

year. A couple in love that kiss under the mistletoe is equivalent to promising to marry and a prediction of long life and happiness together. Nowadays, in many parts of Europe and America, a person

standing under a ball or even a sprig of mistletoe at Christmastime is inviting to be kissed by members of the opposite gender as a sign of friendship and goodwill. There are, actually, more myths and

customs associated with mistletoe. Who would think that a minor parasitic higher plant would excite the imagination of so many others and have so many stories about it.

### BOX 3 Plant diseases as the result of spontaneous generation

Following Theophrastus, other than the proposal by Magnus that the mistletoe was a parasite, there was little useful knowledge that was added about plants or about plant diseases for about 2000 years, although there are reports of famines in several parts of the world. Especially bad were outbreaks in north-central Europe of ergotism, a disease of humans and animals caused from eating grains contaminated with parts of the fungus that causes the ergot disease of cereals (see pages 501–504). People continued to associate plant diseases with sin and the wrath of God and therefore were fatalistic about the occurrence of

plant diseases, the repeated losses of food, and the hunger and famines that followed. References to the ravages of plant diseases appeared in the writings of several contemporary historians, but little was added to the knowledge about the causes and control of plant diseases. People everywhere believed that plant diseases, as well as human and animal diseases, just happened spontaneously. Whatever was observed on diseased plants or on diseased plant produce was considered to be the product or the result of the disease rather than the cause of it. After the invention of the compound microscope in the mid-1600s,

which enabled scientists to see many of the previously invisible microorganisms, scientists, as well as laypeople, became even stronger believers in the spontaneous generation of diseases and of the microorganisms associated with diseased or decaying plant, human, or animal tissues. That is, they came to believe that the mildews, rusts, decay, or other symptoms observed on diseased plants, and any microorganisms found on or in diseased plant parts, were the natural products of diseases that just happened rather than being the cause and effect of the diseases.

### Biology and Plant Pathology in Early Renaissance

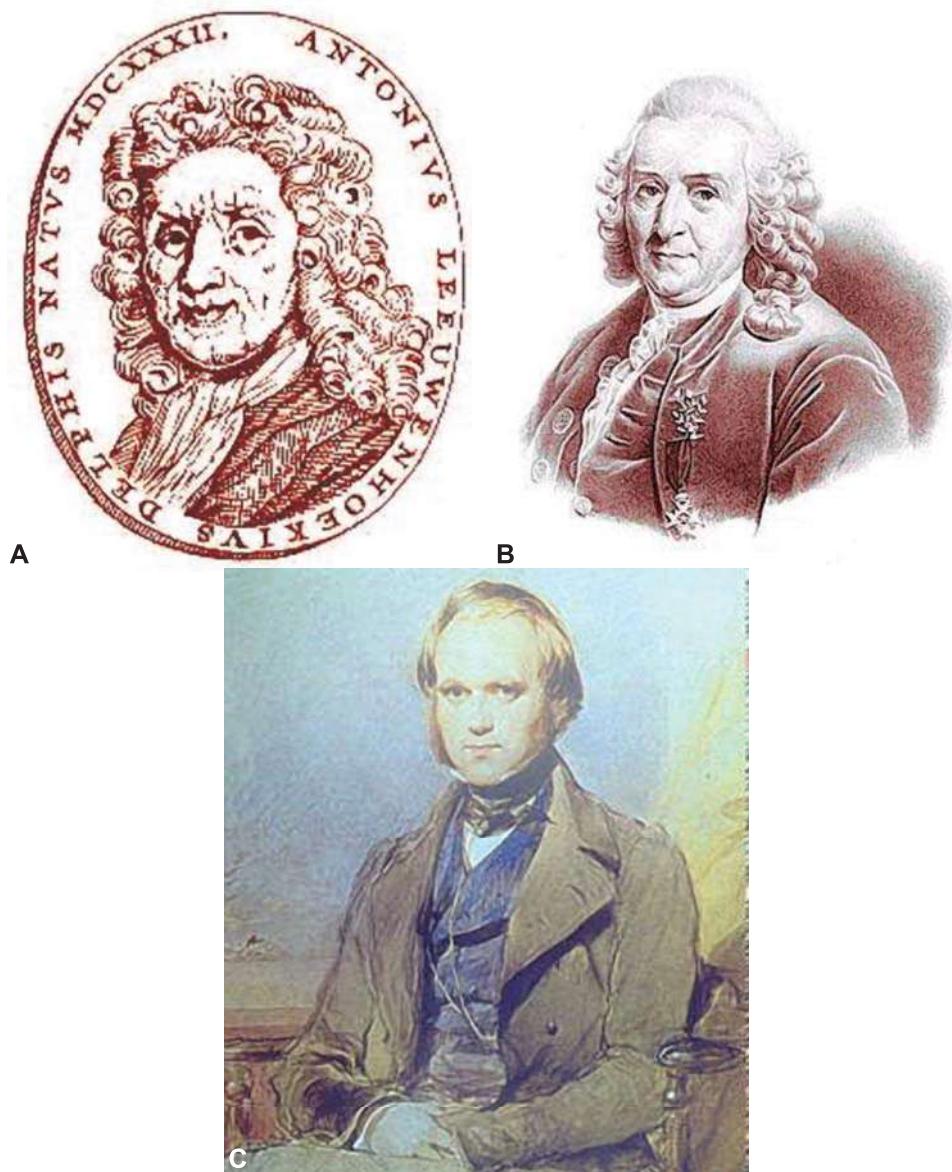
People continued to suffer from hunger and malnutrition due partially at least to diseases destroying their crops and their fruit. They, however, continued to consider plant diseases as the work and wish of their God and, therefore, an event that could neither be understood nor avoided. In the mid-1600s, however, a group of French farmers noted that wheat rust was always more severe on wheat near barberry bushes than away from them (Fig. 1-13). The farmers thought that the rust was produced by the barberry plants from which it moved to wheat. They, therefore, asked the French government to pass the first plant disease regulatory legislation that would force towns to cut and destroy the barberry bushes to protect the wheat crop.

In 1670, the French physician Thouillier observed that ergotism or Holy Fire, a serious and often deadly disease of humans in northcentral Europe (see pages 39 and 559), did not spread from one person to another but seemed to be associated with the consumption of ergot-contaminated grains. At about the same time, Robert Hooke, in England, invented the double-lensed (compound) microscope with which he examined thin slices of cork and called its units “cells.” Soon after, the Dutchman Antonius van Leeuwenhoek (Fig. 1-14A) improved significantly the lenses and the structure of the



**FIGURE 1-13** A bush of barberry (*Berberis vulgaris*) growing at the edge of a wheat field and helping close the dioecious disease cycle of wheat stem rust disease. The fungus, *Puccinia graminis*, overwinters on barberry on which it produces spores that infect wheat plants near the barberry (see photo) from which then spores of the fungus spread to more wheat plants. (Photograph courtesy of USDA Cereal Dis. Lab., St. Paul, MN.)

microscope and began to examine not only the anatomy of plants, but also the body of filamentous fungi and algae, protozoa, sperm cells, blood cells, and even bacteria. All of these microorganisms, of course, were considered to be produced by whatever organism (animal



**FIGURE 1-14** (A) Antonius van Leeuwenhoek. (B) Carl von Linne'. (C) Charles Darwin.

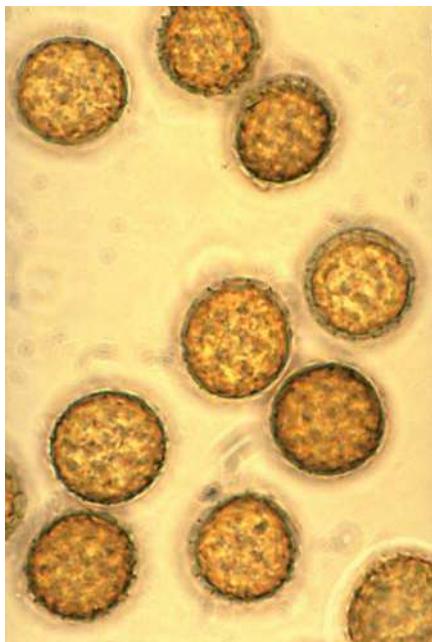
or plant) or medium they happened to be found in and were not thought of as independent, autonomous organisms. In 1735, the Swedish philosopher-botanist Carl von Linne' (Fig. 1-14B) published his main work "Systema Naturae," by which he established the diagnosis of plant species and the binomial nomenclature of plants. Linne's species, however, were rigid and were supposed to have remained unchanged since creation. It was not until more than a century later, in 1859, that the Englishman Charles Darwin (Fig. 1-14C) published his book "The Origin of Species by Means of Natural Selection" and showed that species of all organisms, plants and animals, evolve over time and adapt to changes in their environment for survival.

The discovery and availability of the microscope, however, sparked significant interest in microscopic fungi and, subsequently, their possible association with plant diseases. In 1729, the Italian botanist Pier Antonio Micheli described many new genera of fungi and illustrated their reproductive structures. He also noted that when placed on freshly cut slices of melon, these structures grew and produced the same kind of fungus that had produced them. He proposed, therefore, that fungi arise from their own spores rather than spontaneously, but because the "spontaneous generation" theory was so imbedded in people's minds, nobody believed Micheli's evidence. Similarly, in 1743, the English scientist Needham observed nematodes inside small,

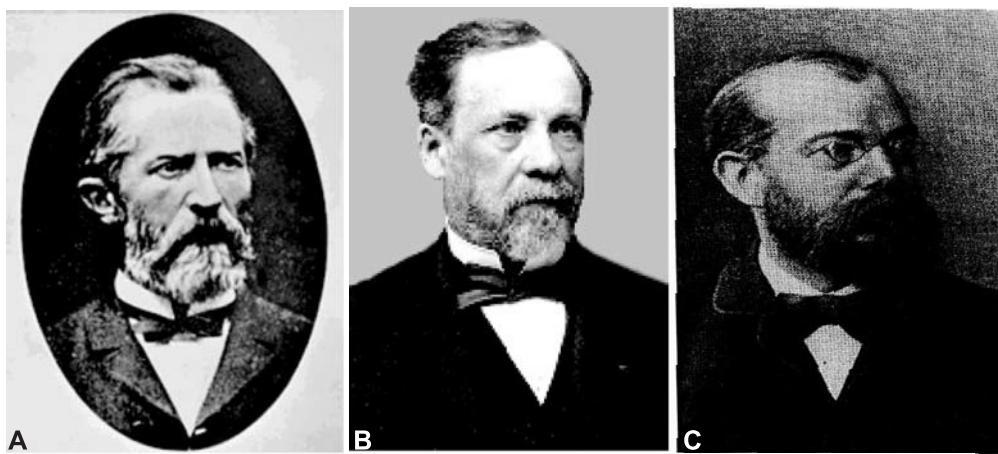
abnormally rounded wheat kernels but he, too, failed to show or suggest that they were the cause of the problem.

In 1755, the Frenchman Tillet, working with smutted wheat, showed that he could increase the number of wheat plants developing covered smut (Figs. 1-8A and 1-8B) by dusting wheat kernels before planting with smut dust, i.e., with smut spores (Fig. 1-15). He also noted that he could reduce the number of smutted wheat plants produced by treating the smut-treated kernels

with copper sulfate. Tillet, too, however, did not interpret his experiments properly and, instead of concluding that wheat smut is an infectious plant disease, he believed that it was a poisonous substance contained in the smut dust, rather than the living spores and fungus coming from them, that caused the disease. More than 50 years later, in 1807, Prevost, another Frenchman, repeated both the inoculation experiments and those in which the seeds were treated with copper sulfate, as done by Tillet, and he obtained the same results. In addition, Prevost observed smut spores from untreated and treated wheat seed under the microscope and noticed that those from untreated seed germinated and grew whereas those from treated seed failed to germinate. He, therefore, concluded correctly that it was the smut spores that caused the smut disease in wheat and that the reduced number of smutted wheat plants derived from copper sulfate-treated seed was due to the inhibition of germination of smut spores by the copper sulfate. Prevost's conclusions, however, were not accepted by the French Academy of Sciences because its scientists and other scientists throughout Europe still believed that microorganisms and their spores formed through spontaneous generation and were the result rather than the cause of disease. In 1855, a nematode was observed in galls of cucumber roots, but again they were thought to have appeared there spontaneously. These beliefs continued to be held and expounded by scientists until the early 1860s, when, in 1861–1863, Anton deBary (Fig. 1-16A) proved that potato late blight was caused by a fungus and Louis Pasteur (Fig. 1-16B) proved that microorganisms were produced from preexisting microorganisms and that most infectious diseases were caused by germs. The latter established the “germ theory of disease,” which changed the way of thinking of scientists and led to tremendous progress. Significant



**FIGURE 1-15** Teliospores of the fungus *Tilletia*, the cause of the covered smut or bunt of wheat. (Photograph courtesy of M. Babadoost, University of Illinois.)



**FIGURE 1-16** (A) Anton deBary. (B) Louis Pasteur. (C) Robert Koch.

impetus to this progress was added by Robert Petri, who developed artificial nutrient media for culturing the microorganisms (Petri dishes), and by Robert Koch (Fig. 1-16C), who established that for proving that a

certain microorganism was the cause of a particular infectious disease, certain necessary steps (Koch's postulates) must be carried out and certain conditions must be satisfied.

#### BOX 4 Potato blight and the irish famine: a deadly mix of ignorance and politics

In about 1800, the potato, which was introduced in Europe from South and Central America around 1570 A.D., was a well-established crop in Ireland. After strong objections against adopting it because (1) it was new and not mentioned in the Bible, (2) it was produced in the ground and, therefore, was unclean, and (3) because parts of it were poisonous, the potato was nevertheless adopted and its cultivation spread rapidly. Adoption of potato cultivation came as a result of it producing much more edible food per unit of land than grain crops, mostly wheat and rye, grown until then. It was adopted also because the ground protected it from the pests and diseases that destroyed above-ground crops and from destruction by the soldiers sent by absentee English landlords to collect overdue land rents.

At that time, most Irish farmers were extremely poor, owned no land, and lived in small windowless, one-room huts. The farmers rented land from absentee English landlords who lived in England, and planted grain and other crops. The yields were poor and, in any case, large portions of them had to be used for paying the exorbitant rent so as to avoid eviction. The Irish farmers also kept small plots of land, usually as small as a quarter of an acre and basically survived the winter with the food they produced on that land. Potato production was greatly favored by the cool, wet climate of Ireland, and the farmers began growing and eating potatoes to the exclusion of other crops and food-stuffs. Irish farmers, therefore, became dependent on potatoes for their sustenance and survival. Lacking proper warehouses, the farmers stored their potato tubers for the winter in shallow ditches in the ground. Periodically, they would open up part of the ditch and remove as many potatoes as they thought they would need for the next few weeks.

The potatoes grew well for many years, free of any serious problems. In the early 1840s, potato crops began to fail to varying extents in several areas of Europe and Ireland. Most of the growing season of 1845 in Ireland was quite favorable for the growth of potato plants and for the formation of tubers. Everything looked as though there would be an excellent yield of potatoes everywhere that year. Then, the weather over northern Europe and Ireland became cloudy, wetter, and cooler and stayed that way for several weeks (Fig. 1-17A). The potato crop, which until then looked so promising, began to show blighted leaves and shoots (Fig. 1-17B), and whole potato plants became blighted and died. In just a few weeks, the potato fields in northern Europe and in Ireland became masses of blighted and rotting vegetation (Fig. 1-17C). The farmers were surprised and worried, especially when they noticed that many of the potatoes still in the ground were rotten and others had rotting areas on their surface (Fig. 1-17D). They did what they could to dig up the healthy-looking potatoes from the affected fields and put them in the ditches to hold them through the winter.

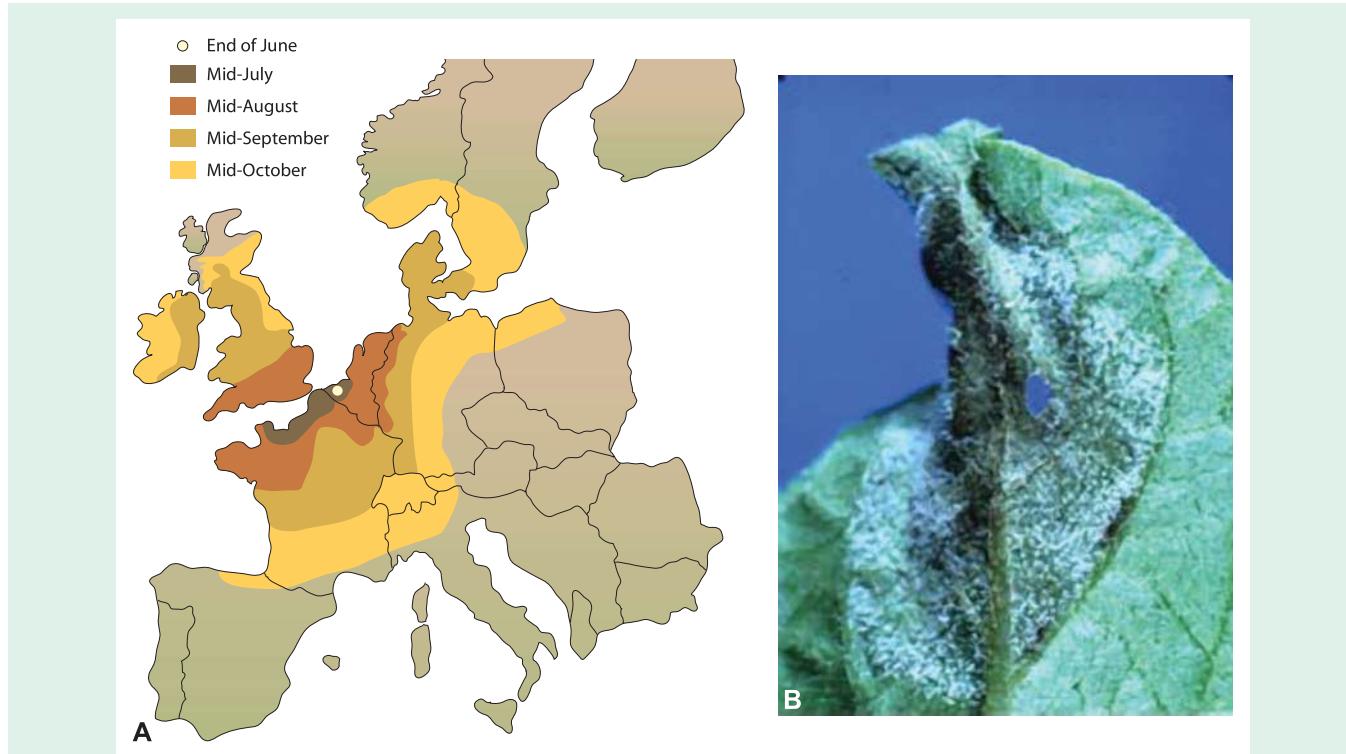
The farmer's worry became horror when later in the fall and winter they began opening the ditches and looking for the potatoes they had put in them at harvest. Alas, instead of potatoes they found only masses of rotting tubers (Figs. 1-17D and 1-17E), totally unfit for consumption by humans or animals. The dependence of Irish farmers on potatoes alone meant that they had nothing else to eat—and neither did any of their neighbors. Hunger (Fig. 1-17F) was quickly followed by starvation, which resulted in the death of many Irish. The famine was exacerbated by the political situation between England and Ireland. The British refused to intervene and help the starving Irish with food for several

months after the blight destroyed the potatoes. Eventually, by February of the next year (1846), food, in the form of corn from the United States, began to be imported and made available to the starving poor who paid for it by working on various government construction projects. Unfortunately, the weather in 1846 was again cool and wet, favoring the potato blight, which again spread into and destroyed the potato plants and tubers. Hunger, dysentery, and typhus spread among the farmers again, and more of the survivors emigrated to North America. It is estimated that one and a half million Irish died from hunger, and about as many left Ireland, emigrating mostly to the United States of America.

The cause of the destruction of the potato plants and of the rotting of the potato tubers was, of course, unknown and a mystery to all. The farmers and other simple folk believed it to have been brought about by "the little people," by the devil himself whom they tried to exorcise and chase away by sprinkling holy water in the fields, by locomotives traveling the countryside at devilish speeds of up to 20 miles per hour and discharging electricity harmful to crops they went by, or to have been sent by God as punishment for some unspecified sin they had committed. The more educated doctors and clergy were so convinced of the truth of the theory of spontaneous generation that even when they saw the mildewy fungus growth on affected leaves and on some stems and tubers, they thought that this growth was produced by the dying plant as a result of the rotting rather than the cause of the death and rotting of the plant.

Some of the educated people, however, began to have second thoughts about the situation. Dr. J. Lindley, a professor of botany in London, proposed incorrectly that the plants, during the rains, overabsorbed water through their roots and because they could not get rid

*continued*



**FIGURE 1-17** The late blight of potato and the Irish famine. (A) Itinerary of the advance of the potato blight between June, when the blight was first detected in Belgium, and the end of October 1845, by which time it spread from Italy to Ireland and from Spain to the Scandinavian countries. (B) A young lesion on a potato leaf covered with sporangiophores and sporangiospores of the fungus (oomycete). (C) A potato plant killed completely by the blight (right) next to a healthy-looking resistant plant (left). (D) External and internal appearance of potato tubers infected with the late blight disease. The oomycete is still found near the surface. (E) Advanced invasion and rotting of potato tuber infected with late blight. (F) A period drawing of a family digging for potatoes to avoid starvation during the Irish famine. [Photographs courtesy of (A) W. E. Fry, Cornell University, (B) D. P. Weingartner, University of Florida, (C and D) Cornell University, (E) USDA, and (F) Illustrated London News, 1849.]

of the excess water, their tissues became swollen and rotted. The Reverend Dr. Miles Berkeley, however, noticed that the mold covering potato plants about to rot was a fungus (oomycete) similar but not identical to a fungus he observed on a sick onion. The fungus on potato, however, was identical to a fungus recovered from sick potato plants in northern Europe. Berkeley concluded that this fungus was the cause of the potato blight, but when he proposed it in a letter to a newspaper, it was considered as an incredible and bizarre theory unsupported by facts. The puzzle of what caused blight of potato continued unanswered for 16 years after the 1845 destruction of potatoes by the blight. Finally, in 1861, Anton deBary (Fig. 1-16A) did a simple experiment that proved that the potato blight was

caused by a fungus. DeBary simply planted two sets of healthy potatoes, one of which he dusted with spores of the fungus collected from blighted potato plants. When the tubers germinated and began to produce potato plants, the healthy tubers produced healthy plants, whereas the healthy tubers dusted with the spores of the fungus produced plants that became blighted and died. No matter how many times deBary repeated the experiment, only tubers treated with the fungus became infected and produced plants that became infected. Therefore, the fungus, which, we know now, is an oomycete was named *Phytophthora infestans* ("infectious plant destroyer" from phyto = plant, phthora = destruction, infestans = infectious), was the cause of the potato blight. DeBary also showed that the fungus did

not just reappear from nowhere the following growing season but instead survived the winter in partially infected potato tubers in the field or storage. In the spring, the fungus infected young plants coming from these partially rotten tubers, produced new spores on these plants, and the spores then spread to other cultivated potato plants that were infected and killed. With this experiment deBary actually disproved the theory of spontaneous generation, which stated that microorganisms are produced spontaneously by dying and dead plants and animals, and ushered in the germ theory of disease. The honor for this proof, however, is reserved for Louis Pasteur, who proved the theories while working with bacteria at about the same time, 1861–1863, that deBary published his work with the potato blight fungus.

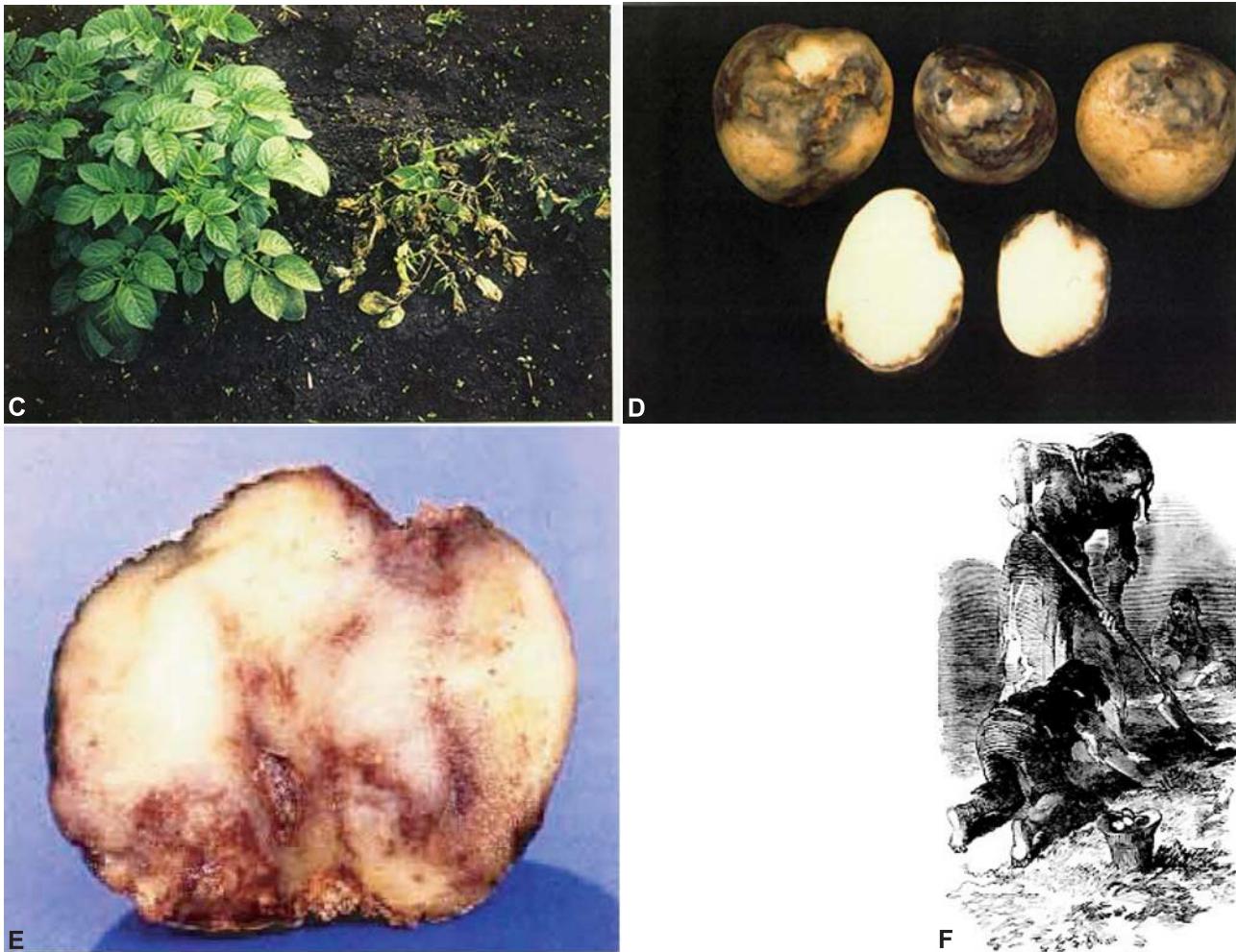


FIGURE 1-17 (Continued)

### The Expanding Role of Fungi as Causes of Plant Disease

Following the observation by French farmers around the mid-1600s and, independently, by Connecticut farmers in the early 1700s that wheat rust was worse near barberry bushes, the farmers came to believe that barberry fathered the rust, which then moved to wheat. The request by farmers for legislation to force towns to eradicate barberries and in that way to protect the wheat plants from rust followed. At about the same time, spores of the rust fungus were observed with the compound microscope for the first time in England (Hooke, 1667). In Italy, Micheli 60 years later (1729) described many new genera of fungi, illustrated their reproductive structures, and noted that when he placed them on freshly cut slices of melon, these fungal struc-

tures generally reproduced the same kind of fungus that produced them. He proposed that fungi arose from their own spores rather than spontaneously, but nobody believed him. New information about plant pathogenic fungi continued to be developed, but most of it was not accepted by the scientists of the time for a long time.

As mentioned previously, in 1755, Tillet in France showed that wheat smut is a contagious plant disease, but even he believed that it was a poisonous substance contained in the smut dust, rather than a living microorganism, that caused the disease. In 1807, Prevost, also in France, repeated and expanded Tillet's experiments and appeared to have demonstrated conclusively that wheat smut was caused by a fungus. His conclusions, however, were not accepted because the scientists were blinded by the belief that microorganisms and their

spores were the result rather than the cause of disease. These beliefs continued to be shared and expounded by scientists for at least another 50 years.

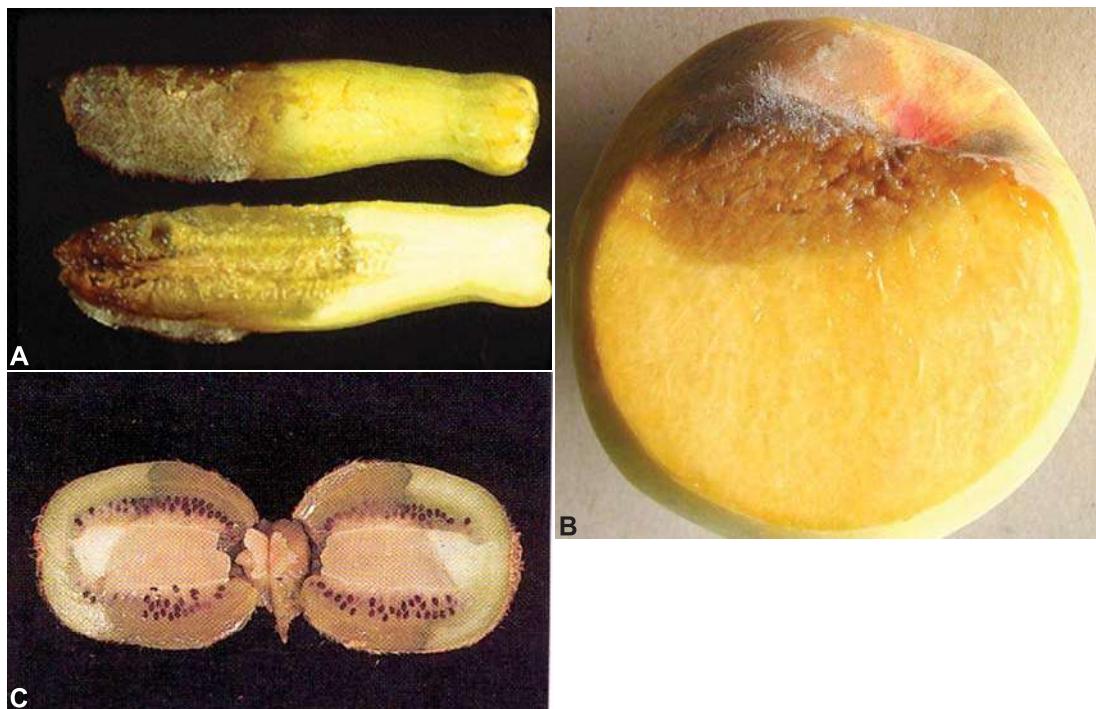
The devastating epidemics of late blight of potato in northern Europe, particularly Ireland, in the 1840s not only dramatized the effect of plant diseases on human suffering and survival, but also greatly stimulated interest in their causes and control. In 1861, deBary finally established experimentally beyond criticism that a fungus (*Ph. infestans*) was the cause of the plant disease known as late blight of potato, a disease that closely resembles the downy mildews.

It is, perhaps, worth noting here that it was during those years (1860–1863) that Louis Pasteur proposed, and finally provided irrefutable evidence, that microorganisms arise only from preexisting microorganisms and that fermentation is a biological phenomenon, not just a chemical one. Pasteur's conclusions, however, were not generally accepted for many years afterward. Nevertheless, the proof for involvement of microorganisms (germs) in fermentation and disease signaled the beginning of the end of the theory of spontaneous generation and provided the basis for the germ theory of disease.

Although fungi had already been the object of study by many scientists, proof that they were causing disease in plants greatly increased interest in them. DeBary

himself also carried out studies of the smut and rust fungi, of the fungi causing downy mildews, and of the fungus *Sclerotinia*, which induces rotting of vegetables. The German Kühn in the 1870s and later contributed significantly to the studies of infection and development of smut in wheat plants and promoted the development and application of control measures, particularly seed treatment for cereals. Kühn also wrote the first book on plant pathology, “*Diseases of Cultivated Crops, Their Causes and Their Control*,” in which he recognized that plant diseases are caused by an unfavorable environment but can also be caused by parasitic organisms such as insects, fungi, and parasitic plants.

During the years of Pasteur and Koch, several scientists also made significant contributions to plant pathology and to biology and medicine. After establishing beyond criticism in 1861 that the potato blight was caused by a fungus, DeBary went on to show conclusively that smut and rust fungi were also the causes and not the results of their respective plant diseases. Moreover, he showed that some rust diseases require two alternate host plants (see Fig. 1-13) to complete their life cycle, e.g., the fungus causing the stem rust of wheat requires wheat and barberry. DeBary also showed (1886) that some fungi induce rotting of vegetables (Fig. 1-18) by secreting substances (enzymes) that diffuse into plant tissues in advance of the pathogen.



**FIGURE 1-18** Infection and advanced internal rotting of summer squash (A) by the fungus *Choanephora*, of peach fruit (B) by the fungus *Rhizopus* sp., and (C) of kiwi fruit by the fungus *Botrytis cinerea*. In all cases, fruit rot is a result of, primarily, pectinolytic enzymes secreted by the fungi and advancing ahead of the mycelium. A small amount of the fungi can be seen on the surface of the fruits. (C) Courtesy of T. Michailides, University of California.

### The Discovery of Other Causes of Infectious Diseases

Although Leeuwenhoek first saw microbes with the microscope he invented in 1674, little progress was made toward the concept of microbes as the cause of disease for almost another 200 years. In 1776, Jenner introduced vaccination against the virus-induced smallpox, an extremely infectious and severe disease that used to kill 10 to 20% of those infected, but could only speculate as to its cause and how it worked. In 1861, however, deBary showed that the potato blight was caused by a fungus while Pasteur formulated the germ theory of fermentation. In 1864, Pasteur invented pasteurization and, in 1880, made the first vaccine against the chicken cholera. In the meantime, in 1876, Koch

identified the anthrax bacillus, *Bacillus anthracis*, as the first bacterium to cause disease in animals and humans. In addition, in 1887, Koch formulated his rules of disease diagnosis that became known as “Koch’s postulates.” These rules became the standard procedure for proving that a disease is caused by a bacterium or any other kind of pathogen.

#### Nematodes

The first report of nematodes associated with a plant disease was made in England by Needham in 1743. He observed nematodes (Fig. 1-19A) within small, abnormally rounded wheat kernels (wheat galls; Fig. 1-19B); however, he did not show or suggest that they were the cause of the disease. It was not until 1855 that a second



**FIGURE 1-19** (A) A typical nematode. (B) Wheat seed galls, each filled with as many as 30,000 nematodes. (C) M. Woronin. (D) Clubroot of cabbage caused by the protozoan *Plasmodiophora brassicae*. [Photographs courtesy of (A and B) USDA Nematology Laboratory, Beltsville, Maryland, and (D) C. M. Ocamp, Oregon State University.]

nematode, the root knot nematode, was observed in cucumber root galls. In the next 4 years two other plant parasitic nematodes, the bulb and stem nematode and the sugarbeet cyst nematode, were reported from infected plant parts. Several more nematodes parasitizing plants were described in the early part of the 20th century by Cobb, who made numerous significant contributions to plant nematology.

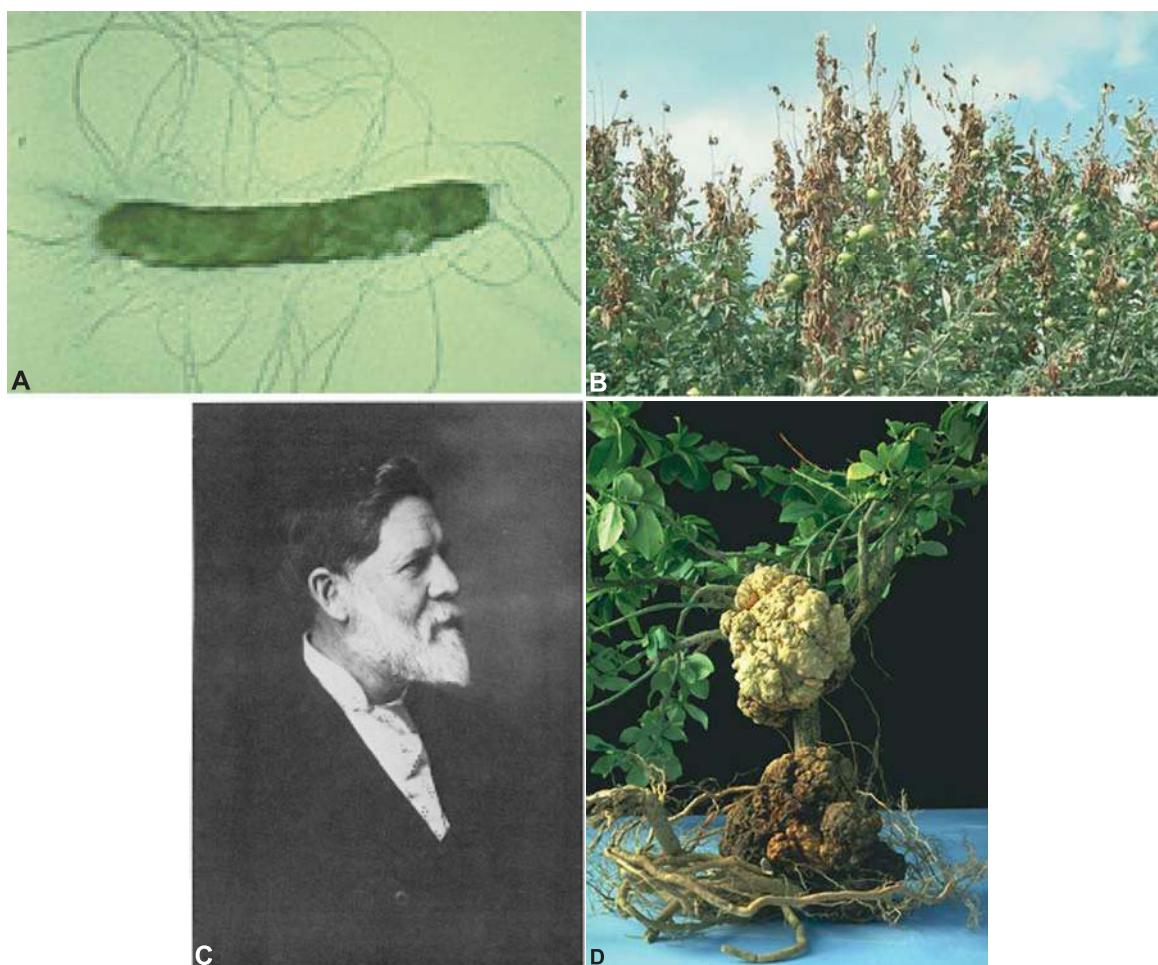
### Protozoan Myxomycetes

In 1878, Woronin (Fig. 1-19C), in Russia, was the first to show that a plant disease, the clubroot disease of cabbage (Fig. 1-19D), was caused by a fungus that has been shown to be a protozoan plasmodiophoromycete. These are fungus-like, single-celled microorganisms that lack a cell wall and, as a result, produce an amoeba-like body called a plasmodium and zoospores. These microorganisms used to be thought of as lower fungi but

are now considered members of a different kingdom, the kingdom protozoa.

### Bacteria

Soon after Koch showed that bacteria cause disease in animals and humans, Burrill in Illinois showed, in 1878, that bacteria (Fig. 1-20A) caused the fire blight disease (Fig. 1-20B) of pear and apple. Following Burrill's discovery, several other plant diseases were shown, particularly by Erwin Smith (Fig. 1-20C) of the U.S. Department of Agriculture (USDA), to be caused by bacteria. In the early 1890s, Smith was the first to show that crown gall disease (Fig. 1-20D), which he considered similar to cancerous tumors of humans and animals, was caused by bacteria. Studies of how this bacterium, known as *Agrobacterium tumefaciens*, caused tumors in plants led to the discovery, almost a century later, that whenever the bacterium infects plants



**FIGURE 1-20** (A) The fire blight bacterium *Erwinia amylovora*. (B) Fire blight on apple trees. (C) Erwin F. Smith. (D) Crown gall, caused by the bacterium *Agrobacterium tumefaciens*. [Photographs courtesy of (A) Oregon State University, and (B) K. Mohan and (D) R. L. Forster, University of Idaho.]

it transfers part of its DNA to the plant and that the DNA is expressed by the plant as if it were plant DNA (see also pages 624–625). The discovery that the bacterium acts as a natural genetic engineer of plants led to the development of this bacterium so that it could be loaded with, and then transfer to plants, DNA segments coding for desirable characteristics, which formed the basis of biotechnology, especially of plants. As with fungal plant pathogens, however, acceptance of bacteria as causes of disease in plants was slow. For example, as late as 1899, Alfred Fischer, a prominent German botanist, rejected the results of Smith and others who claimed to have seen bacteria in plant cells.

### Viruses

At about the same time that more diseases of plants were shown to be caused by bacteria, the Dutchman Adolph Mayer (Fig. 1-21A), in 1886, injected juice obtained from tobacco plant leaves showing various patterns of greenish yellow mosaic (Fig. 1-21B) into healthy tobacco plants and the latter then developed similar mosaic patterns. Because no fungus was present on the plant or in filtered juice, Mayer concluded that the disease was probably caused by bacteria. In 1892, however, Ivanowski showed that whatever caused the tobacco mosaic disease could pass through a filter that retains bacteria, so he concluded that the disease was caused by a toxin secreted by bacteria or, perhaps, by unusually small bacteria that passed through the pores of the filter. In 1898, Beijerinck, by repeating some of these experiments, finally concluded that the tobacco mosaic disease was caused not by a microorganism, but by a “contagious living fluid” that he called a virus.

No one had any idea, however, what a virus was and what it looked like for another 40 years. The true nature, size, and shape of the virus (Fig. 1-21C) remained unknown for several more decades. In 1935, Stanley added ammonium sulfate to tobacco juice extracted from infected tobacco leaves and obtained as a sediment in the flask a crystalline protein that, when rubbed on tobacco, caused the tobacco mosaic disease. This led him to conclude that the virus was an autocatalytic protein that could multiply within living cells. Although his results and conclusions were later proved incorrect, for his discovery Stanley received a Nobel Prize in Chemistry. In 1936, Bawden and colleagues demonstrated that the crystalline preparations of the virus actually consisted of not only protein, but also a small amount of ribonucleic acid (RNA). The first virus (tobacco mosaic virus) particles were seen with the electron microscope in 1939 by Kausche and colleagues. Finally, in 1956, Gierrer and Schramm showed that the protein could be removed from the virus and that the ribonucleic acid carried all the genetic information that enabled it to cause infection and to reproduce the complete virus. It was shown subsequently that although the nucleic acid of most viruses infecting plants is single-stranded RNA, some viruses have double-stranded RNA, some double-stranded DNA, and some single-stranded DNA.

The search for the cause of the many thousands of plant diseases led to the discovery of at least three more kinds of pathogens and it is likely that others remain to be discovered.

### Protozoa

Flagellate trypanosomatid protozoa were observed in the latex-bearing cells of laticiferous plants of the family



**FIGURE 1-21** (A) Adolph Mayer. (B) Tobacco leaf showing symptoms of tobacco mosaic. (C) Particles of *tobacco mosaic virus*.

Euphorbiaceae by Lafont in 1909. Such protozoa, however, were thought to parasitize the plant latex without causing disease on the host plant. In 1931, Stahel found flagellates infecting the phloem of coffee trees, causing abnormal phloem formation and wilting of the trees. In 1963, Vermeulen presented convincing evidence of the pathogenicity of flagellates to coffee trees, and in 1976 flagellates were reported to be associated with several diseases of coconut and oil palm trees in South America and in Africa. In recent years, of course, the Myxomycota and the Plasmodiophoromycota, which were previously thought to be fungi, have been transferred to the kingdom protozoa.

### Mollicutes (Phytoplasmas)

For nearly 70 years after viruses were discovered, many plant diseases were described that showed symptoms of general yellowing or reddening of the plant or of shoots proliferating and forming structures that resembled witches' brooms. These diseases were thought to be caused by viruses, but no viruses could be found in such plants. In 1967, Doi and colleagues in Japan observed mollicutes, i.e., wall-less mycoplasma-like bodies in the phloem of plants exhibiting yellows and witches' broom symptoms. That same year the same group showed that the mycoplasma-like bodies and symptoms disappeared temporarily when the plants were treated with tetracycline antibiotics. Since then, mycoplasma-like organisms (MLOs) that infect plants have been reclassified as phytoplasmas, and some of them that have helical bodies and can be found in other environments besides plants are known as spiroplasmas.

### Viroids

In 1971, studies of the potato spindle tuber disease showed that it was caused by a small, naked, single-

stranded, circular molecule of infectious RNA, which was called a viroid (see later). Viroids have been found to be the cause of several dozen plant diseases. Viroids seem to be the smallest infectious nucleic acid molecules. Although more than 40 viroids have been found to infect plants, no viroids have been found that infect animals or humans.

Apparently, however, an even smaller type of infectious agent, called a prion, exists (see later). Prions apparently consist only of a small (~55,000 Da) protein, which is encoded by a chromosomal gene of the host. Prions have been shown to cause the scrapie disease of sheep, "mad cow" disease, and at least three slow-developing degenerative diseases of humans. So far, no prions have been found to infect plants, but there is no obvious reason why they should not.

### Serious Plant Diseases of Unknown Etiology

Although pathogens as large and complex as fungi and nematodes or as tiny and simple as viroids and prions have been discovered, there are many severe diseases of plants, particularly of trees, for which we still do not know their real cause, despite years of searching and research. Some of them, such as peach short life in the southeastern United States, waldsterben, or forest decline in central Europe and various forest tree declines in the northeastern and northwestern United States, may be caused by more than one pathogen or by combinations of pathogens and adverse environment. Others, such as citrus blight in Florida and South America, spear rot in oil palm in Suriname and Brazil, and mango malformation in India and other mango-growing countries, seem to have a biotic agent as the primary cause, but the activity of the agent seems to be strongly affected by environmental factors such as soil or temperature. Despite more than 100 years of research on some plant diseases, the causes of these diseases remain unknown.

### BOX 5 Koch's postulates

Robert Koch (1843–1910) (Fig. 1-16C) was a medical doctor and a bacteriologist. He was the first to show, in 1876, that anthrax, a disease of sheep and other animals, including humans, was caused by a bacterium that he called *Bacillus anthracis*. He subsequently discovered, in 1882, that tuberculosis and, in 1883, that cholera are each caused by a different bacterium, which led to the general conclusion that each disease is

caused by a specific microbe. These experiments confirmed for the first time the germ theory of disease proposed earlier by Louis Pasteur.

Before Koch's experiments, and while Koch himself was carrying out the work on the diseases mentioned earlier, there was confusion and uncertainty about the occurrence and the cause of each disease. Much of the time when bacteria or fungi were isolated from diseased or dead

human, animal, or plant tissues, the isolated bacteria or fungi were subsequently shown to be saprophytes, i.e., they coexisted with the microorganism that caused the disease but could not by themselves cause the disease for which they were being considered. Based on his experiences, in 1887, Koch set out the four steps or criteria that must be satisfied before a microorganism isolated from a diseased human, animal, or plant

can be considered as the cause of the disease. These four steps, rules, or criteria are known as "Koch's postulates."

1. The suspected causal agent (bacterium or other microorganism) must be present in every diseased organism (e.g., a plant) examined.
2. The suspected causal agent (bacterium, etc.) must be isolated from the diseased host organism (plant) and grown in pure culture.
3. When a pure culture of the suspected causal agent is inoculated into a healthy susceptible host (plant), the host must reproduce the specific disease.
4. The same causal agent must be recovered again from the experimentally inoculated and infected host, i.e., the recovered agent must have the same characteristics as the organism in step 2.

Koch's rules are possible to implement, although not always easy to carry out, with such pathogens as fungi, bac-

teria, parasitic higher plants, nematodes, most viruses and viroids, and the spiroplasmas. These organisms can be isolated and cultured, or can be purified, and they can then be introduced into the plant to see if they cause the disease. With the other pathogens, however, such as some viruses, phytoplasmas, fastidious phloem-inhabiting bacteria, protozoa, and even some plant pathogenic fungi that are obligate parasites of plants (such as the powdery mildew, downy mildew, and rust fungi), culture or purification of the pathogen is not yet possible and the pathogen often cannot be reintroduced into the plant to reproduce the disease. Thus, with these pathogens, Koch's rules cannot be carried out, and their acceptance as the actual pathogens of the diseases with which they are associated is more or less tentative. In most cases, however, the circumstantial evidence is overwhelming, and it is assumed that further improvement of techniques of isolation, culture, and inoculation of pathogens will someday prove that today's assumptions

are justified. However, in the absence of the proof demanded by Koch's rules and as a result of insufficient information, all plant diseases caused by phytoplasmas (e.g., aster yellows) and fastidious vascular bacteria (e.g., Pierce's disease of grape) were for years thought to be caused by viruses.

Despite the difficulties of carrying out Koch's postulates with some causal agents, they have been and continue to be applied, sometimes with certain modifications, in all cases of disease. They have had and continue to have a tremendous effect in deciding and in convincing others that a particular microorganism is the cause of a specific disease. By attempting to carry out Koch's postulates in all newly discovered diseases, a great deal of work with potential saprophytes has been avoided, while, at the same time, doubt and criticism are reduced to a minimum while confidence in and use of the identification increase greatly and quickly.

#### BOX 6 Viruses, Viroids, and Prions

Although they have been with us forever, we know relatively little about how these pathogens operate. There are many common characteristics among viruses and viroids. The relationship of prions to others is only in their small size but they are contrasted to the other two in that they do not depend on any kind of nucleic acid (RNA or DNA). Viruses cause numerous severe diseases in all types of organisms, have been studied the longest, and we know the most about them. Viroids cause more than 40 diseases in plants, some of them lethal. Prions seem to affect only humans and animals in which they cause degenerative diseases of the brain, such as the recently much publicized "mad cow disease."

**Viruses** are submicroscopic spherical, rod-shaped, or filamentous entities (organisms) (Figs. 1-22A–1-22C) that consist of only one type of nucleic acid

(DNA or RNA). The nucleic acid is surrounded by a coat consisting of one or more kinds of protein molecules. Viruses infect and multiply inside the cells of humans, animals, plants, or other organisms and usually cause disease.

**Viroids** were discovered by Diener (Fig. 1-22D) and colleagues in 1971 while they were studying the potato spindle tuber disease (Fig. 1-22E). Viroids are the smallest infectious agents that multiply autonomously in plant cells; they consist only of small, circular RNA molecules (Fig. 1-22F) that are too small to code for even one small protein and therefore lack a protein coat. Viroids infect plant cells and are replicated in their nucleus, using the substances and enzymes of plant cells. Viroids infect only plants and in many of them they usually cause disease. Viroids have not yet been detected in any other kind of organism besides plants.

**Prions** were proposed for the first time in 1972 by Prusiner (Fig. 1-22G) who, for that and subsequent work, received the Nobel Prize in Physiology or Medicine in 1997. Prions are at first normal small protein molecules produced in nerve and other cells of the brain. Prions become pathogenic, i.e., they cannot carry out their normal functions and, instead, have adverse effects on the brain and cause disease. This occurs when prions are forced by conditions in the brain to change shape (Fig. 1-22H). The change in shape signals the onset of infection. Prions are not associated with any nucleic acid. Abnormal prions appear to increase in number and to cause the appearance of amyloid fibrils and plaques, as well as the appearance of small cavities (Fig. 1-22I) in the brain of diseased animals and humans. Prions have not been observed in plants or other organisms.

*continued*