chapter five

HOW PATHOGENS ATTACK PLANTS

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 (Fig. 5-1) 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 (Fig. 5-2). 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. 5-3).

Pathogens attack plants because during their evolutionary development they have acquired the ability to live off the substances manufactured by the host plants, and some of the pathogens depend on these substances for survival. Many substances are contained in the protoplast of the plant cells, however, and if pathogens are to gain access to them they must first penetrate the outer barriers formed by the cuticle and/or cell walls. Even after the outer cell wall has been penetrated, further invasion of the plant by the pathogen necessitates the penetration of more cell walls. Furthermore, the plant cell contents are not always found in forms immediately utilizable by the pathogen and must be broken down to units that the pathogen can absorb and assimilate. Moreover, the plant, reacting to the presence and activities of the pathogen, produces structures and chemical substances that interfere with the advance or the existence of the pathogen; if the pathogen is to survive and to continue living off the plant, it must be able to overcome such obstacles.

Therefore, for a pathogen to infect a plant it must be able to make its way into and through the plant, obtain nutrients from the plant, and neutralize the defense reactions of the plant. Pathogens accomplish these activities mostly through secretions of chemical substances that affect certain components or metabolic mechanisms of

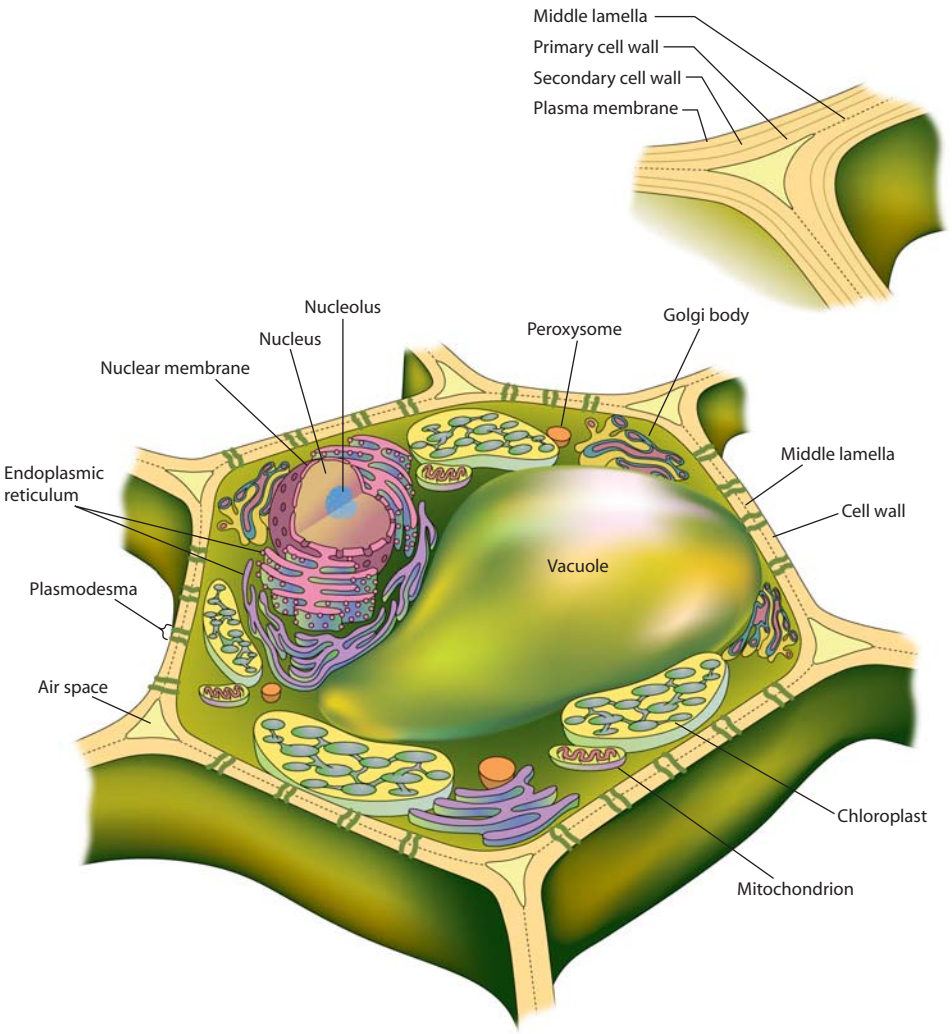


FIGURE 5-1 Schematic representation of a plant cell and its main components.

their hosts. Penetration and invasion, however, seem to be aided by, or in some cases be entirely the result of, the mechanical force exerted by certain pathogens on the cell walls of the plant.

MECHANICAL FORCES EXERTED BY PATHOGENS ON HOST TISSUES

Plant pathogens are, generally, tiny microorganisms that cannot apply a "voluntary" force to a plant surface. Only some fungi, parasitic higher plants, and nematodes appear to apply mechanical pressure to the plant surface they are about to penetrate. The amount of pressure, however, may vary greatly with the degree of "presoftening" of a plant surface by enzymatic secretions of the pathogen.

For fungi and parasitic higher plants to penetrate a plant surface, they must, generally, first adhere to it. Hyphae and radicles are usually surrounded by mucilaginous substances, and their adhesion to the plant seems to be brought about primarily by the intermolecular forces developing between the surfaces of plant and pathogen on close contact with the adhesive substances and with one another. In some cases an adhesion pad forms from the spore when it comes in contact with a moist surface, and cutinase and cellulase enzymes released from the spore surface help the spore adhere to the plant surface. Spores of some fungi carry adhesive substances at their tips that, on hydration, allow spores to become attached to various surfaces.

After contact is established, the diameter of the tip of the hypha or radicle in contact with the host increases and forms the flattened, bulb-like structure called the appressorium (Figs. 2-4 and 2-5). This increases the area of adherence between the two organisms and securely fastens the pathogen to the plant. From the appressorium, a fine growing point, called the penetration peg,

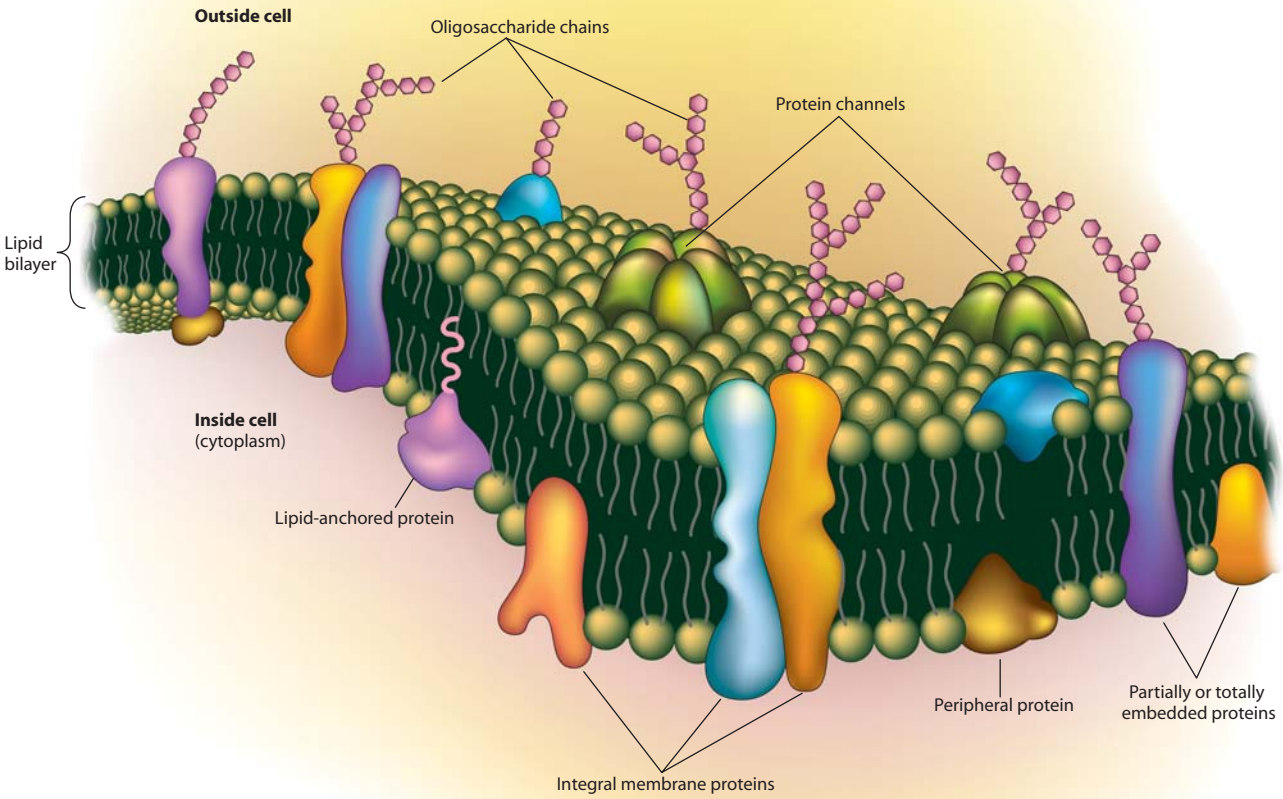


FIGURE 5-2 Schematic representation of a portion of a cell membrane and of the arrangement of protein molecules in relation to the membrane.

arises and advances into and through the cuticle and cell wall. In some fungi, such as *Alternaria*, *Cochliobolus*, *Colletotrichum*, *Gaeumannomyces*, *Magnaporthe*, and *Verticillium*, penetration of the plant takes place only if melanin (dark pigment) accumulates in the appressorial cell wall. It appears that melanin produces a rigid structural layer and, by trapping solutes inside the appressorium, causes water to be absorbed. This increases the turgor pressure in the appressorium and, thereby, the physical penetration of the plant by the penetration peg. If the underlying host wall is soft, penetration occurs easily. When the underlying wall is hard, however, the force of the growing point may be greater than the adhesion force of the two surfaces and may cause separation of the appressorial and host walls, thus averting infection. Penetration of plant barriers by fungi and parasitic higher plants is almost always assisted by the presence of enzymes secreted by the pathogen at the penetration site, resulting in the softening or dissolution of the barrier. It was found, for example, that while appressoria of some powdery mildew fungi developed a maximum turgor pressure of 2–4 MPa, approximately sufficient to bring about host cell penetration, two cellulases were also present: one primarily at the tip of the appressorial germ tube and the other at the tip of the primary germ tube.

While the penetration tube is passing through the cuticle, it usually attains its smallest diameter and

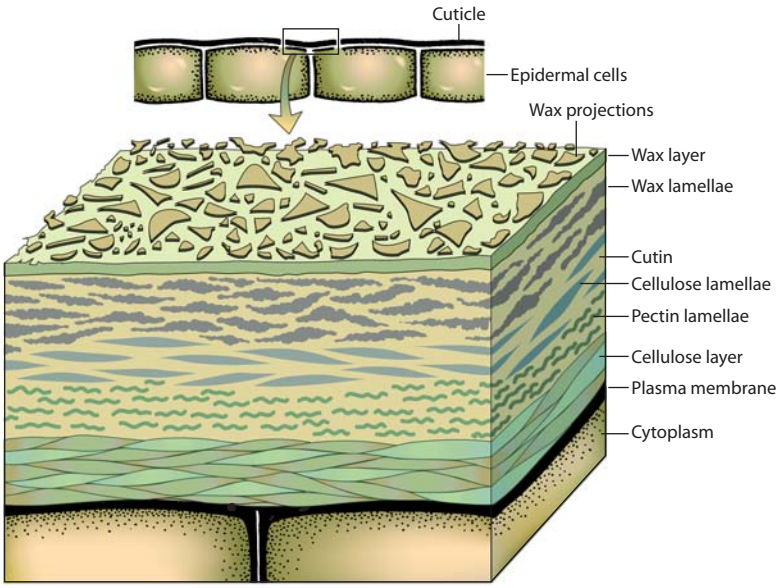


FIGURE 5-3 Schematic representation of the structure and composition of the cuticle and cell wall of foliar epidermal cells. [Adapted from Goodman *et al.* (1967).]

appears thread-like. After penetration of the cuticle, the hyphal tube diameter often increases considerably. The penetration tube attains the diameter normal for the hyphae of the particular fungus only after it has passed through the cell wall (see Figs. 2-5 and 2-9 in Chapter 2).

Nematodes penetrate plant surfaces by means of the stylet, which is thrust back and forth and exerts mechanical pressure on the cell wall (Fig. 2-10). The nematode first adheres to the plant surface by suction, which it develops by bringing its fused lips in contact with the plant. After adhesion is accomplished, the nematode brings its body, or at least the forward portion of its body, to a position vertical to the cell wall. With its head stationary and fixed to the cell wall, the nematode then thrusts its stylet forward while the rear part of its body sways or rotates slowly round and round. After several consecutive thrusts of the stylet, the cell wall is pierced, and the stylet or the entire nematode enters the cell.

Once a fungus or nematode has entered a cell, it generally secretes increased amounts of enzymes that presumably soften or dissolve the opposite cell wall and make its penetration easier. Mechanical force, however, probably is brought to bear in most such penetrations, although to a lesser extent.

Considerable mechanical force is also exerted on host tissues from the inside out by some pathogenic fungi on formation of their fructifications in the tissues beneath the plant surface. Through increased pressure, the sporophore hyphae, as well as fruiting bodies, such as pycnidia and perithecia, push outward and cause the cell walls and the cuticle to expand, become raised in the form of blister-like proturberances, and finally break.

CHEMICAL WEAPONS OF PATHOGENS

Although some pathogens may use mechanical force to penetrate plant tissues, the activities of pathogens in plants are largely chemical in nature. Therefore, the effects caused by pathogens on plants are almost entirely the result of biochemical reactions taking place between substances secreted by the pathogen and those present in, or produced by, the plant.

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). These substances vary greatly as to their importance in pathogenicity, and their relative importance may be different from one disease to another. Thus, in some diseases, such as soft rots, enzymes seem to be by far the most important, whereas in diseases such as crown gall, growth regulators are apparently the main substances involved. However, in the *Bipolaris* blight of Victoria oats, the disease is primarily the result of a toxin secreted in the plant by the pathogen. Enzymes, toxins, and growth regulators, probably in that order, are considerably more common and probably more important in plant disease development than polysaccharides. It has also been shown that some pathogens

produce compounds that act as suppressors of the defense responses of the host plant.

Among the plant pathogens, all except viruses and viroids can probably produce enzymes, growth regulators, and polysaccharides. How many of them produce toxins is unknown, but the number of known toxin-producing plant pathogenic fungi and bacteria increases each year. Plant viruses and viroids are not known to produce any substances themselves, but they induce the host cell to produce either excessive amounts of certain substances already found in healthy host cells or substances completely new to the host. Some of these substances are enzymes, and others may belong to one of the other groups mentioned earlier.

Pathogens produce these substances either in the normal course of their activities (constitutively) or when they grow on certain substrates such as their host plants (inducible). Undoubtedly, natural selection has favored the survival of pathogens that are assisted in their parasitism through the production of such substances. The presence or the amount of any such substance produced, however, is not always a measure of the ability of the pathogen to cause disease. It must also be kept in mind that many substances, identical to those produced by pathogens, are also produced by the healthy host plant.

In general, plant pathogenic enzymes disintegrate the structural components of host cells, break down inert food substances in the cell, or affect components of its membranes and the protoplast directly, thereby interfering with its functioning systems. Toxins seem to act directly on protoplast components and interfere with the permeability of its membranes and with its function. Growth regulators exert a hormonal effect on the cells and either increase or decrease their ability to divide and enlarge. Polysaccharides seem to play a role only in the vascular diseases, in which they interfere passively with the translocation of water in the plants.

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.

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 (Fig. 5-4). The presence and condition of waxes at the leaf surface affect the degree of colonization of leaves and the effect varies with the plant species. Electron microscope studies suggest that several pathogens, e.g., *Puccinia hordei*, produce enzymes that can degrade waxes. 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 (see Fig. 5-3). Cutin is an insoluble polyester of C_{16} and C_{18} hydroxy fatty acids.

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

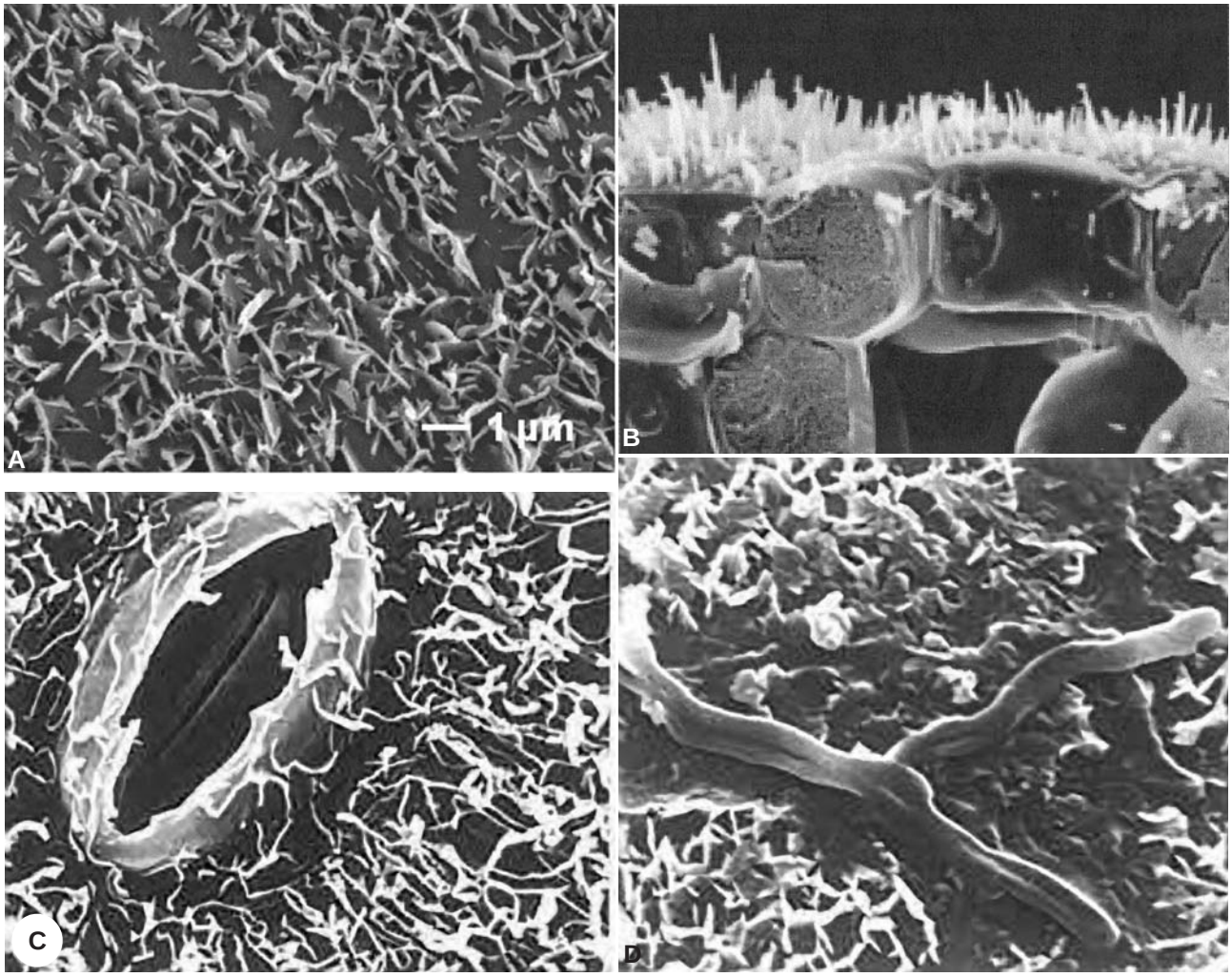


FIGURE 5-4 Morphology of cuticular wax projections on different leaf surfaces. (A) Surface view of wax on corn leaf. (B) Wax projections as seen in cross section of leaf. (C) Wax projections surrounding a stoma. (D) Wax degraded along the passage of fungal mycelium. [Photographs courtesy of (A) L. M. Marcell and G. A. Beattie, Iowa State University, (B) H. V. Davis, United Kingdom, (C and D) P. V. Sangbusen, Hamburg.]

enzymes that can degrade cutin. Cutinases break cutin molecules and release monomers (single molecules) as well as oligomers (small groups of molecules) of the component fatty acid derivatives from the insoluble cutin polymer.

Fungi that penetrate the cuticle directly seem to constantly produce low levels of cutinase, which on contact with cutin releases small amounts of monomers. These subsequently enter the pathogen cell, trigger further expression of the cutinase genes, and stimulate the fungus to produce almost a thousand times more cutinase than before (Fig. 5-5). Cutinase production by the pathogen, however, may also be stimulated by some of the fatty acids present in the wax normally associated with cutin in the plant cuticle. However, the presence of glucose suppresses expression of the cutinase gene and reduces cutinase production drastically.

The involvement of cutinase in the penetration of the host cuticle by plant pathogenic fungi is shown by several facts. For example, the enzyme reaches its highest concentration at the penetrating point of the germ tube and at the infection peg of appressorium-forming fungi. Inhibition of cutinase by specific chemical inhibitors, or by antibodies of the enzyme applied to the plant surface, protects the plant from infection by fungal pathogens. Also, cutinase-deficient mutants show reduced virulence but become fully virulent when cutinase is added on the plant surface. In the brown rot of stone fruits, caused by the fungus *Monilinia fructicola*, fungal cutinase activity seems to be inhibited greatly by

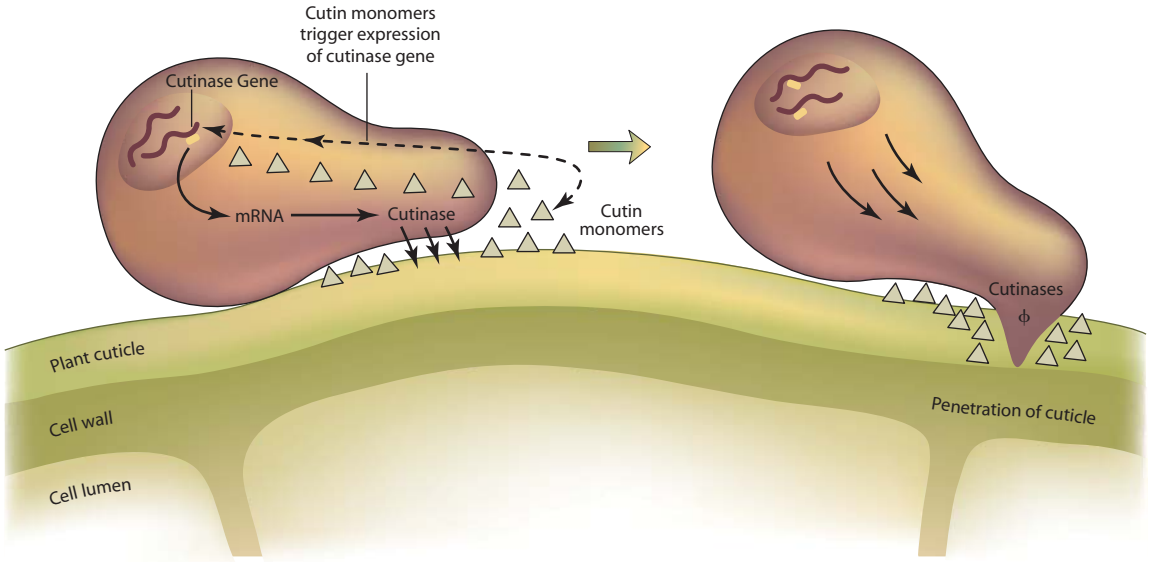


FIGURE 5-5 Diagrammatic representation of cuticle penetration by a germinating fungus spore. Constitutive cutinase releases a few cutin monomers from the plant cuticle. These trigger expression of the cutinase genes of the fungus, leading to the production of more cutinase(s), which macerates the cuticle and allows penetration by the fungus.

phenolic compounds such as chlorogenic and caffeic acids, which are abundant in epidermal cells of young fruit and the fruit is resistant to infection. As the fruit matures, the concentration of these compounds declines sharply, cutinase activity increases, and the fruit is penetrated by the fungus. Moreover, fungi that infect only through wounds and do not produce cutinase acquire the ability to infect directly if a cutinase gene from another fungus is introduced into them and enables them to produce cutinase. Pathogens that produce higher levels of cutinase seem to be more virulent than others. At least one study has shown that the germinating spores of a virulent isolate of the fungus *Fusarium* produced much more cutinase than those of an avirulent isolate of the same fungus and that the avirulent isolate could be turned into a virulent one if purified cutinase was added to its spores. The fungus *Botrytis cinerea*, the cause of numerous types of diseases on many plants, produces a cutinase and a lipase, both of which break down cutin. In the presence of antilipase antibodies, fungal spores failed to penetrate the cuticle and lesion formation was inhibited, indicating that lipase activity is required in at least the early stages of host infection.

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 (Fig. 5-6). Pectic substances also make up a large portion of the primary cell wall in which they form an amorphous gel filling the spaces between the cellulose microfibrils (Fig. 5-7).

Pectic substances are polysaccharides consisting mostly of chains of galacturonan molecules interspersed with a much smaller number of rhamnose molecules and small side chains of galacturonan, xylan, and some other five carbon sugars. Several enzymes degrade pectic substances and are known as **pectinases** or **pectolytic enzymes** (Fig. 5-8). Some of them, e.g., the **pectin methyl esterases**, remove small branches off the pectin chains. Pectin methyl esterases have no effect on the overall chain length, but they alter the solubility of the pectins and affect the rate at which they can be attacked by the chain-splitting pectinases. The latter cleave the pectic chain and release shorter chain portions containing one or a few molecules of galacturonan. Some chain-splitting pectinases, called **polygalacturonases**, split the pectic chain by adding a molecule of water and breaking (hydrolyzing) the linkage between two galacturonan molecules; others, known as **pectin lyases**, split the chain by removing a molecule of water from the linkage, thereby breaking it and releasing products with an unsaturated double bond (Fig. 5-8). Polygalacturonases and pectin lyases occur in types that either can break the pectin chain at random sites (**endopectinases**) and release shorter chains, or can break only the terminal linkage (**exopectinases**) of the chain and release single units of galacturonan. The rhamnose and other sugars that may be forming part or branches of the pectin chain

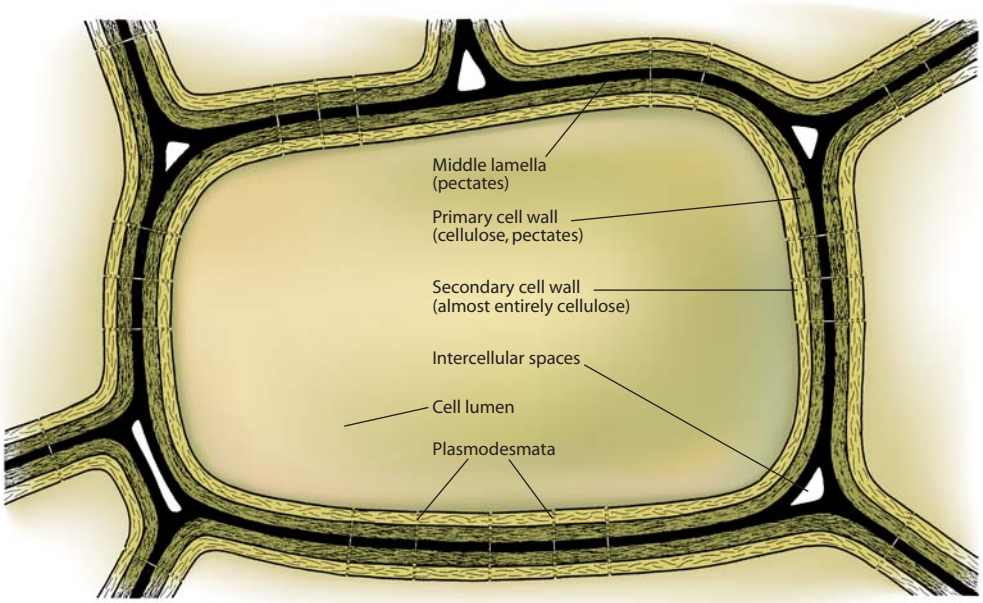


FIGURE 5-6 Schematic representation of the structure and composition of plant cell walls.

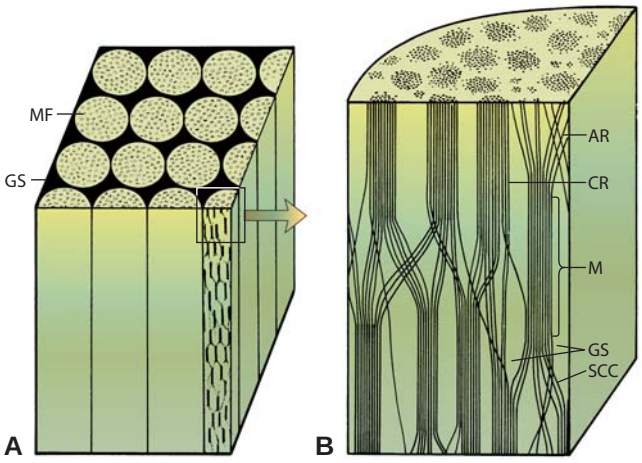


FIGURE 5-7 Schematic diagram of the gross struture of cellulose and microfibrils (A) and of the arrangement of cellulose molecules within a microfibril (B). MF, microfibril; GS, ground substance (pectin, hemicelluloses, or lignin); AR, amorphous region of cellulose; CR, crystalline region; M, micelle; SCC, single cellulose chain (molecule). [Adapted from Brown *et al.* (1949).]

are hydrolyzed by other enzymes that recognize these molecules.

As with cutinases, and with other enzymes involved in the degradation of cell wall substances, the production of extracellular pectolytic enzymes by pathogens is regulated by the availability of the pectin polymer and the released galacturonan units. The pathogen seems to produce at all times small, constitutive, base-level amounts of pectolytic enzymes that, in the presence of pectin, release from it a small number of galacturonan monomers, dimers, or oligomers. These molecules, when absorbed by the pathogen, serve as inducers for the enhanced synthesis and release of pectolytic enzymes (substrate induction), which further increase the amount of galacturonan monomers, etc. The latter are assimilated readily by the pathogen, but at higher concentrations they act to repress the synthesis of the same enzymes (catabolite repression), thus reducing production of the enzymes and the subsequent release of galacturonan monomers. The production of pectolytic enzymes is also repressed when the pathogen is grown in the presence of glucose. However, in some resistant host–pathogen combinations, pectolytic enzymes seem to elicit the plant defense response through the release from the cell wall of pectic fragments that function as endogenous elicitors of the defense mechanisms of the host.

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. Various pathogens produce different sets of pectinases and their isozymes. In some diseases, e.g., the bacterial wilt of solanaceous crops caused by *Ralstonia solanacearum*, pectinolytic enzymes collectively are absolutely essential for disease to develop, although some of them individually seem to not be required for disease but rather for accelerated colonization and enhanced aggressiveness by bacteria. In black rot of cantaloupe caused by the fungus *Didymella bryoniae*, there is a highly positive correlation between the

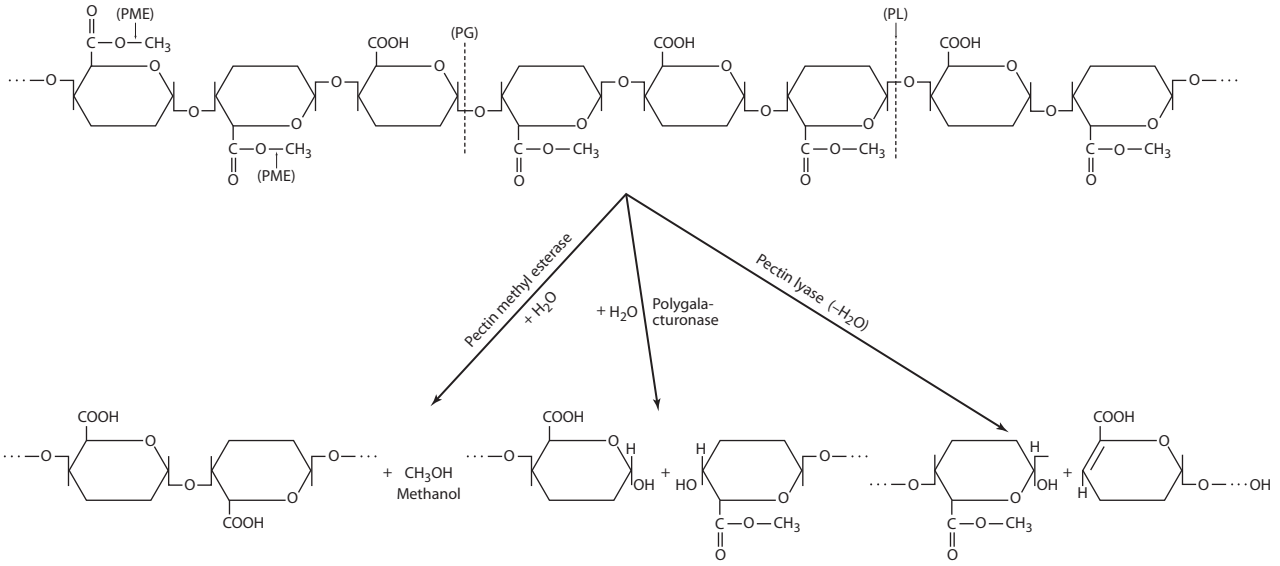


FIGURE 5-8 Degradation of a pectin chain by the three types of pectinases into modified and smaller molecules.

size of the rotting tissue lesion and the total fungal polygalacturonase activity in the rotting tissue.

In some *Colletotrichum*-caused anthracnoses, the fungus produces one pectin lyase that is a key virulence factor in disease development. The amount and activity of the enzyme and the amount of disease increase as the pH at the infection site increase to 7.5–8.0. The fungus maintains the high pH at the infection area by secreting ammonia. Inoculation of nonhost species in the presence of ammonia-releasing compounds enhances pathogenicity to levels similar to those caused by the compatible fungal and host species. Ammonia secretion by the fungus is a virulence factor for the fungus. Pectin–degrading enzymes are produced and play a role in the ability of nematodes, such as the root knot nematode, *Meloidogyne javanica*, for the penetration of root tissues, movement between plant cells along the middle lamella, and possibly in the formation of tee multinucleate giant cells on which the nematode feeds throughout the rest of its life. Some of these enzymes seem to affect the virulence of the pathogen on different hosts, i.e., they affect the degree of host specialization of the pathogen. Pectic enzymes are produced by germinating spores and, apparently, acting together with other pathogen enzymes (cutinases and cellulases), assist in the penetration of the host.

Pectin degradation results in liquefaction of the pectic substances that hold plant cells together and in the weakening of cell walls. This leads to tissue maceration, i.e., softening and loss of coherence of plant tissues and separation of individual cells, which eventually die (Fig. 5-9). The weakening of cell walls and tissue maceration undoubtedly facilitate the inter- or intracellular invasion of the tissues by the pathogen. Pectic enzymes also provide nutrients for the pathogen in infected tissues. Pectic enzymes, by the debris they create, seem to be involved in the induction of vascular plugs and occlusions in the vascular wilt diseases (Fig. 5-11). Although cells are usually killed quickly in tissues macerated by pectic enzymes, how these enzymes kill cells is not yet clear. It is thought that cell death results from the weakening by the pectolytic enzymes of the primary cell wall, which then cannot support the osmotically fragile protoplast, and the protoplast bursts.

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 (see Figs. 5-7, 5-10, and 5-12). Microfibrils, which can be perceived as bundles of iron bars in a reinforced concrete building, are the basic structural units (matrix) of the wall, even though they account for less than 20% of the wall volume in most meristematic cells. The cellulose content of tissues varies from about 12% in the nonwoody tissues of grasses to about 50% in mature wood tissues to more than 90% in cotton fibers. The spaces between microfibrils and between micelles or cellulose chains within the microfibrils may be filled with pectins and hemicelluloses and probably some lignin at maturation. Although the bulk of cell wall polysaccharides is broken down by numerous enzymes produced by fungi and bacteria, a portion of them

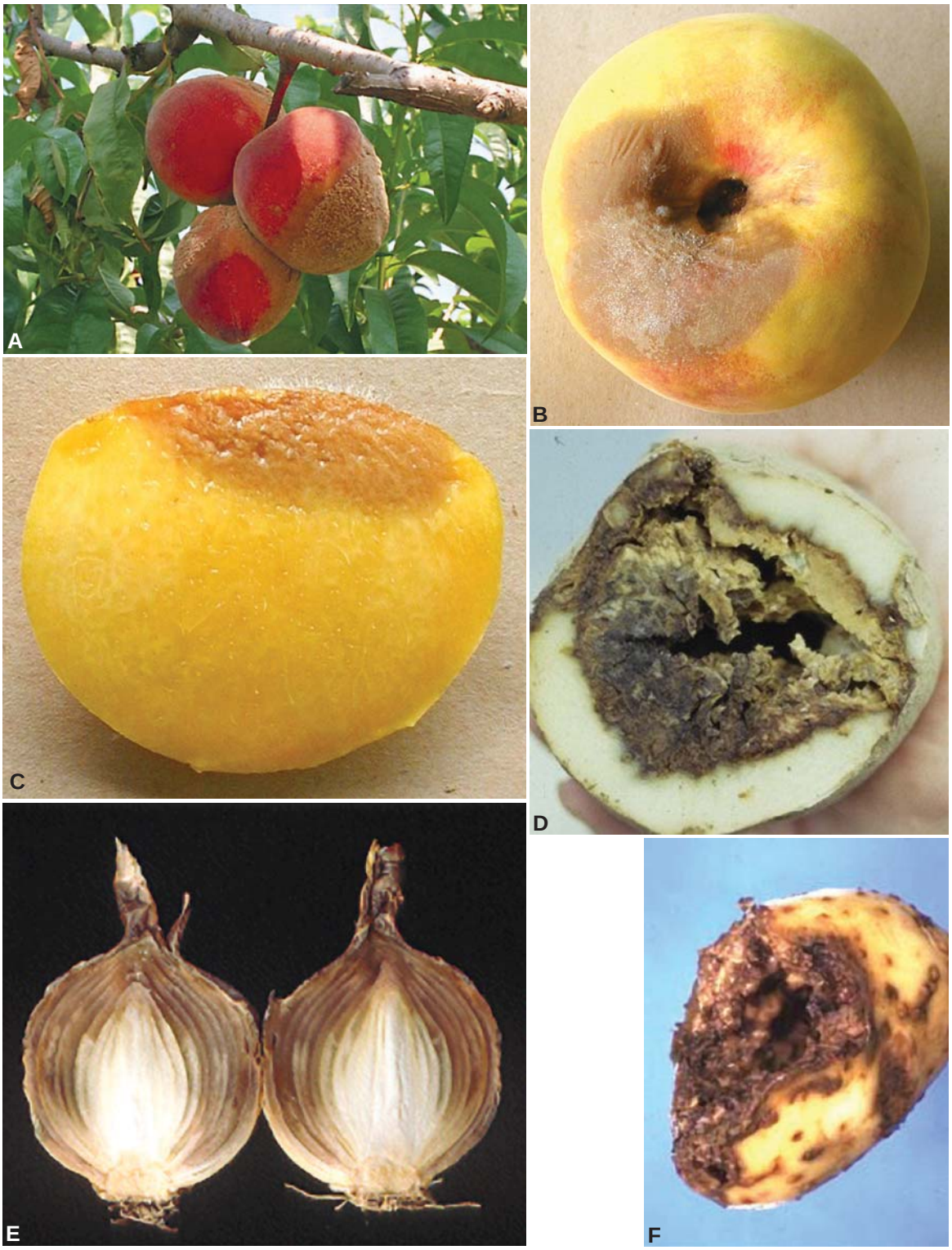


FIGURE 5-9 Involvement of pectolytic enzymes in disease development. Peach tissues infected with the brown rot fungus *Monilinia fructicola* while still on the tree (A) and by *Rhizopus* sp. at harvest (B and C) are macerated by the pectinases of the fungus and subsequently turn brown due to the oxidation 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. [Photographs courtesy of (A) D. Ritchie, North Carolina State University, (D) P. Hamm, Oregon State University, (E) K. Mohan, University of Idaho, and (F) R. Rowe, Ohio State University.]

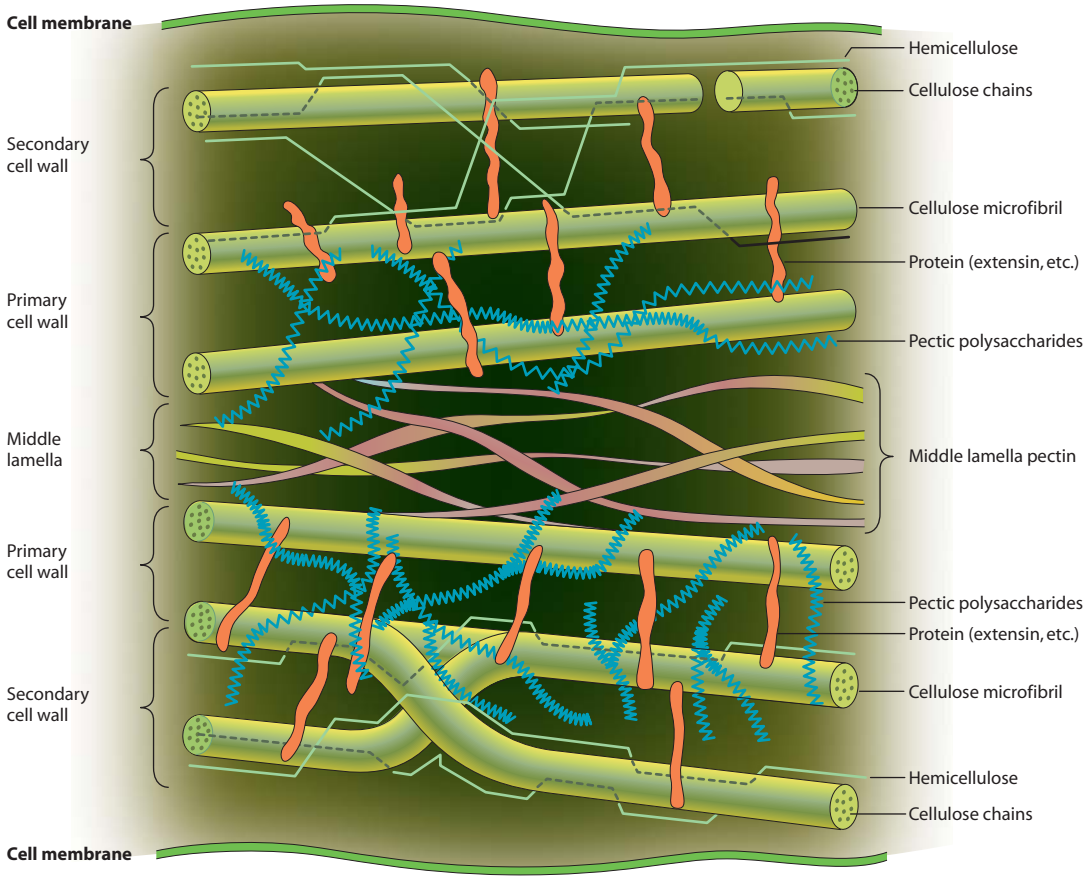


FIGURE 5-10 Schematic diagram of morphology and arrangement of some cell wall components.

appears to be broken down by nonenzymatic oxidative systems, such as activated oxygen and hydroxyl radicals (OH) produced during plant–fungus interactions. **Callose** differs from cellulose in that it consists of (1–3) β-D-glucan chains that can form duplexes and triplexes. Callose is normally made by a few cell types but is made by most cells following wounding and during attempted penetration by invading fungal hyphae.

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.

Cellulose-degrading enzymes (cellulases) have been shown to be produced by several phytopathogenic fungi, bacteria, and nematodes and are undoubtedly produced by parasitic higher plants. Saprophytic fungi, mainly certain groups of basidiomycetes, and, to a lesser degree, saprophytic bacteria cause the breakdown of most of the cellulose decomposed in nature. In living plant tissues, however, cellulolytic enzymes secreted by pathogens play a role in the softening and disintegration of cell wall material (Figs. 5-11 and 5-12). They facilitate the penetration and spread of the pathogen in the host and cause the collapse and disintegration of the cellular structure, thereby aiding the pathogen in the production of disease. Cellulolytic enzymes may further participate indirectly in disease development by releasing, from cellulose chains, soluble sugars that serve as food for the pathogen and, in the vascular diseases, by liberating into the transpiration stream large molecules from cellulose, which interfere with the normal movement of water. In the bacterial wilt of tomato, production of an endocellulase by the bacterium was required for the latter to be pathogenic and induce the disease.

Cross-Linking Glycans (Hemicelluloses)

Cross-linking glycans, known earlier as hemicelluloses, are complex mixtures of polysaccharide

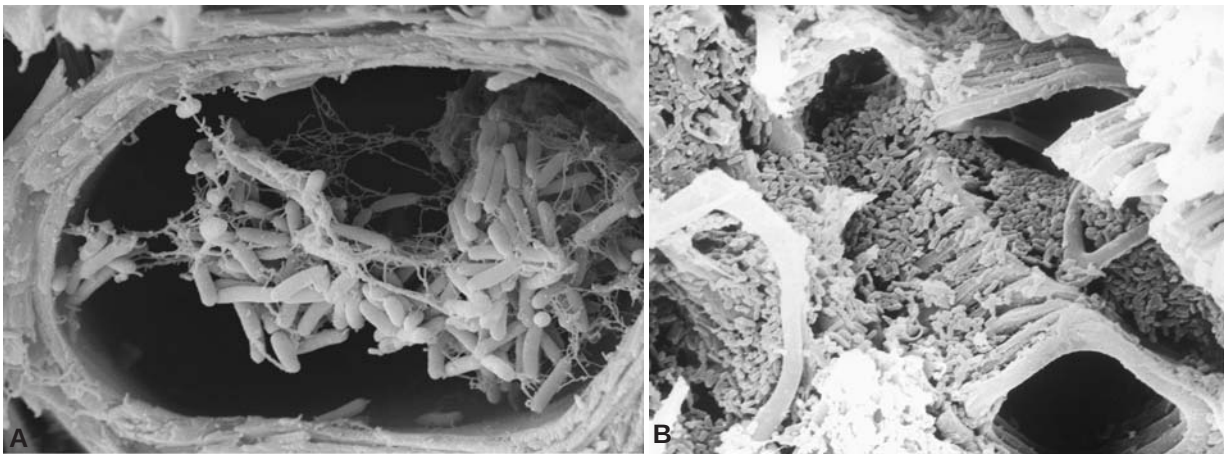


FIGURE 5-11 (A) Xylella bacteria in xylem vessel of citrus leaf. (B) Close-up of cell breakdown and maceration of pectic substances and celluloses of parenchyma cells and xylem vessels caused by enzymes secreted by bacteria of the genus *Pseudomonas*. Only the lignin-impregnated rings of xylem vessels remain intact. 1500×. [Photographs courtesy of (A) E. Alves, Federal University of Lavras, Brazil, and (B) E. L. Mansvelt, I. M. M. Roos, and M. J. Hattingh.]

polymers that can hydrogen-bond to and may cover and link cellulose microfibrils together (Figs. 5-10 and 5-12). Their composition and frequency seem to vary among plant tissues, plant species, and with the developmental stage of the plant. Cross-linking glycans are a major constituent of the primary cell wall and may also make up a varying proportion of the middle lamella and secondary cell wall. Hemicellulosic polymers include primarily xyloglucans and glucuronoarabinoxylans, but also glucomannans, galactomannans, arabinogalactans, and others. Xyloglucan, for example, is made of glucose chains with terminal branches of smaller xylose chains and lesser amounts of galactose, arabinose, and fucose. Cross-linking glycans link the ends of pectic polysaccharides and various points of the cellulose microfibrils.

The enzymatic breakdown of hemicelluloses appears to require the activity of many enzymes. Several hemicellulases seem to be produced by many plant pathogenic fungi. Depending on the monomer released from the polymer on which they act, the particular enzymes are called xylanase, galactanase, glucanase, arabinase, mannase, and so on. The nonenzymatic breakdown of hemicelluloses by activated oxygen, hydroxyl, and other radicals produced by attacking fungi also occurs. Despite the fact that fungal pathogens produce these enzymes and oxidative agents, it is still not clear how they contribute to cell wall breakdown or to the ability of the pathogen to cause disease.

Suberin

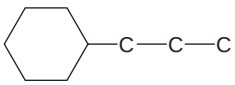
Suberin is found in certain tissues of various underground organs, such as roots, tubers, and stolons, and in periderm layers, such as cork and bark tissues. Suberins are also formed in response to wounding and to pathogen-induced defenses of certain organs and cell types. Typical suberization occurs, for example, in cut potato tubers where browning and encrustation develop in the form of multilamellar areas consisting of alternating polyaliphatic and polyaromatic layers. These layers are impermeable and help strengthen the cell wall and limit water loss through the wound. The aliphatic layer is composed of long chain (20 carbons or more) lipid substances, plus some specialized fatty acids, and is located between the primary cell wall and the plasmalemma. The polyaromatic layer consists of building blocks containing substances derived from hydroxycinnamic acid and is located in the cell wall. The polyaromatic layer also contains several phenolic compounds, such as chlorogenic acid, that act as local disinfectants. Although plants obviously produce enzymes that synthesize suberin, it is not known whether or how pathogens break it down during infection.

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.

Lignin is an amorphous, three-dimensional polymer that is different from both carbohydrates and proteins in composition and properties. The most common basic structural unit of lignin is a phenylpropanoid:

FIGURE 5-12 (A and B) Cellulases, produced by the corn stalk rot fungus *Fusarium* sp., have broken down cellulosic walls of corn cells but did not affect the lignified vascular bundles. (C and D) Ligninases of the basidiomycete fungus *Phellinus* have caused complete disintegration and discoloration of the heartwood in the pine trunk (C) and of the roots and lower stem of the tree, causing it to topple over (D). [Photographs courtesy of (A and B) G. Munkvold, Iowa State University, (C) R. L. Anderson, USDA Forest Service, and (D) R. L. James, USDA Forest Service.]



where one or more of the carbons have a —OH, —OCH_3, or =O group. Lignin forms by oxidative condensation (C—C and C—O bond formation) between such substituted phenylpropanoid units. The lignin polymer is perhaps more resistant to enzymatic degradation than any other plant substance (Figs. 5-11 and 5-12).

It is obvious that enormous amounts of lignin are degraded by microorganisms in nature, as is evidenced by the yearly decomposition of all annual plants and a

large portion of perennial plants. It is generally accepted, however, that 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 (Fig. 5-12).

In addition to wood-rotting basidiomycetes, several other pathogens, primarily several ascomycetes and imperfect fungi and even some bacteria, apparently produce small amounts of lignin-degrading enzymes and cause soft rot cavities in wood they colonize. However, it is not known to what extent the diseases they cause are dependent on the presence of such enzymes.

Cell Wall Flavonoids

Flavonoids are a large class of phenolic compounds that occur in most plant tissues and, especially, in the vacuoles. They also occur as mixtures of single and polymeric components in various barks and heartwoods. Among the various functions of flavonoids, some act as signaling molecules for certain functions in specific plant/microbe combinations. Many of them, however, are inhibitory or toxic to pathogens and some of them, e.g., medicarpin, act as phytoalexins and are involved in the inducible defense in plants against fungi. It is important, therefore, that pathogens be able to survive in the presence of various flavonoids in cell walls or they must be able to neutralize them or to break them down. Little is known how pathogens accomplish this, although the joining of phenolics with sugar molecules (glycosylation) seems to neutralize the toxicity of many phenolics.

Cell Wall Structural Proteins

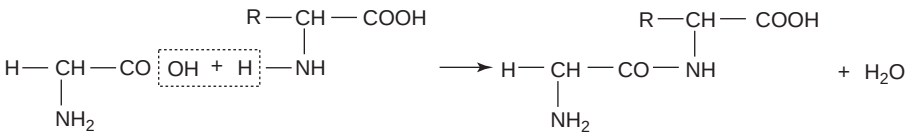
Cell walls consist primarily of polysaccharides, i.e., cellulose fibers embedded in a matrix of hemicellulose and pectin, but structural proteins, in the form of glycoproteins, may also form networks in the cell wall (Fig. 5-2). Four classes of structural proteins have been found in cell walls. Three of them are known by the most abundant amino acid they contain: **hydroxyproline-rich glycoproteins** (**HRGPs**), **proline-rich proteins** (**PRPs**), and **glycine-rich proteins** (**GRPs**). The fourth class is **arabinogalactan proteins** (**AGPs**). Each of these protein groups is coded by a large multigene family. Upon their production they are inserted in the endoplasmic reticulum and, through signal peptides they encode, they are targeted to the cell wall through the secretory pathway. One of the HRGP proteins is **extensin**, which makes up only 0.5% of the cell wall mass in healthy tissue but increases to 5 to 15% of the wall mass on infection with fungi and helps add rigidity to the cell wall. Another group of cell wall proteins are the **lectins**, which bind to specific sugar molecules**.** The role of all of these groups of proteins is not clear, but they are thought to accumulate in response to elicitor molecules released by fungi and to play a role in the plant defense response. The breakdown of structural proteins is presumably advantageous to invading pathogens and is thought to be similar to that of proteins contained within plant cells. This is discussed later.

Enzymatic Degradation of Substances Contained in Plant Cells

Most kinds of pathogens live all or part of their lives in association with or inside the living protoplast. These pathogens obviously derive nutrients from the protoplast. All the other pathogens — the great majority of fungi and bacteria — obtain nutrients from protoplasts after the latter have been killed. Some of the nutrients, e.g., sugars and amino acids, are molecules sufficiently small to be absorbed by the pathogen directly. Some of the other plant cell constituents, however, such as starch, proteins, and fats, can be utilized only after degradation by enzymes secreted by the pathogen.

Proteins

Plant cells contain innumerable different proteins, which play diverse roles as catalysts of cellular reactions (enzymes) or as structural material (in membranes and cell walls). Proteins are formed by the joining together of numerous molecules of about 20 different kinds of amino acids:



Amino Acids and Protein

All pathogens seem to be capable of degrading many kinds of protein molecules. The plant pathogenic enzymes involved in protein degradation are similar to those present in higher plants and animals and are called **proteases** or **proteinases** or, occasionally, peptidases.

Considering the paramount importance of proteins as enzymes, constituents of cell membranes, and structural components of plant cell walls, the degradation of host proteins by proteolytic enzymes secreted by pathogens can profoundly affect the organization and function of the host cells. The nature and extent of such effects, however, have been investigated little so far and their significance in disease development is not known.

Starch

Starch is the main reserve polysaccharide found in plant cells. Starch is synthesized in the chloroplasts and, in nonphotosynthetic organs, in the amyloplasts. Starch is a glucose polymer and exists in two forms: amylose, an essentially linear molecule, and amylopectin, a highly branched molecule of various chain lengths.

Most pathogens utilize starch, and other reserve polysaccharides, in their metabolic activities. The degradation of starch is brought about by the action of enzymes called **amylases**. The end product of starch breakdown is glucose and it is used by the pathogens directly.

Lipids

Various types of lipids occur in all plant cells, with the most important being **phospholipids** and **glycolipids**, both of which, along with protein, are the main constituents of all plant cell membranes. The latter form a hydrophobic barrier that is critical to life by separating cells from their surroundings and keeping organelles such as chloroplasts and mitochondria intact and separate from the cytoplasm. **Oils** and **fats** are found in many cells, especially in seeds where they function as energy storage compounds; **wax lipids** are found on most aerial epidermal cells. The common characteristic of all lipids is that they contain fatty acids, which may be saturated or unsaturated.

Several fungi, bacteria, and nematodes are known to be capable of degrading lipids. Lipolytic enzymes, called **lipases**, **phospholipases**, and so on, hydrolyze liberation of the fatty acids from the lipid molecule. The fatty acids are presumably utilized by the pathogen directly. But Some of them, before or after hyperoxidation by plant lipoxygenases or active oxygen species, provide signal molecules for the development of plant defenses and also act as antimicrobial compounds that inhibit the pathogen directly.

Microbial Toxins in Plant Disease

Living plant cells are complex systems in which many interdependent biochemical reactions are taking place concurrently or in a well-defined succession. These reactions result in the intricate and well-organized processes essential for life. Disturbance of any of these metabolic reactions causes disruption of the physiological processes that sustain the plant and leads to the development of disease. Among the factors inducing such disturbances are substances that are produced by plant pathogenic microorganisms and are called toxins. 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. Many toxins exist in multiple forms that have different potency.

Fungi and bacteria may produce toxins in infected plants as well as in culture medium. Toxins, however, are extremely poisonous substances and are effective in very low concentrations. Some are unstable or react quickly and are bound tightly to specific sites within the plant cell.

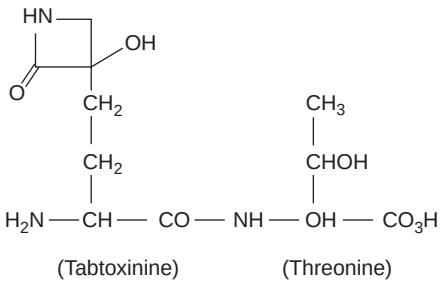
Toxins injure host cells either by affecting the permeability of the cell membrane (Fig. 5-2) or by inactivating or inhibiting enzymes and subsequently interrupting the corresponding enzymatic reactions. Certain toxins act as antimetabolites and induce a deficiency for an essential growth factor.

Toxins 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. Several of these toxins, e.g., tabtoxin and phaseolotoxin, inhibit normal host enzymes, thereby leading to increases in toxic substrates or to depletion of needed compounds. Several toxins affect the cellular transport system, especially H^+/K^+ exchange at the cell membrane. Some, e.g., tagetitoxin, act as inhibitors of transcription in cell organelles, such as the chloroplasts. Others, e.g., cercosporin, act as photosensitizing agents, causing the peroxidation of membrane lipids.

Tabtoxin

Tabtoxin is produced by the bacterium *Pseudomonas syringae*; pv. *tabaci*, which causes the wildfire disease of tobacco; by strains of pv. tabaci occurring on other hosts such as bean and soybean; and by other pathovars (subspecies) of *P. syringae*, such as those occurring on oats, maize, and coffee. Toxin-producing strains cause necrotic spots on leaves, with each spot surrounded by a yellow halo (Figs. 5-13A and 5-13B). 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 (nonhost-specific toxin!). Strains of *P. syringae* pv. *tabaci* sometimes produce mutants that have lost the ability to produce the toxin (they become Tox^-). Tox^- strains show reduced virulence and cause necrotic leaf spots without the yellow halo. Tox^- strains are indistinguishable from *P. angulata*, the cause of angular leaf spot of tobacco, which is now thought to be a nontoxigenic form of *P. syringae* pv. *tabaci*.



Tabtoxin

Tabtoxin is a dipeptide composed of the common amino acid threonine and the previously unknown amino acid tabtoxinine. Tabtoxin as such is not toxic, but in the cell it becomes hydrolyzed and releases tabtoxinine, which is the active toxin. 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. The effects of the toxin lead to a reduced ability of the plant to respond actively to the bacterium.

Phaseolotoxin

Phaseolotoxin is produced by the bacterium *Pseudomonas syringae* pv. *phaseolicola*, the cause of halo blight of bean (Fig. 5-13C) and some other legumes. The localized and systemic chlorotic symptoms produced in infected plants are identical to those produced on plants treated with the toxin alone so they are apparently the results of the toxin produced by the bacteria. Infected plants and plants treated with purified toxin also show reduced growth of newly expanding leaves, disruption of apical dominance, and accumulation of the amino acid ornithine.

Phaseolotoxin is a modified ornithine–alanine–arginine tripeptide carrying a phosphosulfinyl group. Soon after the tripeptide is excreted by the bacterium into the plant, plant enzymes cleave the peptide bonds and release alanine, arginine, and phosphosulfinylornithine. The latter 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. Phaseolotoxin plays a major role in the virulence of the pathogen by interfering with or breaking the disease resistance of the host toward not only the halo blight bacterium, but also several other fungal, bacterial, and viral pathogens.

Tentoxin

Tentoxin is produced by the fungus *Alternaria alternata* (previously called *A. tenuis*), which causes spots and chlorosis (Fig. 5-13D) in plants of many species. Seedlings with more than one-third of their leaf area chlorotic die, and those with less chlorosis are much less vigorous than healthy plants.

Tentoxin is a cyclic tetrapeptide that binds to and inactivates a protein (chloroplast-coupling factor) involved in energy transfer into chloroplasts. The toxin also inhibits the light-dependent phosphorylation of ADP to ATP. Both the inactivation of the protein and the inhibition of photophosphorylation are much greater in plant species susceptible to chlorosis after tentoxin treatment than in species not sensitive to the toxin. In sensitive species, tentoxin interferes with normal chloroplast development and results in chlorosis by disrupting chlorophyll synthesis, but it is not certain that these effects are solely related to tentoxin binding to the chloroplast-coupling factor protein. An additional but apparently unrelated effect of tentoxin on sensitive plants is that it inhibits the activity of polyphenol

FIGURE 5-13 Symptoms caused by nonhost-selective toxins. Early (A) and semiadvanced (B) symptoms of young tobacco leaves showing spots caused by the bacterium *Pseudomonas syringae* pv. *tabaci*. The chlorotic halos surrounding the necrotic white spots are caused by the tabtoxin produced by the bacterium. (C) Leaf spots and halos caused by the toxin phaseolotoxin produced by the bacterium *Pseudomonas phaseolicola*, the cause of halo blight of bean. (D) Leaf spots and chlorosis caused by the *Alternaria alternata* toxin. [Photographs courtesy of (A, B, and D) Reynolds Tobacco Co. and (C) Plant Pathology Department, University of Florida.]

oxidases, enzymes involved in several resistance mechanisms of plants. Both effects of the toxin, namely stressing the host plant with events that lead to chlorosis and suppressing host resistance mechanisms, tend to enhance the virulence of the pathogen. The molecular site of action of tentoxin, however, and the exact mechanism by which it brings about these effects are still unknown.

Cercosporin

Cercosporin is produced by the fungus *Cercospora* and by several other fungi. It causes damaging leaf spot and blight diseases of many crop plants, such as Cercospora leaf spot of zinnia (Fig. 5-14A) and gray leaf spot of corn (Fig. 5-14B).

Cercosporin is unique among fungal toxins in that it is activated by light and becomes toxic to plants by generating activated species of oxygen, particularly single oxygen. The generated active single oxygen destroys the membranes of host plants and provides nutrients for this intercellular pathogen. Cercosporin is a photosensitizing perylenequinone that absorbs light energy, it is converted to an energetically activated state and then reacts with molecular oxygen and forms activated oxygen. The latter reacts with lipids, proteins, and nucleic acids of plant cells and severely damages or kills the plant cells, thereby enhancing the virulence of the pathogen. The

FIGURE 5-14 Leaf spots on zinnia (A) and gray leaf spots on corn (B) caused by the photosensitizing toxin cercosporin, produced by different species of the fungus *Cercospora*. [Photographs courtesy of (A) Plant Pathology Department, University of Florida and (B) G. Munkvold, Iowa State University.]

ability of fungal spores and mycelium to survive the general toxicity of cercosporin is due to the production by the fungus of pyridoxine (vitamin B_6). Pyridoxine reacts with single oxygen atoms and is currently neutralized during that reaction.

Other Nonhost-Specific Toxins

Numerous other nonhost-specific toxic substances have been isolated from cultures of pathogenic fungi and bacteria and have been implicated as contributing factors in the development of the disease caused by the pathogen. Among such toxins produced by fungi are fumaric acid, produced by *Rhizopus* spp. in almond hull rot disease; oxalic acid, produced by *Sclerotium* and *Sclerotinia* spp. in various plants they infect and by *Cryphonectria parasitica*, the cause of chestnut blight; alternaric acid, alternariol, and zinniol produced by *Alternaria* spp. in leaf spot diseases of various plants; ceratoulmin, produced by *Ophiostoma ulmi* in Dutch elm disease; fusicoccin, produced by *Fusicoccum amygdali* in the twig blight disease of almond and peach trees; ophiobolins, produced by several *Cochliobolus* spp. in diseases of grain crops; pyricularin, produced by *Pyricularia grisea* in rice blast disease; fusaric acid and lycomarasmin, produced by *Fusarium oxysporum* in tomato wilt; and many others. Other nonhost-specific toxins produced by bacteria are coronatine, produced by *P. syringae* pv. *atropurpurea* and other forms infecting grasses and soybean; syringomycin, produced by *P. syringae* pv. *syringae* in leaf spots of many plants; syringotoxin, produced by *P. syringae* pv. *syringae* in citrus plants; and tagetitoxin, produced by *P. syringae* pv. *tagetis* in marigold leaf spot disease. One family of toxins essential for pathogenicity, is the thaxtomins, produced by species of the bacterium *Streptomyces* that cause root and tuber roots. Thaxtomins cause dramatic plant cell hypertrophy and/or seedling stunting by altering the development of primary cell walls and the ability of the cells to go through normal cell division cycles.

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. So far, host-specific toxins have been shown to be produced only by certain fungi (*Cochliobolus*, *Alternaria*, *Periconia*, *Phyllosticta*, *Corynespora*, and *Hypoxylon*), although certain bacterial polysaccharides from *Pseudomonas* and *Xanthomonas* have been reported to be host specific.

Victorin, or HV Toxin

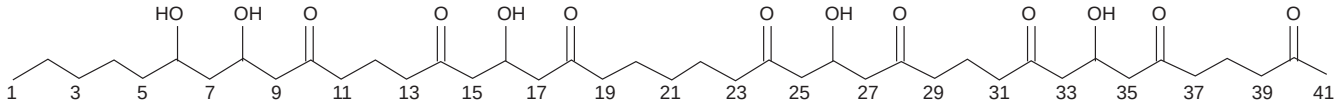
Victorin, or Hv-toxin, is produced by the fungus *Cochliobolus (Helminthosporium) victoriae*. This fungus appeared in 1945 after the introduction and widespread use of the oat variety Victoria and its derivatives, all of which contained the gene V_b for resistance to crown rust disease. *C. victoriae* infects the basal portions of susceptible oat plants and produces a toxin that is carried to the leaves, causes a leaf blight, and destroys the entire plant. All other oats and other plant species tested were either immune to the fungus and to the toxin or their sensitivity to the toxin was proportional to their susceptibility to the fungus. Toxin production in the fungus is controlled by a single gene. Resistance and susceptibility to the fungus, as well as tolerance and sensitivity to the toxin, are controlled by the same pair of alleles, although different sets of these alleles may be involved in cases of intermediate resistance. The toxin not only produces all the external symptoms of the disease induced by the pathogen, but it also produces similar histochemical and biochemical changes in the host, such as changes in cell wall structure, loss of electrolytes from cells, increased respiration, and decreased growth and protein synthesis. Moreover, only fungus isolates that produce the toxin in culture are pathogenic to oats, whereas those that do not produce toxin are nonpathogenic.

Victorin has been purified and its chemical structure has been determined to be a complex chlorinated, partially cyclic pentapeptide. The primary target of the toxin seems to be the cell plasma membrane where victorin seems to bind to several proteins. The possible site of action of victorin seems to be the glycine decarboxylate complex, which is a key component of the photorespiratory cycle. Considerable evidence, however, indicates that victorin functions as an elicitor that induces components of a resistance response that include many of the features of hypersensitive response and lead to programmed cell death.

*T Toxin [*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 (Fig. 5-15A). Race T, indistinguishable from all other *C. heterostrophus* races except for its ability to produce the T toxin, appeared in the United States in 1968. By 1970, it had spread throughout the corn belt, attacking only corn that had the Texas male-sterile (Tms) cytoplasm. Corn with normal cytoplasm was resistant to the fungus and the toxin. Resistance and susceptibility to *C. heterostrophus* T and its toxin are inherited maternally (in cytoplasmic genes). The ability of *C. heterostrophus* T to produce T toxin and its virulence to corn with Tms cytoplasm are controlled by one and the same gene. T toxin does not seem to be necessary for the pathogenicity of *C. heterostrophus* race T, but it increases the virulence of the pathogen.

T toxin is a mixture of linear, long (35 to 45 carbon) polyketols, the most prevalent having the following formula:



T toxin

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. It is now thought that plants exhibiting cytoplasmic male sterility of the Texas type have a slight rearrangement in their mitochondrial DNA comprising gene *T-urf13* that codes for the production of protein URF13. This gene and its protein are absent from maize lines with normal cytoplasm. When the T toxin is present, protein URF13 forms pores in the inner mitochondrial membrane of maize lines with cytoplasmic male sterility. The pores cause loss of mitochondrial integrity, i.e., loss of selective permeability of the mitochondrial membrane, and disease.

HC Toxin

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

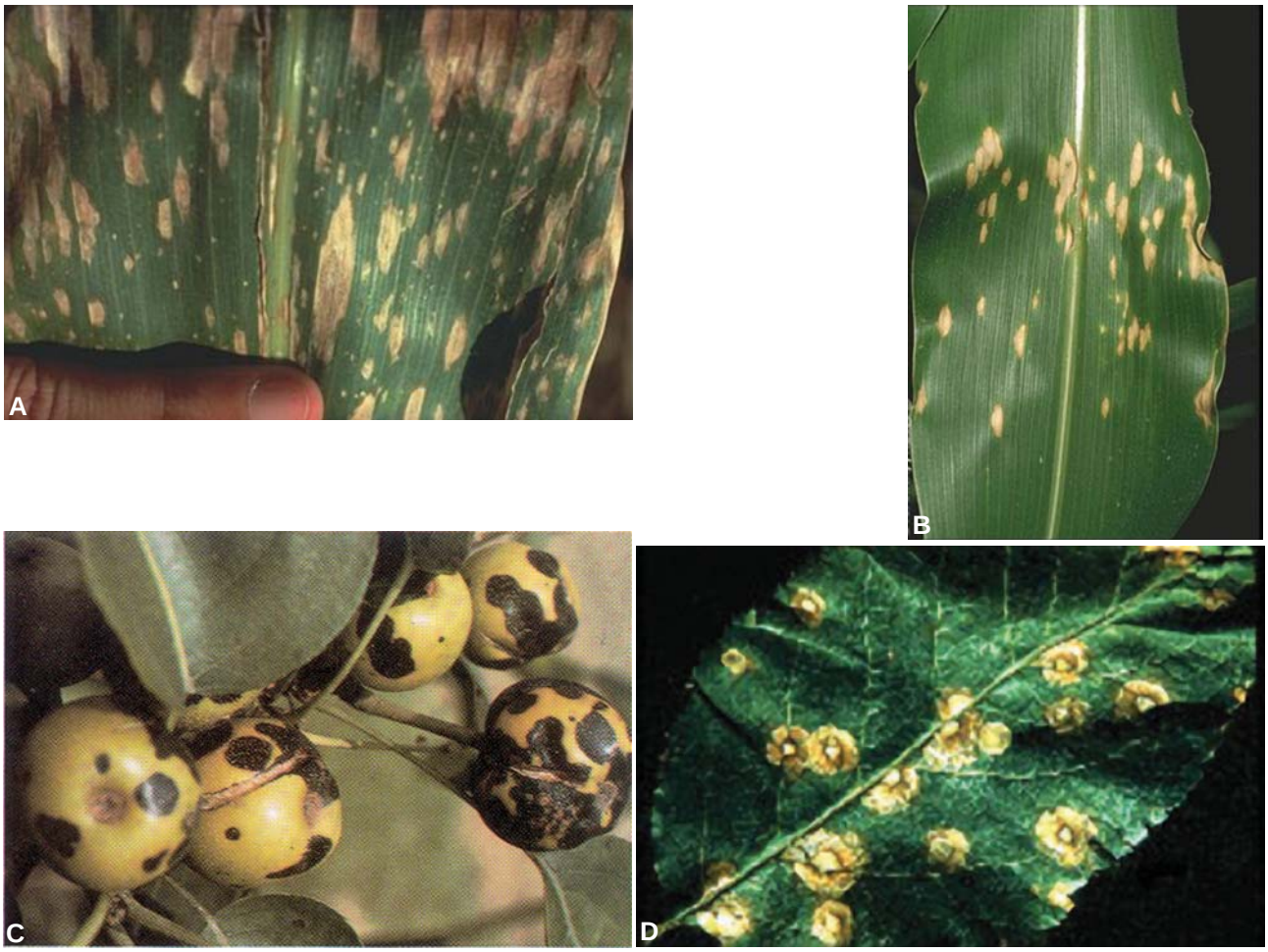


FIGURE 5-15 Symptoms caused by host-selective toxins. (A) Southern corn leaf blight symptoms caused by two race T of the fungus *Cochliobolus (Helminthosporium) heterostrophus* and its toxin, T toxin, on a corn plant containing Texas male-sterile cytoplasm. (B) Northern corn leaf spot symptoms caused by the fungus *Cochliobolus carbonum* and its toxin, HC toxin, on corn. (C) Fruit spots on Japanese pear caused by one of the strains of the fungus *Alternaria alternata* and its toxin, AK toxin. (D) Leaf spots caused by the AM toxin produced by another strain of the fungus *A. alternata* and its toxin, AM toxin, on apple leaves. [Photographs courtesy of (A) C. Martinson and (B) G. Munkvold, Iowa State University, (C) T. Sakuma, and (D) J. W. Travis, Pennsylvania State University.]

toxin. Experimental findings suggest that the HC toxin is not actually toxic in itself, but rather acts as a virulence factor by preventing initiation of the changes in gene expression that are necessary for the establishment of induced defense responses, i.e., it acts as a suppressor of defense responses.

Alternaria alternata *Toxins*

Several pathotypes of *Alternaria alternata* attack different host plants and on each they produce one of several multiple forms of related compounds that are toxic only on the particular host plant of each pathotype. Some of the toxins and the hosts on which they are produced and affect are the AK toxin causing black spot on Japanese peat fruit (Fig. 5-15C), the AAL toxin causing stem canker on tomato, the AF toxin on strawberry, the AM toxin on apple, the ACT toxin on tangerine, the ACL toxin on rough lemon, and the HS toxin on sugar cane.

As an example of *A. alternata* toxins, the AM toxin is produced by the apple pathotype of *A. alternata*, known previously as *A. mali*, the cause of alternaria leaf blotch of apple (Fig. 5-15D). The toxin molecule is a

cyclic depsipeptide and usually exists as a mixture of three forms. The toxin is extremely selective for susceptible apple varieties, whereas resistant varieties can tolerate more than 10,000 times as much toxin without showing symptoms. The AM toxin causes plasma membranes of susceptible cells to develop invaginations, and cells show a significant loss of electrolytes. The initial toxic effect of the toxin seems to occur at the interface between the cell wall and the plasma membrane. However, the AM toxin also causes rapid loss of chlorophyll, suggesting that this toxin has more than one site of action.

Other Host-Specific Toxins

At least two other fungi produce well-known host-specific toxins: *Periconia circinata* produces peritoxin (PC toxin), which causes sorghum rot in sorghum root rot disease; *Mycosphaerella (Phyllosticta) zeae-maydis* produces the PM toxin (T toxin) in corn that has Texas male-sterile cytoplasm; and *Pyrenophora tritici-repentis* produces the Ptr toxin, which causes the tan spot of wheat. Another fungus, *Corynespora cassiicola*, produces the CC toxin in tomato. Toxins produced by some other fungi, e.g., *Hypoxylon mammatum* on poplar and *Perenophora teres* on barley, seem to be species selective rather than host specific. In addition, there are the SV toxins produced by *Stemphylium vesicarium* on European pear and destruxin B from *A. brassicae* on brassicas.

Growth Regulators in Plant Disease

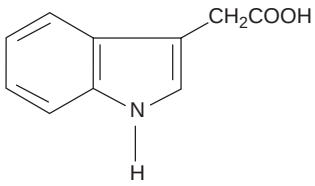
Plant growth is regulated by a small number of groups of naturally occurring compounds that act as hormones and are generally called growth regulators. The most important growth regulators are auxins, gibberellins, and cytokinins, but other compounds, such as ethylene and growth inhibitors, play important regulatory roles in the life of the plant. Growth regulators act in very small concentrations and even slight deviations from the normal concentration may bring about strikingly different plant growth patterns. The concentration of a specific growth regulator in the plant is not constant, but it usually rises quickly to a peak and then declines quickly as a result of the action of hormone-inhibitory systems present in the plant. Growth regulators appear to act, at least in some cases, by promoting the synthesis of messenger RNA molecules. This leads to the formation of specific enzymes, which in turn control the biochemistry and the physiology of the plant.

Plant pathogens may produce more of the same growth regulators as those produced by the plant or more of the same inhibitors of the growth regulators as those produced by the plant. They may produce new and different growth regulators or inhibitors of growth regulators. Alternatively, they may produce substances that stimulate or retard the production of growth regulators or growth inhibitors by the plant.

Whatever the mechanism of action involved, pathogens often cause an imbalance in the hormonal system of the plant and bring about abnormal growth responses incompatible with the healthy development of a plant. That pathogens can cause disease through the secretion of growth regulators in the infected plant or through their effects on the growth regulatory systems of the infected plant is made evident by the variety of abnormal plant growth responses they cause, such as stunting, overgrowths, rosetting, excessive root branching, stem malformation, leaf epinasty, defoliation, and suppression of bud growth. The most important groups of plant growth regulators, their function in the plant, and their role in disease development, where known, are discussed next.

Auxins

The auxin occurring naturally in plants is indole-3-acetic acid (IAA). Produced continually in growing plant tissues, IAA moves rapidly from the young green tissues to older tissues, but is destroyed constantly by the enzyme indole-3-acetic acid oxidase, which explains the low concentration of the auxin.



Indole-3-acetic acid

The effects of IAA on the plant are numerous. It is required for cell elongation and differentiation, and absorption of IAA to the cell membrane also affects the permeability of the membrane. The compound causes a general increase in the respiration of plant tissues and promotes the synthesis of messenger RNA and, subsequently, of proteins/enzymes as well as structural proteins.

Increased auxin (IAA) levels occur in many plants infected by fungi, bacteria, viruses, mollicutes, and nematodes, although some pathogens seem to lower the auxin level of the host. Thus, the basidiomycete *Exobasidium azaleae* causing azalea leaf and flower gall (Fig. 5-16A), the protozoon causing clubroot of cabbage (*Plasmodiophora brassicae*) (Fig. 5-16E), the bacterium

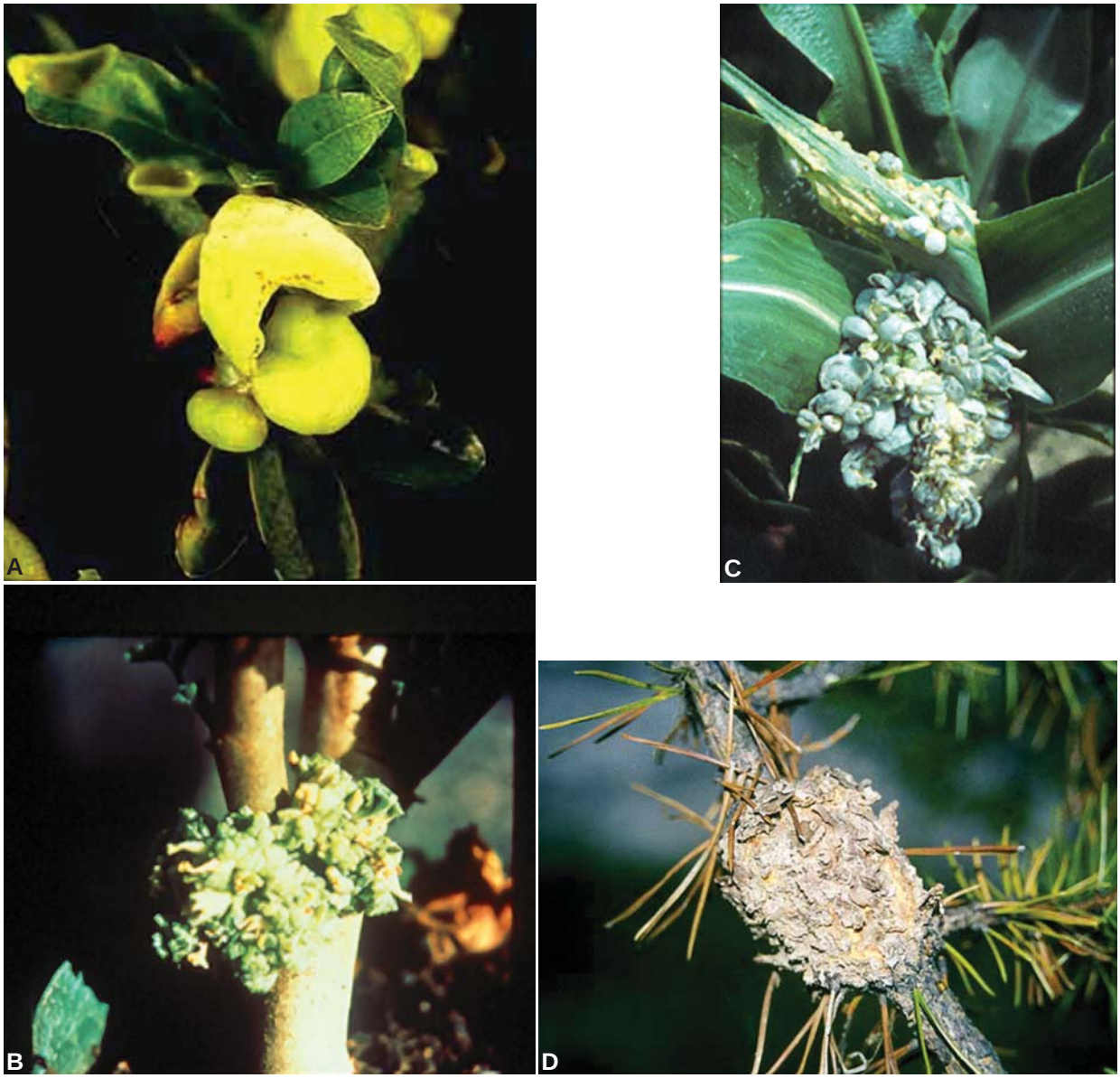


FIGURE 5-16 Plant diseases showing symptoms caused by the excessive production of growth regulators (primarily auxins) by the pathogen. (A) Enlarged and deformed leaf and flower gall of azalea caused by infection of the fungus *Exobasidium azaleae*. (B) Leafy gall produced on a sweet pea plant as a result of infection by the bacterium *Rhodococcus fascians*. (C) Corn ear and tassel showing numerous small galls as a result of infection by the corn smut fungus *Ustilago maydis*. (D) Western pine gall caused by the fungus *Cronartium* sp. (E) Cabbage roots enlarged grotesquely following infection with the clubroot pathogen *Plasmodiophora brassicae*. A few normal, thin roots are still present. (F) Root galls on bean plant infected with the root-knot nematode *Meloidogyne* sp. [Photographs courtesy of (A and B) Oregon State University, (C) K. Mohan, Idaho State University, (D) E. Hansen, Oregon State University, (E) University of Minnesota, and (F) R. T. MacMillan, University of Florida.]

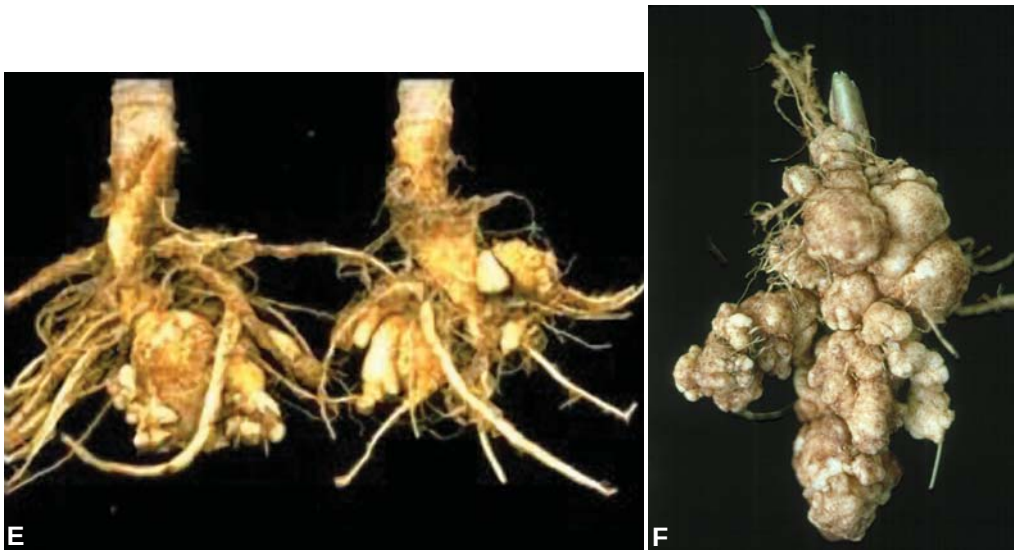


FIGURE 5-16 (*Continued*)

A. tumefaciens causing crown gall (Fig. 5-17A) and the one causing leafy gall of sweet pea and other plants (Fig. 5-16B), the fungi causing corn smut (*Ustilago maydis*) (Fig. 5-16C), cedar apple rust (*Gymnosporangium juniperi-virginianae*), banana wilt (*Fusarium oxysporum f. cubense*), pine western gall rust (Fig. 5-16D), the root-knot nematode (*Meloidogyne* sp.) (Fig. 5-16F), and others not only induce increased levels of IAA in their respective hosts, but are themselves capable of producing IAA. In some diseases, however, increased levels of IAA are wholly or partly due to the decreased degradation of IAA through the inhibition of IAA oxidase, as has been shown to be the case in several diseases, including corn smut and stem rust of wheat.

The production and role of auxin in plant disease have been studied more extensively in some bacterial diseases of plants. *Ralstonia solanacearum*, the cause of bacterial wilt of solanaceous plants, induces a 100-fold increase in the IAA level of diseased plants compared with that of healthy plants. How the increased levels of IAA contribute to the development of wilt of plants is not yet clear, but the 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, and may facilitate their degradation by, the respective enzymes secreted by the pathogen. An increase in IAA levels seems to inhibit the lignification of tissues and may thus prolong the period of exposure of the nonlignified tissues to the cell wall-degrading enzymes of the pathogen. Increased respiratory rates in the infected tissues may also be due to high IAA levels, and because auxin affects cell permeability, it may be responsible for the increased transpiration of the infected plants.

In **crown gall**, a disease caused by the bacterium *A. tumefaciens* on more than a hundred plant species, galls or tumors develop on the roots, stems (Figs. 3-2E, 3-11E, and 5-17A), leaves, ears, tassels, and petioles of host plants. Crown gall tumors develop when crown gall bacteria enter fresh wounds on a susceptible host. Immediately after wounding, cells around the wound produce various phenolic compounds and are activated to divide. *Agrobacterium* bacteria do not invade cells but attach to cell walls, and, in response to phenolic compounds such as acetosyringone and other signals, they become activated and begin processing the DNA in their Ti plasmid (for tumor-inducing plasmid) (Fig. 5-17). During the intense cell division of the second and third days after wounding, the plant cells are somehow conditioned and made receptive to a piece of bacterial plasmid DNA (called T-DNA, for tumor DNA). Proteins coded by genes in the T-DNA virulence (Vir) region cut out a single strand of the T-DNA from the Ti plasmid and transfer it into the plant cell nucleus as a T-DNA–protein complex. The T-DNA then becomes integrated into the nuclear plant DNA (chromosomes) and some of its genes are expressed and lead to the synthesis of auxins and cytokinins, which transform normal plant cells into tumor cells. Tumor cells subsequently grow and divide independently of the bacteria, and their

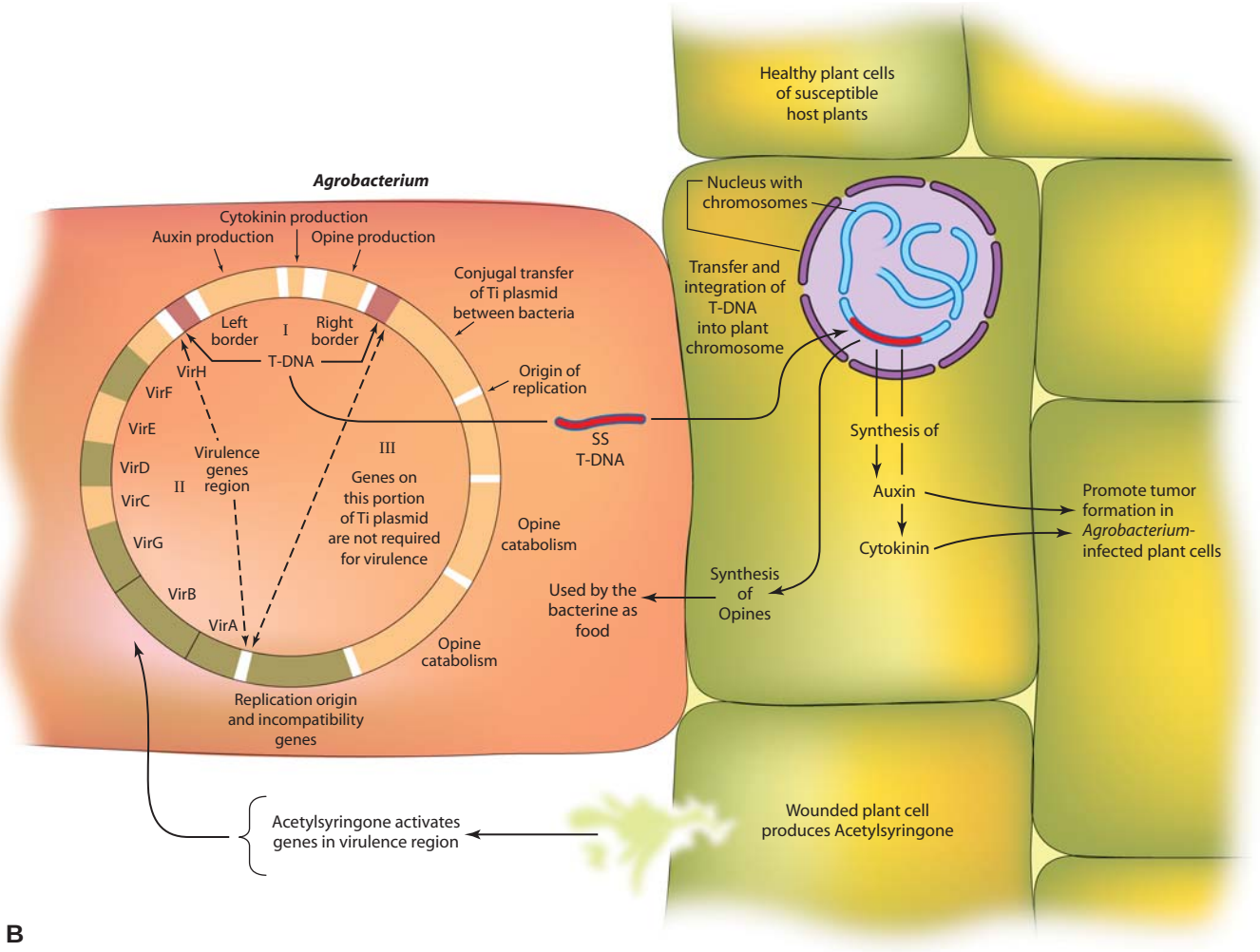


FIGURE 5-17 (A) External and cross-sectional view of crown gall on a rose stem caused by the bacterium *Agrobacterium tumefaciens*. (B) Schematic representation of the structure of Ti plasmid of the bacterium and of the transfer, integration, and expression of T-DNA in an infected plant that results in the production of crown gall tumors. Genes A, B, D, and G are needed for tumor formation on any susceptible plant species. Genes C, E, F, and H affect the host plant range and/or the size of tumors caused by the bacterium. The functions of the proteins of virulence genes are as follows: A, receptor of wound signal; B, codes for proteins that form membrane pores; C, enhances transfer of T-DNA; D, codes for proteins that nick T-DNA at its borders, help transport T-DNA across membranes, and carry signal compounds to the nucleus; E, protects T-DNA from nuclease enzymes and also carries nuclear localization signals; F, may increase host range of tumor induction; G, activates other virulence genes; H, protects the bacterium from toxic plant compounds. The entire diagram presents a simplified scheme of interaction of gene products of host cells and T-DNA that lead to the production of a gall. [Photograph (A) courtesy of Oregon State University.]

organization, rate of growth, and rate of division can no longer be controlled by the host plant.

The integrated T-DNA also contains genes that code for substances known as opines. Transformed plant cells produce opines, which can be used only by the intercellularly growing crown gall bacteria as a source of food. Although the increased levels of IAA and cytokinins of tumor cells are sufficient to cause the autonomous enlargement and division of these cells once they have been transformed to tumor cells, high IAA and cytokinin levels alone cannot cause the transformation of healthy cells into tumor cells. What other conditions or substances are involved in the transformation of healthy cells into tumor cells is not known.

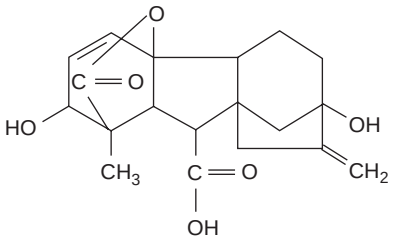
In the **knot disease** of olive, oleander, and privet, another hyperplastic disease caused by the bacterium *Pseudomonas savastanoi*, the pathogen produces IAA, which induces infected plants to produce galls. The more IAA a strain produces, the more severe the symptoms it causes. Strains that do not produce IAA fail to induce the formation of galls. The bacterial genes for IAA production are in a plasmid carried in the bacterium, but some IAA synthesis is also carried out by a gene in the chromosome of the bacterium.

In the **leafy gall disease** of many plants caused by the bacterium *Rhodococcus fascians*, leafy galls are produced that consist of centers of shoot overproductions and shoot growth inhibition. The bacterium exists mostly at the surface of the plant tissues, but it can also grow internally in the plant. Auxin, cytokinins, and other hormonal substances are produced by the bacterium in cultured and by infected tissues. Signals from bacteria involved in the development of symptoms initiate new cell divisions and formation of shoot meristem in tissues already differentiated. The bacterial signals originate in genes located on a linear plasmid and exert activities much more unique and more complex than those of cytokinins alone.

Gibberellins

Gibberellins are normal constituents of green plants and are also produced by several microorganisms. Gibberellins were first isolated from the fungus *Gibberella fujikuroi*, the cause of the foolish seedling disease of rice (Figure 1-37D). The best-known gibberellin is gibberellic acid. Compounds such as vitamin E and helminthosporol also have gibberellin-like activity.

Gibberellins have striking growth-promoting effects. They speed the elongation of dwarf varieties to normal sizes and promote flowering, stem and root elongation, and growth of fruit. Such *elongation* resembles in some respects that caused by IAA, and gibberellin also induces IAA formation. Auxin and gibberellin may also act synergistically. Gibberellins seem to activate genes that have been previously "turned off." The foolish seedling disease of rice, in which rice seedlings infected with the fungus *Gibberella fujikuroi* grow rapidly and become much taller than healthy plants, is apparently the result, to a considerable extent at least, of the gibberellin secreted by the pathogen.

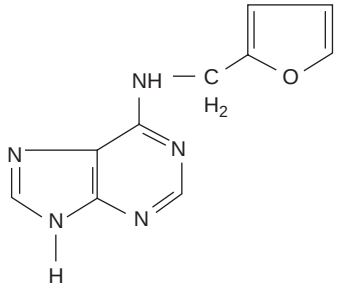


Gibberellins acid

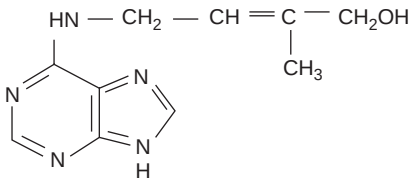
Although no difference has been reported so far in the gibberellin content of healthy and virus- or mollicute-infected plants, spraying of diseased plants with gibberellin overcomes some of the symptoms caused by these pathogens. Thus, stunting of corn plants infected with corn stunt spiroplasma and of tobacco plants infected with severe etch virus was reversed after treatment with gibberellin. Axillary bud suppression, caused by prunus dwarf virus (PDV) on cherry and by leaf curl virus on tobacco, was also overcome by gibberellin sprays. The same treatment also increased fruit production in PDV-infected cherries. In most of these treatments the pathogen itself does not seem to be affected and the symptoms reappear on the plants after gibberellin applications are stopped. It is not known, however, whether the pathogen-caused stunting of plants is actually due to reduced gibberellin concentration in the diseased plant, especially since the growth of even healthy plants is equally increased after gibberellin treatments.

Cytokinins

Cytokinins are potent growth factors necessary for cell growth and differentiation. In addition, they inhibit the breakdown of proteins and nucleic acids, thereby causing the inhibition of senescence, and they have the capacity to direct the flow of amino acids and other nutrients through the plant toward the point of high cytokinin concentration. Cytokinins occur in very small concentrations in green plants, in seeds, and in the sap stream. The first compound with cytokinin activity to be identified was kinetin, which, however, was isolated from herring sperm DNA and does not occur naturally in plants. Several cytokinins, e.g., zeatin and isopentenyl adenosine (IPA), have since been isolated from plants.



Kinetin



Zeatin

Cytokinins act by preventing genes from being turned off and by activating genes that have been previously turned off. The role of cytokinins in plant disease has just begun to be studied. Cytokinin activity increases in clubroot galls, in crown galls, in smut and rust galls, and in rust-infected bean leaves. In the latter, cytokinin activity seems to be related to both the juvenile feature of the green islands around the infection centers and the senescence outside the green island. However, cytokinin activity is lower in the sap and in tissue extracts of cotton plants infected with verticillium wilt and in plants suffering from drought. A cytokinin is partly responsible for several bacterial galls of plants, such as "leafy" gall disease of sweet pea caused by the bacterium *Rhodococcus (Corynebacterium) fascians*, and for the witches' broom diseases caused by fungi and mollicutes.

Treating plants with kinetin before or shortly after inoculation with a virus seems to reduce the number of infections in local lesion hosts and to reduce virus multiplication in systematically infected hosts.

Ethylene: $CH_2{=}CH_2$

Produced naturally by plants, ethylene exerts a variety of effects on plants, including chlorosis, leaf abscission, epinasty, stimulation of adventitious roots, and fruit ripening. Ethylene also causes increased permeability of cell membranes, which is a common effect of infections. However, ethylene production in infected tissues often parallels the formation of phytoalexins and the increased synthesis or activity of several enzymes or signal compounds that may play a role in increasing plant resistance to infection. Never-the-less it has not been shown that ethylene actually provides resistance. Ethylene is produced by several plant pathogenic fungi and bacteria. In the fruit of banana infected with *Ralstonia solanacearum*, the ethylene content increases proportionately with the (premature) yellowing of the fruit, whereas no ethylene can be detected in healthy fruits. Ethylene has also been implicated in the leaf epinasty symptom of the vascular wilt syndromes and in the premature defoliation observed in several types of plant diseases. In Verticillium wilt of tomato, the presence of ethylene at the time of infection inhibits disease development, whereas the presence of ethylene after infection has been established enhances Verticillium wilt development.

Polysaccharides

Fungi, bacteria, nematodes, and possibly other pathogens constantly release varying amounts of mucilaginous substances that coat their bodies and provide the interface between the outer surface of the microorganism and its environment. Exopolysaccharides appear to be necessary for several pathogens to cause normal disease symptoms either by being directly responsible for inducing symptoms or by indirectly facilitating pathogenesis by promoting colonization or by enhancing survival of the pathogen.

The role of slimy polysaccharides in plant disease appears to be particularly important in wilt diseases caused by pathogens that invade the vascular system of the plant. In vascular wilts, large polysaccharide molecules released by the pathogen in the xylem may be sufficient to cause a mechanical blockage of vascular bundles and thus initiate wilting (Figures 3-3E,F and 3-5D,E). Although such an effect by the polysaccharides alone may occur rarely in nature, when it is considered together with the effect caused by the macromolecular substances released in the vessels through the breakdown of host substances by pathogen enzymes, the possibility of polysaccharide involvement in the blockage of vessels during vascular wilts becomes obvious.

Detoxification of Low Molecular Weight Antimicrobial Molecules

Several kinds of low molecular weight antimicrobial molecules are present in plants or are produced by them

in response to infection by pathogens. Some of the most common constitutive such substances are the **saponins**, which include the avenacins and the tomatines. Saponins are glycosylated triterpenoid or steroid alkaloid molecules that provide plants with some degree of protection against fungal pathogens. Saponins are thought to provide antifungal protection by forming complexes with cell membranes, leading to the formation of pores and loss of membrane integrity.

Avenacins are produced in oat roots and leaves and they protect oats from the root-infecting fungus *Gaeumannomyces graminis* while it infects the other cereals that contain no avenacins. A strain of the fungus that infects oats, *G. graminis f. sp. avenae*, produces the avenacin-detoxifying enzyme avenacinase, which is required for pathogenicity on oats. Also, the fungus *Stagonospora avenae* can infect oat leaves, despite the fact that they contain avenacins, by secreting at least three enzymes that degrade and detoxify the avenacins. Another saponin, tomatine, is present in tomatoes, which are protected from infection by some fungi that lack the tomatinase enzyme needed for tomatine detoxification. The fungus *Septoria lycopersici* produces tomatinase and infects tomato plants. Mutants of this fungus, however, that do not produce tomatinase were sensitive to tomatine but could still grow in its presence. They could cause lesions on tomato leaves that actually had more dying mesophyll cells and greater activity of a defense-related enzyme. It is not clear whether this behavior of the host is the result of differences between the mutants and the normal strains or whether the production of tomatinase helps suppress some mechanism(s) of plant defense. In *Botrytis cinerea*, all but 1 of 13 isolates could detoxify tomatine and could severely infect tomato, while one strain that was more sensitive to tomatine was also much less aggressive on tomato.

Promotion of Bacterial Virulence by *avr* Genes

avr genes in bacteria are thought to encode or to direct the production of molecules that are recognized by the host plant and elicit the rapid induction of defense responses on resistant host plants. Their prevalence among pathogens, however, suggests that they may provide some advantage to the pathogen in addition to warning host plants that they are about to be attacked. It has been proposed, therefore, and been demonstrated in many plant–bacteria combinations, that the proteins (Avr proteins) coded for by *avr* genes promote pathogen growth and disease development in susceptible hosts. How Avr proteins accomplish that is not known, but they have been shown to interfere with the resistance mediated by the *avr* gene. Because the Avr proteins are coded for by the *avr* genes, it is apparent that *avr* genes can modify the signaling of host defense pathways in resistant hosts. In some cases, in the absence of a resistance R gene, the particular *avr* gene acts as a virulence factor that not only promotes growth of the particular bacterium in several hosts, including some that exhibit varying degrees of resistance, but transgenic plants that express the *avr* gene actually exhibit enhanced susceptibility to the pathogen and/or aggressiveness of the pathogen. Different *avr* genes, however, even of the same bacterial pathogen, contribute different degrees of susceptibility/aggressiveness to bacteria that provide these genes. This shows that the particular Avr proteins function inside the host plant cell and promote bacterial virulence.

Role of Type III Secretion in Bacterial Pathogenesis

Although the primary determinants of pathogenicity and virulence in many bacteria are secreted enzymes such as pectin lyases, cellulases, and proteases that macerate plant tissues of many species, it is now known that in at least *Erwinia* bacteria, the genes for hypersensitive reaction and pathogenicity (*hrp* genes) determine the potential secondary pathogenesis. In plant pathogens, *hrp* genes code for a type III secretion machinery, which is thought to transport bacterial effector proteins directly into the host cell. *hrp* genes exist in clusters of about 20 genes, one of which codes for a constituent of an outer membrane, whereas many others code for the core secretion machinery, for regulatory genes, for harpins, for the Hrp-pilin, which in some bacteria is required for type III secretion to function, for avirulence (*avr*) genes, and so on. In nonmacerating bacteria *Pseudomonas*, *Ralstonia*, and *Xanthomonas* and in the fire blight bacterium *Erwinia amylovora*, *hrp* genes are essential for virulence and elicitation of a hypersensitive response.

Suppressors of Plant Defense Responses

It has been shown that at least some plant pathogenic fungi, e.g., *Puccinia graminis* f. sp. *tritici*, which causes stem rust of wheat, and *Mycosphaerella pinodes*, which causes a leaf spot on pea, produce substances called **suppressors** that act as pathogenicity factors by suppressing the expression of defense responses in the host plant. The defense suppressor of the wheat stem rust fungus has been found in the fungus germination fluid and in the intercellular fluid of rust-infected wheat leaves. This suppressor interacts with the wheat cell

plasma membrane and reduces binding of the pathogen's 67-kDa glycoprotein elicitor of host defenses to the plasma membrane. In this way, the suppressor molecule suppresses the activity of phenylalanine lyase (PAL) and the normal development of defense responses. The pea-infecting fungus produces two suppressors in the spore germination fluid. Both suppressors are glycopeptides, counteract the elicitor of phytoalexin biosynthesis, and temporarily suppress the expression of all defense reactions of the host plant. The *Mycosphaerella* suppressors seem to reduce the proton-pumping activity of the host cell membrane ATPase and thereby temporarily lower the ability of the cell to function and to defend itself. A different mechanism of suppression of plant defense responses has been reported in the ergot disease of rye caused by the fungus *Claviceps purpurea*. In that disease the fungus produces the enzyme catalase, which reacts with and neutralizes the hydrogen peroxide that is produced as one of the first defense responses of plants against infecting pathogens. The fungal catalase concentration is greatest at hyphal walls and hyphal surfaces and is secreted by the fungus into the host apoplast at the host–pathogen interface, where the host H_2O_2 is produced. By inactivating active oxygen species produced by the host through catalase, the fungus suppresses the host defenses.

Pathogenicity and Virulence Factors in Viruses and Viroids

Until recently, little was known about the intrinsic factors of viruses and viroids that determine their pathogenicity and/or virulence. Viruses have a few, usually less than 10, genes, yet they are very capable pathogens. This requires that viral genes and gene products have multitask functions. Some of the most basic functions viral genes control are infectivity on a particular host, replication of the virus, movement of the virus from cell to cell, long-distance transport of the virus in the plant, transmissibility of the virus from plant to plant, and production of the coat protein of the virus. All of these functions are necessary for the pathogenicity and survival of the virus, although the variation in the degree most of these functions are carried out affects the virulence of the virus, i.e., the level of disease and symptoms it can cause in a host plant, rather than its pathogenicity, i.e., its ability to infect a plant.

Plant viruses have no genes that allow them to produce macerating enzymes, toxins, growth regulators, or other biologically active compounds by which to affect plant cells. However, different viruses manage to induce the plant to develop symptoms that appear to be the result of action and interaction of numerous such compounds present in the cell, despite the fact that no such compound can be found in infected cells. How viruses cause disease remains, therefore, pretty much a mystery but some facts are beginning to emerge.

One of the most important proteins coded by viruses that plays an important role in their pathogenicity and virulence is their coat protein. In addition to protecting the viral nucleic acid from external damaging factors, the coat protein plays important roles in practically everything pertaining to viral replication and dissemination. Thus, the coat protein plays a role in host recognition, uncoating and release of the nucleic acid, assistance in replication of the nucleic acid, movement of the virus between cells and organs, movement of the virus via a vector between plants, and modification of symptoms. Again, little is known on the mechanisms by which the coat protein affects these functions.

Another viral protein that has been studied extensively is the so-called movement protein, which enables viruses to move between cells and/or through the phloem system of the plant by altering the properties of plasmodesmata. However, some movement proteins not only open movement channels for the virus, they also block a defense molecule, the suppressor of virus silencing by the plant cell activated by the viral infection. Some viroids seem to form complexes with certain host proteins that help the viroids pass through plasmodesmata and with plant lectins that help viroids move through the phloem of host plants.

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Chapter six

HOW PLANTS DEFEND THEMSELVES AGAINST PATHOGENS

Each plant species is affected by approximately 100 different kinds of fungi, bacteria, mollicutes, viruses, and nematodes. Frequently, a single plant is attacked by hundreds, thousands, and, in leafspot diseases of large trees, probably hundreds of thousands of individuals of a single kind of pathogen. Although such plants may suffer damage to a lesser or greater extent, many survive all these attacks and, not uncommonly, manage to grow well and to produce appreciable yields.

In general, plants defend themselves against pathogens by a combination of weapons from two arsenals: (1) structural characteristics that act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant and (2) biochemical reactions that take place in the cells and tissues of the plant and produce substances that are either toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant. The combinations of structural characteristics and biochemical reactions employed in the defense of plants are different in different host–pathogen systems. In addition, even within the same host and pathogen, the combinations vary with the age of the plant, the kind of plant organ and tissue attacked, the nutritional condition of the plant, and the weather conditions.

WHATEVER THE PLANT DEFENSE OR RESISTANCE, IT IS CONTROLLED BY ITS GENES

One concept that must be made clear at the outset is that whatever the kind of defense or resistance a host plant employs against a pathogen or against an abiotic agent, it is ultimately controlled, directly or indirectly, by the genetic material (genes) of the host plant and of the pathogen (Fig. 6-1).

Nonhost Resistance

A plant may find it easy to defend itself, i.e., to stay resistant (immune) when it is brought in contact with a pathogenic biotic agent to which the plant is not a host. This is known as nonhost resistance and is the most common form of resistance (or defense from attack) in nature. For example, apple trees are not affected by pathogens of tomato, of wheat, or of citrus trees because the genetic makeup of apple is in some way(s) different from that of any other kinds of host plants, which, of course, are attacked by their own pathogens. However, apple can be attacked by its own pathogens, which, in turn, do not attack tomato, wheat, citrus, or anything else. Similarly, the fungus that causes powdery mildew on wheat (*Blumeria graminis* f. sp. *tritici*) does not infect barley and vice versa, the fungus that causes powdery mildew on barley (*B. graminis* f. sp. *hordei*) does not infect wheat, and so on. All such unsuccessful plant/pathogen interactions are thought to represent nonhost resistance. It has been shown recently however, that in at least some related pairings, e.g., the wheat, powdery mildew fungus inoculated on barley, the fungus produces haustoria and the host reacts by producing hydrogen peroxide (H_2O_2), cell wall appositions under the appressoria, and a hypersensitive response in which epidermal cells die rapidly in response to fungal attack.

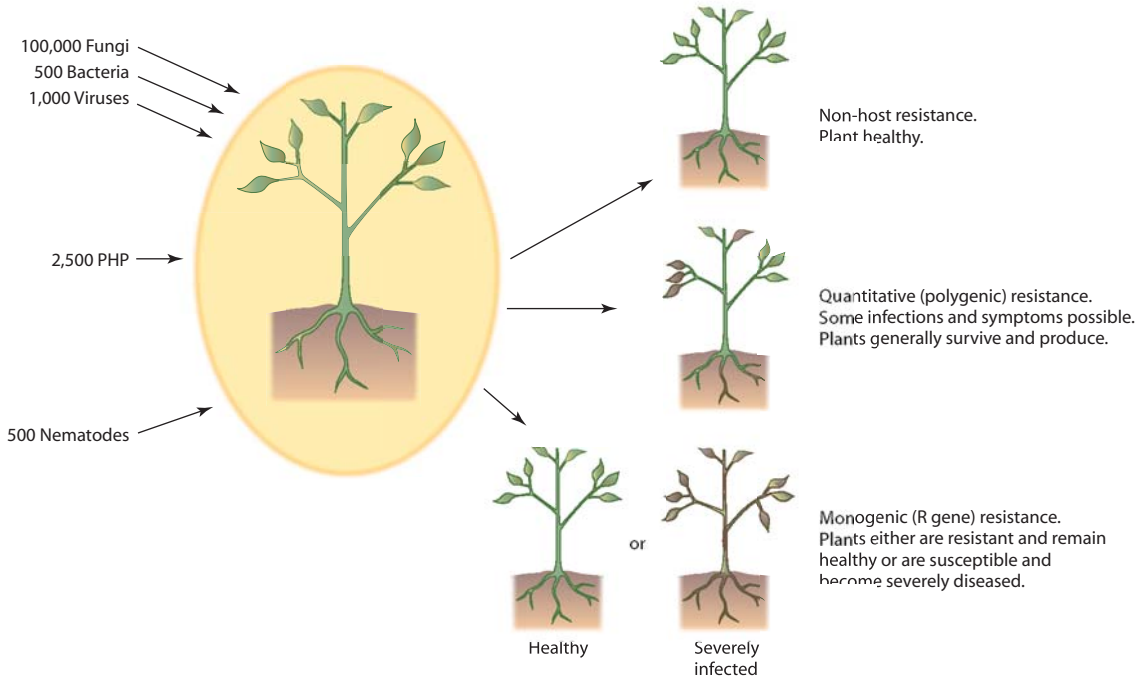


FIGURE 6-1 Types of reaction of plants to attacks by various pathogens in relation to the kind of resistance of the plant.

Partial, Polygenic, Quantitative, or Horizontal Resistance

Each plant, of course, is attacked by its own pathogens, but there is often a big difference in how effectively the plant can defend itself (how resistant the plant is) against each pathogen. Even when conditions for infection and disease development are favorable, a plant, upon infection with a particular pathogen, may develop no disease, only mild disease, or severe disease, depending on the specific genetic makeup of the plant and of the pathogen that attacks it. Many genes are involved in keeping a plant protected from attack by pathogens. Many of these genes provide for the general upkeep and well-being functions of plants, but plants also have many genes whose main functions seem to be the protection of plants from pathogens. Some of the latter plant genes code for chemical substances that are toxic to pathogens or neutralize the toxins of the pathogens, and these substances may be present in plants regardless of whether the plant is under attack or not. Plants also have genes that produce and regulate the formation of structures that can slow down or stop the advance of a pathogen into the host and cause disease. These structures can also be present in a plant throughout its life or they may be produced in response to attack by one of several pathogens or following injury by an abiotic agent. Preexisting defense structures or toxic chemical substances, and many of those formed in response to attack by a pathogen or abiotic agent, are important in the defense of most plants against most pathogens.

When a pathogen attacks a host plant, the genes of the pathogen are activated, produce, and release all their weapons of attack (enzymes, toxins, etc.) against the plants that they try to infect. With the help of different combinations of preexisting or induced toxic chemical substances or defense structures, most plants manage to defend themselves partially or nearly completely. Such plants show sufficient resistance that allows them to survive the pathogen attacks and to produce a satisfactory yield. This type of defense or resistance is known as polygenic, general, or quantitative resistance because it depends on many genes for the presence or formation of the various defense structures and for preexisting or induced production of many substances toxic to the pathogen. This type of resistance is present at different levels against different pathogens in absolutely all plants and is also known as partial, quantitative, horizontal, multigenic, field, durable, or minor gene resistance.

Most plants depend on general resistance against their pathogens, especially nonobligate parasites, e.g., the semibiotrophic or nectrotrophic oomycetes *Pythium* and *Phytophthora*, the fungi *Botrytis*, *Fusarium*, *Sclerotinia*, and *Rhizoctonia*, and most bacteria, nematodes, and so on. In at least some polygenic plant–pathogen combinations, such as the early blight of tomato caused by the

necrotrophic fungus *Alternaria solani*, the more resistant the varieties are, the higher the constitutive concentration and the more rapid the accumulation in them of pathogen-induced pathogenesis related (PR) proteins, than in susceptible varieties. These PR proteins include some of the specific antifungal isozymes of chitinase and β-1,3-glucanase. Also, total enzyme preparations from resistant varieties were able to release elicitors of the hypersensitive response (HR) (see later) from purified fungal cell walls, whereas enzymes from susceptible varieties could not. Furthermore, partially purified chitinases from tomato leaves could release HR elicitors from germinating *A. solani* spores but not from mature intact cell walls. This suggests that, perhaps, constitutively produced hydrolytic enzymes may act as a mechanism of elicitor release in tomato resistance to the early blight disease. Quantitative resistance has also been shown to increase in transgenic plants carrying introduced R genes and matching avirulence genes, even though the latter do not express the hypersensitive cell death.

Race-Specific, Monogenic, R Gene, or Vertical Resistance

In many plant–pathogen combinations, especially those involving biotrophic oomycetes (downy mildews), fungi (powdery mildews, rusts), and many other fungi, e.g., *Cochliobolus*, *Magnaporthe*, *Cladosporium*, many bacteria, nematodes, and viruses, defense (resistance) of a host plant against many of its pathogens is through the presence of matching pairs of juxtaposed genes for disease in the host plant and the pathogen. The host plant carries one or few resistance genes (R) per pathogen capable of attacking it, while each pathogen carries matching genes for avirulence (A) for each of the R genes of the host plant. As explained in some detail later, the avirulence gene of the pathogen serves to trigger the host R gene into action. This then sets in motion a series of defense reactions that neutralize and eliminate the specific pathogen that carries the corresponding (matching) gene for avirulence (A), while the attacked and a few surrounding cells die. This type of defense or resistance is known as race-specific, hypersensitive response (HR), major gene, R gene, or vertical resistance. However, some R genes, e.g., Xa21 of rice, do not induce a visible HR.

PREEXISTING STRUCTURAL AND CHEMICAL DEFENSES

Preexisting Defense Structures

The first line of defense of a plant against pathogens is its surface, which the pathogen must adhere to and penetrate if it is to cause infection. Some structural defenses are present in the plant even before the pathogen comes in contact with the plant. Such structures include the amount and quality of wax and cuticle that cover the epidermal cells, the structure of the epidermal cell walls, the size, location, and shapes of stomata and lenticels, and the presence of tissues made of thick-walled cells that hinder the advance of the pathogen on the plant.

Waxes on leaf and fruit surfaces form a water-repellent surface, thereby preventing the formation of a film of water on which pathogens might be deposited and germinate (fungi) or multiply (bacteria). A thick mat of hairs on a plant surface may also exert a similar water-repelling effect and may reduce infection.

A thick cuticle may increase resistance to infection in diseases in which the pathogen enters its host only through direct penetration. Cuticle thickness, however, is not always correlated with resistance, and many plant varieties with cuticles of considerable thickness are invaded easily by directly penetrating pathogens.

The thickness and toughness of the outer wall of epidermal cells are apparently important factors in the resistance of some plants to certain pathogens. Thick, tough walls of epidermal cells make direct penetration by fungal pathogens difficult or impossible. Plants with such walls are often resistant, although if the pathogen is introduced beyond the epidermis of the same plants by means of a wound, the inner tissues of the plant are invaded easily by the pathogen.

Many pathogenic fungi and bacteria enter plants only through stomata. Although the majority of pathogens can force their way through closed stomata, some, like the stem rust of wheat, can enter only when stomata are open. Thus, some wheat varieties, in which the stomata open late in the day, are resistant because the germ tubes of spores germinating in the night dew desiccate due to evaporation of the dew before the stomata begin to open. The structure of the stomata, e.g., a very narrow entrance and broad, elevated guard cells, may also confer resistance to some varieties against certain of their bacterial pathogens.

The cell walls of the tissues being invaded vary in thickness and toughness and may sometimes inhibit the advance of the pathogen. The presence, in particular, of bundles or extended areas of sclerenchyma cells, such as are found in the stems of many cereal crops, may stop the further spread of pathogens such as stem rust fungi. Also, the xylem, bundle sheath, and sclerenchyma cells of the leaf veins effectively block the spread of some fungal, bacterial, and nematode pathogens that cause various “angular” leaf spots because of their spread only into areas between, but not across, veins. Xylem vessels seem to be involved more directly in the resistance and susceptibility to vascular diseases. For example, xylem vessel diameter and the proportion of large

vessels were strongly correlated with the susceptibility of elm to Dutch elm disease caused by the fungus *Ophiostoma novo-ulni*.

Preexisting Chemical Defenses

Although structural characteristics may provide a plant with various degrees of defense against attacking pathogens, it is clear that the resistance of a plant against pathogen attacks depends not so much on its structural barriers as on the substances produced in its cells before or after infection. This is apparent from the fact that a particular pathogen will not infect certain plant varieties even though no structural barriers of any kind seem to be present or to form in these varieties. Similarly, in resistant varieties, the rate of disease development soon slows down, and finally, in the absence of structural defenses, the disease is completely checked. Moreover, many pathogens that enter nonhost plants naturally or that are introduced into nonhost plants artificially, fail to cause infection, although no apparent visible host structures inhibit them from doing so. These examples suggest that defense mechanisms of a chemical rather than a structural nature are responsible for the resistance to infection exhibited by plants against certain pathogens.

Inhibitors Released by the Plant in Its Environment

Plants exude a variety of substances through the surface of their aboveground parts as well as through the surface of their roots. Some of the compounds released by certain kinds of plants, however, seem to have an inhibitory action against certain pathogens. **Fungitoxic exudates** on the leaves of some plants, e.g., tomato and sugar beet, seem to be present in sufficient concentrations to inhibit the germination of spores of fungi *Botrytis* and *Cercospora*, respectively, that may be present in dew or rain droplets on these leaves. Similarly, in the case of onion smudge, caused by the fungus *Colletotrichum circinans*, resistant varieties generally have red scales and contain, in addition to the red pigments, the phenolic compounds protocatechuic acid and catechol. In the presence of water drops or soil moisture containing conidia of the onion smudge fungus on the surface of red onions, these two fungitoxic substances diffuse into the liquid, inhibit the germination of the conidia, and cause them to burst, thus protecting the plant from infection. Both fungitoxic exudates and inhibition of infection are missing in white-scaled, susceptible onion varieties (Fig. 6-2). It was noticed that applications of acibenzolar-*S*-methyl (ASM) on sunflower reduced infection by the rust fungus *Puccinia helianthi* through the reduction of spore germination and appressorium formation. It was subsequently shown that ASM accomplished this by increasing the production and secretion by the plant on the leaf surface of coumarins and other toxic phenolics that inhibit spore germination and appressorium formation on the leaf surfaces on which they are present.

FIGURE 6-2 Onion smudge, caused by the fungus *Colletotrichum circinans*, develops on white onions but not on colored ones, which, in addition to the red or yellow pigment, also contain the phenolics protocatechuic acid and catechol, both of which are toxic to the fungus. (Photograph courtesy of G. W. Simone.)

Inhibitors Present in Plant Cells before Infection

It is becoming increasingly apparent that some plants are resistant to diseases caused by certain pathogens because of one or more inhibitory antimicrobial compounds, known as phytoanticipins, which are present in the cell before infection. 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, have been proposed as responsible for the resistance of young tissues to pathogenic microorganisms such as *Botrytis*. For example, increased 9-hexadecanoic acid in cutin monomers in transgenic tomato plants led to resistance of such plants to powdery mildew because these cutin monomers inhibit the germination of powdery mildew spores. Many such compounds are potent inhibitors of many hydrolytic enzymes, including the pectolytic-macerating enzymes of plant pathogens. As the young tissues grow older, their inhibitor content and their resistance to infection decrease steadily. Strawberry leaves naturally contain **(+)-catechin**, which inhibits infection by *Alternaria alternata* by blocking the formation of infection hyphae from haustoria although it allows both spore germination and appressoria formation. Several other types of preformed compounds, such as the saponins (glycosylated steroidal or triterpenoid compounds) **tomatine** in tomato and **avenacin** in oats, not only have antifungal membranolytic activity, they actually exclude fungal pathogens that lack enzymes

(saponinases) that break down the saponin from infecting the host. In this way, the presence or absence of saponin in a host and of saponinase in a fungus determines the host range of the fungus.

In addition to the simple molecule antifungal compounds listed earlier, several preformed plant proteins have been reported to act as inhibitors of pathogen proteinases or of hydrolytic enzymes involved in host cell wall degradation, to inactivate foreign ribosomes, or to increase the permeability of the plasma membranes of fungi.

For example, in a number of plants there is a family of low molecular weight proteins called phytocystatins that inhibit cysteine proteinases carried in the digestive system of nematodes and are also secreted by some plant pathogenic fungi. Constitutively present or transgenically introduced phytocystatins in plants reduce the size of nematode females and the number of eggs produced by females, thereby providing effective or significant control of several plants to root knot, cyst, reniform, and lesion nematodes.

Another type of compounds, the 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. However, plant surface cells also contain variable amounts of hydrolytic enzymes, some of which, such as glucanases and chitinases, may cause the breakdown of pathogen cell wall components, thereby contributing to resistance to infection. The importance of either of these types of inhibitors to disease resistance is not currently known, but some of these substances are known to increase rapidly upon infection and are considered to play an important role in the defense of plants to infection.

DEFENSE THROUGH LACK OF ESSENTIAL FACTORS

Lack of Recognition between Host and Pathogen

A plant species either is a host for a particular pathogen, e.g., wheat for the wheat stem rust fungus, or it is not a host for that pathogen, e.g., tomato for wheat stem rust fungus. How does a pathogen recognize that the plant with which it comes in contact is a host or nonhost? Plants of a species or variety may not become infected by a pathogen if their surface cells lack specific **recognition factors** (specific molecules or structures) that can be recognized by the pathogen. If the pathogen does not recognize the plant as one of its host plants, it may not become attached to the plant or may not produce infection substances, such as enzymes, or structures, such as appressoria, penetration pegs, and haustoria, necessary for the establishment of infection. It is not known what types of molecules or structures are involved in the recognition of plants and pathogens, but it is thought that they probably include various types of oligosaccharides and polysaccharides, and proteins or glycoproteins. Also, it is not known to what extent these recognition phenomena are responsible for the success or failure of initiation of infection in any particular host–pathogen combination.

Lack of Host Receptors and Sensitive Sites for Toxins

In host–pathogen combinations in which the pathogen (usually a fungus) produces a host-specific toxin, the toxin, which is responsible for the symptoms, is thought to attach to and react with specific receptors or sensitive sites in the cell. Only plants that have such sensitive receptors or sites become diseased. Plants of other varieties or species that lack such receptors or sites remain resistant to the toxin and develop no symptoms.

Lack of Essential Substances for the Pathogen

Species or varieties of plants that for some reason do not produce one of the substances essential for the survival of an obligate parasite, or for development of infection by any parasite, would be resistant to the pathogen that requires it. Thus, for *Rhizoctonia* to infect a plant it needs to obtain from the plant a substance necessary for formation of a hyphal cushion from which the fungus sends into the plant its penetration hyphae. In plants in which this substance is apparently lacking, cushions do not form, infection does not occur, and the plants are resistant. The fungus does not normally form hyphal cushions in pure cultures but forms them when extracts from a susceptible but not a resistant plant are added to the culture. Also, certain mutants of *Venturia inaequalis*, the cause of apple scab, which had lost the ability to synthesize a certain growth factor, also lost the ability to cause infection. When, however, the particular growth factor is sprayed on the apple leaves during inoculation with the mutant, the mutant not only survives but it also causes infection. The advance of the infection, though, continues only as long as the growth factor is supplied externally to the mutant. In some host–pathogen combinations, disease develops but the amount of disease may be reduced by the fact that certain host substances are present in lower concentrations. For example, bacterial soft rot of potatoes, caused

by *Erwinia carotovora* var. *atroseptica*, is less severe on potatoes with low-reducing sugar content than on potatoes high in reducing sugars.

INDUCED STRUCTURAL AND BIOCHEMICAL DEFENSES

Recognition of the Pathogen by the Host Plant

Early recognition of the pathogen by the plant is very important if the plant is to mobilize the available biochemical and structural defenses to protect itself from the pathogen. The plant apparently begins to receive signal molecules, i.e., molecules that indicate the presence of a pathogen, as soon as the pathogen establishes physical contact with the plant (Fig. 6-3).

Pathogen Elicitors

Various pathogens, especially fungi and bacteria, release a variety of substances in their immediate environment that act as nonspecific elicitors of pathogen recognition by the host. Such nonspecific elicitors include toxins, glycoproteins, carbohydrates, fatty acids, peptides, and extracellular microbial enzymes such as proteases and pectic enzymes. In various host–pathogen combinations, certain substances secreted by the pathogen, such as *avr* gene products, *hrp* gene products, and suppressor molecules, act as specific pathogen elicitors of recognition by the specific host plant. In many cases, in which host enzymes break down a portion of the polysaccharides making up the pathogen surface or pathogen enzymes break down a portion of the plant surface polysaccharides, the released oligomers or monomers of the polysaccharides act as recognition elicitors for the plant.

Host Plant Receptors

The location of host receptors that recognize pathogen elicitors is not generally known, but several of those studied appear to exist outside or on the cell membrane, whereas others apparently occur intracellularly. In the powdery mildew of cereals, a soluble carbohydrate that acts as an elicitor from the wheat powdery mildew fungus *Blumeria graminis* f. sp. *tritici* is recognized by a broad range of cereals (barley, oat, rye, rice, and maize) in which it induces the expression of all defense-related genes tested and also induced resistance to subsequent attacks with the fungus. The elicitor alone, in

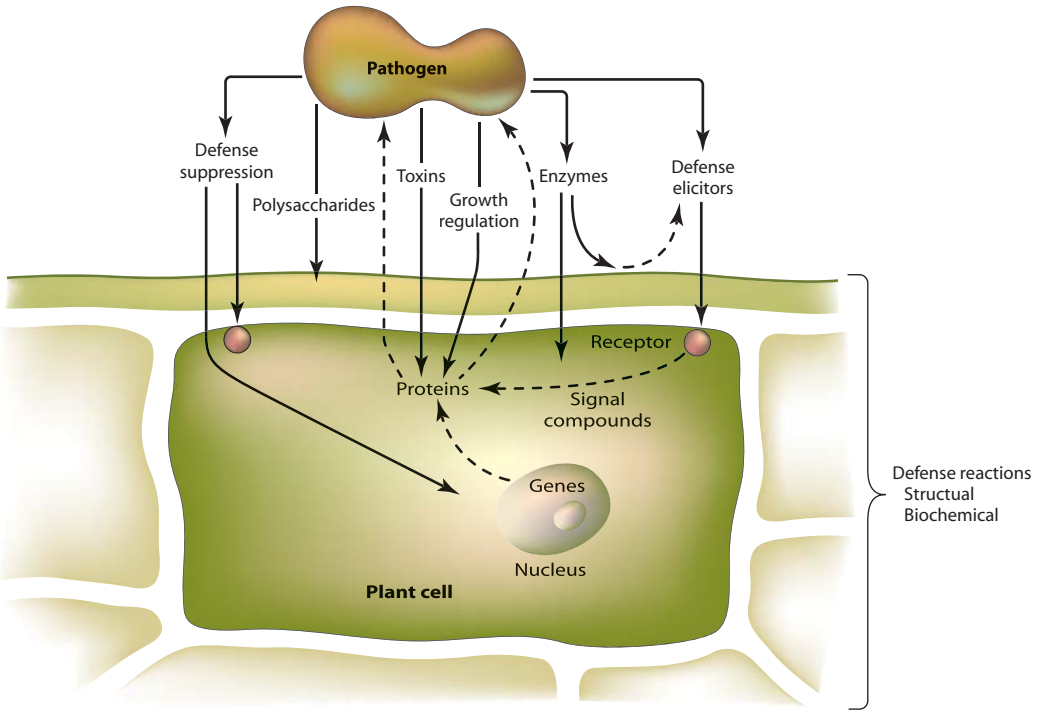


FIGURE 6-3 Schematic representation of pathogen interactions with host plant cells. Depending on its genetic makeup, the plant cell may react with numerous defenses, which may include cell wall structural defenses (waxes, cutin, suberin, lignin, phenolics, cellulose, callose, cell wall proteins) or biochemical wall, membrane, cytoplasm, and nucleus defense reactions. The latter may involve bursts of oxidative reactions, production of elicitors, hypersensitive cell death, ethylene, phytoalexins, pathogenesis-related proteins (hydrolytic enzymes, β-1,3-glucanases, chitinases), inhibitors (thionins, proteinase inhibitors, thaumatin-like proteins), and so on.

absence of the powdery mildew fungus, did not induce a hypersensitive response but it did induce an accumulation of thaumatin-like proteins in the various cereals.

Mobilization of Defenses

Once a particular plant molecule recognizes and reacts with a molecule (elicitor) derived from a pathogen, it is assumed that the plant "recognizes" the pathogen. Following such recognition, a series of biochemical reactions and structural changes are set in motion in the plant cell(s) in an effort to fend off the pathogen and its enzymes, toxins, etc. How quickly the plant recognizes the (presence of a) pathogen and how quickly it can send out its alarm message(s) and mobilize its defenses determine whether hardly any infection will take place at all (as in the hypersensitive response) or how much the pathogen will develop, i.e., how severe the symptoms (leaf spots, stem, fruit, or root lesions, etc.) will be, before the host defenses finally stop further development of the pathogen.

Transmission of the Alarm Signal to Host Defense Providers: Signal Transduction

Once the pathogen-derived elicitors are recognized by the host, a series of alarm signals are sent out to host cell proteins and to nuclear genes, causing them to become activated, to produce substances inhibitory to the pathogen, and to mobilize themselves or their products toward the point of cell attack by the pathogen. Some of the alarm substances and signal transductions are only intracellular, but in many cases the signal is also transmitted to several adjacent cells and, apparently, the alarm signal is often transmitted systemically to most or all of the plant.

The chemical nature of the transmitted signal molecules is not known with certainty in any host–pathogen combination. Several types of molecules have been implicated in intracellular signal transduction. The most common such signal transducers appear to be various protein kinases, calcium ions, phosphorylases and phospholipases, ATPases, hydrogen peroxide (H_2O_2), ethylene, and others. Systemic signal transduction, which leads to systemic acquired resistance, is thought to be carried out by salicylic acid, oligogalacturonides released from plant cell walls, jasmonic acid, systemin, fatty acids, ethylene, and others. Some natural or synthetic chemicals, such as salicylic acid and the synthetic dichloroisonicotinic acid, also activate the signaling pathway that leads to systemic acquired resistance against several diverse types of plant pathogenic viruses, bacteria, and fungi.

INDUCED STRUCTURAL DEFENSES

Despite the preformed superficial or internal defense structures of host plants, most pathogens manage to penetrate their hosts through wounds and natural openings and to produce various degrees of infection. Even after the pathogen has penetrated the preformed defense structures, however, plants usually respond by forming one or more types of structures that are more or less successful in defending the plant from further pathogen invasion. Some of the defense structures formed involve the cytoplasm of the cells under attack, and the process is called **cytoplasmic defense reaction;** others involve the walls of invaded cells and are called **cell wall defense structures**; and still others involve tissues ahead of the pathogen (deeper into the plant) and are called **histological defense structures**. Finally, the death of the invaded cell may protect the plant from further invasion. This is called the **necrotic** or **hypersensitive defense reaction** and is discussed here briefly, with more detailed treatment a little later.

Cytoplasmic Defense Reaction

In a few cases of slowly growing, weakly pathogenic fungi, such as weakly pathogenic *Armillaria* strains and the mycorrhizal fungi, that induce chronic diseases or nearly symbiotic conditions, the plant cell cytoplasm surrounds the clump of hyphae and the plant cell nucleus is stretched to the point where it breaks in two. In some cells, the cytoplasmic reaction is overcome and the protoplast disappears while fungal growth increases. In some of the invaded cells, however, the cytoplasm and nucleus enlarge. The cytoplasm becomes granular and dense, and various particles or structures appear in it. Finally, the mycelium of the pathogen disintegrates and the invasion stops.

Cell Wall Defense Structures

Cell wall defense structures involve morphological changes in the cell wall or changes derived from the cell wall of the cell being invaded by the pathogen. The effectiveness of these structures as defense mechanisms seems to be rather limited, however. Three main types of such structures have been observed in plant diseases. (1) The outer layer of the cell wall of parenchyma cells coming in contact with incompatible bacteria swells and produces an amorphous, fibrillar material that surrounds and traps the bacteria and prevents them from multiplying. (2) Cell walls thicken in response to several pathogens by producing what appears to be a cellulosic material. This material, however, is often infused with

phenolic substances that are cross-linked and further increase its resistance to penetration. (3) Callose **papillae** are deposited on the inner side of cell walls in response to invasion by fungal pathogens (see Figs. 2-8C and 2-8D). Papillae seem to be produced by cells within minutes after wounding and within 2 to 3 hours after inoculation with microorganisms. Although the main function of papillae seems to be repair of cellular damage, sometimes, especially if papillae are present before inoculation, they also seem to prevent the pathogen from subsequently penetrating the cell. In some cases, hyphal tips of fungi penetrating a cell wall and growing into the cell lumen are enveloped by cellulosic (callose) materials that later become infused with phenolic substances and form a sheath or lignituber around the hypha (Fig. 6-4).

Histological Defense Structures

Formation of Cork Layers

Infection by fungi or bacteria, and even by some viruses and nematodes, frequently induces plants to form several layers of cork cells beyond the point of infection (Figs. 6-5 and 6-6), apparently as a result of stimulation of the host cells by substances secreted by the pathogen. The cork layers inhibit further invasion by the pathogen beyond the initial lesion and also block the spread of any toxic substances that the pathogen may secrete. Furthermore, cork layers stop the flow of nutrients and water from the healthy to the infected area and deprive the pathogen of nourishment. The dead tissues, including the pathogen, are thus delimited by the cork layers

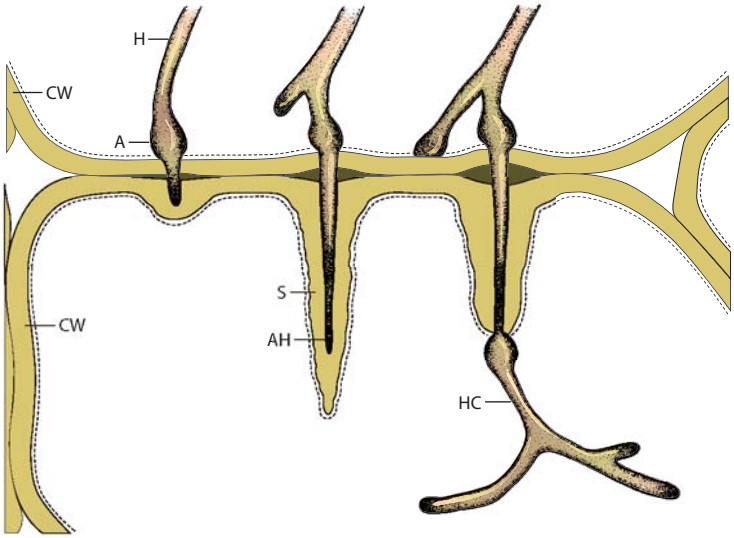


FIGURE 6-4 Formation of a sheath around a hypha (H) penetrating a cell wall (CW). A, appressorium; AH, advancing hypha still enclosed in sheath; HC, hypha in cytoplasm; S, sheath.

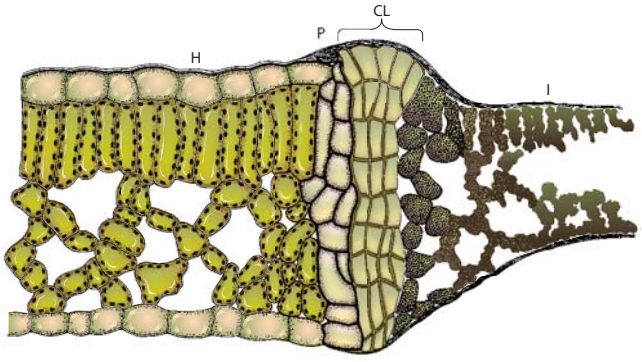


FIGURE 6-5 Formation of a cork layer (CL) between infected (I) and healthy (H) areas of leaf. P, phellogen. [After Cunningham (1928). *Phytopathology* **18**, 717–751.]

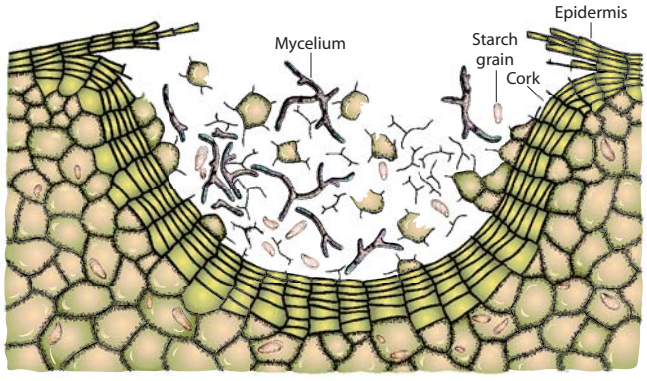
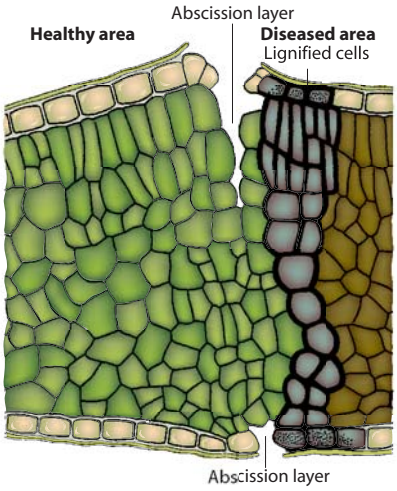


FIGURE 6-6 Formation of a cork layer on a potato tuber following infection with *Rhizoctonia*. [After Ramsey (1917). *J. Agric. Res.* **9**, 421–426.]

and may remain in place, forming necrotic lesions (spots) that are remarkably uniform in size and shape for a particular host–pathogen combination. In some host–pathogen combinations the necrotic tissues are pushed outward by the underlying healthy tissues and form scabs that may be sloughed off, thus removing the pathogen from the host completely. In tree cankers, such as those caused by the fungus *Seiridium cardinale* on cypress trees, resistant plant clones restrict growth of the fungus by forming ligno-suberized boundary zones, which included four to six layers of cells with suberized cell walls. In contrast, susceptible clones have only two to four layers of suberized cells and these are discontinuous, allowing repeated penetration by the fungus past the incomplete barrier.

Formation of Abscission Layers

Abscission layers are formed on young, active leaves of stone fruit trees after infection by any of several fungi, bacteria, or viruses. An abscission layer consists of a gap formed between two circular layers of leaf cells surrounding the locus of infection. Upon infection, the middle lamella between these two layers of cells is dissolved throughout the thickness of the leaf, completely cutting off the central area of the infection from the rest of the leaf (Fig. 6-7). Gradually, this area shrivels, dies, and sloughs off, carrying with it the pathogen. Thus, the plant, by discarding the infected area along with a few yet uninfected cells, protects the rest of the leaf tissue from being invaded by the



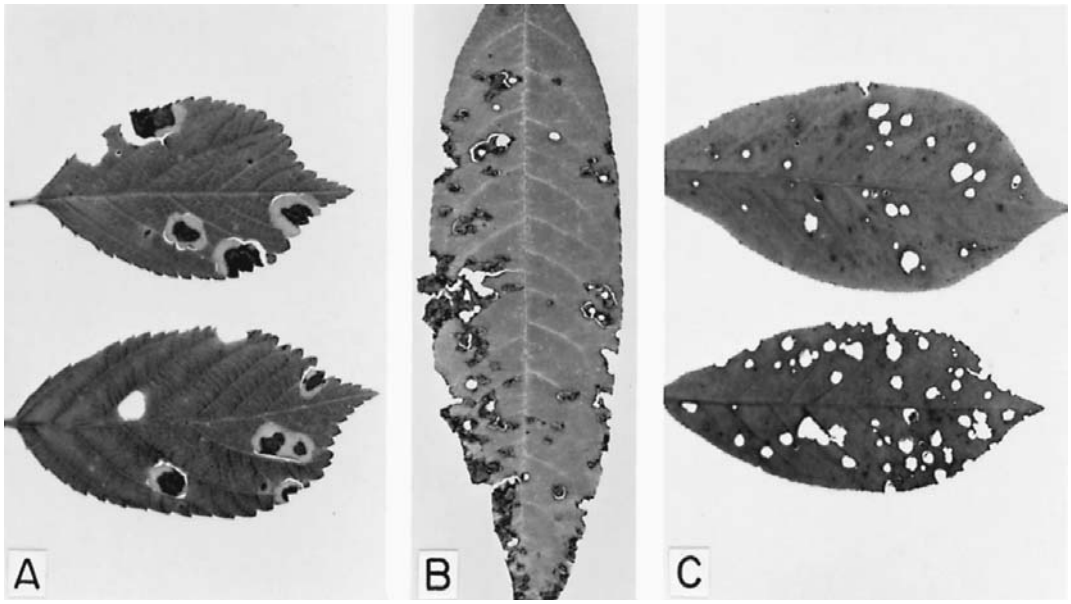


FIGURE 6-7 Schematic formation of an abscission layer around a diseased spot of a *Prunus* leaf. [After Samuel (1927).] (A–C) Leaf spots and shot holes caused by *Xanthomonas arboricola* pv. *pruni* bacteria on (A) ornamental cherry leaves; characteristic broad, light green halos form around the infected area before all affected tissue falls off, (B) on peach, and (C) on plum. The shot hole effect is particularly obvious on the plum leaves.

pathogen and from becoming affected by the toxic secretions of the pathogen.

Formation of Tyloses

Tyloses form in xylem vessels of most plants under various conditions of stress and during invasion by most of the xylem-invading pathogens. Tyloses are overgrowths of the protoplast of adjacent living parenchymatous cells, which protrude into xylem vessels through pits (Fig. 6-8). Tyloses have cellulosic walls and may, by their size and numbers, clog the vessel completely. In some varieties of plants, tyloses form abundantly and quickly ahead of the pathogen, while the pathogen is still in the young roots, and block further advance of the pathogen. The plants of these varieties remain free of and therefore resistant to this pathogen. Varieties in which few, if any, tyloses form ahead of the pathogen are susceptible to disease.

Deposition of Gums

Various types of gums are produced by many plants around lesions after infection by pathogens or injury. Gum secretion is most common in stone fruit trees but occurs in most plants. The defensive role of gums stems from the fact that they are deposited quickly in the intercellular spaces and within the cells surrounding the locus of infection, thus forming an impenetrable barrier that completely encloses the pathogen. The pathogen then becomes isolated, starves, and sooner or later dies.

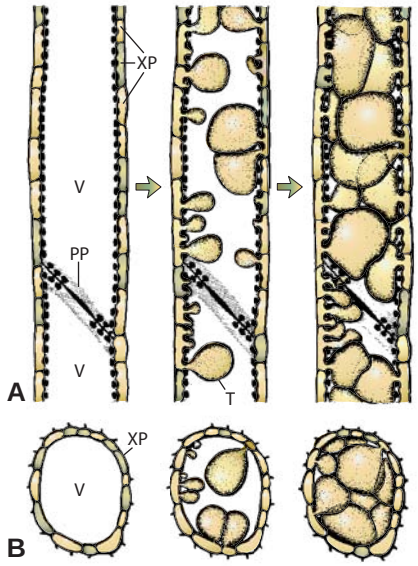


FIGURE 6-8 Development of tyloses in xylem vessels. Longitudinal (A) and cross section (B) views of healthy vessels (left) and of vessels with tyloses. Vessels at right are completely clogged with tyloses. PP, perforation plate; V, xylem vessel; XP, xylem parenchyma cell; T, tylosis.

Necrotic Structural Defense Reaction: Defense through the Hypersensitive Response

The hypersensitive response is considered a biochemical rather than a structural defense mechanism but is described here briefly because some of the cellular responses that accompany it can be seen with the naked eye or with the microscope. 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. At the same time, brown, resin-like granules form in the cytoplasm, first around the point of penetration of the pathogen and then throughout the cytoplasm. As the browning discoloration of the plant cell cytoplasm continues and death sets in, the invading hypha begins to degenerate (Fig. 6-9). In most cases the hypha does not grow out of such cells, and further invasion is stopped. In bacterial infections of leaves, the hypersensitive response results in the destruction of all cellular membranes of cells in contact with bacteria, which is followed by desiccation and necrosis of the leaf tissues invaded by the bacteria.

Although it is not quite clear whether the HR is the cause or the consequence of resistance, this type of necrotic defense is quite common, particularly in diseases caused by obligate fungal parasites and by viruses (Fig. 6-10A), bacteria (Fig. 6-10B), and nematodes. Apparently, the necrotic tissue not only isolates the parasite from the living substance on which it depends for its nutrition and, thereby, results in its starvation and death, but, more importantly, it signifies the concentration of numerous biochemical cell responses and antimicrobial substances that neutralize the pathogen. The faster the host cell dies after invasion, the more resistant to infection the plant seems to be. Moreover, through the signaling compounds and pathways developed during the hypersensitive response, the latter serves as the springboard for localized and systemic acquired resistance.

INDUCED BIOCHEMICAL DEFENSES

Induced Biochemical Nonhost Resistance

As mentioned earlier, nonhost resistance is the resistance that keeps a plant protected from pathogens that are, through evolution, incompatible with that host. Although the nature of nonhost resistance is unknown, for a pathogen it can be as big a gap to bridge as the difference between the features of a potato plant and an oak tree, or as close as the difference between the features of potato and tomato, or barley and wheat. It appears, however, that in some plant/pathogen

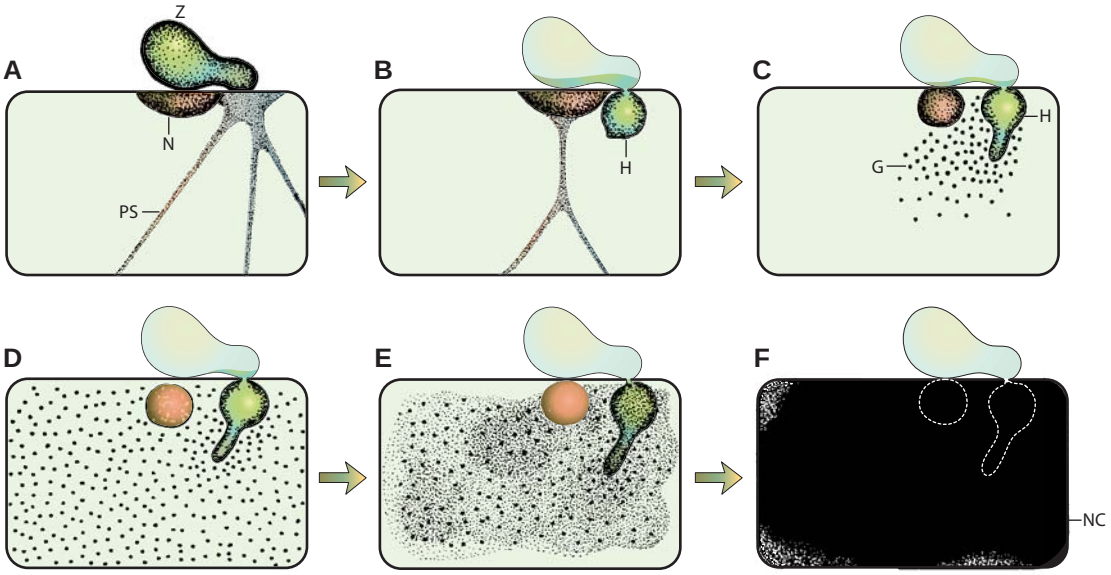


FIGURE 6-9 Stages in the development of the necrotic defense reaction in a cell of a very resistant potato variety infected by *Phytophthora infestans*. N, nucleus; PS, protoplasmic strands; Z, zoospore; H, hypha; G, granular material; NC, necrotic cell. [After Tomiyama (1956). *Ann. Phytopathol. Soc. Jpn.* **21**, 54–62.]

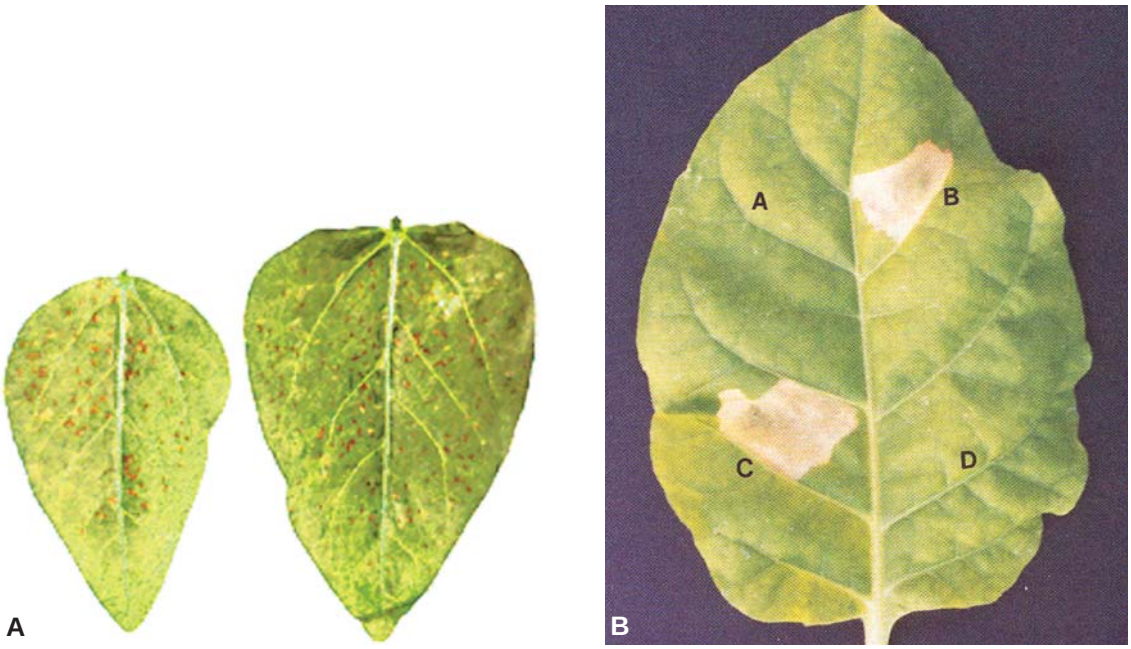


FIGURE 6-10 (A) Hypersensitive response (HR) expressed on leaves of a resistant cowpea variety following sap inoculation with a strain of a virus that causes local lesions (in this case, *alfalfa mosaic virus*). The virus remains localized in the lesions. (B) Tobacco leaf showing typical hypersensitive responses (white areas) 24 hours after injection with water (A) or with preparations of bacterial strains B, C, and D. Strain (B), which does not infect tobacco, and (C), which carries a *hrp* (hypersensitive response and pathogenicity) gene, both induced the hypersensitive response, whereas the third strain (D), a mutant of C that lacked the *hrp* gene, did not. [From Mukherjee *et al.* (1997). *Mol. Plant-Microbe Interact.* **10**, 462–471.]

interactions of taxonomically unrelated plants (e.g., potato and oak or oak and wheat), nonhost resistance is controlled by constitutive defenses and/or defenses induced by nonspecific stimuli in a nonspecific manner. Such defenses include physical topography and the structures present on the plant, the presence of toxic or the absence of essential compounds, and so on. In other plant/pathogen combinations, in which the plants are taxonomically related (e.g., potato and tomato, barley and wheat), nonhost resistance involves primarily inducible defenses elicited by the recognition of pathogen-specific molecules. Some cases of nonhost resistance, however, seem to be controlled by a single gene.

Some examples of questionable nonhost resistance include the resistance of the nonhost pea to the *Pseudomonas syringae* pv. *syringae* bacterium, which infects bean but not pea. The reaction occurs when that bacterium carries a gene that is responsible for elicitation of a potentially defensive response in the normally nonhost pea, that is expressed as a visible hypersensitive response. In another example, the potato late blight fungus *Phytophthora infestans*, normally does not infect the tobacco species *Nicotiana benthamiana*. The nonhost resistance of the tobacco species, however, is lost if the pathogen does not carry an "avirulence-like gene," which produces a protein that elicits cell death in the tobacco. This is unique in that in other plant/pathogen combinations, the absence of a single "nonhost avirulence gene" does not make the nonhost plant susceptible. It would appear, therefore, that if the cell death response to the elicitor controlled by the avirulence gene really contributes to resistance, then the nonhost resistance in such situations is controlled by more than one component. In still another case, nonhost resistance in some cereals [wheat to powdery mildew strains from another cereal (barley), or in barley to *Puccinia* rust races from wheat], involves similar gene-for-gene interactions and nonhost resistance occurs through defense mechanisms involving recognition of an elicitor and development of a hypersensitive response. Disease resistance does not always involve pathogen recognition events, but, especially in polygenic or quantitative resistance, it may involve directly various structural or chemical defense mechanisms. This also happens in some cases of nonhost resistance, e.g., in oat roots to the wheat fungus *Gaeumannomyces graminis* f. sp. *tritici*, while they are susceptible to the oat fungus *G. graminis* f. sp. *avenae*. The nonhost resistance of oat roots to the wheat fungus is caused by the presence of the saponin compound avenacin in the oat roots, which is toxic to the fungus. This compound is also toxic to the oat fungus, but the latter produces an enzyme that detoxifies the saponin in oat roots and can infect them. The nonhost resistance to the wheat fungus, however, is compromised in saponin-deficient mutants in which the wheat fungus causes a successful infection. This shows that nonhost resistance in some plant/microbe interactions is caused by a direct defense mechanism rather than by recognition events.

In all these examples, the pathogen or the host is already closely related and nearly fully adopted to the characteristics of nonhost resistance presented to it. In less related plants or pathogens, however, in which true nonhost resistance is found routinely, it is more likely to be the result of effective nonspecific defenses such as physical characteristics and nonspecific responses to wounding and damage done by the pathogen during attempted invasion than to defenses elicited by specific recognition events. There is also, however, the case of pathogens that have alternate hosts, such as wheat stem rust and barberry and cedar apple rust on apple and cedar. These are, perhaps, interesting from an evolutionary point of view because, presumably, before the second of the alternate hosts that became a host, it was surely a nonhost. How the rust fungus bridged the two taxonomically extremely different hosts is not known. The change in ploidy (from haploid to diploid and back to haploid) was probably involved, but how the fungus broke the nonhost resistance of the other host and how it used the nonresistant host as a completely cooperative host is still a mystery.

The present consensus is that plants that exhibit nonhost resistance against pathogens of other plants do not need to carry resistance genes that recognize these pathogens because they carry genes that provide the plants with nonspecific defenses that are fully effective in protecting the plant from these pathogens. However, it may be possible that nonhost resistance, along with polygenic and monogenic host resistance, forms a continuum of resistance that begins to overlap as the taxonomic (evolutionary) distance between host and nonhost plants becomes closer and results in a complex and continuous network of plant/pathogen interactions.

Induced Biochemical Defenses in Quantitative (Partial, Polygenic, General, or Horizontal) Resistance

In quantitative (partial, polygenic, multigenic, general, field, durable, or horizontal) resistance, plants depend on the action of numerous genes, expressed constitutively or upon attack by a pathogen (induced resistance). These genes provide the plants with defensive structures or toxic substances that slow down or stop the advance of the pathogen into the host tissues and reduce the damage caused by the pathogen. Quantitative resistance is particularly common in diseases caused

by nonbiotrophic pathogens. Quantitative resistance may vary considerably, in some cases being specific against some of the strains of a pathogen, in others being effective against all strains of a pathogen, or providing resistance against more than one pathogen. Genes for quantitative resistance are present and provide a basal level of resistance to all plants against all pathogens regardless of whether the plant also carries major (or R) genes against a particular pathogen.

Function of Gene Products in Quantitative Resistance

Unlike most major (or R) genes involved in monogenic resistance, which appear to code for components that help the host recognize the pathogen and to subsequently express the hypersensitive response, genes for quantitative resistance seem to be involved directly in the expression or production of some sort of structural or biochemical defense. Quantitative resistance defenses are basically the same ones that follow the hypersensitive response in monogenic resistance; in quantitative resistance, however, defenses generally do not follow a hypersensitive response and cell death because the latter do not usually occur in quantitative resistance. Genes involved in quantitative resistance are present in the same areas of plant chromosomes that contain the genes involved in defense responses, such as the production of phenylalanine ammonia lyase, hydroxyproline-rich glycoproteins, and pathogenesis–related proteins. The defenses in quantitative resistance, however, develop slower and perhaps reach a lower level than those in the race-specific (R gene) resistance. Quantitative resistance is also affected much more by changes in the environment, mostly of changes in temperature during the various stages of development of resistance.

Mechanisms of Quantitative Resistance

Studies of defense mechanisms in diseases with quantitative resistance are few and far between. For example, in the early blight of tomato caused by the fungus *Alternaria solani*, all resistant tomato lines had higher constitutive levels of the pathogenesis-related proteins chitinase and β-1,3-glucanase than the susceptible lines. Also, preparations of constitutive enzymes from quantitatively resistant, but not from susceptible, tomato plants could release elicitors of plant cell death, and possibly of a hypersensitive response, from the cell walls of the fungus. These results show that, in this host–plant interaction, the defense responses involve the production of higher levels of pathogenesis-related proteins in resistant plants, and the same plants may also induce the pathogen to produce elicitor molecules that potentiate a more aggressive defense response through the induction of cell death and a hypersensitive-like response. The latter defenses are produced in a manner not unlike that in a specific host–pathogen interaction, but in the absence of host R genes. In the quantitatively controlled resistance of the soybean–*Phytophthora* interaction, soybean tissues actually caused the release of phytoalexin elicitors from the cell walls of the fungus, again showing that the plant can play an important role in forcing the release of defense-triggering signals from the pathogen. Finally, when five cabbage varieties of different resistance levels were inoculated with a strain of the cabbage black rot bacterium *Xanthomonas campestris* pv. *campestris*, two varieties were resistant, one was partially resistant, and two were susceptible. In all varieties there was an increase in the total oxidant activity of peroxidase and superoxide dismutase, accumulation of peroxidases, and lignin deposition. The increases, however, were greater and generally occurred earlier in resistant than in susceptible varieties. However, activity of the antioxidant catalase decreased in both resistant and susceptible varieties, but it decreased more in the resistant variety. The resistant varieties also produced new isozymes of peroxidase and superoxide dismutase that were not produced by the susceptible variety. These results suggest that in the cabbage–*X. campestris* pv. *campestris* system there is a multilevel resistance similar to a hypersensitive response, although the onset of this response was delayed when compared to the classical HR. In barley leaves infected with the fungus *Drechslera teres*, as many as eight pathogenicity-related proteins with thaumatin-like activity were detected.

Effect of Temperature on Quantitative Resistance

Quantitative resistance is often affected greatly by the temperature in the environment. This effect, however, is not unique to plants with quantitative resistance, as even in plants with monogenic (R) gene resistance, the resistance of the host may be changed drastically by changes in temperature. For example, in R resistance-carrying wheat, a change in temperature from 18 to 30°C changes the reaction of wheat plants carrying the Sr6 R gene from rust resistant to rust susceptible. Also, resistance to rust and powdery mildew was increased in pea and barley, respectively, by low-temperature hardening of these grain crops. However, a brief "heat shock" may cause a brief period of susceptibility of wheat plants to rust, while it induces resistance to powdery mildew in barley and to cucumber scab, caused by the fungus *Cladosporium cucumerinum*, in cucumber, in which it also causes an increase in peroxidase activity. There are numerous reports of different plants synthesizing a

variety of pathogenesis-related (PR) proteins in response to abiotic (low temperature, drought, pollution, wounding) as well as to biotic (fungi, bacteria, etc.) stresses. Some of the PR proteins include PR-1, PR-2 (β-1,3-glucanases), PR-3 (chitinases), and PR-5 (thaumatin-like proteins), as well as peroxidases. Stressed plants also increase the production of phenylalanine ammonia lyase (PAL), which is involved in the production of phytoalexins.

In a detailed study of the effect of cold hardening of wheat on its quantitative resistance to infection by the snow mold fungi, it was found that cold hardening increases the resistance of wheat to snow mold and also induces changes in the expression (activity) of genes associated with PR proteins and other defense responses, some of them associated with induced systemic resistance. The most abundant PR proteins produced were chitinase, followed by PAL, β-1,3-glucanase, PR-1, and peroxidase. Similar PR proteins were produced by plants receiving cold treatment only, but the level of these proteins was lower and appeared later than when the plants were also infected by the snow mold fungi. It is apparent, therefore, that this biotic stress induces resistance and that the resistance is further augmented by the fungal infection. This type of resistance has characteristics similar to those of pathogen- and salicylic acid-induced resistance, including the expression of PR genes and further enhancement of defense-associated genes following the infection by a pathogen.

It should be noted in the aforementioned paragraphs that all plants produce PR and other defense-associated proteins constitutively and/or following induction by biotic and abiotic agents. In some host/pathogen combinations the level of constitutively produced PR proteins can be correlated with the level of partial resistance of the cultivars to the pathogen. There is no proof, however, that this correlation is meaningful, especially since some varieties lack the constitutive production of certain PR proteins and yet the plants exhibit partial resistance. It is possible, of course, that plants in the latter varieties have a means of upregulating PR gene expression upon infection that the other varieties lack. As was mentioned already, quantitative resistance depends (a) on the preexisting and induced structural and biochemical defenses provided by dozens and, probably, hundreds of defense-associated genes, (b) on PR proteins, which may provide another significant portion of the overall defenses, and (c) on the possible ability of PR proteins to potentiate a more aggressive response by plant cells to the pathogen invasion by inducing the pathogen to release molecules eliciting host defenses in the absence of a gene-for-gene relationship between host and pathogen.

INDUCED BIOCHEMICAL DEFENSES IN THE HYPERSENSITIVE RESPONSE (RACE-SPECIFIC, MONOGENIC, R GENE, OR VERTICAL) RESISTANCE

The Hypersensitive Response

The hypersensitive response, often referred to as HR, is a localized induced cell defense in the host plant at the site of infection by a pathogen (Fig. 6-10A). HR is the result of quick mobilization of a cascade of defense responses by the affected and surrounding cells and the subsequent release of toxic compounds that often kill both the invaded and surrounding cells and, also, the pathogen. The hypersensitive response is often thought to be responsible for limiting the growth of the pathogen and, in that way, is capable of providing resistance to the host plant against the pathogen. An effective hypersensitive response may not always be visible when a plant remains resistant to attack by a pathogen, as it is possible for the hypersensitive response to involve only single cells or very few cells and thereby remain unnoticed. Under artificial conditions, however, injection of several genera of plant pathogenic bacteria into leaf tissues of nonhost plants results in the development of a hypersensitive response. The artificially induced HR consists of large leaf sectors becoming water soaked at first and, subsequently, necrotic and collapsed within 8 to 12 hours after inoculation (Fig. 6-10B). The bacteria injected in the tissues are trapped in the necrotic lesions and generally are killed rapidly. The HR may occur whenever virulent strains of plant pathogenic bacteria are injected into nonhost plants or into resistant varieties and when avirulent strains are injected into susceptible cultivars. Although not all cases of resistance are due to the hypersensitive response, HR-induced resistance has been described in numerous diseases involving obligate parasites (fungi, viruses, mollicutes, and nematodes), as well as nonobligate parasites (fungi and bacteria).

The hypersensitive response is the culmination of the plant defense responses initiated by the recognition by the plant of specific pathogen-produced signal molecules, known as elicitors. Recognition of the elicitors by the host plant activates a cascade of biochemical reactions in the attacked and surrounding plant cells and leads to new or altered cell functions and to new or greatly activated defense-related compounds (Fig. 6-11). The most common new cell functions and compounds include a rapid burst of reactive oxygen species, leading to a dramatic increase of oxidative reactions; increased ion movement, especially of K^+ and H^+ through the cell membrane; disruption of membranes and loss of

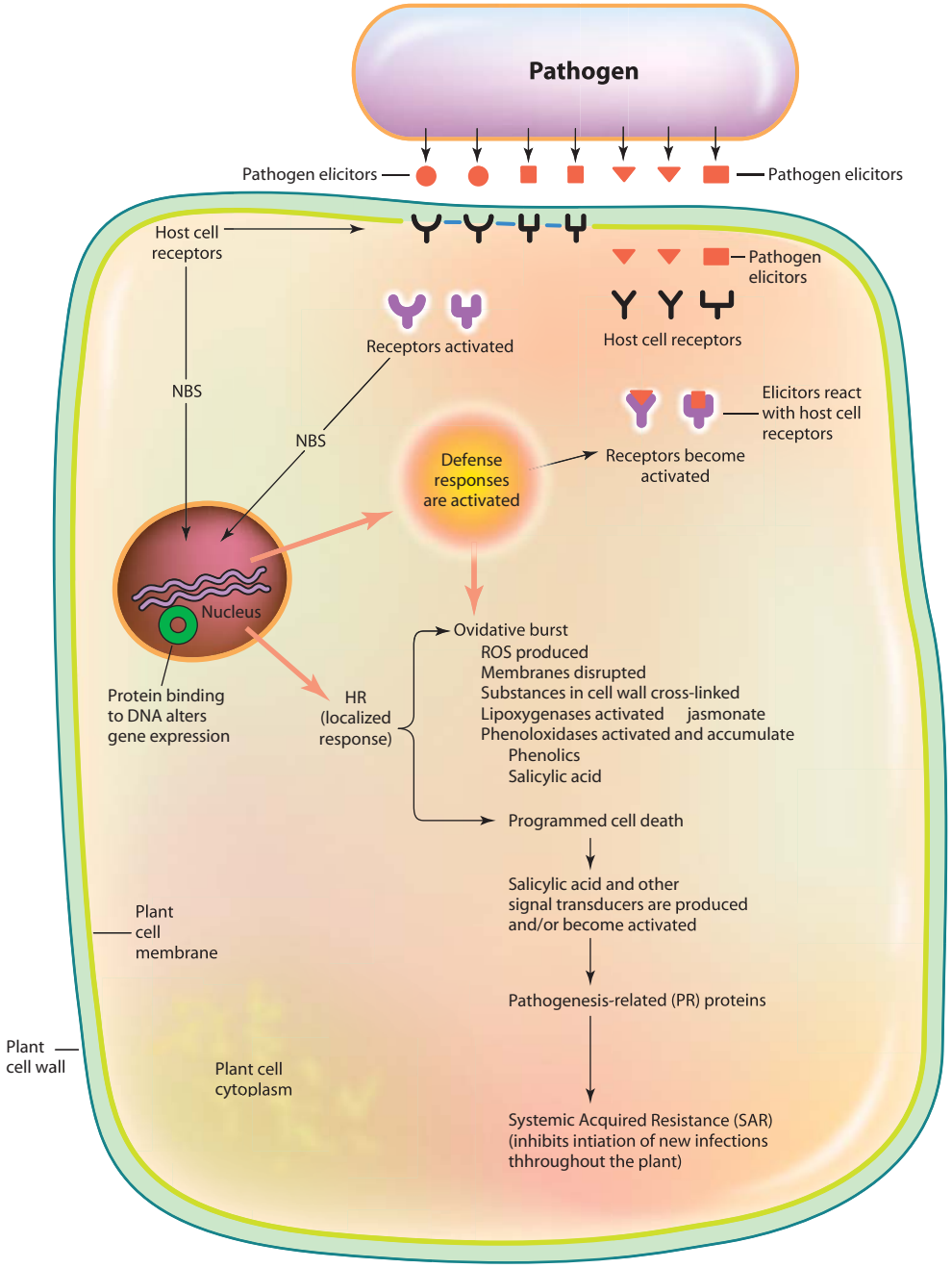


FIGURE 6-11 Diagram of the hypothetical steps in the hypersensitive response defense of plants following interaction of an elicitor molecule produced by a pathogen avirulence gene with a receptor molecule produced by the matching host R gene.

cellular compartmentalization (Fig. 6-12); cross-linking of phenolics with cell wall components and strengthening of the plant cell wall; transient activation of protein kinases (wounding-induced and salicylic acid-induced kinases); production of antimicrobial substances such as phenolics (phytoalexins); and formation of antimicrobial so-called pathogenesis-related proteins such as chitinases.