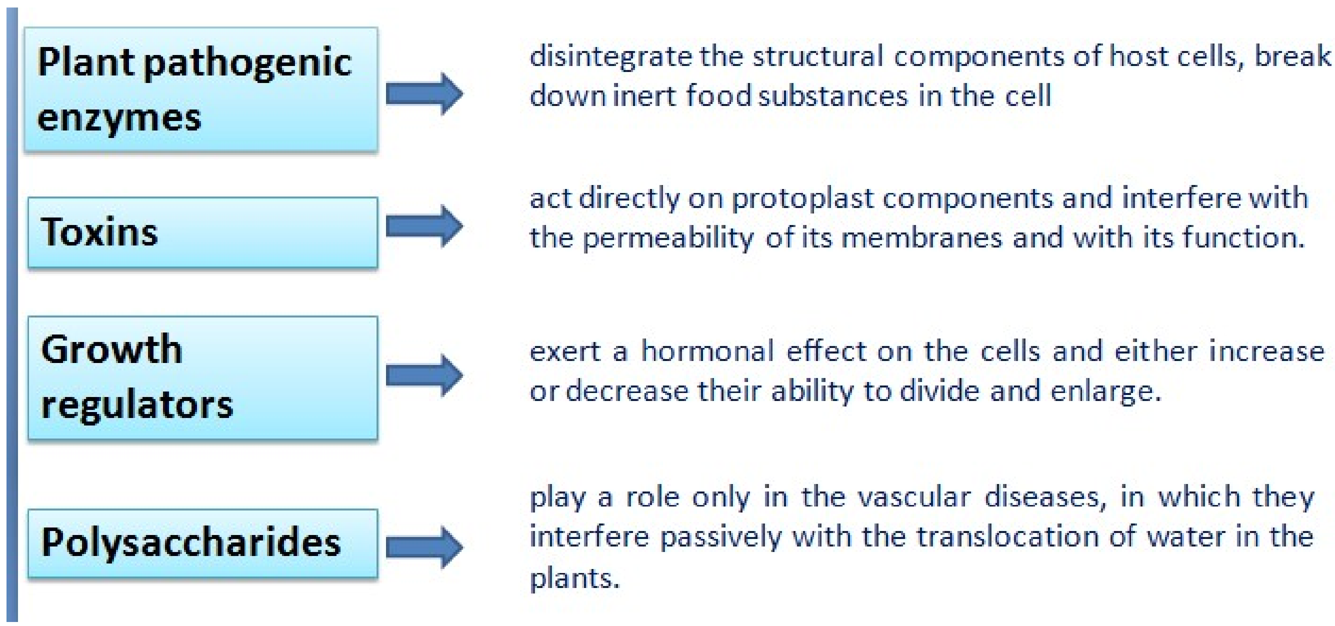


Fig.3 Schematic representation of the structure and composition of the cuticle and cell wall of foliar epidermal cells.

CHEMICAL WEAPONS OF PATHOGENS

The main groups of substances secreted by pathogens in plants that seem to be involved in production of disease, either directly or indirectly, are enzymes, toxins, growth regulators, and polysaccharides (plugging substances).



Enzymes in Plant Disease

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

Enzymatic Degradation of Cell Wall Substances

Usually, the first contact of pathogens with their host plants occurs at a plant surface. Aerial plant part surfaces consist primarily of cuticle and/or cellulose, whereas root cell wall surfaces consist only of cellulose. Cuticle consists primarily of cutin, more or less impregnated with wax and frequently covered with a layer of wax. The lower part of cutin is intermingled with pectin and cellulose lamellae and lower yet there is a layer consisting predominantly of pectic substances; below that there is a layer of cellulose. Polysaccharides of various types are often found in cell walls. Proteins of many different types, both structural, e.g., elastin, which helps loosen the cell wall, and extensin, which helps add rigidity to the cell wall, some enzymes, and some signal molecules that help receive or transmit signals inward or outward, are normal constituents of cell walls. Finally, epidermal cell walls may also contain suberin and lignin. The penetration of pathogens into parenchymatous tissues is facilitated by the breakdown of the internal cell walls, which consist of cellulose, pectins, hemicelluloses, and structural proteins, and of the middle lamella, which consists primarily of pectins. In addition, complete plant tissue disintegration involves the breakdown of lignin. The degradation of each of these substances is brought about by the action of one or more sets of enzymes secreted by the pathogen.

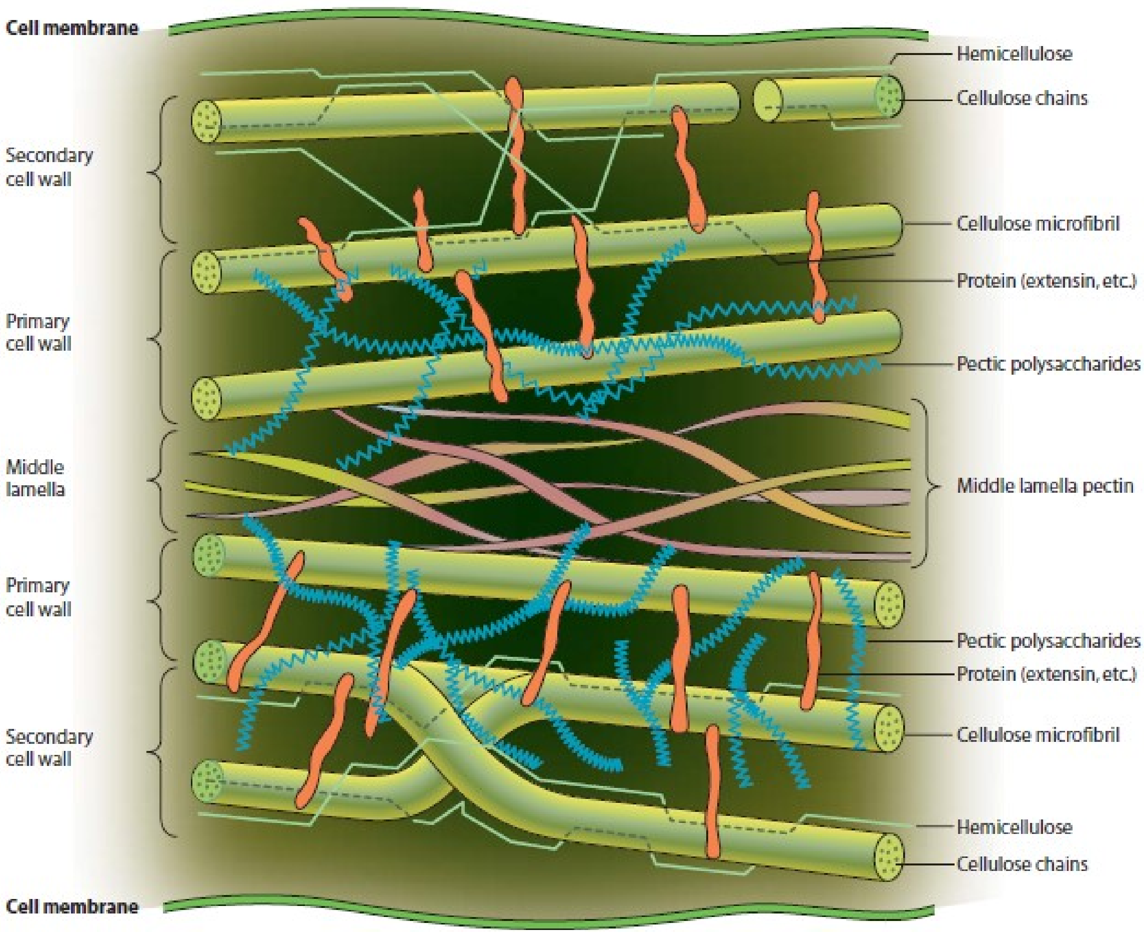


Fig. Schematic diagram of morphology and arrangement of some cell wall components

Cuticular Wax

Plant waxes are found as granular, blade, or rod-like projections or as continuous layers outside or within the cuticle of many aerial plant parts.

Several pathogens, e.g., *Puccinia hordei*, produce enzymes that can degrade waxes. Another fungus, *Pestalotia malicola*, which attacks fruit of Chinese quince, grows on, within, and beneath the fruit cuticle.

Fungi and parasitic higher plants, however, apparently can penetrate wax layers by means of mechanical force alone

Cutin

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

Cutin is an insoluble polyester of C16 and C18 hydroxy fatty acids.

Many fungi and a few bacteria have been shown to produce cutinases and/or nonspecific esterases, i.e., enzymes that can degrade cutin.

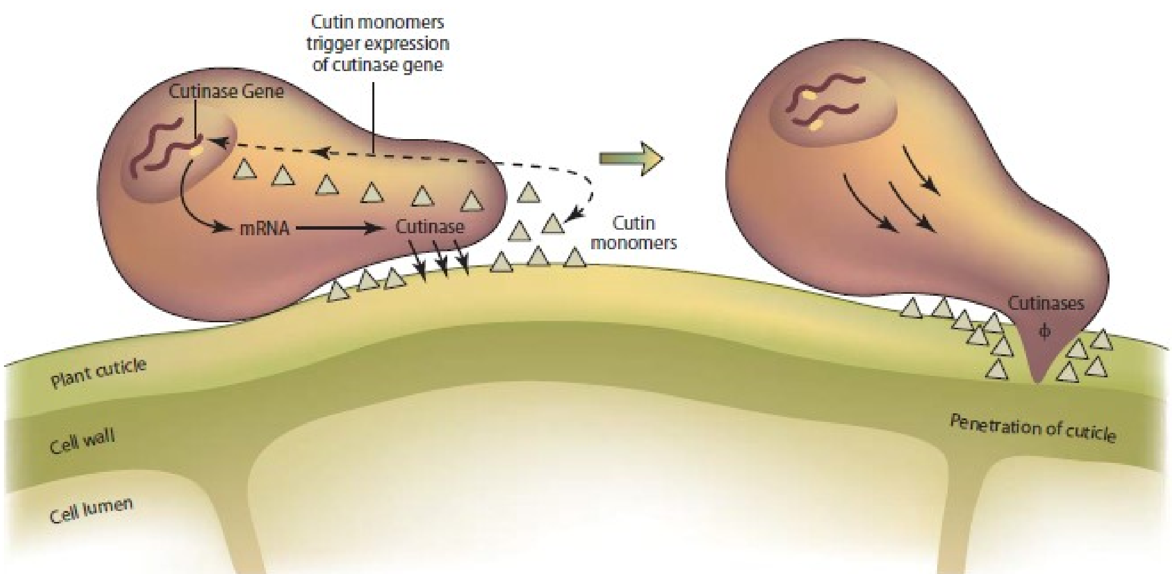


Figure Diagrammatic representation of cuticle penetration by a germinating fungus spore. Constitutive cutinase releases a few cutin monomers from the plant cuticle. These trigger expression of the cutinase genes of the fungus, leading to the production of more cutinase(s), which macerates the cuticle and allows penetration by the fungus.

Pectic Substances

Pectic substances constitute the main components of the middle lamella, i.e., the intercellular cement that holds in place the cells of plant tissues.

Several enzymes degrade pectic substances and are known as pectinases or pectolytic enzymes

Pectin methyl esterases, remove small branches off the pectin chains.

Some chain splitting pectinases, called **polygalacturonases**, split the pectic chain by adding a molecule of water and breaking (hydrolyzing) the linkage between two galacturonan molecules;

Others, known as **pectin lyases**, split the chain by removing a molecule of water from the linkage, thereby breaking it and releasing products with an unsaturated double bond.

Pectin-degrading enzymes have been shown to be involved in the production of many fungal and bacterial diseases, particularly those characterized by the soft rotting of tissues.

Figure Involvement of pectolytic enzymes in disease development. Peach tissues infected with the brown rot fungus *Monilinia fructicola* while still on the tree (A) and by *Rhizopus* sp. at harvest (B and C) are macerated by the pectinases of the fungus and subsequently turn brown due to the oxidation of phenolic compounds released during maceration. Subsequent loss of water results in shrinking of the fruit. (D) Potato tuber, part of which has been macerated by the enzymes of the fungus *Fusarium* and subsequently has lost some of the water. An onion bulb (E) and a potato tuber (F) macerated by the enzymes of the fungus *Botrytis* and the bacterium *Erwinia*, respectively.

Cellulose

Cellulose is also a polysaccharide, but it consists of chains of glucose (1–4) β-d-glucan molecules. The glucose chains are held to one another by a large number of hydrogen bonds. Cellulose occurs in all higher plants as the skeletal substance of cell walls in the form of microfibrils.

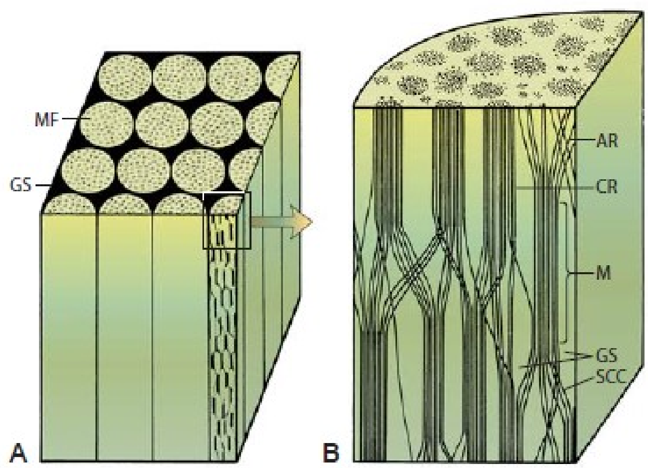


Figure Schematic diagram of the gross struture of cellulose and microfibrils (A) and of the arrangement of cellulose molecules within a microfibril (B). MF, microfibril; GS, ground substance (pectin, hemicelluloses, or lignin); AR, amorphous region of cellulose; CR, crystalline region; M, micelle; SCC, single cellulose chain (molecule).

The enzymatic breakdown of cellulose results in the final production of glucose molecules. The glucose is produced by a series of enzymatic reactions carried out by several cellulases and other enzymes.

One cellulase (C1) attacks native cellulose by cleaving cross-linkages between chains.

A **second cellulase (C2)** also attacks native cellulose and breaks it into shorter chains.

These are then attacked by a third group of **cellulases (Cx)**, which degrade them to the disaccharide cellobiose.

Finally, cellobiose is degraded by the enzyme **β-glucosidase** into glucose.

Lignin

Lignin is found in the middle lamella, as well as in the secondary cell wall of xylem vessels and the fibers that strengthen plants. It is also found in epidermal and occasionally hypodermal cell walls of some plants. The lignin content of mature woody plants varies from 15 to 38% and is second only to cellulose in abundance.

Only a small group of microorganisms is capable of degrading lignin. Actually, only about 500 species of fungi, almost all of them basidiomycetes, have been reported so far as being capable of decomposing wood. About one-fourth of these fungi (the brown rot fungi) seem to cause some degradation of lignin but cannot utilize it.

Most of the lignin in the world is degraded and utilized by a group of basidiomycetes called white rot fungi. It appears that white rot fungi secrete one or more enzymes (ligninases), which enable them to utilize lignin.

Microbial Toxins in Plant Disease

Toxins act directly on living host protoplasts, seriously damaging or killing the cells of the plant. Some toxins act as general protoplasmic poisons and affect many species of plants representing different families. Others are toxic to only a few plant species or varieties and are completely harmless to others.

Toxins injure host cells either by affecting the permeability of the cell membrane or by inactivating or inhibiting enzymes and subsequently interrupting the corresponding enzymatic reactions. Certain toxins act as antimetabolites and induce a deficiency for an essential growth factor.

A. Toxins That Affect a Wide Range of Host Plants

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

These toxins increase the severity of disease caused by a pathogen, i.e., **they affect the virulence of the pathogen,** but are not essential for the pathogen to cause disease, i.e., **they do not determine the pathogenicity of the pathogen.**

1. Tabtoxin

Produced by the bacterium ***Pseudomonas syringae*; pv. *tabaci***, which causes the wildfire disease of tobacco.

Toxin-producing strains cause necrotic spots on leaves, with each spot surrounded by a yellow halo.

Sterile culture filtrates of the organism, as well as purified toxin, produce symptoms identical to those characteristic of wildfire of tobacco not only on tobacco, but in a large number of plant species belonging to many different families.

Tox^{-} strains show reduced virulence and cause necrotic leaf spots without the yellow halo.

Tabtoxin, through tabtoxinine, is toxic to cells because it inactivates the enzyme glutamine synthetase, which leads to depleted glutamine levels and, as a consequence, accumulation of toxic concentrations of ammonia. The latter uncouples photosynthesis and photorespiration and destroys the thylakoid membrane of the chloroplast, thereby causing chlorosis and eventually necrosis.

2. Phaseolotoxin

Phaseolotoxin is produced by the bacterium *Pseudomonas syringae* pv. *phaseolicola*, the cause of halo blight of bean and some other legumes.

Phaseolotoxin is a modified ornithine–alanine– arginine tripeptide carrying a phosphosulfinyl group.

Phosphosulfinylornithine is the biologically functional moiety of phaseolotoxin. The toxin affects cells by binding to the active site of and inactivating the enzyme ornithine carbamoyltransferase, which normally converts ornithine to citrulline, a precursor of arginine. By its action on the enzyme, the toxin thus causes the accumulation of ornithine and depleted levels of citrulline and arginine.

Phaseolotoxin, however, seems to also **inhibit pyrimidine nucleotide biosynthesis, reduce the activity of ribosomes, interfere with lipid synthesis, change the permeability of membranes, and result in the accumulation of large starch grains in the chloroplasts**.

B. Host-Specific or Host-Selective Toxins

A **host-specific** or **host-selective** toxin is a substance produced by a pathogenic microorganism that, at physiological concentrations, is toxic only to the hosts of that pathogen and shows little or no toxicity against nonsusceptible plants.

Most host-specific toxins must be present for the producing microorganism to be able to cause disease.

1. Victorin, or HV Toxin

Victorin, or Hv-toxin, is produced by the fungus *Cochliobolus* (*Helminthosporium*) *victoriae*.

The primary target of the toxin seems to be the cell plasma membrane where victorin seems to bind to several proteins. The possible site of action of victorin seems to be the glycine decarboxylate complex, which is a key component of the photorespiratory cycle.

2. T Toxin [Cochliobolus (Helminthosporium) heterostrophus Race T Toxin]

T toxin is produced by race T of *C. heterostrophus* (*Bipolaris maydis*), the cause of southern corn leaf blight.

The T toxin apparently acts specifically on mitochondria of susceptible cells, which are rendered nonfunctional, and inhibits ATP synthesis. The T toxin reacts with a specific receptor protein molecule (URF13) that is located on the inner mitochondrial membrane of sensitive mitochondria.

3. HC Toxin

Race 1 of *Cochliobolus* (*Helminthosporium*) *carbonum* (*Bipolaris zeicola*) causes northern leaf spot and ear rot disease in maize. It also produces the hostspecific HC toxin, which is toxic only on specific maize lines.

Resistant corn lines have a gene (Hm1) coding for an enzyme called HC toxin reductase that reduces and thereby detoxifies the toxin. Susceptible corn lines lack this gene and, therefore, cannot defend themselves against the