chapter two

# PARASITISM *AND* DISEASE DEVELOPMENT



The pathogens that attack plants belong to the same groups of organisms that cause diseases in humans and animals. Moreover, plants are attacked by a number of other plants. With the exception of some insect-transmitted plant pathogens, however, which cause diseases in both their host plants and their insect vectors, none of the pathogen species that attack plants is known to affect humans or animals.

**Infectious diseases** are those that result from infection of a plant by a pathogen. In such diseases, the pathogen can grow and multiply rapidly on diseased plants, it can spread from diseased to healthy plants, and it can cause additional plants to become diseased, thereby leading to the development of a small or large epidemic.

## PARASITISM AND PATHOGENICITY

An organism that lives on or in some other organism and obtains its food from the latter is called a **parasite.** The removal of food by a parasite from its host is called **parasitism**. A **plant parasite** is an organism that becomes intimately associated with a plant and multiplies or grows at the expense of the plant. The removal by the parasite of nutrients and water from the host plant usually reduces efficiency in the normal growth of the plant and becomes detrimental to the further development and reproduction of the plant. In many cases, parasitism is intimately associated with **pathogenicity**, i.e., the ability of a pathogen to cause disease, as the ability

of the parasite to invade and become established in the host generally results in the development of a diseased condition in the host.

In some cases of parasitism, as with the root nodule bacteria of legume plants and the mycorrhizal infection of feeder roots of most flowering plants, both the plant and the microorganism benefit from the association. This phenomenon is known as **symbiosis**.

In most plant diseases, however, the amount of damage caused to plants is often much greater than would be expected from the mere removal of nutrients by the parasite. This additional damage results from substances secreted by the parasite or produced by the host in response to stimuli originating in the parasite. Tissues affected by such substances may show increased respiration, disintegration or collapse of cells, wilting, abscission, abnormal cell division and enlargement, and degeneration of specific components such as chlorophyll. These conditions in themselves do not seem directly to improve the welfare of the parasite. It would appear, therefore, that the damage caused by a parasite is not always proportional to the nutrients removed by the parasite from its host. **Pathogenicity**, then, is the ability of the parasite to interfere with one or more of the essential functions of the plant, thereby causing disease. Parasitism frequently plays an important, but not always the most important, role in pathogenicity.

Of the large number of groups of living organisms, only a few members of a few groups can parasitize plants: fungi, bacteria, mollicutes, parasitic higher plants, parasitic green algae, nematodes, protozoa, viruses, and viroids. These parasites are successful because they can invade a host plant, feed and proliferate in it, and withstand the conditions in which the host lives. Some parasites, including viruses, viroids, mollicutes, some fastidious bacteria, nematodes, protozoa, and fungi causing downy mildews, powdery mildews, and rusts, are **biotrophs**, i.e., they can grow and reproduce in nature only in living hosts, and they are called **obligate parasites**. Other parasites (most fungi and bacteria) can live on either living or dead hosts and on various nutrient media, and they are therefore called **nonobligate parasites**. Some nonobligate parasites live most of the time or most of their life cycles as parasites, but, under certain conditions, may grow saprophytically on dead organic matter; such parasites are **semibiotrophs** and are called **facultative saprophytes**. Others live most of the time and thrive well on dead organic matter (**necrotrophs**) but, under certain circumstances, may attack living plants and become parasitic; these parasites are called **facultative parasites**. Usually no correlation exists between the degree of parasitism of a pathogen and the severity of disease it can cause, as many diseases caused by weakly parasitic pathogens are much more damaging to a plant than others caused even by obligate parasites. Moreover, certain pathogens, e.g., slime molds and those causing sooty molds, can cause disease by just covering the surface of the plant without parasitizing the plant.

Obligate and nonobligate parasites generally differ in the ways in which they attack their host plants and procure their nutrients from the host. Many nonobligate parasites secrete enzymes that bring about the disintegration of the cell components of plants, and these alone or with the toxins secreted by the pathogen result in the death and degradation of the cells. The invading pathogen then utilizes the contents of the cells for its growth. Many fungi and most bacteria act in this fashion, growing as necrotrophs on a nonliving substrate within a living plant. This mode of nutrition is like that of saprophytes. However, all obligate (and some nonobligate) parasites do not kill cells in advance but get their nutrients either by penetrating living cells or by establishing close contact with them. The association of these pathogens with their host cells is an intimate one and results in continuous absorption or diversion of nutrients, which would normally be utilized by the host, into the body of the parasite. The depletion of nutrients, however, although it restricts the growth of the host and causes symptoms, does not always kill the host. In the case of obligate parasites, death of the host cells restricts the further development of the parasite and may result in its death.

Parasitism of cultivated crops is a common phenomenon. In North America, for example, more than 8,000 species of fungi cause nearly 100,000 diseases, and at least 200 bacteria, about 75 mollicutes, more than 1,000 different viruses and 40 viroids, and more than 500 species of nematodes attack crops. Although about 2,500 species of higher plants are parasitic on other plants, only a few of them are serious parasites of crop plants. A single crop, e.g., the tomato, may be attacked by more than 40 species of fungi, 7 bacteria, 16 viruses, several mollicutes, and several nematodes. This number of diseases is average as corn has 100, wheat 80, and apple and potato each are susceptible to about 80–100 diseases. Fortunately, however, in any given location, only a fraction of the diseases affecting a crop are present and, in any given year, only a small number of these diseases become severe.

## HOST RANGE OF PATHOGENS

Pathogens differ with respect to the kinds of plants that they can attack, with respect to the organs and tissues that they can infect, and with respect to the age of the organ or tissue of the plant on which they can grow.

Some pathogens are restricted to a single species, others to one genus of plants, and still others have a wide range of hosts, belonging to many families of higher plants. Some pathogens grow especially on roots, others on stems, and some mainly on the leaves or on fleshy fruits or vegetables. Some pathogens, e.g., vascular parasites, attack specifically certain kinds of tissues, such as phloem or xylem. Others may produce different effects on different parts of the same plant. With regard to the age of plants, some pathogens attack seedlings or the young tender parts of plants, whereas others attack only mature tissues.

Many obligate parasites are quite specific as to the kind of host they attack, possibly because they have evolved in parallel with their host and require certain nutrients that are produced or become available to the pathogen only in these hosts. However, many viruses and nematodes, although obligate parasites, attack many different host plants. Nonobligate parasites, especially root, stem, and fruit-attacking fungi, usually attack many different plants and plant parts of varying age, possibly because these pathogens depend on nonspecific toxins or enzymes that affect substances or processes found commonly among plants for their attack. Some nonobligate parasites, however, produce disease on only one or a few plant species. In any case, the number of plant species currently known to be susceptible to a single pathogen is smaller than the actual number in nature, as only a few species out of thousands have been studied for their susceptibility to each pathogen. Furthermore, because of genetic changes, a pathogen may be able to attack hosts previously immune to it. It should be noted, however, that each plant species is susceptible to attack by only a relatively small number of all known plant pathogens.

## DEVELOPMENT OF DISEASE IN PLANTS

A plant becomes diseased in most cases when it is attacked by a pathogen or when it is affected by an abiotic agent. Therefore, in the first case, for a plant disease to occur, at least two components (plant and pathogen) must come in contact and must interact. If at the time of contact of a pathogen with a plant, and for some time afterward, conditions are too cold, too hot, too dry, or some other extreme, the pathogen may be unable to attack or the plant may be able to resist the attack, and therefore, despite the two being in contact, no disease develops. Apparently then, a third component, namely a set of environmental conditions within a favorable range, must also occur for disease to develop. Each of the three components can display considerable variability; however, as one component changes it affects the degree of disease severity within an individual plant and within a plant population. For example, the plant may be of a species or variety that may be more or less resistant to the pathogen or it may be too young or too old for what the pathogen prefers, or plants over a large area may show genetic uniformity, all of which can either reduce or increase the rate of disease development by a particular pathogen. Similarly, the pathogen may be of a more or less virulent race, it may be present in small or extremely large numbers, it may be in a dormant state, or it may require a film of water or a specific vector. Finally, the environment may affect both the growth and the resistance of the host plant and also the rate of growth or multiplication and degree of virulence of the pathogen, as well as its dispersal by wind, water, vector, and so on.

The interactions of the three components of disease have often been visualized as a triangle (Fig. 2-1), generally referred to as the "**disease triangle**." Each side of the triangle represents one of the three components. The length of each side is proportional to the sum total of the characteristics of each component that favor disease. For example, if the plants are resistant, the wrong age, or widely spaced, the host side — and the amount of disease — would be small or zero, whereas if the plants are susceptible, at a susceptible stage of growth, or planted densely, the host side would be long and the potential amount of disease could be great. Similarly, the more virulent, abundant, and active the pathogen, the longer the pathogen side would be and the greater the potential amount of disease. Also, the more favorable the environmental conditions that help the pathogen (e.g., temperature, moisture, and wind) or that reduce host resistance, the longer the environment side would be and the greater the potential amount of disease. If the three components of the disease triangle could be quantified, the area of the triangle would represent the amount of disease in a plant or in a plant population. If any of the three components is zero, there can

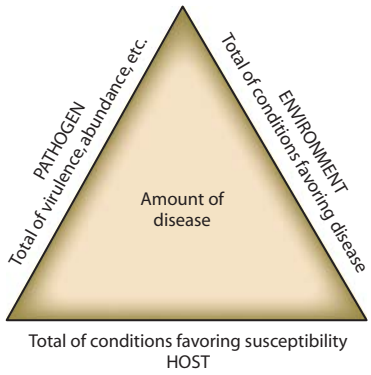


**FIGURE 2-1** The disease triangle.

be no disease. The disease triangle is also represented as a triangle with the words of the three components (host plant, pathogen, environment) placed at the peaks of the triangle rather than along its sides.

## STAGES IN THE DEVELOPMENT OF DISEASE: THE DISEASE CYCLE

In every infectious disease a series of more or less distinct events occurs in succession and leads to the development and perpetuation of the disease and the pathogen. This chain of events is called a **disease cycle**. A disease cycle sometimes corresponds fairly closely to the **life cycle** of the pathogen, but it refers primarily to the appearance, development, and perpetuation of the disease as a function of the pathogen rather than to the pathogen itself. The disease cycle involves changes in the plant and its symptoms as well as those in the pathogen and spans periods within a growing season and from one growing season to the next. The primary events in a disease cycle are inoculation, penetration, establishment of infection, colonization (invasion), growth and reproduction of the pathogen, dissemination of the pathogen, and survival of the pathogen in the absence of the host, i.e., overwintering or oversummering (overseasoning) of the pathogen (Fig. 2-2). In some diseases there may be several **infection cycles** within one disease cycle.

### Inoculation

**Inoculation** is the initial contact of a pathogen with a site of plant where infection is possible. The pathogen(s) that lands on or is otherwise brought into contact with the plant is called the **inoculum**. The inoculum is any part of the pathogen that can initiate infection. Thus, in fungi the inoculum may be spores (Figs. 2-3A–2-3C), **sclerotia** (i.e., a compact mass of mycelium), or fragments of mycelium. In bacteria, mollicutes, protozoa, viruses, and viroids, the inoculum is always whole individuals of bacteria (Fig. 2-3D), mollicutes, protozoa, viruses, and viroids, respectively. In nematodes, the inoculum may be adult nematodes, nematode juveniles, or eggs. In parasitic higher plants, the inoculum may be plant fragments or seeds. The inoculum may consist of a single individual of a pathogen, e.g., one spore or one multicellular sclerotium, or of millions of individuals of a pathogen, e.g., bacteria carried in a drop of water. One unit of inoculum of any pathogen is called a **propagule**.

#### Types of Inoculum

An inoculum that survives dormant in the winter or summer and causes the original infections in the spring or in the autumn is called a **primary inoculum**, and the infections it causes are called **primary infections**. An inoculum produced from primary infections is called a **secondary inoculum** and it, in turn, causes **secondary infections**. Generally, the more abundant the primary inoculum and the closer it is to the crop, the more severe the disease and the losses that result.

#### Sources of Inoculum

In some fungal and bacterial diseases of perennial plants, such as shrubs and trees, the inoculum is produced on the branches, trunks, or roots of the plants. The inoculum sometimes is present right in the plant

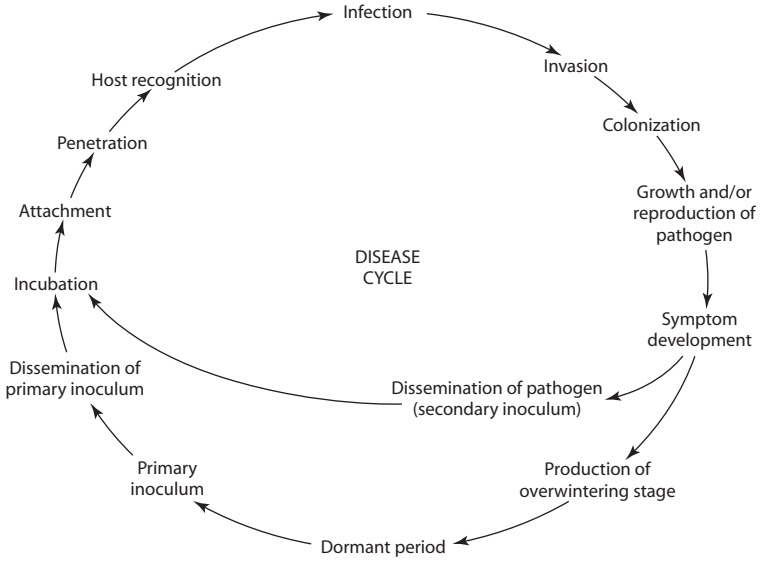


**FIGURE 2-2** Stages in development of a disease cycle.

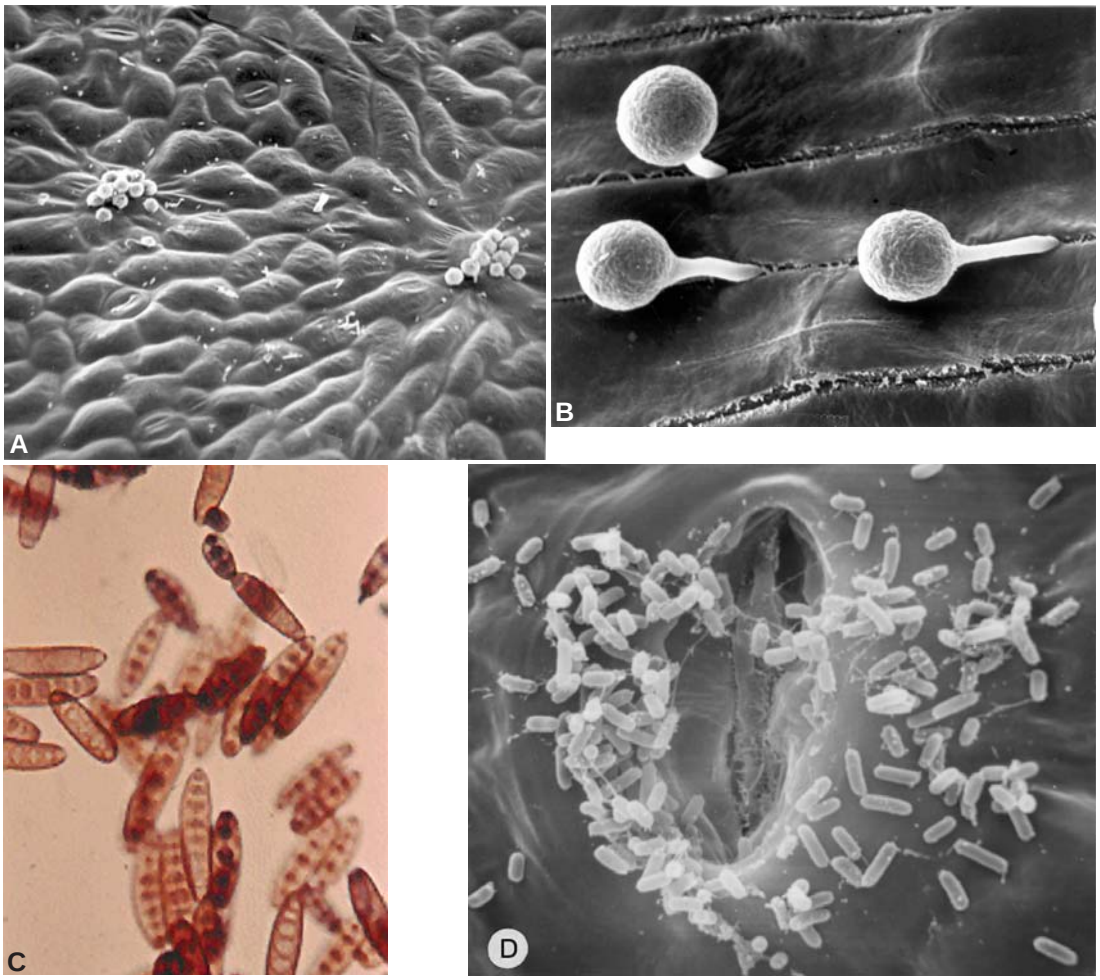


**FIGURE 2-3** Types of inoculum and ways in which some pathogens enter a host plant. (A) Two groups of zoospores of the grape downy mildew oomycete have gathered over two leaf stomata. (B) Encysted zoospores of the soybean root rot pathogen *Phytophthora sojae* germinating and penetrating the root. (C) Mitospores (conidia) of a fungus that causes a corn leaf spot disease. (D) Bacteria of *Pseudomonas syringae* that causes bacterial spot and canker of stone fruits are seen in and surrounding a stoma of a cherry leaf. [Photographs courtesy of (A) D. J. Royle, (B) C. W. Mims and K. Enkerli, University of Georgia, and (D) E. L. Mansvelt, Stellenbosch, South Africa.]

debris or soil in the field where the crop is grown; other times it comes into the field with the seed, transplants, tubers, or other propagative organs or it may come from sources outside the field. Outside sources of inoculum may be nearby plants or fields or fields many miles away. In many plant diseases, especially those of annual crops, the inoculum survives in perennial weeds or alternate hosts, and every season it is carried from them to the annual and other plants. Fungi, bacteria, parasitic higher plants, and nematodes either produce their inoculum on the surface of infected plants or their inoculum reaches the plant surface when the infected tissue breaks down. Viruses, viroids, mollicutes, fastidious bacteria, and protozoa produce their inoculum within the plants; such an inoculum almost never reaches the plant surface in nature and, therefore, it can be transmitted from one plant to another almost entirely by some kind of vector, such as an insect.

### Landing or Arrival of Inoculum

The inoculum of most pathogens is carried to host plants passively by wind, water, and insects. An airborne inoculum usually gets out of the air and onto the plant surface not just by gravity but by being washed out by rain. Only a tiny fraction of the potential inoculum produced actually lands on susceptible host plants; the bulk of the produced inoculum lands on things that cannot

become infected. Some types of inoculum in the soil, e.g., zoospores and nematodes, may be attracted to the host plant by such substances as sugars and amino acids diffusing out of the plant roots. Vector-transmitted pathogens are usually carried to their host plants with an extremely high efficiency.

## Prepenetration Phenomena

### Attachment of Pathogen to Host

Pathogens such as mollicutes, fastidious bacteria, protozoa, and most viruses are placed directly into cells of plants by their vectors and, in most cases, they are probably immediately surrounded by cytoplasm, cytoplasmic membranes, and cell walls. However, almost all fungi, bacteria, and parasitic higher plants are first brought into contact with the external surface of plant organs. Before they can penetrate and colonize the host, they must first become attached to the host surface (Figs. 2-3–2-6). Attachment takes place through the adhesion of spores, bacteria, and seeds through adhesive materials that vary significantly in composition and in the environmental factors they need to become adhesive. Disruption of adhesion by nontoxic synthetic compounds results in failure of the spores to infect leaves.

The propagules of these pathogens have on their surface or at their tips mucilaginous substances consisting of mixtures of water-insoluble polysaccharides, glycoproteins, lipids, and fibrillar materials, which, when moistened, become sticky and help the pathogen adhere to the plant. In some fungi, hydration of the spore by moist air or dew causes the extrusion of preformed mucilage at the tip of the spore that serves for the immediate adherence of the spore to the hydrophobic plant surface and resistance to removal by flowing water. However, in powdery mildew fungi, which do not require free water for infection, adhesion is accomplished by release from the spore of the enzyme cutinase, which makes the plant and spore areas of attachment more hydrophilic and cements the spore to the plant surface. In other cases, propagule adhesion requires on-the-spot synthesis of new glycoproteins and it may not reach maximum levels until 30 minutes after contact. In some fungi causing vascular wilts, spores fail to adhere after hydration but become adhesive after they are allowed to respire and to synthesize new proteins.

How exactly spores adhere to plant surfaces is not known, but it seems to either involve a very specific interaction of the spore with a host plant surface via lectins, ionic interactions, or hydrophobic contact with the plant cuticle, or involve stimulation of the spore by physical rather than chemical signals. The extracellular matrix surrounding the propagules of many pathogens contains several enzymes, including cutinases, which are expected to play an important role in spore attachment. In any case, the act of attachment often seems necessary for the subsequent transmission of signals for germ tube extension and production of infection structure. It is now clear that many proteins of the fungal cell wall, in addition to their structural role, play an important role in the adhesion of fungi, as well as in the host-surface perception by the fungus.

### Spore Germination and Perception of the Host Surface

It is not clear what exactly triggers spore germination, but stimulation by the contact with the host surface, hydration and absorption of low molecular weight ionic material from the host surface, and availability of nutrients play a role. Spores also have mechanisms that prevent their germination until they sense such stimulations or when there are too many spores in their vicinity. Once the stimulation for germination has been received by the spore, the latter mobilizes its stored food reserves, such as lipids, polyoles, and carbohydrates, and directs them toward the rapid synthesis of cell membrane and cell wall toward the germ tube formation and extension (Figs. 2-4 and 2-5). The germ tube is a specialized structure distinct from the fungal mycelium, often growing for a very short distance before it differentiates into an appressorium. The germ tube is also the structure and site that perceives the host surface and, if it does not receive the appropriate external stimuli, the germ tube remains undifferentiated and, when the nutrients are exhausted, it stops growing. When appropriate physical and chemical signals, such as surface hardness, hydrophobicity, surface topography, and plant signals, are present, germ tube extension and differentiation take place.

The perception of signals from plant surfaces by pathogenic fungi (Fig. 2-6) seems to be the result of signaling pathways mediated by cyclic adenosine monophosphate (cAMP) and mitogen-activated protein kinase (MAPK), which have been implicated in regulating the development of infection-related phenomena in many different fungi. In response to a signal from the host plant, e.g., the presence of a hydrophobic plant surface, which transmits a cue for appressorium formation, the fungus perceives the extracellular signal and its transmission via the plasma membrane and, as a first step, it accumulates intracellular signaling molecules and induces a phosphorylation cascade. In some fungi, the receptor of the signal is a protein in the plasma membrane of the fungal spore. Transmission of the cAMP signal proceeds via the cAMP-dependent activity of

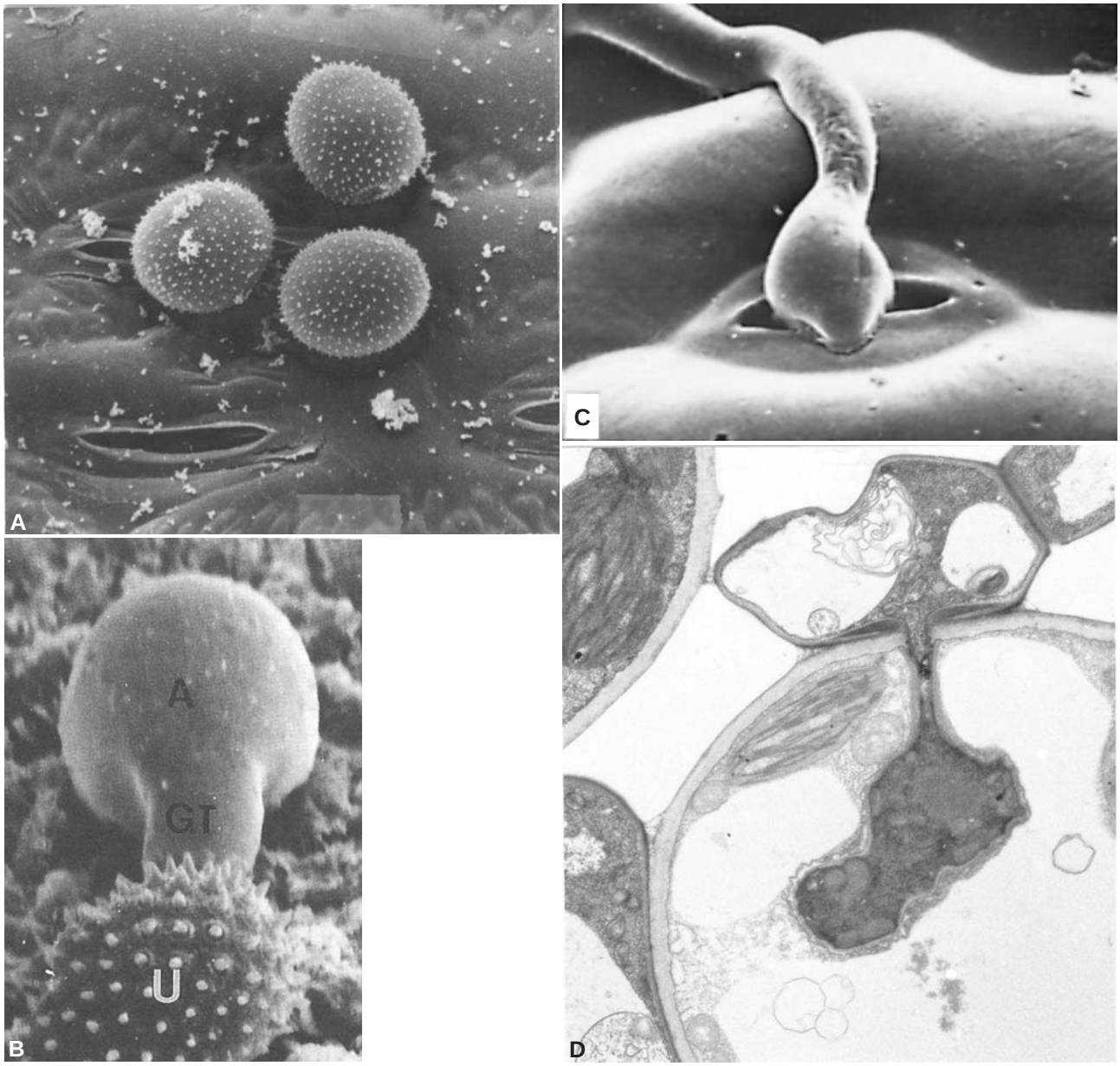


**FIGURE 2-4** Methods of germination and penetration by fungi. (A) Uredospores of a rust fungus on a grass leaf next to open stomata. (B) A rust uredospore (U) that has germinated and produced a dome-like appressorium. (C) Uredospore germination, germ tube elongation, and appressorium penetration through a stoma. (D) A haustorium of a rust fungus inside a host cell. (E) A spore of the apple black rot fungus that has germinated directly into mycelium. (F) Two multicellular conidia of *Alternaria* sp. (G) A germinating conidium of *Alternaria* with a germ tube covered with extracellular material. [Photographs courtesy of (A) Plant Pathology Department, University of Florida, (B and C) W. K. Wynn and (D) C. W. Mims, University of Georgia, (E) J. Rytter and J. W. Travis, Pennslyvania State University, (F and G) Mims *et al.* (1997). *Can. J. Bot.* 75, 252–260.]

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protein kinase A (= PKA) and subsequent phosphorylation of target proteins. The major activity of PKA in developing germ tubes is the mobilization of carbohydrates and lipids to the appressorium site and is, therefore, pivotal to the production of functional appressoria. In some fungi, cAMP signaling is required for the initiation of appressorium development, at which time intracellular cAMP concentrations rise during differentiation of conidia and emergence of the appressorium germ tube. Subsequently, cAMP levels fall as the germ tube extends and, if more cAMP is added at this point, further development of the germ tube is inhibited.

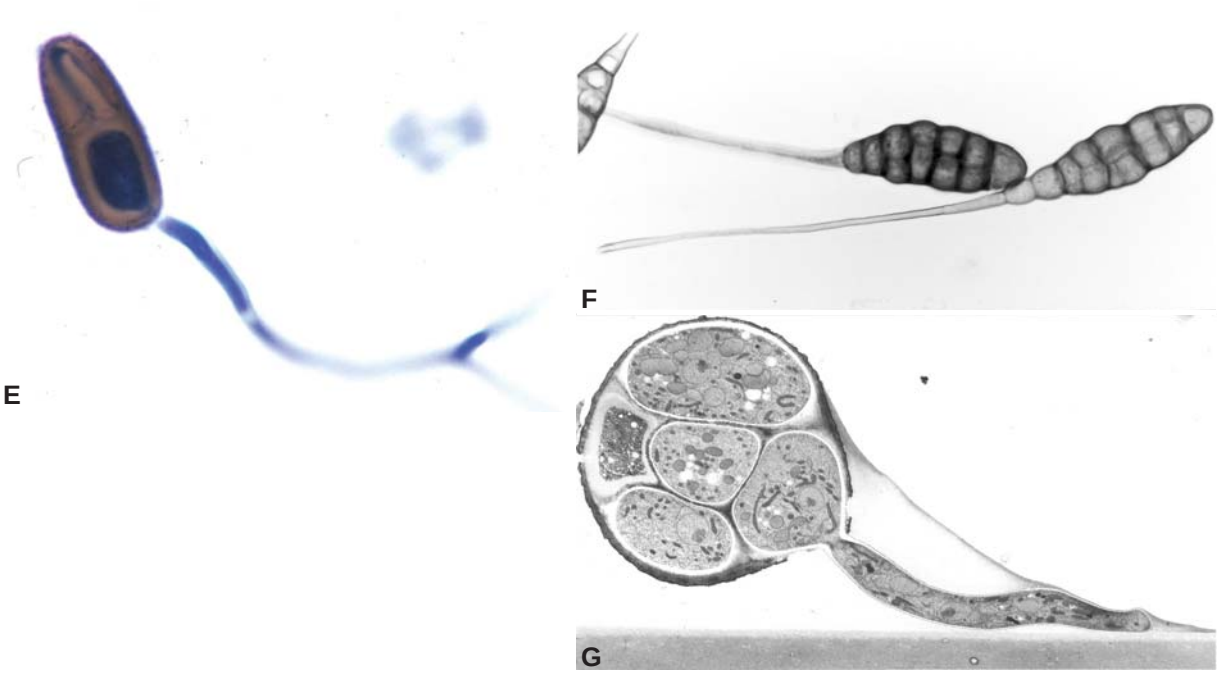



FIGURE 2-4 (*Continued*)

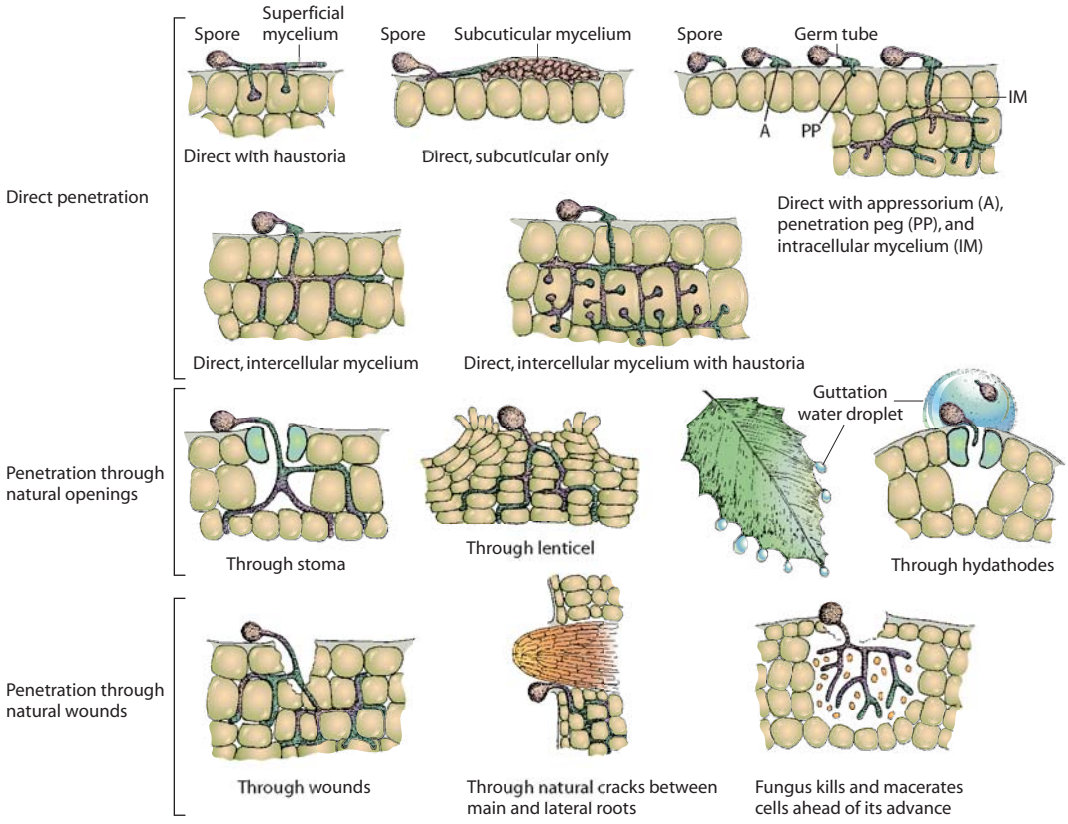



FIGURE 2-5 Methods of penetration and invasion by fungi.

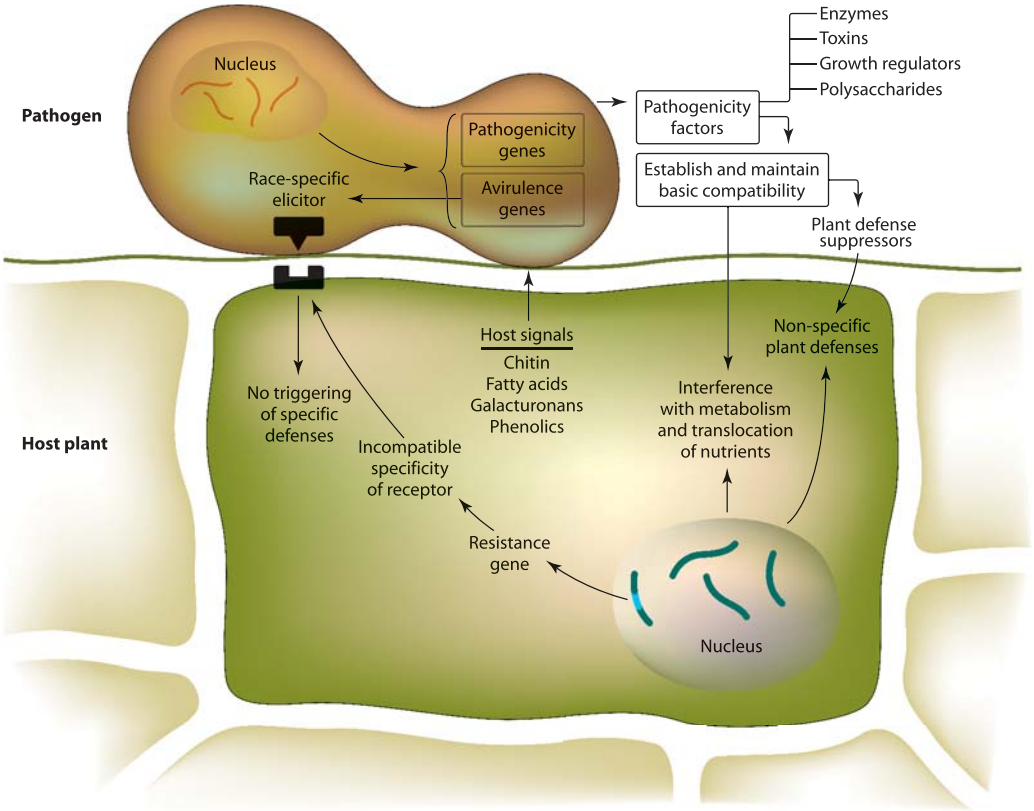



**FIGURE 2-6** Establishment of infection in a compatible reaction between a pathogen and its host plant.

Signaling pathways for infection-related development are also achieved through mitogen-activated protein kinases (MAPKs) and their upstream regulatory kinases. All of these together comprise a functional unit that transmits input signals from the periphery of the cell to the cell nucleus to elicit the expression of appropriate genes. A MAP kinase, K1 or P1, regulates appressorium formation in response to a signal from the plant surface but it is also required for invasive growth or viability in its host plant.

After attachment of the propagule to the host surface, as spores and seeds germinate, germ tubes also produce mucilaginous materials that allow them to adhere to the cuticular surface of the host, either along their entire length or only at the tip of the germ tube. In regions of contact with the germ tube, the structure of the host cuticle and cell walls often appears altered, presumably as a result of degradative enzymes contained in the mucilaginous sheath.

### Appressorium Formation and Maturation

Once appressoria are formed, they adhere tightly to the leaf surface (Figs. 2-4 and 2-9). Subsequently, appressoria secrete extracellular enzymes, generate physical force, or both to bring about penetration of the cuticle by the fungus. Appressoria must be attached to the host plant surface strongly enough to withstand the invasive physical force applied by the fungus and to resist the chemical action of the enzymes secreted by the fungus. Appressoria of some fungi contain lipids, polysaccharides, and proteins. Fungi that produce melanin-pigmented appressoria produce a narrow penetration hypha from the base of the appressorium and use primarily physical force to puncture the plant cuticle with that hypha.

The size of the turgor pressure inside an appressorium has been measured and found to be 40 times greater than the pressure of a typical car tire. The turgor pressure of an appressorium is due to the enormous accumulation of glycerol in the appressorium, which, due to its high osmotic pressure, draws water into the cell and generates hydrostatic pressure that pushes the thin hypha (appressorial penetration peg) outward through the host cuticle. Mobilization of spore-stored products to the developing appressorium and glycerol biosynthesis in it is regulated by the cAMP signaling pathway, whereas the initial movement of lipid and glycogen reserves to the developing appressorium was also found to be regulated by the K1 MAP. This

indicates that the maturation of appressoria and their specific biochemical activity are intimately associated with genetic control of the initial development of appressoria.

The production of penetration hyphae by appressoria, or directly from germ tubes, is not well understood at the genetic level. Production of the penetration peg requires the localization of actin to the hyphal tip and rapid biosynthesis of the cell wall as the hypha grows through the cuticle and the layers of the epidermal cell walls. Production of penetration hyphae appears to be regulated by a MAP kinase pathway.

### Recognition between Host and Pathogen

It is still unclear how pathogens recognize their hosts and vice versa. It is assumed that when a pathogen comes in contact with a host cell, an early event takes place that triggers a fairly rapid response in each organism that either allows or impedes further growth of the pathogen and development of disease. The nature of the "early event" is not known with certainty in any host–parasite combination, but it may be one of many biochemical substances, structures, and pathways. These may include specific host signal compounds or structures, or specific pathogen elicitor molecules, and either of them may induce specific actions or formation of specific products by the other organism (Fig. 2-6).

Host components acting as signals for recognition by and activation of pathogens are numerous. They may include fatty acids of the plant cuticle that activate production by the pathogen of the cutinase enzyme, which breaks down cutin; galacturonan molecules of host pectin, which stimulate the production of pectin lyase enzymes by the fungus or bacterium; certain phenolic compounds, such as strigol, which stimulate activation and germination of propagules of some pathogens; and isoflavones and other phenolics, amino acids, and sugars released from plant wounds that activate a series of genes in certain pathogens leading to infection. A host plant may also send cues for recognition by some of its pathogens by certain of its surface characteristics such as ridges or furrows, hardness, or release of certain ions such as calcium.

Pathogen components that act as elicitors of recognition by the host plant and subsequent mobilization of plant defenses are still poorly understood. Elicitor molecules may be released from attacking pathogens before or during entry into the host, and they may have a narrow host range, e.g., the elicitins. Some elicitors may be components of the cell surface of the pathogen (e.g., β-glucans, chitin, or chitosan) that are released by the action of host enzymes (e.g., β-glucanase and/or chitinase) and have broad host ranges; some may be synthesized and released by the pathogen after it enters the host in response to host signals. The latter elicitors include the harpin proteins of bacteria that induce development of the hypersensitive response, certain hydroxylipids, and certain peptides and carbohydrates that induce specific host defense responses such as the production of phytoalexins. Elicitors are considered as determinants of pathogen avirulence, as by their presence they elicit the hypersensitive (resistance) response and initiation of transcription of the plant genes that encode the various components of the defense response. These defense measures by the host plant, in turn, result in the pathogen appearing as avirulent.

When the initial recognition signal received by the pathogen favors growth and development, disease may be induced; if the signal suppresses pathogen growth and activity, disease may be aborted. However, if the initial recognition elicitor received by the host triggers a defense reaction, pathogen growth and activity may be slowed or stopped and disease may not develop; if the elicitor either suppresses or bypasses the defense reaction of the host, disease may develop.

### Germination of Spores and Seeds

Almost all pathogens in their vegetative state are capable of initiating infection immediately. Fungal spores and seeds of parasitic higher plants, however, must first germinate (Figs. 2-4 and 2-5). Spores germinate by producing a typical mycelium (Figs. 2-4E and 2-4G) that infects and grows into host plants or they produce a short germ tube that produces a specialized infectious structure, the haustorium (Figs. 2-4B–2-4D). In order to germinate, spores require a favorable temperature and also moisture in the form of rain, dew, or a film of water on the plant surface or at least high relative humidity. The moist conditions must last long enough for the pathogen to penetrate or else it desiccates and dies. Most spores can germinate immediately after their maturation and release, but others (so-called resting spores) require a dormancy period of varying duration before they can germinate. When a spore germinates it produces a germ tube, i.e., the first part of the mycelium, that can penetrate the host plant. Some fungal spores germinate by producing other spores, e.g., sporangia produce zoospores and teliospores produce basidiospores.

Spore germination is often favored by nutrients diffusing from the plant surface; the more nutrients (sugars and amino acids) exuded from the plant, the more spores germinate and the faster they germinate. In some cases, spore germination of a certain pathogen is stimulated only by exudates of plants susceptible to that particular pathogen. In other cases, spore germination may be inhibited to a lesser or greater extent by materials

released into the surrounding water by the plant, by substances contained within the spores themselves, especially when the spores are highly concentrated ("quorum sensing"), and by saprophytic microflora present on or near the plant surface.

Fungi in soil coexist with a variety of antagonistic microorganisms that cause an environment of starvation and of toxic metabolites. As a result, spores of many soilborne fungi are often unable to germinate in some soils, and this phenomenon is called **fungistasis**, or their germ tubes lyse rapidly. Soils in which such events occur are known as **suppressive soils**. Fungistasis, however, is generally counteracted by root exudates of host plants growing nearby, and the spores are then able to germinate and infect.

After spores germinate, the resulting germ tube must grow, or the motile secondary spore (zoospore) must move, toward a site on the plant surface at which successful penetration can take place (Figs. 2-3A and 2-3B). The number, length, and rate of growth of germ tubes, or the number and mobility of motile spores, may be affected by physical conditions, such as temperature and moisture, by the kind and amount of exudates the plant produces at its surface, and by the saprophytic microflora.

The growth of germ tubes in the direction of successful penetration sites seems to be regulated by several factors, including greater humidity or chemical stimuli associated with such openings as wounds, stomata, and lenticels; thigmotropic (contact) responses to the topography of the leaf surface, resulting in germ tubes growing at right angles to cuticular ridges that generally surround stomata and thus eventually reaching a stoma; and nutritional responses of germ tubes toward greater concentrations of sugars and amino acids present along roots. The direction of movement of motile spores (zoospores) is also regulated by similar factors, namely chemical stimuli emanating from stomata, wounds, or the zone of elongation of roots, physical stimuli related to the structure of open stomata, and the nutrient gradient present in wound and root exudates.

Seeds germinate by producing a radicle, which either penetrates the host plant directly or first produces a small plant that subsequently penetrates the host plant by means of specialized feeding organs called haustoria. Most conditions described earlier as affecting spore germination and the direction of growth of germ tubes also apply to seeds. Haustoria are also produced by many fungi.

### Hatching of Nematode Eggs

Nematode eggs also require conditions of favorable temperature and moisture to become activated and hatch. In most nematodes, the egg contains the first juvenile stage before or soon after the egg is laid. This juvenile immediately undergoes a molt and gives rise to the second juvenile stage, which may remain dormant in the egg for various periods of time. Thus, when the egg finally hatches, it is the second-stage juvenile that emerges, and it either finds and penetrates a host plant or undergoes additional molts that produce further juvenile stages and adults.

Once nematodes are in close proximity to plant roots, they are attracted to roots by certain chemical factors associated with root growth, particularly carbon dioxide and some amino acids. These factors may diffuse through soil and may have an attractant effect on nematodes present several centimeters away from the root. Nematodes are generally attracted to roots of both host and nonhost plants, although there may be some cases in which nematodes are attracted more strongly to the roots of host plants.

## Penetration

Pathogens penetrate plant surfaces by direct penetration of cell walls, through natural openings, or through wounds (Figs. 2-3–2-5). Some fungi penetrate tissues in only one of these ways, others in more than one. Bacteria enter plants mostly through wounds, less frequently through natural openings, and never directly through unbroken cell walls (Fig. 2-5). Viruses, viroids, mollicutes, fastidious bacteria, and protozoa enter through wounds made by vectors, although some viruses and viroids may also enter through wounds made by tools and other means. Parasitic higher plants enter their hosts by direct penetration. Nematodes enter plants by direct penetration and, sometimes, through natural openings (Fig. 2-10).

Penetration does not always lead to infection. Many organisms actually penetrate cells of plants that are not susceptible to these organisms and that do not become diseased; these organisms cannot proceed beyond the stage of penetration and die without producing disease.

### Direct Penetration through Intact Plant Surfaces

Direct penetration through intact plant surfaces is probably the most common type of penetration by fungi, oomycetes, and nematodes and the only type of penetration by parasitic higher plants. None of the other pathogens can enter plants by direct penetration.

Of the fungi that penetrate their host plants directly, the hemibiotrophic, i.e., nonobligate parasitic ones, do so through a fine hypha produced directly by the spore or mycelium (Figs. 2-3B, 2-5, and 2-8), whereas the

obligately parasitic ones do so through a penetration peg produced by an **appressorium** (Figs. 2-4B–2-4D and 2-9). The fine hypha or appressorium is formed at the point of contact of the germ tube or mycelium with a plant surface. The fine hypha grows toward the plant surface and pierces the cuticle and the cell wall through mechanical force and enzymatic softening of the cell wall substances. Most fungi, however, form an appressorium at the end of the germ tube, with the appressorium usually being bulbous or cylindrical with a flat surface in contact with the surface of the host plant (Figs. 2-4, 2-9Ab, and 2-9B). Then, a **penetration peg** grows from the flat surface of the appressorium toward the host and pierces the cuticle and the cell wall. The penetration peg grows into a fine hypha generally much smaller in diameter than a normal hypha of the fungus, but it regains its normal diameter once inside the cell. In most fungal diseases the fungus penetrates the plant cuticle and the cell wall, but in some, such as apple scab (Fig. 2-11A), the fungus penetrates only the cuticle and stays between the cuticle and the cell wall.

Parasitic higher plants also form an appressorium and penetration peg at the point of contact of the radicle with the host plant, and penetration is similar to that in fungi. Direct penetration in nematodes is accomplished by repeated back-and-forth thrusts of their stylets. Such thrusts finally create a small opening in the cell wall; the nematode then inserts its stylet into the cell or the entire nematode enters the cell (Fig. 2-12).

### Penetration through Wounds

All bacteria, most fungi, some viruses, and all viroids can enter plants through various types of wounds (Fig. 2-5). Some viruses and all mollicutes, fastidious vascular bacteria, and protozoa enter plants through wounds made by their vectors. The wounds utilized by bacteria and fungi may be fresh or old and may consist of lacerated or killed tissue. These pathogens may grow briefly on such tissue before they advance into healthy tissue. Laceration or death of tissues may be the result of environmental factors such as wind breakage and hail; animal feeding, e.g., by insects and large animals; cultural practices of humans, such as pruning, transplanting, and harvesting; self-inflicted injuries, such as leaf scars; and, finally, wounds or lesions caused by other pathogens. Bacteria and fungi penetrating through wounds germinate or multiply in the wound sap or in a film of rain or dew water present on the wound. Subsequently, the pathogen invades adjacent plant cells or it secretes enzymes and toxins that kill and macerate the nearby cells.

The penetration of viruses, mollicutes, fastidious bacteria, and protozoa through wounds depends on the deposition of these pathogens by their vectors in fresh wounds created at the time of inoculation. All four types of pathogens are transmitted by certain types of insects. Some viruses are also transmitted by certain nematodes, mites, and fungi. Some viruses and viroids are transmitted through wounds made by human hands and tools. In most cases, however, these pathogens are carried by one or a few kinds of specific vectors and can be inoculated successfully only when they are brought to the plant by these particular vectors.

### Penetration through Natural Openings

Many fungi and bacteria enter plants through stomata, and some enter through hydathodes, nectarthodes, and lenticels (Figs. 2-3, 2-4, 2-5, and 2-7). Stomata are most numerous on the lower side of leaves. They measure about 10–20 by 5–8 μm and are open in the daytime but are more or less closed at night. Bacteria present in a film of water over a stoma and, if water soaking occurs, can swim through the stoma easily (Fig. 2-3D) and into the substomatal cavity where they can multiply and start infection. Fungal spores generally germinate on the plant surface, and the germ tube may then grow through the stoma (Figs. 2-3A, 2-4B, and 2-5). Frequently, however, the germ tube forms an appressorium that fits tightly over the stoma, and usually one fine hypha grows from it into the stoma (Figs. 2-4 and 2-5). In the substomatal cavity the hypha enlarges, and from it grow one or several small hyphae that actually invade the cells of the host plant directly or by means of haustoria (Fig. 2-5). Although some fungi can apparently penetrate

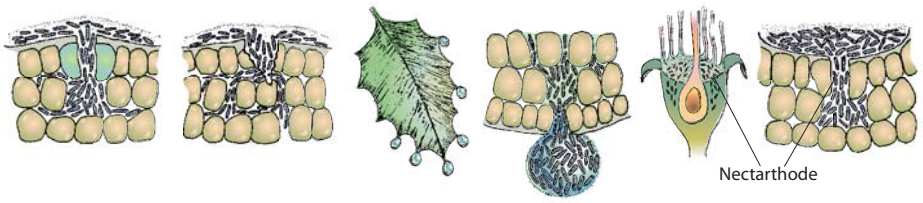



**FIGURE 2-7** Methods of penetration and invasion by bacteria.

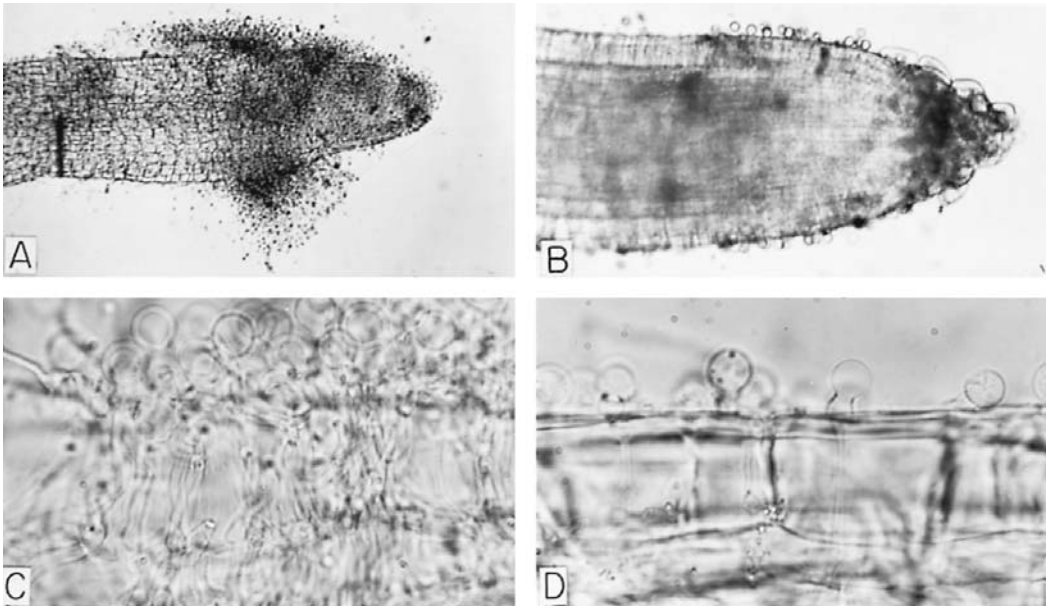



**FIGURE 2-8** Attraction of zoospores of *Phytophthora cinnamomi* to roots of susceptible (A and C) and resistant (B and D) blueberry varieties, and infection of the roots by the zoospores. (A and B) Attraction of zoospores to roots 1 hour after inoculation. (C and D) Infection and colonization of the root after 24 hours are greater in the susceptible highbush blueberry (A and C) than in the more resistant rabbit-eye blueberry (B and D). (Photographs courtesy of R. D. Milholland.)

even closed stomata, others penetrate stomata only while they are open. Certain fungi, e.g., the powdery mildew fungi, may grow over open stomata without entering them.

**Hydathodes** are more or less permanently open pores at the margins and tips of leaves; they are connected to the veins and secrete droplets of liquid, called guttation drops, containing various nutrients (Fig. 2-5). Some bacteria use these pores as a means of entry into leaves, but few fungi seem to enter plants through hydathodes. Some bacteria also enter blossoms through the nectarthodes or nectaries, which are similar to hydathodes (Fig. 2-7).

**Lenticels** are openings on fruits, stems, and tubers that are filled with loosely connected cells that allow the passage of air. During the growing season, lenticels are open, but even so, relatively few fungi and bacteria penetrate tissues through them, growing and advancing mostly between the cells (Fig. 2-5). Most pathogens that penetrate through lenticels can also enter through wounds, with lenticel penetration being apparently a less efficient, secondary pathway.

## Infection

**Infection** is the process by which pathogens establish contact with susceptible cells or tissues of the host and procure nutrients from them. Following infection, pathogens grow, multiply, or both within the plant tissues and invade and colonize the plant to a lesser or greater extent. Growth and/or reproduction of the pathogen (colonization) in or on infected tissues are actually two concurrent substages of disease development (Fig. 2-2).

Successful infections result in the appearance of symptoms, i.e., discolored, malformed, or necrotic areas on the host plant. Some infections, however, remain latent, i.e., they do not produce symptoms right away but at a later time when the environmental conditions or the stage of maturity of the plant become more favorable.

All the visible and otherwise detectable changes in the infected plants make up the **symptoms** of the disease. Symptoms may change continuously from the moment of their appearance until the entire plant dies or they may develop up to a point and then remain more or less unchanged for the rest of the growing season. Symptoms may appear as soon as 2 to 4 days after inoculation, as happens in some localized viral diseases of herbaceous plants, or as late as 2 to 3 years after inoculation, as in the case of some viral, mollicute, and other diseases of trees. In most plant diseases, however, symptoms appear from a few days to a few weeks after inoculation.

The time interval between inoculation and the appearance of disease symptoms is called the **incubation period**. The length of the incubation period of various diseases varies with the particular pathogen–host

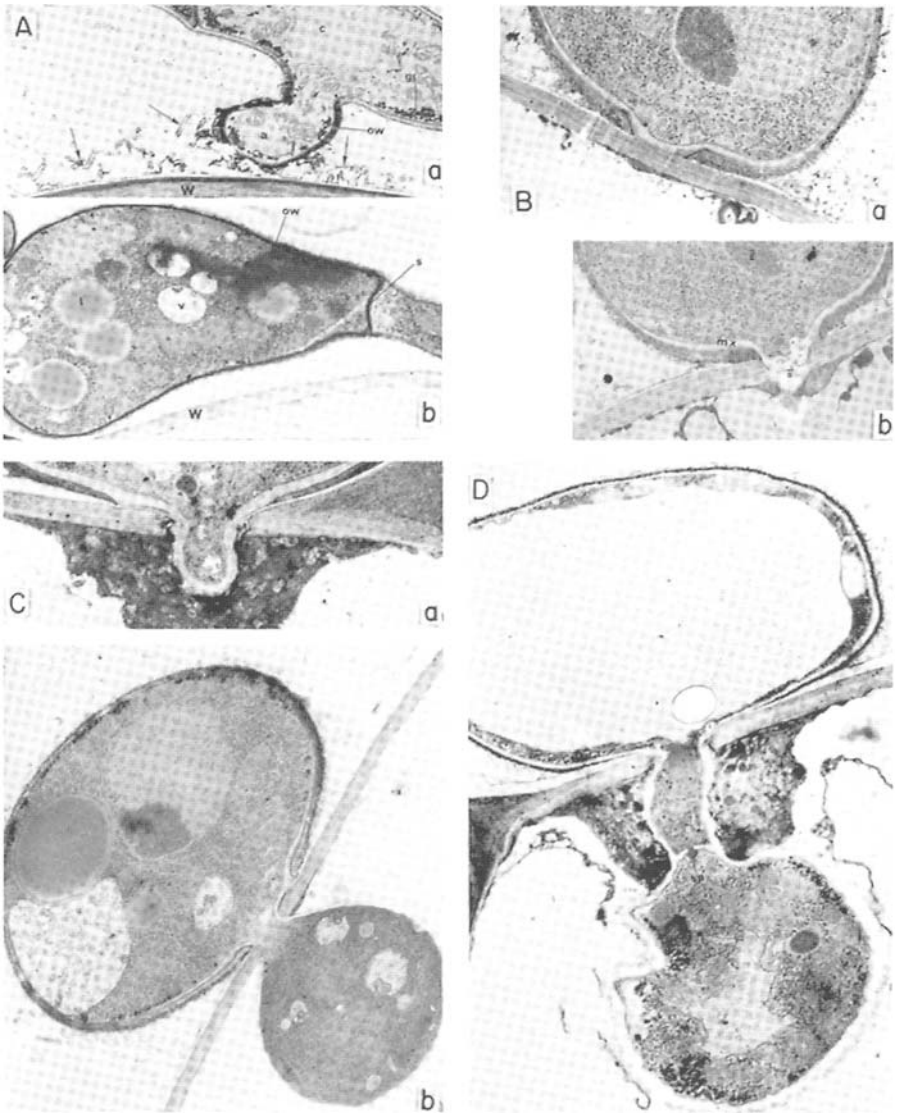
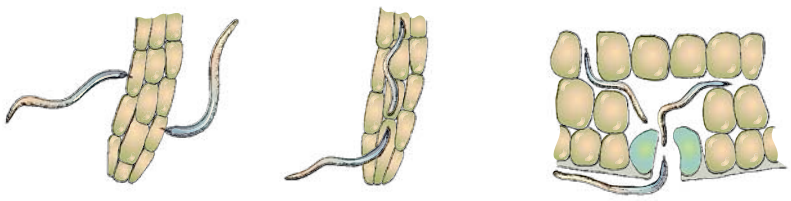



**FIGURE 2-9** Electron micrographs of direct penetration of a fungus (*Colletotrichum graminicola*) into an epidermal leaf cell. (A) (a) Developing appressorium from a conidium. Note wax rods (arrows) on leaf surface. (b) Mature appressorium separated by a septum from the germination tube. (B) (a) Formation of penetration peg at the central point of contact of appressorium with the cell wall. (b) Structures in the penetration peg, which has already penetrated the cell wall, and papilla produced by the invaded cell. (C) Development of infection hypha. (a) Infection peg penetrating the papilla. (b) Appressorium and swollen infection hypha after penetration. (D) On completion of penetration and establishment of infection, the appressorium consists mostly of a large vacuole and is cut off from the infection hypha by a septum. (Photographs courtesy of D. J. Politis and H. Wheeler.)



Direct penetration
Ectoparasitic nematode

Direct penetration
Endoparasitic nematode

Penetration through stoma
Endoparasitic nematode

**FIGURE 2-10** Methods of penetration and invasion by nematodes.

combination, with the stage of development of the host, and with the temperature in the environment of the infected plant.

During infection, some pathogens obtain nutrients from living cells, often without killing the cells or at least not for a long time; others kill cells and utilize their contents as they invade them; and still others kill cells and disorganize surrounding tissues. During infection, pathogens release a number of biologically active substances (e.g., enzymes, toxins, and growth regulators) that may affect the structural integrity of the host cells or their physiological processes. In response, the host reacts with a variety of defense mechanisms, which result in varying degrees of protection of the plant from the pathogen.

As mentioned earlier, for a successful infection to occur it is not sufficient that a pathogen comes in contact with its host; rather, several other conditions must also be satisfied. First of all, the plant variety must be susceptible to the particular pathogen and at a susceptible stage. The pathogen must be in a pathogenic stage that can infect immediately without requiring a resting (dormancy) period first, or infective juvenile stages or adults of nematodes. Finally, the temperature and moisture conditions in the environment of the plant must favor the growth and multiplication of the pathogen. When these conditions occur at an optimum, the pathogen can invade the host plant up to the maximum of its potential, even in the presence of plant defenses, and, as a consequence, disease develops.

### Invasion

Various pathogens invade hosts in different ways and to different extents (Figs. 2-4, 2-5, 2-9, and 2-12). Some fungi, such as those causing apple scab and black spot of rose, produce mycelium that grows only in the area between the cuticle and the epidermis (subcuticular colonization) (Fig. 2-11A); others, such as those causing powdery mildews, produce mycelium only on the surface of the plant (Fig. 2-11B) but send haustoria into the epidermal cells. Most fungi spread into all the tissues of the plant organs (leaves, stems, and roots) they infect, either by growing directly through the cells as an **intracellular mycelium** or by growing between the cells as an **intercellular mycelium** (Figs. 2-11C and 2-11D). Fungi that cause vascular wilts invade the xylem vessels of plants (Fig. 2-11E).

Bacteria invade tissues intercellulary, although when parts of the cell walls dissolve, bacteria also grow intracellularly. Bacteria causing vascular wilts, like the vascular wilt fungi, invade the xylem vessels (Fig. 2-11E). Most nematodes invade tissues intercellularly, but some can invade intracellularly as well (Fig. 2-12). Many nematodes do not invade cells or tissues at all but feed by piercing epidermal cells with their stylets.

Viruses, viroids, mollicutes, fastidious bacteria, and protozoa invade tissues by moving from cell to cell intracellularly. Viruses and viroids invade all types of living plant cells, mollicutes and protozoa invade phloem sieve tubes and perhaps a few adjacent phloem parenchyma cells, and most fastidious bacteria invade xylem vessels and a few invade only phloem sieve tubes.

Many infections caused by fungi, bacteria, nematodes, viruses, and parasitic higher plants are local, i.e., they involve a single cell, a few cells, or a small area of the plant. These infections may remain localized throughout the growing season or they may enlarge slightly or very slowly. Other infections enlarge more or less rapidly and may involve an entire plant organ (flower, fruit, leaf), a large part of the plant (a branch), or the entire plant.

Infections caused by fastidious xylem- or phloem-inhabiting bacteria, mollicutes, and protozoa and natural infections caused by viruses and viroids are **systemic**, i.e., the pathogen, from one initial point in a plant, spreads and invades most or all susceptible cells and tissues throughout the plant. Vascular wilt fungi and bacteria invade xylem vessels internally, but they are usually confined to a few vessels in the roots, the stem, or the top of infected plants; only in the final stages of the disease do they invade most or all xylem vessels of the plant. Some downy mildew pathogens and some fungi, primarily among those causing smuts and rusts, also invade their hosts systemically, although in most cases the older mycelium degenerates and disappears and only the younger mycelium survives in actively growing plant tissues.

### Growth and Reproduction of the Pathogen (Colonization)

Individual fungi and parasitic higher plants generally invade and infect tissues by growing on or into them from one initial point of inoculation. Most of these pathogens, whether inducing a small lesion, a large infected area, or a general necrosis of the plant, continue to grow and branch out within the infected host indefinitely so that the same pathogen individual spreads into more and more plant tissues until the spread of the infection is stopped or the plant is dead. In some fungal infections, however, while younger hyphae continue to grow into new healthy tissues, older ones in the already infected areas die out and disappear so that a diseased plant may have several points where separate units of the mycelium are active. Also, fungi causing vascular wilts often invade plants by producing and releasing spores within the vessels, and as the spores are carried

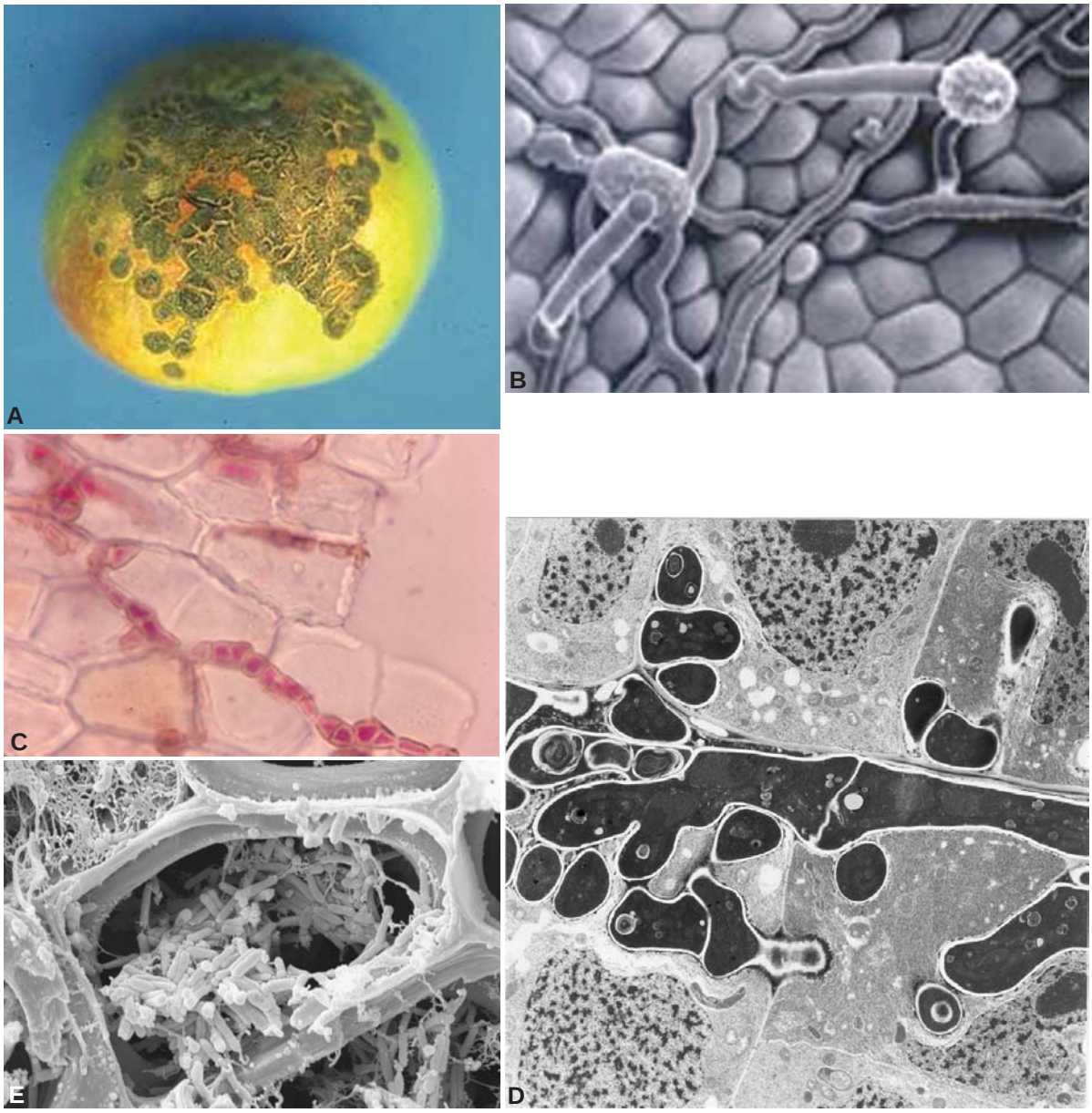



**FIGURE 2-11** Types of invasion of pathogens in infected plants. (A) In apple scab disease, the pathogenic fungus grows only between the cuticle and the epidermal cells of leaves and fruit. (B) In powdery mildews the fungal mycelium grows only on the surface of host plants, but sends haustoria into the epidermal cells. (C) In many diseases the fungal mycelium (stained red here) grows only intercellularly (between the cells). (D) Hyphae of the smut fungus *Ustilago* in an infected leaf. (E) In bacterial vascular diseases, bacteria grow in and may clog the xylem vessels. [Photographs courtesy of (A) University of Oregon, (B) G. Celio, APS, (D) Mims *et al.* (1992). *Intern. J. Plant Sci.* **153**, 289–300, and (E) E. Alves, Federal University of Lavras, Brazil.]

in the sap stream they invade vessels far away from the mycelium, germinate there, and produce a mycelium, which invades more vessels.

All other pathogens, namely bacteria, mollicutes, viruses, viroids, nematodes, and protozoa, do not increase much, if at all, in size with time, as their size and shape remain relatively unchanged throughout their existence. These pathogens invade and infect new tissues within the plant by reproducing at a rapid rate and increasing their numbers tremendously in the infected tissues. The progeny may then be carried passively into new cells and tissues through plasmodesmata (viruses and viroids only), phloem (viruses, viroids, mollicutes, some fastidious bacteria, protozoa), or xylem (some bacteria); alternatively, as happens with protozoa and nematodes (Fig. 2-12) and somewhat with bacteria, they may move through cells on their own power.

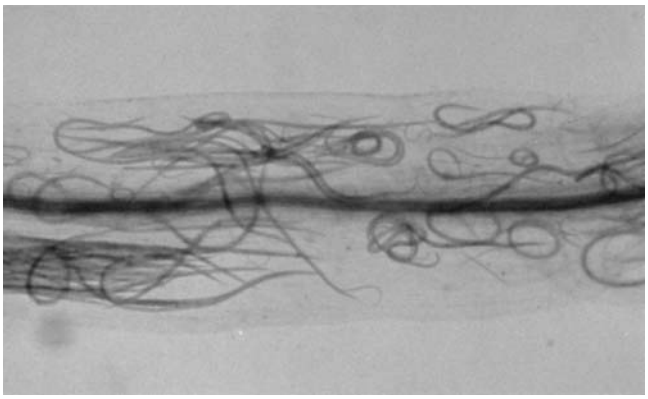


**FIGURE 2-12** Alfalfa shoot invaded by plant parasitic nematodes (*Ditylenchus dipsaci*). (Photograph courtesy of J. Santo.)

Plant pathogens reproduce in a variety of ways (see Fig. 1-3 in Chapter 1). Fungi reproduce by means of spores, which may be either asexual (**mitospores**, i.e., products of mitosis, roughly equivalent to the buds on a twig or the tubers of a potato plant), or sexual (**meiospores**, i.e. products of meiosis, roughly equivalent to the seeds of plants). Parasitic higher plants reproduce just like all plants, i.e., by seeds. Bacteria and mollicutes reproduce by fission in which one mature individual splits into two equal, smaller individuals. Viruses and viroids are replicated by the cell, just as a page placed on a photocopying machine is replicated by the machine as long as the machine is operating and paper supplies last. Nematodes reproduce by means of eggs.

The great majority of plant pathogenic fungi and oomycetes produce a mycelium only within the plants they infect. Relatively few fungi and oomycetes produce a mycelium on the surface of their host plants, but most powdery mildew fungi produce a mycelium only on the surface of, and none within, their hosts (Figs. 2-13A–2-13C). The great majority of fungi and oomycetes


**FIGURE 2-13** Means of reproduction of fungi and bacteria. (A–E) Mycelium [white material on leaf (A, B)], chains of conidia (C), and cleistothecium (B and D) (containing four asci, each containing ascospores) on the leaf surface. (E) Apple trees having numerous branches killed by the fire blight bacterium. (F) Large numbers of bacteria inside a xylem vessel of a bacterial wilt-infected plant. [Photographs courtesy of (A and B) D. Legard, University of Florida, (C) D. Mathre, Montana State University, (D) M. Hoffman, Oregon State University, (E) A. Jones, Michigan State University, and (F) B. Bruton, USDA, Lane, Oklahoma.]

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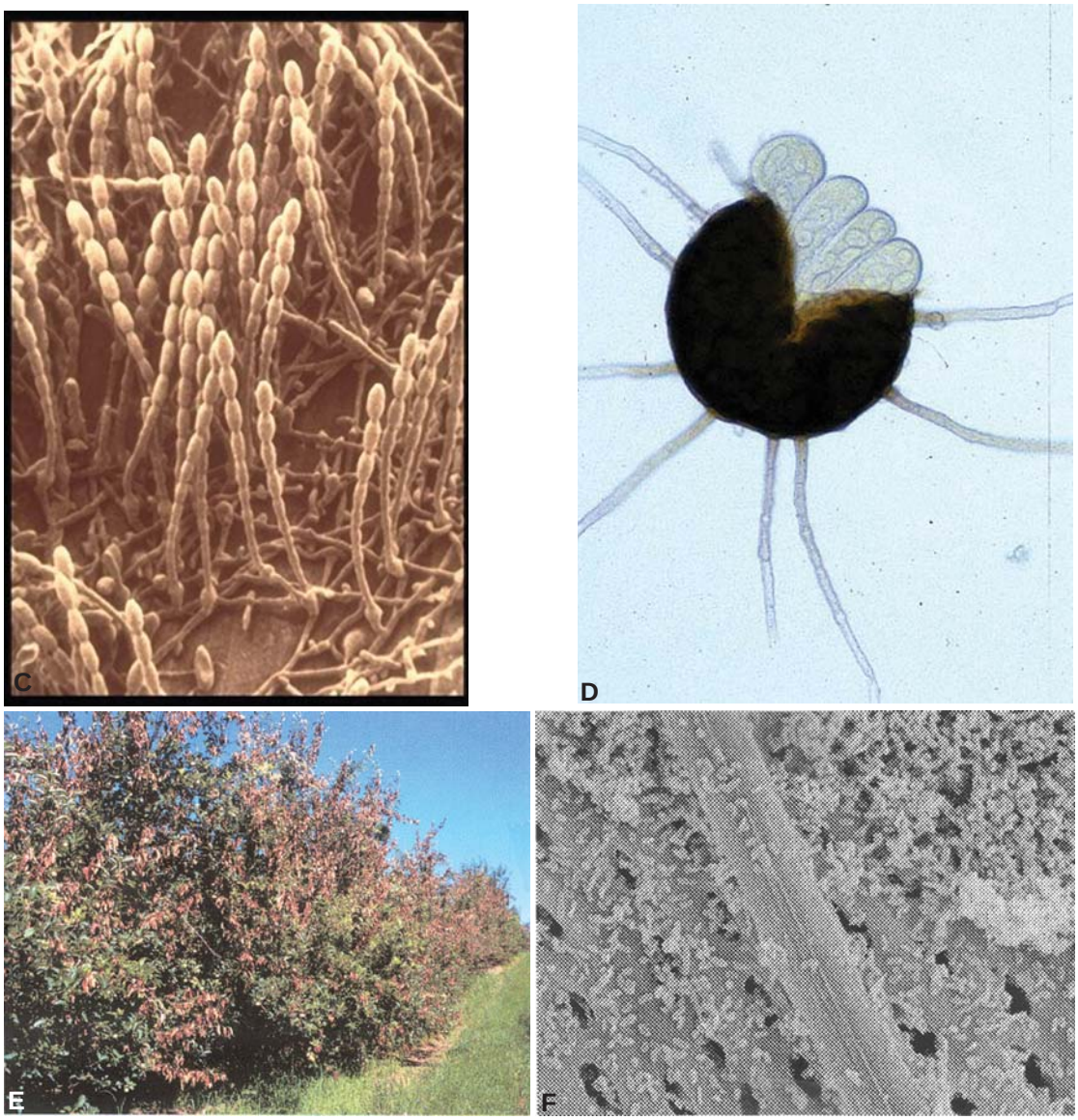


**FIGURE 2-13** (*Continued*)

produce spores on, or just below, the surface of the infected area of the host, and the spores are released outward into the environment. Plant pathogenic plasmodiophoromycetes, however, such as the clubroot pathogen and fungi causing vascular wilts, produce spores within the host tissues, and these spores are not released outward until the host dies and disintegrates. Parasitic higher plants produce their seeds on aerial branches, and some nematodes lay their eggs at or near the surface of the host plant. Bacteria reproduce between or, in xylem- or phloem-inhabiting bacteria, within host cells (Fig. 2-13F), generally inside the host plant; they come to the host surface only through wounds, cracks, stomata, and so on. Viruses, viroids, mollicutes, protozoa, and fastidious bacteria reproduce only inside cells and apparently do not reach or exist on the surface of the host plant.

The rate of reproduction varies considerably among the various kinds of pathogens, but in all types, one or a few pathogens can produce tremendous numbers of individuals within one growing season. Some fungi produce spores more or less continuously (Fig. 2-14), whereas others produce them in successive crops. In either case, several thousand to several hundreds of

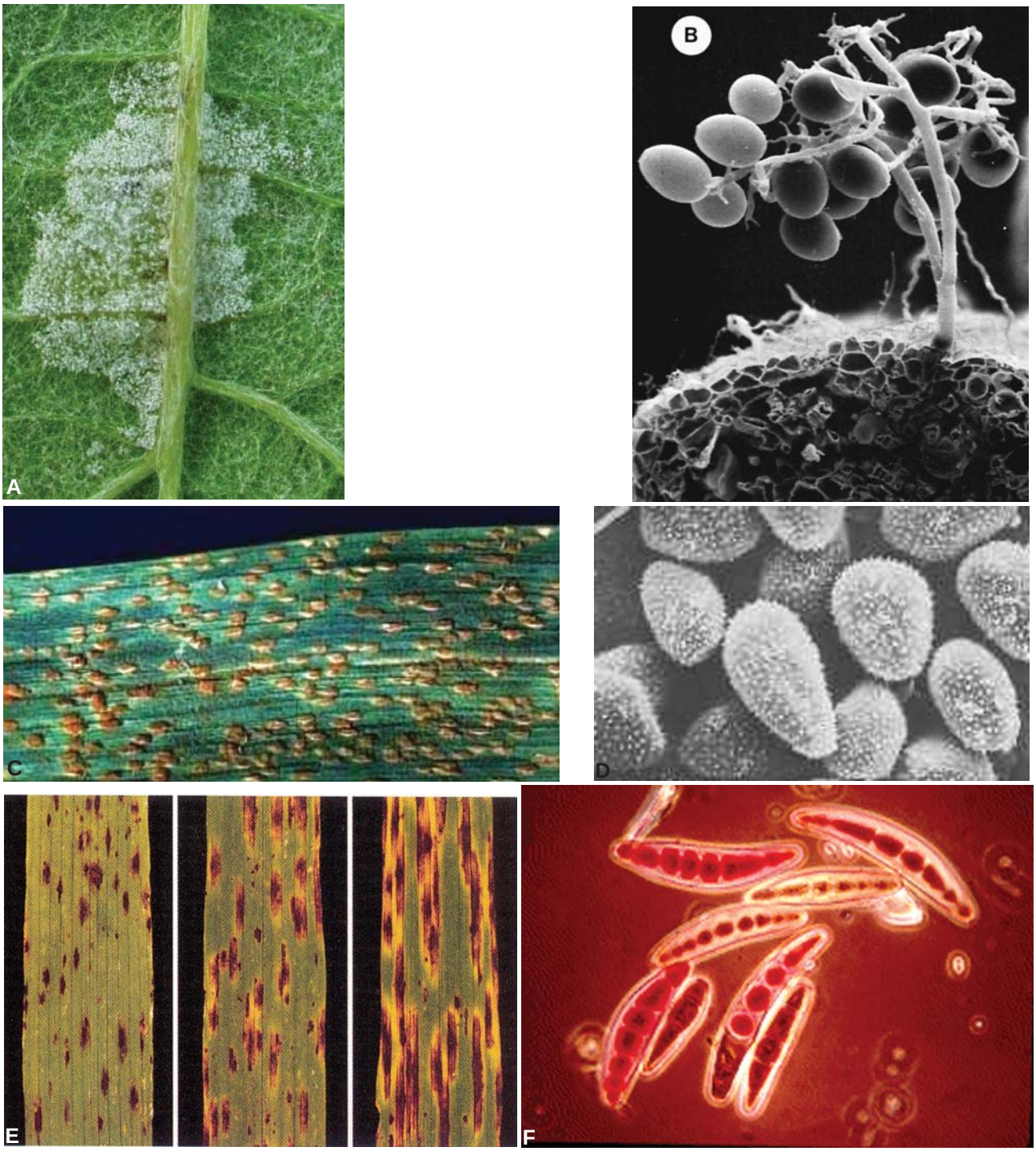


**FIGURE 2-14** Invasion and reproduction of oomycete and fungal plant pathogens. Sporangiophores and sporangia (A) on the underside of a grape leaf infected with the grape downy mildew pathogen *Plasmopara viticola* and (B) on the root of a lettuce plant infected with *Plasmopara lactucae-radicis*. (C) A wheat leaf showing numerous infection lesions (uredia) of the leaf rust fungus. (D) Uredospores of the soybean rust. (E) Leaves of three barley varieties showing infection lesions, the severity (number and size) of which are inversely proportional to the degree of resistance of each variety to the fungal pathogen. (F) Spores of the fungus *Cochliobolus* that cause leaf spot on barley. [Photographs courtesy of (A) J. Rytter and J. W. Travis, Pennsylvania State University, (B) M. E. Stanghellini, University of California, Riverside, and (E) B. Steffenson, University of Minnesota.]