CHAPTER

1

PEST ORIGINS, PESTICIDES, AND THE HISTORY OF BIOLOGICAL CONTROL

HUMAN NEEDS AND THE ORIGINS OF PESTS

The human population is large and still expanding. To gain more farmland, native ecosystems are being rapidly converted to human use, destroying forests, soil, and native plants and animals. To produce sufficient food, commercial and subsistence farming systems must be highly productive, but sustainable and nonpolluting. However, to preserve the world for the future, space must be left for wild animals and wild places. To do both of these things is the great challenge of the early twenty-first century. Part of the solution to this problem is biological control, the foundation on which sustainable, nonpolluting pest control for tomorrow's farms must be built.

Where do pests come from? Some pests are created because of how we grow crops. Crop defenses such as toughness or repellent compounds are decreased by selection. Crops are grown in large patches, with uniform planting and harvesting schedules. These practices have potential to reduce plant defenses against herbivores. Other pests arise because movement of organisms around the world creates new species combinations. Sometimes, local herbivores adopt crop species that are introduced into a country. In South Africa for example, 68% of 188 arthropod pests of 14 introduced crops are native species not previously in contact with the crops they now attack (Dennill and Moran 1989). In other cases, pest herbivores that attack crops accompany crop cultivars as stowaways when cultivars are moved to new locations. The natural enemies that suppress the herbivores, however, are likely to be left behind (Fig. 1.1), allowing herbivore populations to thrive in an enemy-free environment, reaching high, pest densities. Since 1850, the number of nonnative insect species in the United States has expanded over tenfold (Fig. 1.2). Of these, over 200 have become severe pests, and over 500 are lesser pests (Fig. 1.3). Origins of the adventive arthropods in the United States are discussed by Sailer (1983). In the United States, adventive species makes up 39% of the insect pests on crops, 27% of the insect pests of forests, 7% of the animal pests, 31% of the plant pathogens of vegetables, 73% of the weeds of cultivated crops, and 41% of the weeds of pastures (Pimentel 1993). Origins of many weeds are similar to those of pest arthropods in that many are adventive species no longer suppressed by specific herbivores. Unlike arthropods, however, many pest weeds are species deliberately introduced for various reasons (Foy et al. 1983).

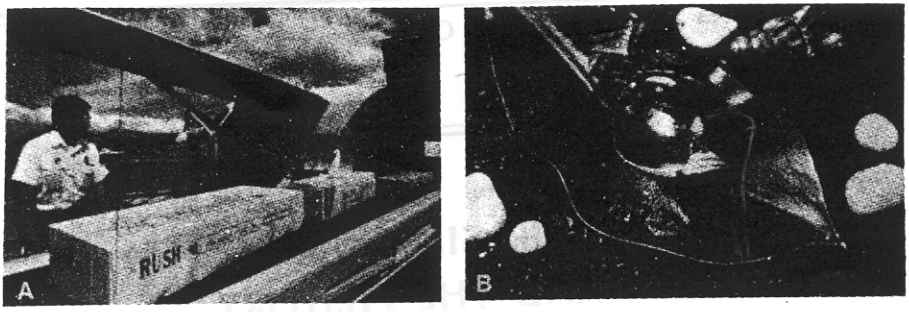


Figure 1.1 Invasions of some adventive species are rooted in commerce, both as unintentional stowaways during shipment (as in the case of plant products [A]), or as intentionally introduced species with unrecognized harmful potential, (as in the case of the giant African snail, *Achatina fulica* Bowditch, sold in some locations as a pet [B]). (Photographs courtesy of USDA-APHIS.)

Consideration of the origins of pests suggests that solutions for our pest problems lie in the modification of agriculture systems (where possible without loss of productivity) to conserve natural enemies of crop pests and in the reconnecting of herbivores with their natural enemies, where these have become separated. Methods to achieve these ends and to protect native species and ecosystems from the effects of aggressive adventive species, via conservation, augmentation, and introduction of natural enemies, are the subjects of this book.

PROBLEMS WITH PESTICIDES

Chemical pesticides are commonly used for the control of many pests. In contrast to biological control methods, use of such pesticides does not require information on the ecological origins of pests. Pest suppression is achieved temporarily by killing (or for plant pathogens, preventing the growth of) as many members of the pest population as possible through repeated applications of chemical products, as needed. Worldwide pesticide use has increased twelvefold since the early 1950s (Fig. 1.4), and costs paid by farmers in the United States for pesticides increased sixfold between 1951 and 1976 (Eichers 1981). Chemical pesticides have proved effective in many cases, particularly in controlling weeds and plant diseases, but have been ineffective

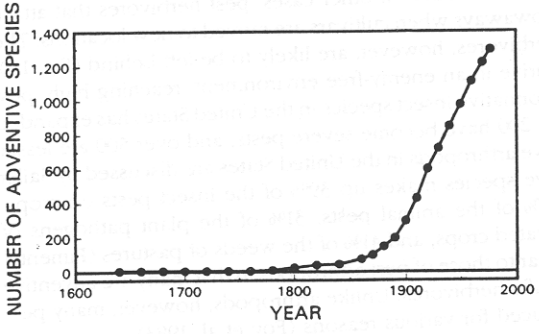
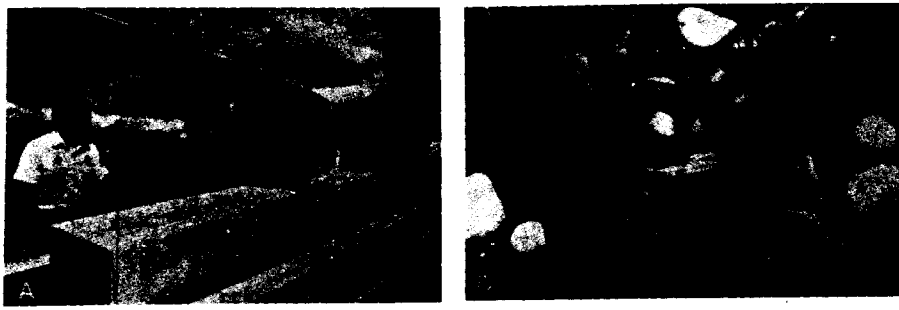


Figure 1.2 Total number of adventive insect species in the United States from 1640 to 1977 (after Sailer 1978).



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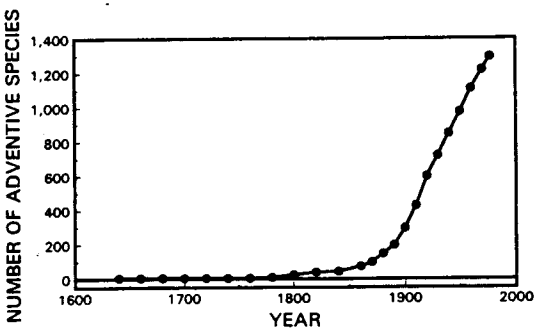

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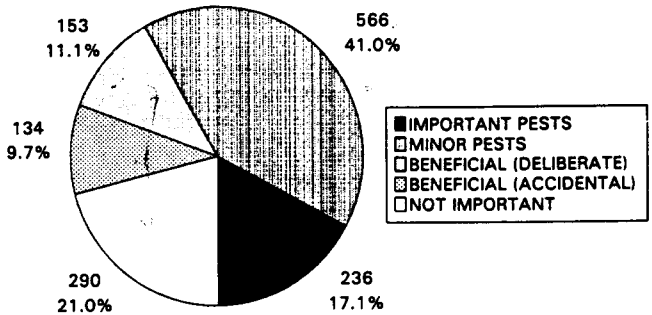

Figure 1.3 Numbers of adventive insect species in the United States in various categories in 1977 (after Sailer 1978).

in others, for example, controlling cotton insects in many countries (Bottrell and Adkisson 1977). Some of the problems associated with using pesticides include failure of pest control, contamination of the environment, and damage to human health. Concern over these issues has prompted some countries to seek to reduce pesticide use.

Pesticides create pest control problems when they fail to control the target pest or when they create new pests. Resistance to pesticides is the main way in which pesticide use can lead to pest control failure. Resistance develops in pest populations through the differential survival of members of the pest population that best detoxify or avoid exposure to the pesticide. Over several generations, pest populations may develop that can no longer easily be killed by one or more pesticides. Since 1945, the number of insects, weeds, and, most recently, plant pathogens that have become resistant to pesticides has increased dramatically (Brent 1987) (Fig. 7.5).

Another way in which pesticide use can foster outbreaks of pests is the destruction of the target pest's natural enemies (Trichilo and Wilson 1993). Many pests and potential pests are held in check, at least partially, by various predacious, parasitic, pathogenic, or antagonistic species. Most pesticides kill natural enemies, which often are more sensitive to the pesticide

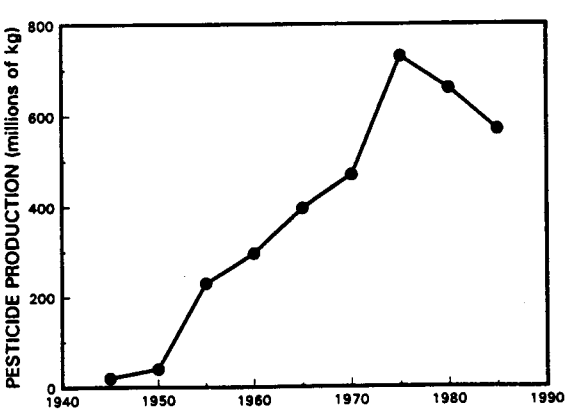

Figure 1.4 Pesticide production in the United States (after Pimentel 1991).

than is the pest itself (Croft 1990). Insecticides, for example, kill beneficial insects. Acaricides kill predacious mites. Fungicides suppress growth of fungi that protect plants against plant diseases and also reduce fungi that kill pest arthropods. When these natural enemies are destroyed, the pest individuals that remain after the pesticide application survive longer, causing their numbers to rebound to yet higher levels. Outbreaks of rice brown planthopper, *Nilaparvata lugens* (Stål), in Asia, for example, have been shown to be greatly increased by the destruction of spiders and other natural enemies by pesticides (Heinrichs et al. 1982).

Pesticides also create new pests when natural enemies of ordinarily harmless species (or minor pests) are destroyed by chemicals (Kerns and Gaylor 1993). These "secondary pests" then reach higher densities than normal and begin to cause economic damage. They are then considered pests by farmers and become targets of pesticides themselves. Spider mites on many crops are examples of species that have become pests because of the use of pesticides. The leaf miner *Phyllonorycter crataegella* (Clemens) in apple (*Malus pumilla* Miller) is a pest because its parasitoids are suppressed by pesticides used in apple orchards for control of other pests (Maier 1982).

In areas in which pesticide use is common, small quantities of some pesticides may move out of the treated area and end up in soil, water, birds, and other wild animals, as well as being found in the crop itself. The consequences of these residues varies from none to serious. Herbicides and soil fumigants have contaminated groundwater supplies; hydrochlorinated pesticides such as DDT have caused regional extinctions of some raptorial bird species; and the more toxic organophosphate pesticides have caused human poisonings (see Graham 1970; Metcalf 1980; Dempster 1987; Newton 1988). Accidental poisonings and contamination are especially likely when farmers do not understand the toxicity of the materials they are using, when they cannot read product instructions, or do not have the necessary protective equipment. As farmers and the public at large gain increased knowledge of these problems, the demand for other forms of pest control, in particular biological control, increases.

Biological control, however, is not simple. Its successful use requires highly trained specialists to conduct research on natural enemies, and well-informed extension workers and farmers to implement their use.

DEFINITION OF BIOLOGICAL PEST CONTROL

The scope of biological control—what kinds of targets it may be used against, what kinds of natural enemies exist to aid in biological control, and what methods work for employing them—are discussed in detail in Chapter 2. Here, we define the process and set the limits of what the remaining chapters will address. Biological control is the use of parasitoid, predator, pathogen, antagonist, or competitor populations to suppress a pest population, making it less abundant and thus less damaging than it would otherwise be. Insects, mites, weeds, plant diseases, and vertebrates all may be targets of biological control. Biological control may be the result of purposeful actions by man or may result from the unassisted action of natural forces. Biological control may be employed either for suppression of crop or forest pests, or for restoration of natural systems affected by adventive (nonnative) pests. Not all nonchemical control is biological control. Plant breeding, cultural control, and use of semiochemicals, if directly intended to influence the pest, are not biological control. These same approaches, however, may in some cases have a role to play in biological control if they are directed not at the pest but at conserving or enhancing its natural enemies. Breeding plants to directly resist pests, for example, by being toxic or having some other pest-suppressive features, is not biological control. But breeding plants to make them better sites for parasitoids or predators to search and attack pests is biological control. Chemicals extracted from plants or microbes and

used for pest control are not biological controls. Biological control is a population-level process in which one species' population lowers the numbers of another species by mechanisms such as predation, parasitism, pathogenicity, or competition. In this book, we discuss principles of biological control broadly with examples drawn from a variety of target taxa; consequently, we do not address biological control of particular taxa (insects, weeds, and so on) in separate chapters, except for biological control of plant pathogens. Plant pathogens involve some considerable differences from biological control of other taxa and requires separate treatment.

Some Terminology

Because movement of organisms to new locations (with consequent loss of natural enemies) is important to many aspects of biological control, it is often necessary to describe the origins of organisms. The terms (native, exotic, endemic, immigrant, introduced) commonly employed in such descriptions, however, present some difficulties because of conflicts in their meanings or unintended implications. If, for example, we refer to a pest that has arrived to a new location as an "introduced pest" (as is commonly done), we prompt the question "Who introduced it?", when in many cases no one did.

To resolve questions of terminology, in this text we follow Frank and McCoy (1990) and use the following terminology:

(1) **indigenous (or native)** organisms are those organisms in a specified area, that arose evolutionarily in their current taxa in that location

(2) **precinctive** organisms are the subset of the indigenous organisms of a given location that occur nowhere else

(3) **adventive** organisms are those species in a specified location that did not evolve there, but arrived there from elsewhere (i.e., the opposite of native)

(4) **immigrants** are those adventive organisms in a specified location that arrived there without the deliberate, purposeful aid of man. (This group includes both actively dispersing organisms, and ones arriving as stowaways on plants or other commodities moved by man.)

(5) **introduced** organisms, which are those brought to a location by the conscious choice of man (i.e. food crop species, ornamental or forage plants, pets and domestic animals, biological control agents).

In this text we distinguish between **parasites** and **parasitoids** (see Chapter 2 for definitions), but employ **parasitism** and **parasitized** when referring to the action of either parasites or parasitoids.

HISTORY OF BIOLOGICAL CONTROL

Like all human advances, the development of biological control followed no master plan, but surged or stagnated at the whim of insights, luck, personal endeavor and, in more recent decades, institutional momentum. The history of the development of the method is really seamless, but for discussion can be divided into four parts.

Early Observations and Development of Key Concepts

In this section we trace the earliest intuitive uses of natural enemies. We discuss the development of the concepts of predation, parasitism, and disease in invertebrates; weed control

agents; and antagonists and parasites of plant pathogens. We point out the first suggestions and attempts for practical use of such agents. This brief presentation has been summarized from an excellent account of the topic by DeBach and Rosen (1991) to which the reader is referred for more detail.

Insect Predators. Before the formal development of natural history as a science in western Europe during the Renaissance period, farmers in other parts of the world were already making use of some types of predacious arthropods. In both China and Yemen, ant colonies were moved between sites for control of pests in tree crops (citrus [*Citrus* spp.] and dates [*Phoenix dactylifera Linnaeus*]) (Coulson et al. 1982). Also, in China, spiders were manipulated for pest control (Sparks et al. 1982). These practices, dating back several thousand years, were developed by farmers through direct observation of these predators, which are large enough to be visible and whose life cycles are easily understood. In Europe, one of the first written proposals to use predacious insects for pest control was made in 1752 by the taxonomist Carl Linnaeus who remarked that "Every insect has its predator which follows and destroys it. Such predatory insects should be caught and used for disinfesting crop-plants" (see Hörstadius 1974). By the early 1800s, naturalists such as Erasmus Darwin and various American entomologists were suggesting that predators such as syrphid flies and coccinellid beetles should be employed to combat aphids in greenhouses and certain outdoor crops (Kirby and Spence 1815). The first international movement of a predacious invertebrate was made in 1873 by the American entomologist C.V. Riley, who sent the mite *Tyroglyphus phylloxerae* Riley and Planchon to France to combat the grape phylloxera (*Daktulosphaira vitifolii* [Fitch]). The mite established but unfortunately had no practical value.

Insect Parasitism. In contrast to insect predation, which was easily understood (at least in concept) by comparison to predacious mammals, insect parasitism was more difficult to correctly understand. In fact, early observers (for example, Aldrovandi in 1602) who witnessed parasitoids emerging from butterfly larvae misunderstood the process as a transformation in which the parasitoids were another stage of the larva produced by a type of metamorphosis (Fig. 1.5) (Bodenheimer 1931). The first person to publish a correct interpretation of insect parasitism was the British physician Martin Lister who in 1685 noted that the ichneumon wasps emerging from caterpillars were the result of eggs laid in the caterpillars by adult female ichneumons. In 1700, Antoni van Leeuvenhoek, the Dutch microscopist, correctly interpreted parasitism of aphids by a species of *Aphidius* wasp.

Following these initial observations, many other workers studied other parasitoids and described their biologies. The nineteenth century saw an explosive increase in the number of scientific works produced on the taxonomy, biology, and ecology of insect parasitoids and predators by scientists such as M.M. Spinola, J.W. Dalman, J.L.C. Gravenhorst, J.O. Westwood, Francis Walker, C. Rondani, A. Förster, J.T.C. Ratzeburg, and many others (DeBach and Rosen 1991). This immense new body of knowledge provided the practical tools needed to begin to turn concepts about biological control into a usable technology.

The first suggestion to move parasitoids between countries for pest control was made in 1855 by the New York entomologist Asa Fitch, who proposed to import parasitoids of the wheat midge, *Sitodiplosis mosellana* (Géhin), from Europe. No importations, however, were made. Not until almost 30 years later, in 1883, was the first parasitoid species, *Cotesia glomerata* (Linnaeus) (= *Apanteles glomeratus* Linnaeus), successfully moved between continents (from England to the United States) and established where released (Riley 1885).

Figure 1.5 A woodcut depicting parasitoid wasps emerging from a lepidopteran pupa (after Goedaert 1662). (Photograph by Bancroft Library, University of California, Berkeley.)

Insect Diseases. Knowledge of insect diseases also developed significantly in the nineteenth century. The foundation for understanding insect diseases was laid by William Kirby's chapter on "Diseases of Insects" in *An Introduction to Entomology* (Kirby and Spence 1815). The understanding of insect disease began, not in relation to insect pest control, but out of need to control damage from diseases caused to commercially important insects such as the silkworm, *Bombyx mori* (Linnaeus).

Agostino Bassi of Lodi, Italy, was the first to demonstrate experimentally the infectious nature of insect disease in his study of the white muscardine diseases (caused by the fungus *Beauveria bassiana* [Balsamo] Vuillemin) of silkworm in 1835. Further work on silkworm diseases was done in 1865–70 by Louis Pasteur in France which correctly identified both vertical and horizontal transmission routes for infection (for an explanation of terms see Chapter 16 on the biology of pathogens).

The first suggestion to use insect pathogens for pest control was made by Bassi in 1836 when he proposed that liquids from putrefied cadavers of diseased insects could be mixed with water and sprayed on foliage to kill insects. The first field trials of pathogens, however, were not conducted until 1884, when the Russian entomologist Elie Metchnikoff developed a facility to mass produce spores of the pathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin

and used them in field tests against larvae of the sugar-beet curculio, *Cleonus punctiventris* (Germar), causing 55–80% mortality of larvae in the field.

Arthropods for Control of Weeds. The first suggestion of using insects to control weedy plants was made by the American entomologist Asa Fitch in 1855, who noted that some European weeds found in America such as toadflax (*Linaria vulgaris* Miller) had no American insects feeding on them and suggested that importing insects from Europe might solve the problem. The first actual use of insects for weed control occurred in 1863 in southern India when an imported cochineal insect, *Dactylopius ceylonicus* (Green), was moved into the region from northern India where it had been observed decimating the pest cactus *Opuntia vulgaris* Miller (Goeden 1978). The first international movement of a biological weed control agent happened soon afterwards when this same species was moved to Sri Lanka.

Biological Control Agents of Plant Pathogens. Sanford (1926) was one of the first to recognize competition between saprophytic and pathogenic organisms for nutrients at the site of initial infection as a form of biological control of plant disease. He observed that potato scab (caused by *Streptomyces scabies* [Thaxter] Waksman and Henrici) could be reduced by adding organic matter (grass clippings) to soil. He speculated, correctly, that this was due to antagonistic effects from saprophytic organisms. Early attempts to use augmentative applications of desirable competitive organisms were, however, often unsuccessful, and it is now recognized that environmental conditions are crucial in affecting the balance between species, either in the soil or on the phylloplane (leaf surface) (Faull 1988; Blakeman 1988). Massive doses of inoculum gradually diminish unless habitat conditions are altered so as to confer a selective advantage on the population growth of the antagonist. Nevertheless, in some circumstances, the approach has been made to work. For example, inoculation of conifer stumps after timber felling with the antagonist fungus *Phanerochaete gigantea* (Fries: Fries) Rattan et al. suppressed the ability of the pathogen *Heterobasidion annosum* (Fries: Fries) Brefeld to attack stumps, with a subsequent lower rate of infection of new trees at the site (Rishbeth 1963).

Biological control of plant diseases can occur via several distinct mechanisms, including competition for nutrients between a pathogen and a harmless species, parasitism, and production of antibiotics (Faull 1988). Concepts of plant disease biological control have been articulated by Baker (1985), Cook and Baker (1983), and Campbell (1989). Relative to other forms of biological control, control of plant diseases is new and has a briefer history.

First Successes: Impetus for Creation of Institutions

By the 1880s, enough knowledge had accumulated concerning natural enemies of arthropods and weeds for their practical use. In this section, we discuss a series of successful and highly visible biological control projects that impressed the societies and governments involved and, in several locations, led to the creation of formal institutions charged with carrying out more biological control projects. These institutions provided greater continuity and increased resources, leading to an expansion in the number of biological control projects undertaken. While many projects could be cited (see DeBach and Rosen 1991 and Clausen 1978 for a fuller listing), those discussed here forcefully demonstrate the value of the method.

Whether or not a given project was catalytic in causing the formation of a biological control institution was influenced by several factors. (See Sawyer 1990 for a history of the unsuccessful effort to develop strong biological control institutions within the United States Department of

Agriculture in the 1888–1951 period.) First, the project needed to provide dramatically visible control. The pest had to be easy to see and the control had to happen rapidly after natural enemy releases began, so the two were easy to connect as cause and effect. Second, the pest itself had to be very serious and well known. Biological control of pests that had only recently invaded an area and still had a limited distribution—however large their potential for damage might have been—seems to have made less of an impression on society than control of pests that were widespread and already causing important losses. Third, the society in which the project took place had to have suitable resources and governmental structure to be receptive and able to react to the success and create institutions to conduct further work.

Introduction of Predacious Arthropods: The Cottony Cushion Scale and Vedalia Beetle in California (U.S.A.). In about 1868, a pest of citrus, the cottony cushion scale, *Icerya purchasi* Maskell, was found in California. By 1886, this pest was on the verge of destroying the citrus industry in southern California. Chemical measures were tried (cyanide fumigation of trees) and had only limited effect. Production was being severely reduced. In or about 1887, it was learned that the native home of the scale was Australia, where *I. purchasi* was not considered a pest. In the next two years, two efforts were made to secure natural enemies from Australia, one well known and one less well-recognized. The famous story is that Albert Koebele, a representative of the United States Department of Agriculture, went to Australia in 1888 and collected a parasitic fly, *Cryptochetum iceryae* (Williston), and a predacious coccinellid, *Rodolia* (formerly *Vedalia*) *cardinalis* (Mulsant), since known as the vedalia beetle (Fig. 1.6). The less famous story is that W.G. Klee, a California State Inspector of Fruit Pests, through correspondence with

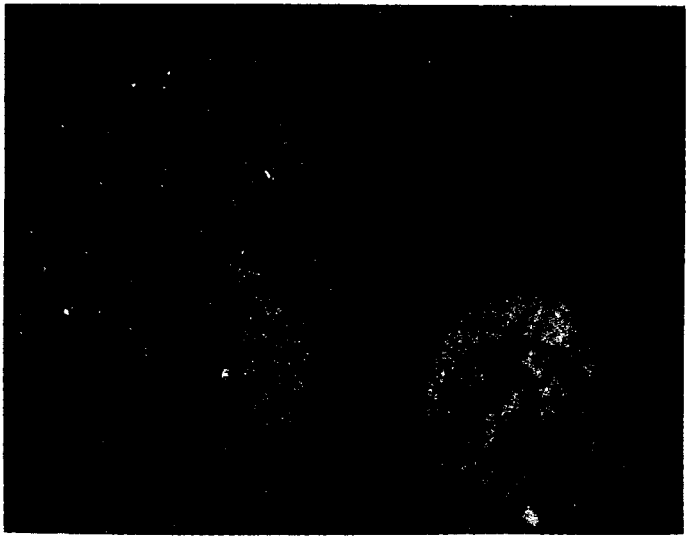

Figure 1.6 Introduction of natural enemies. Importation of the Vedalia beetle (*Rodolia cardinalis* [Mulsant]) into California led to control of the cottony cushion scale, *Icerya purchasi* Maskell. (Photograph by M. Badgley.)

an entomologist in Australia, Frazer Crawford, imported the same parasitic fly in 1887. Of these two insects, the beetle became the center of attention in southern California, since it provided immediate and dramatic control. The vedalia beetle multiplied profusely on scale-infested trees and was soon widely distributed by growers. Within two years, the beetle controlled the pest throughout the state. The fly also established and eventually became the dominant control agent for the pest in the coastal areas of the state.

The cottony cushion scale project had far-reaching effects on biological control by demonstrating that arthropod predators could be manipulated in ways that solved serious problems and produced large economic benefits. Because the economic benefit from the work was so dramatically obvious to the influential citrus growers in the state, it stimulated the official pursuit of biological control by state government, leading ultimately to a long series of projects by entomologists employed by the University of California. In addition, contacts between entomologists from California and entomologists in Australia (during collection of the beetle) and in countries such as Chile (one of many countries to which the beetle was later sent) stimulated interest in introductions of natural enemies in many countries (Caltagirone and Doutt 1989). Such early introductions of natural enemies required methods to preserve small colonies of natural enemies for extended periods; modern transportation has greatly shortened the travel times required to ship natural enemies, which can be transported in 2–5 days between most locations, without need for rearing colonies (Fig. 1.7).

Introduction of Parasitic Arthropods: The Sugarcane Leafhopper in Hawaii (U.S.A.). In 1900, a pest of sugarcane (*Saccharum officinarum* Linnaeus), the leafhopper *Perkinsiella saccharicida* Kirkaldy, was discovered in the Hawaiian Islands. By 1903, sugar yields were being depressed by the pest. It was learned that the species could be found in Queensland, Australia, and caused no important damage there. Inspired by the Vedalia project in California, the entomologists R.C.L. Perkins and A. Koebele went to Australia to search for natural enemies. Between 1904 and 1916, six species of egg parasitoids were imported into Hawaii, of which the most effective turned out to be *Anagrus optabilis* (Perkins). These six parasitoids established and together significantly reduced the problem. Later, an egg predator, *Tytthus mundulus* (Breddin), was also imported and this predator, in combination with the parasitoids, provided complete control. This project, and others, demonstrated the role of parasitoids in control of pest arthropods and stimulated the development of a very active state program that currently makes Hawaii an important center for biological control.

Introduction of Arthropod Herbivores for Weed Control: Prickly Pear and Cactoblastis Moth in Australia. In the 1800s, several species of ornamental cacti were introduced into Australia. Some species, especially the prickly pear species *Opuntia inermis* de Candolle and *Opuntia stricta* (Haworth), spread rapidly in forest and grazing lands such that, by 1925, 24 million hectares of land were densely infested and rendered economically valueless. In 1920, the Commonwealth Prickly Pear Board was formed to send entomologists to South America to search for insects that attacked the weed, and to establish quarantine laboratories in Australia to facilitate the testing of candidate agents. Ultimately, some 50 species of insects were collected and sent to Australia. (See pp. 51–55 of Wilson [1960] for details of this project.) Twelve species established and exerted some control prior to importation of the agent that ultimately solved the problem, the moth *Cactoblastis cactorum* (Bergroth) (and its associated plant pathogens). This agent was collected in 1925 in Argentina and colonized in the field in Australia in 1926. By

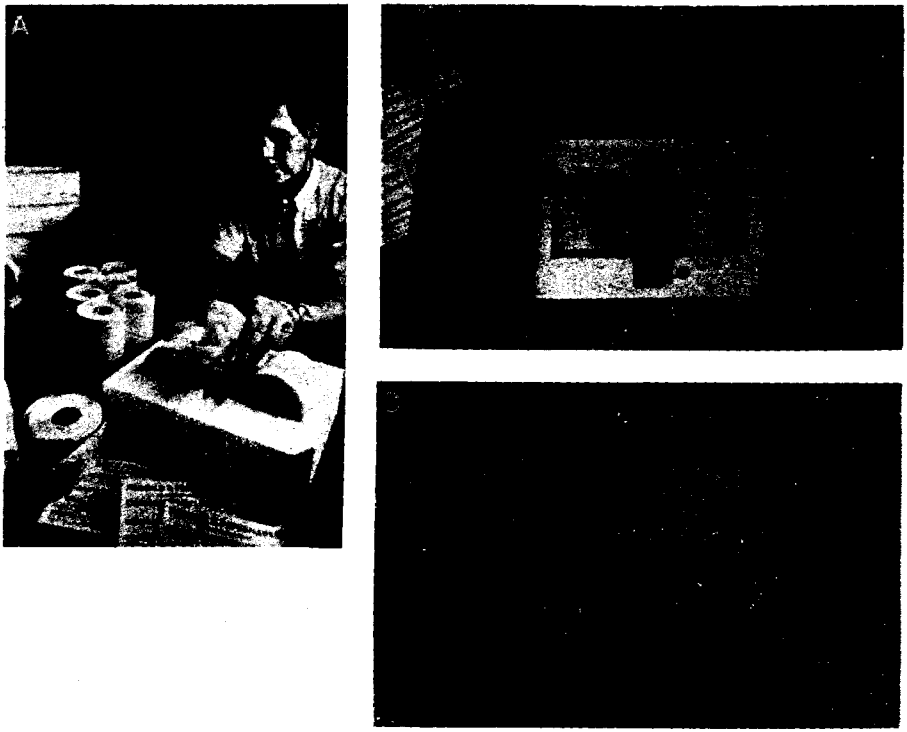

Figure 1.7 A container for international shipment of natural enemies; note use of Styrofoam insulation and pack of artificial ice (in center) for cooling (foam top of box removed for photograph) (A,B); assembled package and associated shipping labels (C). (Photographs courtesy of USDA/BIRL, M. Heppner [A], S.R. Bauer [B], and R.M. Hendrickson [C].)

1930–32, a general collapse of cactus stands at the original release sites had occurred, and the moth quickly spread and controlled the cacti throughout the infested area. The dramatic collapse of such large plants, so rapidly and over such a large area exceeded all expectations. The project so deeply impressed the Australian government that strong biological control institutions were created that today make Australia one of the world leaders in the biological control of weeds. This project, together with the control in the 1950s of Klamath weed, *Hypericum perforatum* Linnaeus, in California (Huffaker and Kennett 1959) convincingly demonstrated the practical ability of imported insects to suppress adventive weeds.

Introduction of Arthropod Pathogens: The Rhinoceros Beetle and Its Baculovirus. The first intentional, successful use of an introduced arthropod pathogen for pest control occurred in 1967. The rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus), is an important pest of coconut (*Cocos nucifera* Linnaeus) and oil palms. The beetle is native to southeast Asia and during the present century spread to many Pacific islands. In 1963, a baculovirus of the larvae and adults

was discovered in Malaysia and in 1967 the virus was introduced into Western Samoa, where it controlled the pest (Bedford 1980, 1986). The virus was subsequently moved to other islands with similar success. In the Maldives (in the Indian Ocean), for example, the pereentage of damaged palms fell from 40–60% before introduction to about 10% afterwards (Zelazny et al. 1990, 1992). One of the reasons for the success of this virus is that infected adult beetles remain alive for many weeks and act as vectors, introducing the virus to larvae living in breeding sites. This project demonstrated the potential for pathogens to be successful as biological control agents through introduction, however, successful movements of arthropod pathogens have been rare, and consequently biological control work with pathogens has focused on the augmentative use of artificially produced and applied pathogens. (Methods of use of natural enemies—introduction, conservation, augmentation—are discussed in Chapter 2).

Augmentation of Parasitoids and Predators: The Greenhouse Whitefly and Two-Spotted Spider mite. Unlike the preceding examples, all of which are based on the intentional international movement of natural enemies, their liberation, and subsequent spread as self-sustaining populations, in glasshouse crops another approach was needed. Because glasshouse crops are partially isolated from the outside environment, natural enemies often have to be introduced in each cropping cycle. Among the most important pests of protected cultivation are the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), and the two-spotted spider mite, *Tetranychus urticae* Koch.

The first step toward the development of a biological control system for pests in glasshouse crops occurred in 1926 when a grower in England noted dark-colored (parasitized) whiteflies in his glasshouse and called them to the attention of the entomologist E.R. Speyer. The responsible parasitoid, *Encarsia formosa* Gahan, was subsequently found to provide control of the pest and commercial production was begun (Speyer 1927; Hussey 1985). Production lapsed in 1949 due to competition from newly developed pesticides and did not resume again until 1967. While several groups were involved in the early 1970s in the production of natural enemies for use in glasshouses, a critical event was the decision in 1965 by a Mr. Koppert in Holland to begin to produce beneficial predator mites and insects for greenhouse use. His business developed a number of improved methods for rearing, sorting, and shipping both *Encarsia formosa* and predacious mites. Several large insect-rearing firms now supply a variety of beneficial arthropods to growers for augmentative releases in glasshouses.

Whitefly control with parasitoids required that all other pests in glasshouse crops also be controlled with natural enemies or chemicals compatible with *Encarsia formosa*. Another important pest on tomatoes (*Lycopersicon lycopersicum* [Linnaeus] Karsten ex Farwell) and cucumbers (*Cucumis sativus* Linnaeus) was the two-spotted spider mite. While several species of natural enemies were considered for control of this pest, the species that ultimately proved the most effective was discovered in 1960 on a shipment of orchids sent from Chile to Germany (Bravenboer and Dosse 1962). This species, *Phytoseiulus persimilis* Athias-Henriot, proved voracious and easy to rear and is now widely used in greenhouses (Fig. 1.8).

Extensive research was required to develop the methods to rear these natural enemies and to use them effectively (Hussey 1985). These agents made the commercial application of biological control in glasshouses possible. By 1994, *Phytoseiulus persimilis* was being applied on over 8000 ha of glasshouse crops annually and *Encarsia formosa* on nearly 5000 ha (van Lenteren 1995), demonstrating that predacious and parasitic arthropods can be reared efficiently to provide cost-effective biological control. This success provided the incentive for the development of the commercial insectary business throughout much of the world.

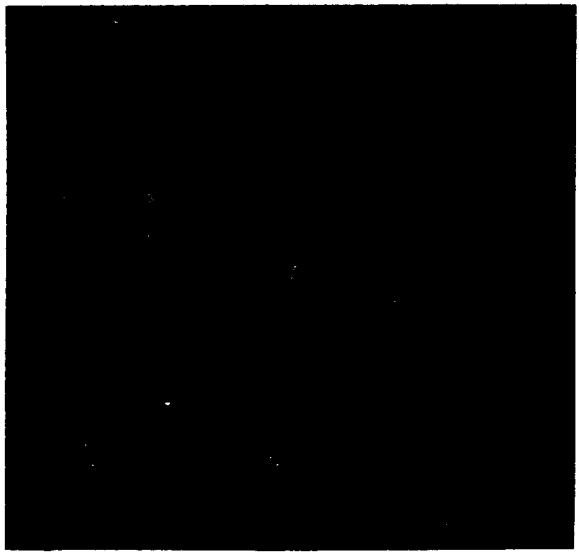

Figure 1.8 Augmentation of natural enemies. Seasonal augmentative releases of the predacious mite *Phytoseiulus persimilis* Athias-Henriot provides control of phytophagous mites in glasshouses. (Photograph courtesy of M. Badgley.)

Augmentation of Arthropod Pathogens: Bacillus thuringiensis Berliner. Early in this century, a bacterial disease of larvae of the flour moth *Anagasta kuehniella* (Zeller) was noted by Berliner (1911) and the causative agent described as *Bacillus thuringiensis*. Tests were conducted on various Lepidoptera and, by 1938, the first commercial preparation based on this organism was marketed under the name Sporeine in France (Jacobs 1951). Many strains of this organism were isolated from various hosts, but prior to the 1980s commercial products were limited in their activity to larvae of certain Lepidoptera. Isolates since have been obtained that are effective against many other taxa, including Diptera and Coleoptera (van Essen and Hembree 1980; Herrnstadt et al. 1987). The effectiveness and selectivity of this pathogen are based on its production of one or more of several toxins that paralyze and kill hosts, with certain toxins affecting some host groups and not others. This pathogen represents the earliest commercially successful use of a microbial pathogen as a formulated pesticide (Fig. 1.9). The toxins of this bacterium are stomach poisons that kill only certain groups of insects, permitting the development of integrated pest management systems in which natural enemies are conserved while selectively suppressing particular pests. The success of this product has been important in stimulating the development of commercial interest in the location, production, and use of microbes as pest control products. Information about this pathogen and its use are summarized by Entwistle et al. (1993).

Conservation of Native Natural Enemies: The Rice Brown Planthopper. The rice brown planthopper, *Nilaparvata lugens*, is an occasional pest of rice in many parts of Asia. In the 1960s and 1970s, cultural changes in rice (*Oryza sativa* Linnaeus) varieties, fertilization, and cropping practices occurred which encouraged farmers to use pesticides to suppress this minor pest. The pesticides destroyed spiders and other generalist natural enemies and led to a cycle of more

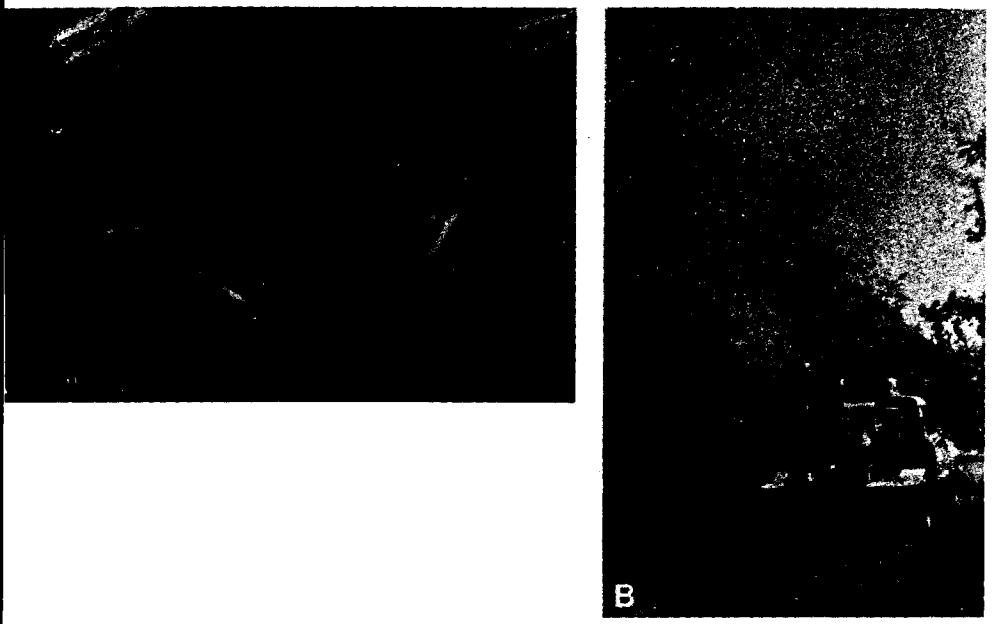

Figure 1.9 Use of pathogens as biopesticides. The bacterium *Bacillus thuringiensis* Berliner (A) can be cultured and applied to crops (B) for control of certain pests, such as the navel orangeworm, *Amyelois transitella* (Walker). (Photograph courtesy of W. F. Burke [A] and P. V. Vail [B].)

frequent and more devastating pest outbreaks, as demonstrated experimentally by Heinrichs et al. (1982). The cycle was broken when farmers were taught to understand the importance of spiders and other predators and to avoid routine pesticide applications (Fig. 1.10). Following such an educational campaign in Indonesia, reduced pesticide use on rice was accompanied by stable or increased rice yields. This major biological control success demonstrated the importance of native natural enemies and the value of extension systems that educate farmers as to the merits of biological control and the risks of pesticide use (Kenmore 1988). This project has influenced agricultural policy in developing countries and international aid organizations, placing emphasis on conserving native biological control agents, rather than encouraging pesticide use.

Recent Successes

Documenting the long list of successful biological control projects is not our purpose. However, discussions of some recent highly successful cases of biological control help illustrate that biological control has not been superseded by pesticides, biotechnology, or other technological developments, and is still an effective solution to many important pest control problems. We have chosen four projects to illustrate some of the recent successes of biological control through the introduction of new natural enemies. Each of these projects was highly effective and economically valuable to the countries involved.

Figure 1.10 Conservation of natural enemies. Field training of farmers in Narshingdi, Bangladesh, in recognition of natural enemies of pests of rice. (Photograph courtesy of P. Kenmore, FAO.)

Alfalfa Weevil. The alfalfa weevil, *Hypera postica* (Gyllenhal), invaded the eastern part of the United States in about 1945 (Day 1981). By the mid-1960s it had spread throughout most of the alfalfa-producing areas in the eastern half of the country. Larval densities were so high that most alfalfa (*Medicago sativa* Linnaeus) growers regularly made one or more pesticide applications per year to suppress damage. Because alfalfa is grown on nearly one million ha in the northeastern United States, pesticide use was extensive, especially in the key dairy states of New York and Wisconsin. Starting in 1959, the United States Department of Agriculture introduced a series of parasitoids of this pest, collected in Europe (Day 1981). These suppressed the pest to such low levels that farmers were able to reduce their use of pesticides against the pest by over 73% (Day 1981). The most effective species were two ichneumonid larval parasitoids in the genus *Bathyplectes* and a parasitoid of the adult weevil, *Microctonus aethiopoides* Loan. This project, over a twenty year period, cost about one million dollars, but by 1968 paid back annual benefits of over $8,000,000 (Day 1981), demonstrating that introduction of natural enemies remains a cost effective approach to solving insect problems

Cassava Mealybug in Africa. The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, invaded West Africa from South America in about 1973. By the early 1980s, it was found in much of tropical Africa and was spreading up to 300 km per year (Fig. 1.11), greatly reducing yields of cassava (*Manihot esculenta* Crantz), the basic subsistence food of nearly 200 million people. A parasitoid, *Epidinocarsis lopezi* (De Santis), of the mealybug was collected in South America after the mealybug was correctly identified and finally located in Paraguay. This parasitoid was introduced into Africa and proved to be highly effective (Norgaard 1988; Neuenschwander et al. 1989) (Fig. 3.9). A massive rearing and release program was conducted, and the pest was soon under biological control in nearly all parts of its range in Africa, and a basic food crop was again productive and its yields secure. More than any other recent project, this success has caused international development agencies to seriously consider using biological control based on the introduction of new species of natural enemies in their projects.

Floating Fern in Papua New Guinea. *Salvinia molesta* D.S. Mitchell is a floating fern from Brazil that was introduced into many tropical countries in the early part of the twentieth century

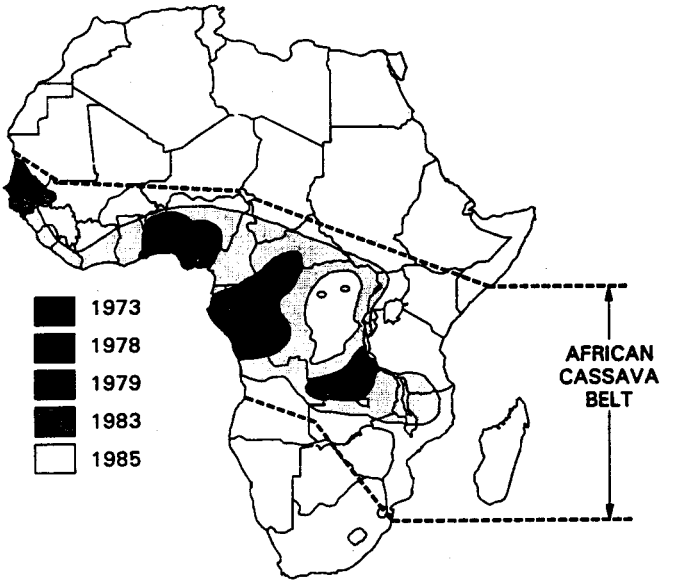

Figure 1.11 Spread of the cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero) in Africa following its invasion from South America (after Herren 1987).

as an aquarium plant and botanical curiosity. The plant reached Papua New Guinea in the early 1970s and rapidly spread over the surface of the Sepik River and associated oxbow lakes. Mats of weed formed up to 1 m thick, preventing boats from moving freely along the river and isolating villages (Mitchell et al. 1980). In the 1960s and 1970s, there were efforts to locate natural enemies of the weed in South America because of problems in other regions. However, little was achieved because mis-identification of the plant species led to the collection of ineffective herbivores from related plants. After the weed was correctly identified and its native range discovered in 1978, more appropriate natural enemies were obtained. The weevil *Cyrtobagous salviniae* Calder and Sands proved highly effective in controlling the weed (Thomas and Room 1986; Room 1990) (Figs. 8.1, 8.2). Promising control programs have since been initiated in several additional countries including India and Namibia (Thomas and Room 1986; Forno 1987). This project achieved its objective of permanently ridding large areas of a damaging aquatic weed at moderate cost where other forms of control would have been totally infeasible.

Narrow-Leaf Skeletonweed in Australian Wheat. Skeletonweed, *Chondrilla juncea* Linnaeus, is an immigrant weed infesting dryland wheat (*Triticum aestivum* Linnaeus) areas in Australia. Three genetic types exist (narrow, broad, and intermediate leaf). The narrow-leaf form (the most abundant type) was controlled by a rust pathogen, *Puccinia chondrillina* Bubak and Sydow, imported to Australia from Italy. Later, a second rust strain was introduced for control of the intermediate-leaf form (Cullen 1978, 1985; Hasan and Wapshere 1973; Hasan

1981; Watson 1991). This project was the first successful use of an introduced plant pathogen for the control of an immigrant pest, and the first successful biological control of a weed in an annual crop.

THE HISTORICAL RECORD OF BIOLOGICAL CONTROL EFFORTS

Success Rates of Biological Control Projects Employing Natural Enemy Introduction

The historical record of past biological control projects is important because it documents the frequency of past success. This record can be evaluated with respect to particular cases (which agent has been most successful for the control of a particular pest), or for rates of success across many projects. The former is an important source of specific information, useful when a new location faces a problem dealt with successfully in the past in another area. The latter provides perspective on the probability of suppressing the target pest of any particular new project. The world database on biological weed control through natural enemy introduction is given by Julien (1992), who summarizes releases by agent and target, with notes on establishment and effects on the target plant. Clausen (1978) summaries worldwide introductions of parasitoids and predators for arthropod targets up through 1968, and Luck (1981) provides a more up-to-date listing of introductions of parasitoids for arthropod control. A worldwide, comprehensive database for predators and parasitoids for arthropod targets (BIOCAT) is maintained by the Commonwealth Agricultural Bureaux in the United Kingdom (Greathead and Greathead 1992). Hall et al. (1980) analyzed data in Clausen (1978) and found that 16% of all biological control projects resulted in complete control of the target pest in the project's location and an additional 42% resulted in partial control. In considering analyses of success rates, it is important to consider whether rates are calculated on a per project (involving potentially many agents) or per agent basis (which necessarily is lower than the rate per project). The figures of Hall et al. (1980) indicate that biological control, while not guaranteed to solve any one particular problem, is on average an effective strategy. Since benefit/cost ratios of biological control projects are large (3:1 to 100 or more:1) project success rates of 16% or more are sufficient for national programs of biological control to be highly cost effective as a form of social investment of public funds (Bellows 1993).

The historical record of projects of biological control through augmentation or conservation have not been summarized, although Frank and McCoy (1994) provide a list of 49 biological control agents imported into Florida (U.S.A.) by commercial organizations.

Regional Summaries of Past Projects

The historical records of biological control introductions in various countries have been summarized and provide an important source of information. These include: for insects worldwide (Clausen 1978; Luck 1981), for weeds worldwide (Goeden 1978; Julien 1992), the Pacific region (Waterhouse and Norris 1987), New Zealand (Cameron et al. 1989), Australia (Wilson 1960), southeast Asia and the Pacific region (Rao et al. 1971), Thailand (Napompeth 1990), Malaysia (Ghee 1990), Taiwan (Chen and Chiu 1986), South Korea (Choi and Lee 1990), Hawaii (Funasaki et al. 1988a), western and southern Europe (Greathead 1976), the British Commonwealth Caribbean and Bermuda (Cock 1985), Latin America (Altieri et al. 1989), sub-Saharan Africa (Greathead 1971), South Africa (Hoffmann 1991), Israel (Argov and Rossler 1988), Canada (Anon. 1971; Kelleher and Hulme 1984); and western North America (Nechols et al. 1995).

BIOLOGICAL CONTROL AND INTEGRATED PEST MANAGEMENT

Biological control projects may exist in their own right, independent of other control efforts. This is particularly true of pests in uncultivated areas, aquatic weeds, rangeland weeds, pests of ornamental plants, and forest insects. Many crop pests, however, are members of pest complexes all of which must be controlled simultaneously for effective crop production. In such cases, integration of potentially conflicting controls (biological control and chemical controls, but also perhaps biological control and plant breeding or agronomic practices) must be achieved. This subject is developed in Chapter 14. Basic questions in such integration are which controls are essential and which are optional. In the period following 1945, when pesticide use was extremely popular, chemical controls came to be considered the basic controls and biological controls were viewed as secondary or unnecessary. These biological controls were expected to play some (usually minor) role, if they could function despite chemical applications. If the biological control agents were unable to function in crops receiving pesticide applications, it was taken as proof that biological control agents were unimportant.

In recent decades (1980s to present), a change has gradually taken place with respect to this perception of priorities. The concept of integrated pest management systems based on biological control (as opposed to merely including some biological control) has arisen. Under this concept, biological control agents are seen as essential and of first priority in building pest control systems. Unlike in the past, biological control-based pest management does not permit the inclusion of control practices that destroy the biological control basis for pest suppression in the crop. Efforts to develop pest control systems based on biological control are in their early stages in many systems and well developed in others and represent the current evolution of biological control implementation. In this book, we explore both biological control through natural enemy introductions and the building of biological control-based integrated pest management systems by a range of methods, including introductions, augmentation, and conservation.

CHAPTER

2

KINDS OF BIOLOGICAL CONTROL TARGETS, AGENTS, AND METHODS

TARGETS OF BIOLOGICAL CONTROL

Biological control was first used to control insects, mites, and weeds (DeBach 1964a; Huffaker and Messenger 1976; Clausen 1978; Waterhouse and Norris 1987; Cameron et al. 1989; Julien 1992). Application of the method broadened with time and other invertebrates, plant pathogens, and even some vertebrates are now considered as likely targets (Cook and Baker 1983; Ross and Tittensor 1986; Campbell 1989; Madeiros 1990; Madsen 1990; Singleton and McCallum 1990; Stirling 1991).

Insects

Pest insects have been the most common type of organism against which biological control has been employed (Laing and Hamai 1976). Worldwide, over 543 species of insects have been targets of more than 1200 programs of biological control introductions (Greathead and Greathead 1992) and more are targeted through programs of natural enemy conservation and augmentation. These have included members of most of the important herbivorous orders, Homoptera, Diptera, Hymenoptera, Coleoptera, Lepidoptera, as well as smaller numbers in other groups. Homoptera has been the order against which biological control through introduction has been successfully employed most frequently (Greathead 1986a). This pattern of successful use of biological control is caused by the high frequency with which scales, aphids, and whiteflies move internationally on plants in trade (due to their small size and inconspicuousness), the large number of species in these groups which are important pests, and the frequency with which parasitoids and predators are significant factors in restraining the densities of such insects.

Mites

Several families of mites have been targets of biological control efforts. These include rust mites of the family Eriophyidae (Gruys 1982; Abou-Awad and El-Banhawy 1986), tarsonemid mites (Huffaker and Kennett 1956), and, most frequently, Tetranychidae, the spider mites (McMurtry 1982). Actions have included introduction of mite predators (principally Phytoseiidae and

Coccinellidae), conservation of native mite predators, and augmentative release of reared mite predators (McMurtry 1982).

Other Invertebrates

After insects and mites, snails have been the invertebrate group against which biological control efforts have been most frequently directed. Snails of concern have been either herbivorous species which damage crops or medically important snails which are intermediate hosts for pathogens which cause diseases of humans or domestic animals. Among the crop pests, the principal concern has been with edible species such as the giant African snail, *Achatina fulica* Bowdich, which has been spread to many areas by deliberate introduction for use as food (Waterhouse and Norris 1987). Of the medically important snails, those which are intermediate hosts for schistosomiasis have been of greatest concern (Greathead 1980; Pointier and McCullough 1989; Madsen 1990).

Biological control efforts against other types of invertebrates have been very rare. Clausen (1978) records the introduction of an egg parasitoid of a poisonous spider, *Latrodectus mactans* (Fabricius), to Hawaii. The parasitic fly *Pelidnoptera nigripennis* (Fabricius) has been imported into Australia for release against the adventive millipede *Ommatoiulus moreletii* (Lucas) (Bailey 1989).

Weeds

Plants in many taxonomic groups have become pest weeds in a variety of habitats, including forest, agricultural and rangeland, and native ecosystems, both terrestrial and aquatic. At least 116 species of plants in 34 families have been targets for biological control (Table 2.1) through introduction of invertebrate herbivores or plant pathogens (Julien 1992). About half (47%) of the weed species involved have been in the three families of Asteraceae, Cactaceae, and Mimosaceae. Other families, however, have contained individual species of great economic

TABLE 2.1 Taxonomic Range of Weeds Against which Biological Control Has Been Attempted via Introduction of Invertebrates

Plant Family	No. of Weed Species	Plant Family	No. of Weed Species
Amaranthaceae	1	Hydrocharitaceae	1
Anacardiaceae	1	Lamiaceae	1
Araceae	1	Loranthaceae	1
Asclepiadaceae	1	Malvaceae	2
Asteraceae	32	Melastomataceae	2
Boraginaceae	2	Mimosaceae	9
Cactaceae	22	Myricaceae	1
Caesalpiniaceae	1	Passifloraceae	1
Caryophyllaceae	1	Polygonaceae	3
Clusiaceae	2	Pontederiaceae	1
Chenopodiaceae	2	Proteaceae	2
Convolvulaceae	1	Rosaceae	4
Cuscutaceae	4	Salviniaceae	1
Cyperaceae	1	Scrophoulariaceae	3
Ehretiaceae	1	Solanaceae	1
Euphorbiaceae	2	Verbanceae	2
Fabaceae	4	Zygophyllaceae	2

importance and have been the focus of intensive efforts; among these are the Clusiaceae (*Hypericum perforatum*, St. John's wort), Salviniaceae (*Salvinia molesta*, water fern), and Verbenaceae (*Lantana camara* Linnaeus, lantana). Grasses have not been a group against which biological control has been commonly applied, but some needs and opportunities exist for biological control of pest grass species (Wapshere 1990).

Plant Diseases

Antagonist organisms have been used to prevent or suppress some plant diseases. Many plant pathogens are potentially affected by biological control agents (Cook and Baker 1983; Campbell 1989). Examples of plant pathogens against which antagonists have been directed include species of *Agrobacterium*, *Fusarium*, *Heterbasidion*, *Pythium*, *Erwinia*, *Pseudomonas*, *Sclerotinia*, *Rhizoctonia*, and *Cryphonectria* (Schroth and Hancock 1985; Campbell 1989). Lindow (1985a) discusses antagonists for foliar pathogens.

Vertebrates

Feral populations of vertebrates, such as rats, pigs, goats, sheep, rabbits and opossums, are important pests damaging grazing lands, forests, and nature conservation in many areas (see Chapter 21). Many of these species, however, also are desirable species in other contexts. Biological control efforts targeted at vertebrates must use agents that are sufficiently specific to protect other vertebrates. Such projects can only be undertaken in locations where conflicts between the need to control feral populations and protect domestic populations of the same species either do not exist, or have been judged in favor of the control efforts.

In addition to the introduction of pathogens with narrow host ranges or enhancement of habitats for native predators, genetic methods for vertebrate control have been developed. These are based on the use of genetically-modified vertebrate pathogens, such as the myxoma virus of rabbits, to cause infected females to develop antibodies against the species' sperm, preventing conception (Deeker 1992; Barlow 1994).

KINDS OF BIOLOGICAL CONTROL AGENTS

Natural enemies, the agents used in biological control, are the fundamental resource with which biological control success is achieved. Agents come from an array of taxonomic groups and have diverse biological and populational properties. These characteristics play a large role in the success or failure associated with the use of any particular group of natural enemies and a detailed appreciation of the biologies of many different natural enemy groups is of great value.

Insect Parasitoids

Parasitoids are arthropods that kill their hosts (unlike true parasites such as fleas or tapeworms) and which are able to complete their development on a single host (unlike predators which generally must consume several prey to complete their development) (Doutt 1959; Askew 1971; Vinson 1976; Vinson and Iwantsch 1980a; Waage and Greathead 1986; Godfray 1994). Parasitoids have been the most common type of natural enemy introduced for biological control of insects (Hall and Ehler 1979; Greathead 1986a). Most parasitoids that have been used in biological control are in the orders Hymenoptera (Fig. 2.1) and, to a lesser degree, Diptera.

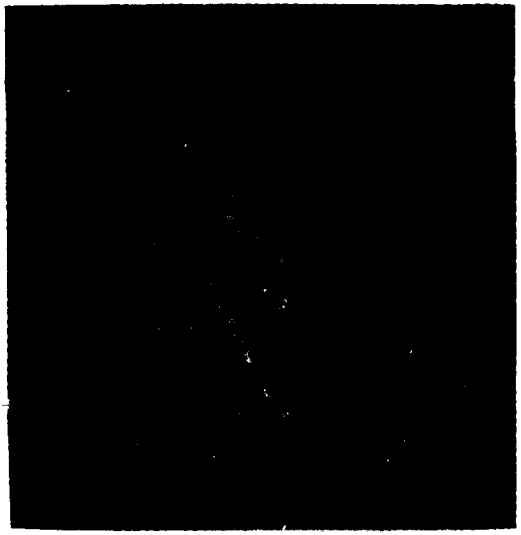

Figure 2.1 The braconid parasitoid *Aphidius matricariae* Haliday parasitizing its aphid host *Diuraphis noxia* (Mordvilko). (Photograph by M. Badgley.)

While use has been made of parasitoids in at least 26 families, certain groups stand out as having more species employed in biological control projects than others. The most frequently used groups in the Hymenoptera have been the Braconidae and Ichneumonidae in the Ichneumonoidea, and the Eulophidae, Pteromalidae, Encrytidae, and Aphelinidae in the Chalcidoidea (Table 2.2). In the Diptera the most frequently employed group has been the Tachinidae (Greathead 1986a). Parasitoids are also found in the insect orders Strepsiptera and Coleoptera (some members of such families as the Staphylinidae, Meloidae, Rhipiphoridae), although parasitism is not typical of the Coleoptera (Askew 1971).

Predators of Arthropods and Other Invertebrates

The predacious habit is extremely widespread among insects and can be found in most orders and a large number of families (Fig. 2.2) (Hagen et al. 1976a; Borror et al. 1989). Predacious insects have been introduced for control of immigrant pests, and native predators are of major importance in the suppression of both native and immigrant herbivores. Groups of predators most frequently recognized as significant for pest suppression in agriculture and forestry include some 32 families (Table 2.3). Of these, the Anthocoridae, Pentatomidae, Reduviidae, Carabidae, Coccinellidae, Staphylinidae, Chrysopidae, Cecidomyiidae, Syrphidae, and Formicidae are among the predators most commonly found preying on pest species in crops. Spiders are virtually all predacious (Foelix 1982) and while usually not specialized as to prey species, do show habitat specialization. The role of spider complexes in suppressing groups of pests in crops and other habitats has become better recognized in recent years (Clarke and Grant 1968; Mansour et al. 1980; Riechert and Lockley 1984; Nyffeler and Benz 1987; Bishop and Riechert 1990). Spider mites, an important agricultural pest group, have no parasitoids and are held in check to a large degree by predators. These include predacious thrips, some coccinellid beetles and, most importantly, mites in the family Phytoseiidae. Slugs and

TABLE 2.2 Number of Times Parasitoids Have Been Established in Biological Control Introduction Programs in Families from which at Least One Species Has Been Released[1]

	No. Parasitoid		No.	No.	No. Effective
	Genera	Species	Pest Species	Occasions	Control Cases
Hymenoptera					
Evanoidea					
Stephanidae	1	1	1	1	—
Ichneumonoidea					
Braconidae	23	66	59	158	53
Ichneumonidae	30	45	28	72	22
Proctotrupoidea					
Proctotrupidae	—	—	—	—	—
Scelionidae	4	12	11	23	6
Platygasteridae	4	7	6	12	5
Diapriidae	—	—	—	—	—
Cynipoidea	3	4	4	7	1
Chalcidoidea					
Trichogrammatidae	2	12	12	24	—
Eulophidae	21	36	47	72	23
Mymaridae	4	7	9	15	9
Chalcididae	2	3	2	4	—
Eurytomidae	—	—	—	—	—
Torymidae	1	1	1	2	—
Pteromalidae	15	26	221	49	17
Encyrtidae	34	61	401	132	53
Aphelinidae	13	59	32	185	90
Eupelmidae	2	4	3	4	—
Bethyloidea					
Bethylidae	2	2	2	3	—
Dryinidae	2	2	1	2	—
Scolioidea	3	13	10	21	3
Diptera					
Pygotidae	—	—	—	—	—
Cryptochetidae	1	2	2	5	5
Tachinidae	27	30	27	69	35
Muscidae (*Acridomyia*)	—	—	—	—	—
Sarcophagidae	—	—	—	—	—
Strepsiptera	—	—	—	—	—
Totals	194	393	2741*	860	216*

*Totals are reduced because more than one parasitoid has often been established on the same host in a single country.
[1]Greathead (1986a).

snails are attacked by predacious snails, sciomyzid flies (whose larvae find and kill one or several snails during their development), and some carabid beetles.

Vertebrate predators which attack insect pests are diverse and include insectivorous birds, small mammals, lizards, amphibians, and fish, some of which have been used in the past as agents of biological control (Davis et al. 1976). While birds and mammals are generally not used as agents for introduction against immigrant pests, indigenous species are believed to be important sources of mortality for some pests, particularly in stable environments such as forests (Bruns 1960; Bellows et al. 1982a; Campbell and Torgersen 1983; Nuessly and Goeden 1984; Atlegrim 1989; Crawford and Jennings 1989; Higashiura 1989; Zhi-Qiang Zhang 1992).

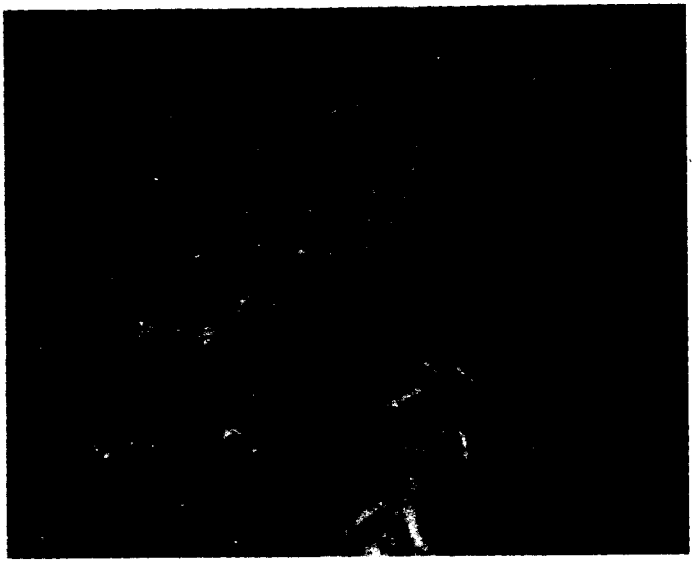

Figure 2.2 The pentatomid *Podisus maculiventris* (Say) attacking a larva of the chrysomelid *Leptinotarsa decemlineata* (Say). (Photograph courtesy of D. Ferro.)

Table 2.3 Some Important Families of Predacious Arthropods

Thysanoptera (thrips)
- Aeolothripidae
- Phloeothripidae
- Thripidae

Hemiptera (true bugs)
- Anthocoridae (minute pirate bugs)
- Gerridae (water striders)
- Miridae (plant bugs)
- Nabidae (damsel bugs)
- Pentatomidae (stink bugs)
- Reduviidae (assassin bugs)
- Veliidae (broad shouldered water striders)
- Phasmatidae (ambush bugs)

Coleoptera (beetles)
- Carabidae (ground beetles)
- Cicindelidae (tiger beetles)
- Dytiscidae (predacious water beetles)
- Cleridae (checkered beetles)
- Coccinellidae (lady bird beetles)
- Cybocephalidae
- Staphylinidae (rove beetles)

Neuroptera (nerve-winged insects)
- Chrysopidae (green lacewings)
- Hemerobiidae (brown lacewings)

Diptera (true flies)
- Cecidomyiidae (gall flies)
- Chamaemyiidae (aphid flies)
- Sciomyzidae (marsh flies)
- Syrphidae (flower flies)

Hymenoptera
- Formicidae (ants)
- Vespidae (yellow jackets and paperwasps)
- Sphecidae (hunting wasps)

Acari (mites)
- Phytoseiidae
- Stigmaeidae
- Hemisarcoptidae

Araneae (spiders)

Fish, in contrast, have mainly been utilized through augmentative releases into water bodies for the suppression of mosquito larvae (Miura et al. 1984).

Pathogens and Predators of Vertebrates

When sufficiently selective agents can be identified for safe use, pathogens are sometimes employed to suppress pest vertebrates. Agents that would be unacceptable in settled areas may be acceptable on oceanic islands where no human settlements exist to create conflict between suppression of feral populations of the species (seen as pests) and husbandry of domestic populations of the same species (seen as economically valuable resources). Thus, the release of the feline panleucopaenia pathogen for control of feral house cats on unsettled islands which are home to seabird colonies (van Rensburg et al. 1987) is acceptable, but in other areas, use of this pathogen would be unacceptable. Other vertebrate pathogens employed for biological control include the myxoma virus of rabbits (Fenner and Ratcliffe 1965; Ross and Tittensor 1986) and some helminths that attack rodents (Singleton and McCallum 1990). Interest also exists in the possible use of venereal diseases to suppress population growth rates of feral goats on uninhabited oceanic islands where goats cause extensive damage to native vegetation (Dobson 1988).

Vertebrate species that are predators of vertebrates are rarely sufficiently specific to allow their safe introduction to areas outside of their natural range. However, some native vertebrates have been successfully enhanced through habitat modification or provision of nesting boxes, as for example the barn owl, *Tyto alba* Linnaeus, for control of rats on oil palm plantations in Malaysia (Madeiros 1990; Mohd 1990) (Fig. 2.3).

Pathogens and Nematodes Attacking Arthropods

Insect pathogens include a range of bacteria, viruses, fungi, and protozoa (Brady 1981; Miller et al. 1983; Maramorosch and Sherman 1985; Moore et al. 1987; Burge 1988; Tanada and Kaya 1993). Natural epizootics of certain of these agents are important sources of mortality for some species (Fuxa and Tanada 1987). In addition, some pathogens have been formulated and commercially marketed as insecticides (Cherwonogrodzky 1980; Falcon 1985).

Bacteria. Of the various pathogen groups, bacteria have been most successfully brought into commercial use. Three species of spore-forming bacteria in the genus *Bacillus* are currently used for the control of several groups of pests: *Bacillus popilliae* Dutky (larvae of scarabaeid Coleoptera), *Bacillus thuringiensis* (Lambert and Peferoen 1992), including var. *kurstaki* (larvae of Lepidoptera), var. *israelensis* (larvae of Diptera, in several families), and var. *tenebrionis* (larvae of chrysomelid Coleoptera), and *Bacillus sphaericus* Neide (larvae of culicid Diptera) (Falcon 1985; Lüthy 1986; Osborne et al. 1990). Bacteria are more amenable to commercial use than viruses because many species can be grown in fermentation media and often do not require the expensive culturing of live insect hosts as do the viruses. Most emphasis has been placed on *B. thuringiensis*, of which about 30 subspecies and more than 700 strains have been isolated. Some *Bacillus thuringiensis* products contain both live bacteria and associated toxic proteins; others contain no live material, but only the toxic chemical proteins the bacteria have produced in culture. Products containing no live spores were initially used for application in regions in Asia where silkworm culture was important and where live bacterial products were viewed as a potential risk (Aizawa 1987).

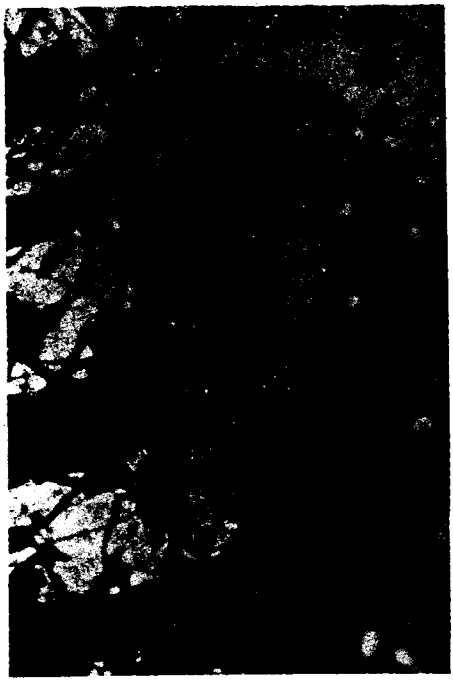

Figure 2.3 The barn owl, *Tyto alba* Linnaeus, a predacious vertebrate. (Photography by M. Badgley.)

Viruses. At least sixteen families of viruses have been found to be pathogens of insects (Entwistle 1983; Moore et al. 1987; Tanada and Kaya 1993). Of these, members of the Baculoviridae (Fig. 2.4) have been the focus of attempted commercial use because they frequently cause lethal infections and are only known to cause disease in insects (Payne 1986). This family contains the nuclear polyhedrosis and granulosis viruses. The nonoccluded viruses, formerly placed in the Baculoviridae, are now unclassified. The biology of this family has been reviewed by Granados and Federici (1986). Few viruses have been successfully marketed as commercial products because of high production costs and narrow host specificity. Existing products are mostly ones supported at public expense (Falcon 1976, 1985; Morris 1980).

Fungi. Most fungi that attack insects are in the family Entomophthoraceae within the subdivision Zygomycotina or in the Deuteromycotina (species known only from asexual forms) (Brady 1981; Zimmermann 1986). The higher classification of fungi varies between authors. In this text, we follow the system outlined by Ainsworth (1971) and Ainsworth et al. (1973). Fungal epidemics occur periodically and can cause high levels of mortality in the affected arthropod populations (see Goh et al. 1989). The fungus *Zoophthora radicans* (Brefeld) Batko from Israel was introduced to Australia to aid in the suppression of the lucerne aphid, *Therioaphis trifolii* (Monell) f. *maculata* (Milner et al. 1982). Other fungi have been of interest for the development of commercial biological pesticides (Ferron 1978; Gillespie 1988). To date, successful development of mycoinsecticides has been extremely limited, because of narrow host range and high humidity requirements for germination or sporulation (Moore and Prior 1993). Fungal pesti-

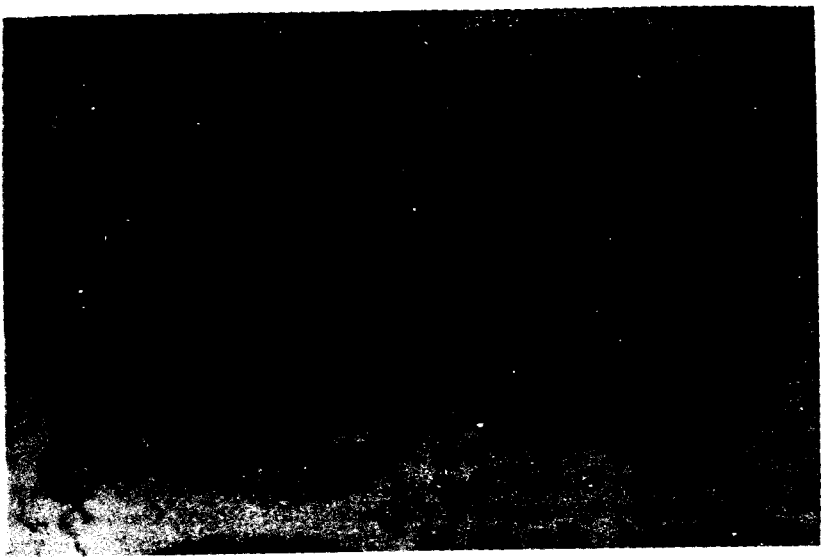

Figure 2.4 Transmission electron micrograph of a thin section of an epidermal cell from a spruce budworm larva, *Choristoneura fumiferana* (Clemens), infected with a nuclear polyhedrosis virus. The polyhedral inclusion bodies are visible in the cell nucleus. Nucleocapsids within virions in these occlusion bodies appear as dark circles or rods, depending on the orientation to the cell section. (Photograph courtesy of J.A. MacDonald, Forestry Canada.)

cides have their greatest potential for use in humid climates or moist environmental strata such as soil. However, new formulation methods such as employing vegetable oils in place of water in spray solutions appear to have potential to widen the range of climates and circumstances under which fungi may be employed successfully (Bateman et al. 1993).

Protozoa. Various protozoa infect insects (Brooks 1988). These protozoa include the microsporidians (Kluge and Caldwell 1992) and the eugregarines (Brooks and Jackson 1990). (See Chapter 4 for an overview of the taxonomy of the protozoa that are pathogens of arthropods.) Some protozoans have been considered for use as microbial insecticides. Species of *Nosema* have been tested as potential biological control agents for grasshoppers (Henry and Onsager 1982) and maize (*Zea mays* Linnaeus) pests (Lublinkhof and Lewis 1980).

Nematodes. Nematodes which have shown the greatest potential for the control of agricultural pests are found in the families Steinernematidae and Heterorhabditidae (Gaugler and Kaya 1990; Kaya 1993), which are mutualistically associated with bacteria that kill the nematode's host through septicemia (Kaya 1985). Nematodes in some other families also attack insects killing their hosts through their growth, much as do parasitoids. These include some mermithids (e.g., *Romanomermis*), phaenopsitylenchids (e.g., *Beddingia* [=*Deladenus*]), iotonchiids (e.g., *Paraiotonchium*), sphaerulariids (e.g., *Tripius*) and tetradonematids (e.g., *Tetradonema plicans* Cobb) (Poinar 1986; Kaya 1993).

Some nematodes have broad host ranges and efforts to use such species for biological control have been through augmentative application of reared nematodes to sites where pests are present. Research for this type of use has been focused on species in the genera *Steinernema* and *Heterorhabditis*. (Literature on nematodes in these genera is confused by many synonyms. See Poinar [1990], Doucet and Doucet [1990], and Kaya [1993] for details on relationships between names.) Nematodes in these families often require high moisture conditions for survival and are sensitive to ultraviolet light, which limits their effectiveness to protected sites such as soil or inside plant tissues.

In addition to augmentative use of nematodes, some species are specific enough for use as introduced agents against immigrant pests. Examples include the phaenopsitylenchid *Beddingia siricidicola* (Bedding), that was introduced into Australia and successfully controlled the European wood wasp *Sirex noctilio* (Fabricius) (Poinar 1986), and the steinernematid *Steinernema scapterisci* Nguyen and Smart that was introduced to Florida to control immigrant mole crickets (Parkman et al. 1993).

Weed-Attacking Herbivores and Pathogens

Herbivores intentionally released for weed control have been primarily insects due to their high degree of host specialization and rapid rates of multiplication (Fig. 2.5) (Andres et al. 1976). Within the Insecta, most releases have been either of beetles (Coleoptera) or moths and butterflies (Lepidoptera) (Chapter 5, see also Julien 1992). The families Chrysomelidae, Curculionidae, Cerambycidae, Pyralidae, Dactylopiidae, and Tephritidae have been used most frequently. In addition to insects, some mites in the Eriophyidae and, more recently, Tetranychidae (Hill et al. 1991) have been employed.

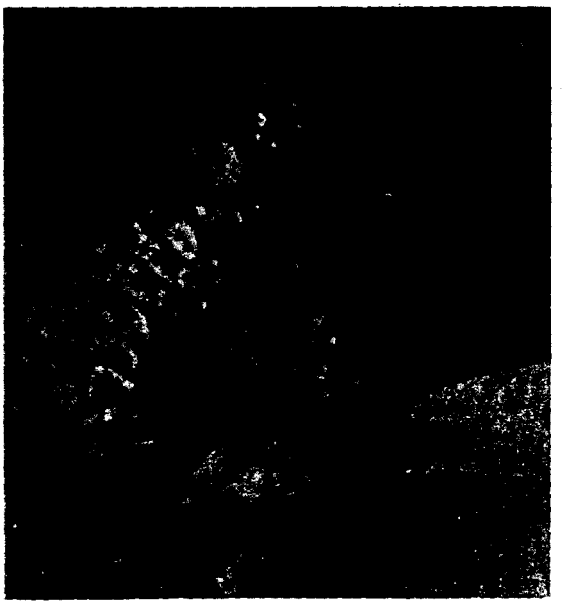

Figure 2.5 Larva of the chrysomelid *Chrysolina sp.*, an invertebrate weed control agent. (Photography courtesy of USAD/ARS.)

In contrast to highly specialized insects targeted at single weed species, some fish have been used as generalist herbivores to control weed complexes in certain habitats, often artificial ones such as canals and water tanks. Among these fish have been several species in the families Cyprinidae, Cichlidae, and Osphronemidae (Julien 1992).

Until recently, few plant pathogens had been used as biological weed control agents (Wilson 1969). Since 1970, however, precedents have been set in both the introduction of new pathogens for control of adventive weeds and the formulation of pathogens for use as biological herbicides. For example, the autoecious rust fungus *Puccinia chondrillina* was introduced in 1971 to Australia from Europe where it controlled narrow-leaf populations of skeletonweed, *Chondrilla juncea* (Hasan and Wapshere 1973; Hasan 1981; Watson 1991). The indigenous fungal pathogen *Colletotrichum gloeosporioides* (Penzig) and Saccardo in Penzig, which attacks the native weed northern jointvetch, *Aeschynomene virginica* (Linnaeus) Brittan, Sterns and Poggenberg, was marketed as a biological herbicide in 1982 (Templeton et al. 1984). TeBeest (1991) reviews the use of pathogens as weed control agents.

Biological Control Agents Suppressing Plant Pathogens

Mechanisms leading to biological control of plant pathogens are complex and may occur by many routes. Consequently, several kinds of agents may be involved (Snyder et al. 1976; Cook and Baker 1983; Campbell 1989; Stirling 1991). Plant pathogens may be suppressed by events that reduce the potential inoculum level of the pathogen in the environment, or by competitive or parasitic interactions among organisms, or by competition for limiting resources. Competition may occur at any point in the infection cycle, from its initiation outside the host, through invasion and growth inside the plant. Some fungi such as species of *Trichoderma* exhibit mycoparasitism, attacking and killing hyphae or other parts of pathogenic fungi (Fig. 2.6). Some bacteria, actinomycetes, and fungi produce chemicals (antibiotics) that actively repress the growth of other species, including pathogens. Some fungi repress the growth of pathogens by outcompeting them for key resources such as minerals, nutrients, oxygen, or water, either at or away from the site of initial infection.

PRINCIPAL BIOLOGICAL CONTROL METHODS

All biological control involves the use, in some manner, of natural enemies to suppress pest population densities to levels lower than they would otherwise be. Three major methods exist for the use of natural enemies: conservation, introduction, and augmentation. Of these, introduction has been the method that has successfully solved the greatest number of insect and weed problems; but has rarely been used to control plant pathogens.

Conservation

Human activities can greatly influence the extent to which natural enemies are able to realize their potential to suppress pests. Conservation as a form of biological control is the study and application of such influences. This approach seeks to identify and rectify negative influences that suppress natural enemies and to enhance agricultural fields (or other sites) as habitats for natural enemies. Fundamental to biological control through conservation is the assumption that species of natural enemies already exist locally that have the potential to effectively suppress the pest if given an opportunity to do so. This assumption is likely to be true for many (but not all) indigenous pests but for adventive pests is usually true only if appropriate natural enemy introductions have already been made.

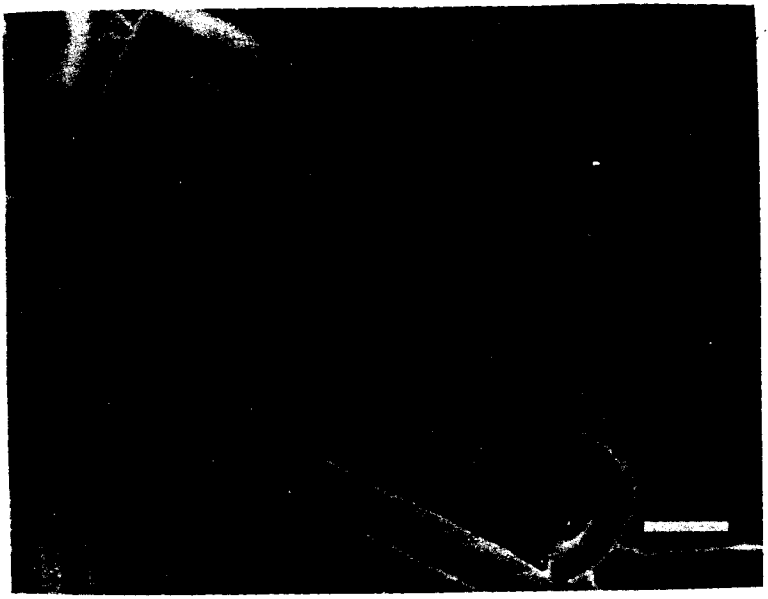

Figure 2.6 Hyphae of the mycoparasitic fungus *Trichoderma* sp. are shown here coiled around the thicker hypha of the fungal root pathogen *Rhizoctonia* sp. Scale bar is 10 μm. (Photograph courtesy of A. Beckett, Department of Botany, University of Bristol.)

Foremost among the negative influences that harm natural enemies are chemical pesticides, especially those with broad spectrum and long residual action (Croft 1990). Other negative forces that affect some kinds of natural enemies are dust on foliage (DeBach 1958; Flaherty and Huffaker 1970) and ants that actively defend such insects as aphids or scales and harvest the honeydew they excrete (DeBach and Huffaker 1971). Other practices that may be negative influences for some natural enemies include date and manner of turning soil, destruction of crop residues, size and placement of crop patches, and removal of natural enemy overwintering sites such as hedgerows.

Positive forms of conservation include efforts to enhance the requisites that natural enemies need to flourish in a system. Such efforts may involve creating or maintaining physical refuges, alternative hosts, or sources of carbohydrates, moderating physical conditions through the use of ground covers, provision of sheltering sites, or use of strip-harvesting methods (van den Bosch et al. 1967; Hance and Gregoire-Wibo 1987; Heidger and Nentwig 1989).

Conservation methods depend primarily on knowing how effective a particular conservation practice is under local conditions. This may require considerable local research and field trials. Once such information is available the method often can be implemented on individual farms independently of the actions of the community as a whole.

Introduction of New Natural Enemy Species

In many areas, adventive ("exotic" or "introduced") species comprise a high proportion of the major pests (Sailer 1978; Van Driesche and Carey 1987). In the United States, for example,

immigrant arthropods make up only 2% of the total arthropod fauna, but account for 35% of the most important 700 pest species (Knutson et al. 1990). For such nonnative pests, conservation is likely to be inadequate because sufficiently effective natural enemies will be absent. In such cases, introducing new natural enemy species that are effective against the pest is absolutely essential, and is an approach that historically has been extremely successful. The needed natural enemy species are most often obtained by examining populations of the pest in its native homeland and observing which species of natural enemies attack it there. These natural enemies are then collected, shipped to the country which the pest has invaded and, after being subjected to appropriate quarantine and testing to ensure safety, are released and established. Introduction as a method of biological control has provided complete or partial control of more than 200 pest species (DeBach 1964a; Laing and Hamai 1976; Clausen 1978; Goeden 1978; Julien 1992; Greathead and Greathead 1992). Introduction has a major advantage over other forms of biological control in that it is self-maintaining and, thus, less expensive over the long term. After new natural enemies have been obtained, conservation measures may be required for the new species to be fully effective. To be conducted safely, introduction biological control programs require a high degree of scientific skill and, therefore, typically are conducted by public institutions, using public resources to solve problems for the common good.

Augmentation

When natural enemies are missing (glasshouses, mushroom houses), or late to arrive at new plantings (some row crops), or simply too scarce to provide control, their numbers may be increased through releases (Ridgway and Vinson 1977; King et al. 1985). Such an approach is termed augmentation. Augmentation covers several situations. **Inoculative releases** are those in which small numbers of a natural enemy are introduced early in the crop cycle with the expectation that they will reproduce in the crop and their offspring will continue to provide pest control for an extended period of time. **Inundation**, or **mass-release**, is used when insufficient reproduction of the released natural enemies is likely to occur, and pest control will be achieved exclusively by the released individuals themselves. Inundation with nematodes and pathogens is distinct from mass release of parasitoids and predators only in that pathogens and nematodes resemble pesticides in their packaging, handling, storage, and application methods. However, when pathogen products (such as toxins) are used in lieu of pathogens themselves, it more closely resembles chemical control than biological control, because populations of live organisms are not involved.

Augmentation may be directed against indigenous or adventive pests. The principal limitations on the method have to do with cost, quality, and field effectiveness of the reared organisms. The cost of rearing natural enemies may limit the use of the method either to situations where the natural enemies are inexpensive to rear or to circumstances where crops have relatively high cash value and less expensive alternatives are not available. Only in such circumstances can private companies recoup their production costs and compete economically with alternative methods. Somewhat broader applications are possible when public institutions rear the necessary natural enemies. In both cases, production of high quality natural enemies is essential, as are prior research studies assessing the degree of pest control provided by the reared agent under field conditions.

Bacillus popilliae and related species are pathogens of scarab larvae and are of interest as potential agents against turf and pasture pests, such as the Japanese beetle, *Popillia japonica*. Infections by this pathogen are referred to as "milky spore disease" because of the milky color of the hemolymph of diseased hosts (Fig. 4.1). *Bacillus popilliae* does not produce spores (the stage used in pesticide formulations) abundantly *in vitro* (Lüthy 1986). The requirement for living insect hosts for spore production has made production of this pathogen costly and limited its commercial use. *Bacillus popillae* is difficult to separate from closely related species. Species determinations are based on the morphology of the sporulated cell and the host spectrum. A review of the taxonomy of this group of species is given by Splittstoesser and Kawanishi (1981).

Bacillus sphaericus occurs as different strains, some of which are pathogenic to insects. Singer (1990) provides a table of strains and a historical review of studies on *Bacillus sphaericus*, and the properties of this bacterium and its toxins have been reviewed by Baumann et al. (1991). Pathogenic strains have been isolated in a number of countries and are amenable to commercial production because the organism can be produced via fermentation. Insecticidal activity is due to crytalline toxins associated with the cell wall. These are released by digestion after the host insect has consumed bacterial spores. Bacterial reproduction occurs saprophytically after the host has died (Singer 1987). The host range of this bacterium is very narrow and appears to be limited to some genera of mosquitoes (Wraight et al. 1981; Singer 1987; see Osborne et al. 1990 for a review). The molecular biology of genes coding for the toxin has been explored by Baumann et al. (1987, 1988), and the gene has been expressed in *Bacillus subtilis* (Ehrenberg) Cohn (Baumann and Baumann 1989).

Bacillus thuringiensis is the most extensively marketed bacterial pathogen of arthropods (Fig. 4.2). Comprehensive information on this important species has been assembled by Entwistle et al. (1993), and the history of research on this species is summarized by Beegle and Yamamoto (1992). Watkinson (1994) and a series of companion articles synthesize current knowledge on many topics related to *B. thuringiensis*. Many isolates have been collected and classified into 34 serovars based on flagellar antigens by de Barjac and Frachon (1990). Most serovars of this species affect Lepidoptera and have been used to suppress a number of fruit, vegetable, and forest pests. More recently, serovars or isolates have been encountered that affect insects in other orders, expanding the range of targets for *B. thuringiensis* products. The serovar *israelensis* is effective against dipteran larvae, including mosquitoes, blackflies, sewage flies, and fungus gnats, among others (de Barjac 1978; van Essen and Hembree 1980; Mulla et al. 1982). An isolate, *tenebrionis* (serovar *morrisoni*), has been found that is effective against Coleoptera in the family Chrysomelidae and has been developed as a commercial control

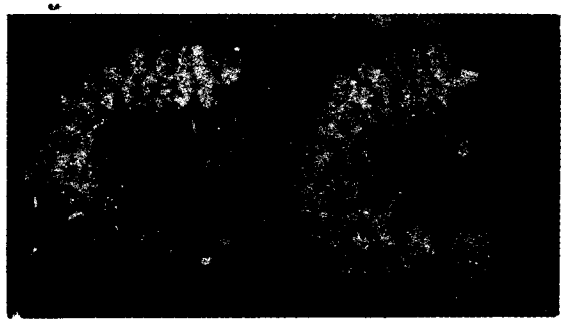

Figure 4.1. *Bacillus popilliae* Dutky infections in scarabaeid larvae are termed "milky spore diseases" because host hemolymph becomes cloudy, as seen here in the infected host on the right, compared to the healthy one on the left. (Photograph courtesy of R. Milner.)

CHAPTER
4

PATHOGENS AND NEMATODES OF ARTHROPODS AND PATHOGENS OF VERTEBRATES

PATHOGENS OF PEST ARTHROPODS

More than 1500 species of pathogens are known to attack arthropods (Miller et al. 1983). These include bacteria, viruses, fungi, protozoa, and nematodes. Historical developments in the recognition and understanding of pathogens of arthropods are summarized by Steinhaus (1956) and are briefly reviewed in Chapter 1. Detailed treatment of the taxonomy and biology of arthropod pathogens is given by Tanada and Kaya (1993) and, for nematodes, Kaya (1993). Much of the research on these pathogens is directed at development of methods for their culture, mass production, formulation, and use through augmentation as biological pesticides (see Chapter 11).

Bacterial Pathogens of Arthropods

The taxonomy of bacteria is outlined in Bergy's Manual of Determinative Bacteriology and Bergy's Manual of Systematic Bacteriology (Buchanan and Gibbons 1974; Holt 1984, 1986, 1989ab). For a description of the general life cycle of entomopathogenic bacteria and symptoms of bacterial disease in insects, see Chapter 16. While many species of bacteria that cause disease in arthropods are certainly still undiscovered, those studied to date can be divided into two broad groups. One group is the non-spore-forming species in such families as Pseudomonadaceae (*Pseudomonas*) and Enterobacteriaceae (e.g., *Aerobacter*, *Cloaca*, *Serratia*) that are found in soil and also occur in the gut of arthropods. These bacteria can become pathogenic, particularly in conjunction with other pathogens or when the host is physiologically stressed. Some of these organisms can infect mammals and so have not been pursued in most cases as commercial pest control agents against arthropods. The only exception to this is *Serratia entomophila* Grimmont, Jackson, Ageron, and Noonan which is marketed in New Zealand for control of the pasture pest scarab *Costelytra zealandica* (White) under the name Invadet (Jackson 1990). The second group are spore-forming bacteria in the Bacillaceae (*Bacillus*, *Clostridium*), of which most attention has been focused on several species of *Bacillus*, including *B. popilliae*, *B. sphaericus*, and *B. thuringiensis* (Cherwonogrodsky 1980; Lüthy 1986; Tanada and Kaya 1993).

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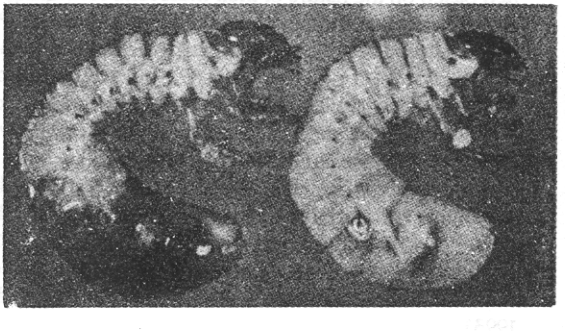

Figure 4.1. *Bacillus popilliae* Dutky infections in scarabaeid larvae are termed "milky spore diseases" because host hemolymph becomes cloudy, as seen here in the infected host on the right, compared to the healthy one on the left. (Photograph courtesy of R. Milner.)

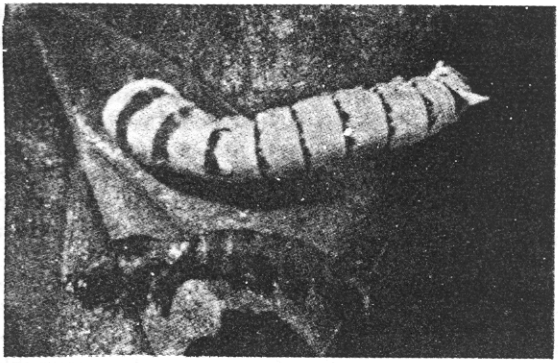

Figure 4.2. Larva (bottom) of the silkworm, *Bombyx mori* (Linnaeus), killed by *Bacillus thuringiensis* Berliner, a spore-forming bacterium that produces toxic proteins. (Photograph courtesy of H.K. Kaya.)

for the vegetable pest *Leptinotarsa decemlineata* and other species (Herrnstadt et al. 1987). Some isolates of *B. thuringiensis* are effective against nonarthropods, including plant-parasitic nematodes and snails (Feitelson et al. 1992; Osman et al. 1992). Several methods are available for detection of new strains of *B. thuringiensis*, including characterization of flagellar antigens, extrachromosomal plasmids, morphology of crystalline inclusion bodies, and gene sequences (Carlton et al. 1990).

Death of hosts which ingest *B. thuringiensis* cells results from the combined effects of poisoning by toxins and bacterial multiplication. Most commercial products of *B. thuringiensis* contain living spores, but some, such as the tablet formulation of the toxin from *B. thuringiensis israelensis*, do not (Becker et al. 1991).

Genes for *B. thuringiensis* toxins have been isolated and incorporated into other bacteria for the commercial production of toxins and also into crop plants, causing the plants to produce toxins at levels adequate to provide pest protection (Vaeck et al. 1987). Populations of pests resistant to the toxins of *B. thuringiensis* have developed in some species of insects subjected to frequent applications of the material (Tabashnik et al. 1990).

Viral Pathogens of Arthropods

More than 1270 insect-virus associations had been recognized by 1981 (Martignoni and Iwai 1981), most (70%) involving Lepidoptera as hosts. See Chapter 16 for a description of a generalized virus infection and its symptoms. Viruses pathogenic in arthropods may be classified based on the nature of their molecular form (single- or double-stranded DNA or RNA) and other characteristics into 16 families: Baculoviridae, Polydnaviridae, Poxviridae, Ascoviridae, Iridoviridae (all five families of which are double-stranded DNA forms), Parvoviridae (a single-stranded DNA form), Reoviridae, Nodaviridae, Picornaviridae, Tetraviridae, Birnaviridae, Rhabdoviridae, Caliciviridae, Togaviridae, Bunyaviridae, and Flaviviridae (all of which are RNA viruses, all single-stranded forms except the Reoviridae and Birnaviridae) (Entwistle 1983; Moore et al. 1987; Tanada and Kaya 1993). Moore et al. (1987) and Tanada and Kaya (1993) provide descriptions of many of these families. Viral nomenclature is different from that of zoological and botanical nomenclature and is set forth in the Fifth Report of the International Committee on Taxonomy of Viruses (Francki et al. 1991). This system has no hierarchical levels above family and does not imply phylogenetic relationships. Phylogenetic relationships between selected baculoviruses have been investigated using DNA sequencing (Zanotto et al. 1993).

The Baculoviridae and Tetraviridae are limited in their host ranges to arthropods. Most other families contain viruses associated with mammals or other nonarthropod groups and thus are of less interest as potential insect control agents because of concerns over possible effects on mammals. All viruses are obligate parasites of living cells. Virus production, therefore, can be achieved only in living hosts or cell cultures.

Baculoviridae have been of greatest interest as potential biological control agents. This family includes the nuclear polyhedrosis viruses (Fig. 4.3ab), the granulosis viruses (Fig. 4.3cd), and, formerly, the nonoccluded viruses (e.g., *Oryctes* virus, now unclassified at the family level) (Entwistle 1983; Tanada and Kaya 1993). Bilimoria (1986) discusses the taxonomy and identification of members of the Baculoviridae. The biology of baculoviruses is discussed by Granados and Federici (1986). Blissard and Rohrmann (1990) present information on the molecular aspects of the baculovirus infection cycle and the organization of the baculovirus genome. Fuxa (1990a) lists 28 cases in which baculoviruses have been used in attempts to suppress insect pests. Of these, 15 cases involve introduction of a virus followed by its permanent establishment and 13 cases were based on augmentative use. Most of these cases have involved nuclear polyhedrosis viruses, four being granulosis viruses and one a nonoccluded virus. Some of the viruses that have been employed successfully for biological control are: a nuclear polyhedrosis virus of *Gilpinia hercyniae* (Hartig), a forest sawfly in Canada which was permanently suppressed after the accidental introduction of the virus (Balch and Bird 1944); the

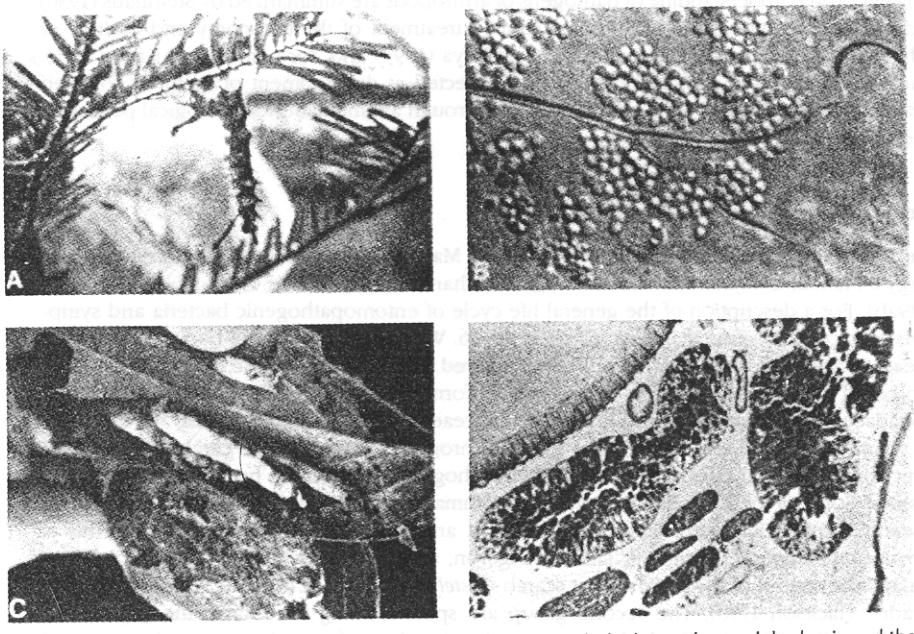

Figure 4.3. Important members of the family Baculoviridae include the nuclear polyhedrosis and the granulosis viruses: (A) larva of Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), killed by nuclear polyhedrosis virus; (B) micrograph of nuclear polyhedrosis virus in hypodermis tissue of beet armyworm, *Spodoptera exigua* (Hübner); (C) larva of a geometrid (*Sabulodes* sp.) killed by granulosis virus; (D) micrograph of granulosis virus in fat body tissue of fall armyworm, *Spodoptera frugiperda* (J.E. Smith). (Photographs courtesy of C.G. Thompson [A], J.V. Maddox [B], B. Federici [C], and J.J. Hamm [D].)

nonoccluded virus of *Oryctes rhinoceros*, a coleopteran pest of coconut palms which was suppressed for up to 3.5 years following deliberate introduction of the virus (Zelazny et al. 1990; Mohan and Pillai 1993); and the nuclear polyhedrosis virus of *Anticarsia gemmatalis* Hübner, a lepidopteran defoliator of soybeans (*Glycine max* [Linnaeus] Merrill) in Brazil, which is managed by augmentative applications of virus (Moscardi 1983).

Another family of viruses that is important to biological control is the Polydnaviridae, which are mutualistic associates of parasitoids in the Ichneumonoidea (Fleming and Fleming 1992). These viruses replicate only in the ovarial calyx epithelium of their wasp hosts, but cause no noticeable pathology. They are injected into the wasp's lepidopteran hosts during oviposition by the wasp. They are important to successful parasitism because they help suppress the defensive response of the host by depressing its immune system (see Chapter 15; see also Fleming and Fleming 1992; Tanada and Kaya 1993).

Fungal Pathogens of Arthropods

The fungi have generally been regarded as comprising the Division Eumycota within the plant kingdom (Ainsworth 1971; Ainsworth et al. 1973). In Chapter 16 a generalized fungal life cycle and disease symptoms are described. Entomopathogenic species are found in five subdivisions: Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina, and Deuteromycotina. Brady (1981) and McCoy et al. (1988) provide overviews of the taxonomy of fungal groups known to attack arthropods. Fungal biology, pathology, and use for pest control is covered by Steinhaus (1963), Müller-Kögler (1965), Ferron (1978), Burges (1981a), McCoy et al. (1988), and Tanada and Kaya (1993). Burge (1988) covers a broad range of topics related to biological control uses of fungi, both through introduction and augmentation. The role of naturally-occurring entomopathogenic fungi in the population dynamics of insects is reviewed by Carruthers and Hural (1990).

While over 400 species of entomopathogenic fungi have been recognized (Hall and Papierok 1982), most attention with respect to potential as biological control agents has been focused on about 20 species (Zimmermann 1986). Most of these are included in 12 genera: *Lagenidium*, *Entomophaga*, *Neozygites*, *Entomophthora*, *Erynia*, *Aschersonia*, *Verticillium*, *Nomuraea*, *Hirsutella*, *Metarhizium*, *Beauveria*, and *Paecilomyces* (Roberts and Wraight 1986). Some important entomophagous genera in these subdivisions are as follows:

Mastigomycotina. This subdivision includes two classes with entomopathogenic species. In the Chytridiomycetes are found *Coelomomyces* spp. which attack mosquito larvae. Members of this genus have complicated life cycles with a required alternate copepod or ostracod host. Species in this group have not been used commercially for insect control. In the Oomycetes is the genus *Lagenidium*, whose members are also pathogens of mosquito larvae but which require only one host, the mosquito. *Lagenidium giganteum* Couch (Fig. 4.4) is registered as a pest control product in the United States.

Zygomycota. The family Entomophthoraceae includes important insect pathogens, such as species of *Entomophthora* (Fig. 4.5), *Entomophaga*, *Erynia*, and *Neozygites*. Information on the taxonomy and biology of this family is found in MacLeod (1963), Waterhouse (1973), Remaudière and Keller (1980), Humber (1981), Ben-Ze'ev et al. (1981), and Wolf (1988). The taxonomic organization of the group has undergone extensive restructuring since the 1960s, which is reviewed by McCoy et al. (1988) and Humber (1990). Hosts of Entomophthoraceae include various Lepidoptera, Coleoptera, aphids, and mites. Spore production for many of the

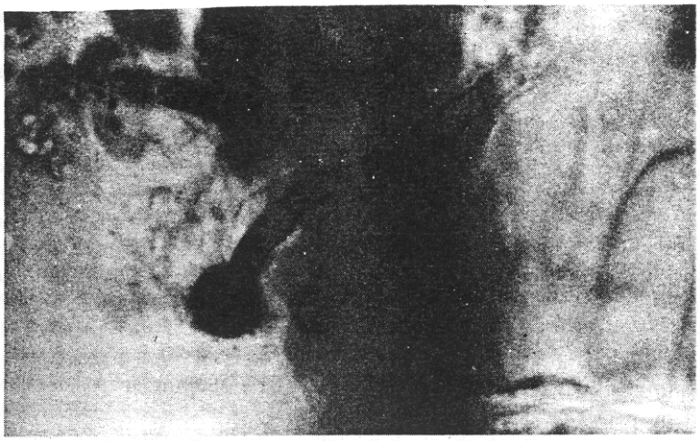

Figure 4.4. Encysted zoospore of *Lagenideum giganteum* Couch with a germ tube penetrating its host, a larva of the culicid *Culex pipiens* Linnaeus. (Photograph courtesy of A.J. Domnas.)

species in these groups has not been obtained independent of living hosts, a factor limiting their mass production and their use as agents of augmentative biological control. Spore production on nonliving media, however, has been achieved for some species such as *Zoophthora radicans*, a species which has been considered for use as a mycoinsecticide (Soper 1985). Recent advances in the use of this group are discussed by Wilding (1990).

Ascomycotina. This subdivision includes *Ascosphaera apis* (Maasen *ex* Claussen) Olive and Spiltoir, the causative agent of chalk brood disease of honey bees (*Apis melifera* Linnaeus). It also includes species of *Cordyceps* that are well-known historically because of the showy nature of the fruiting bodies that grow from infected hosts. No member of this group is of major importance as a pest control agent.

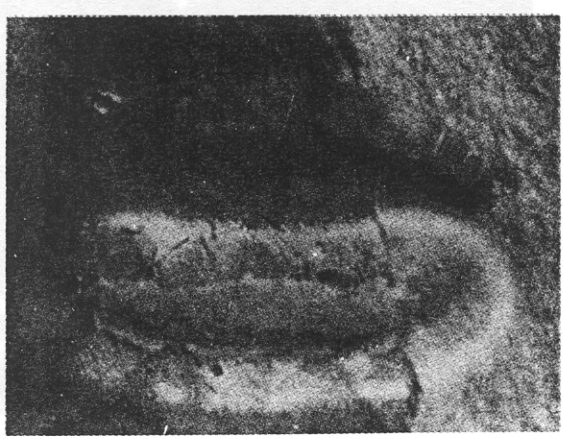

Figure 4.5. Diamond-back moth (*Plutella xylostella* [Linnaeus]) larva killed by *Entomophthora* sp., showing "halo" of conidia. (Photograph courtesy of C.W. McCoy.)

Basidiomycotina. The entomophagous forms within this subdivision are principally members of the genus *Septobasidium*, which are associated with various species of scale insects. Scale-fungus relations are often symbiotic, but verge into parasitism in some species. Entomopathogenic species are also found in the genera *Filobasidiella* and *Uredinella*.

Deuteromycotina. This group (also called the Imperfect Fungi) is comprised of species known only from asexual forms. It is a heterogenous group, the true placement of whose members can only be determined if sexual forms are eventually encountered and correctly associated with the asexual form. The majority of genera of entomopathogenic fungi of importance to pest control are found in this group. These include the genera: *Hirsutella*, in which *H. thompsonii* Fisher is a well-studied pathogen of eriophyid rust mites (McCoy 1981); *Beauveria*, a genus with a wide host range, which includes *B. bassiana* (de Hoog 1972), a species with important biological control potential; *Metarhizium*, a group with a wide host range; *Nomuraea*; *Paecilomyces*, members of which attack important species of soil insects and an important species of whitefly; *Verticillium*, species of which attack whiteflies and aphids; and *Aschersonia*, some of which attack whiteflies and scales (Fig. 4.6). Information on the biologies of many of these fungal pathogens can be found in Ferron (1978), McCoy et al. (1988), and Carruthers and Hural (1990). Information on the commercial development of such fungi as pest control products is given by Roberts and Wraight (1986) and, with special reference to pests in tropical areas, by Maniania (1991).

Protozoan Pathogens of Arthropods

The higher classification of protozoa has changed dramatically in recent years. In 1980, protozoa were raised to a subkingdom, with seven phyla. More recently, Margulis et al. (1990) have combined the protozoa, together with other groups, into the kingdom Protoctista. Entomopathogenic protozoa occur in six phyla: Zoomastigina (flagellates), Rhizopoda (amoebas), Apicomplexa (gregarines, eugregarines, neogregarines, and coccidia), Microspora (micro-

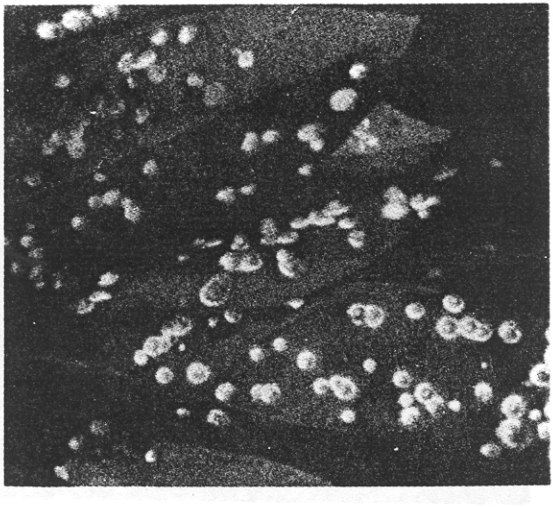

Figure 4.6. Citrus whitefly (*Dialeurodes citri* [Ashmead]) killed by the fungus *Aschersonia aleyrodis* Webber. (Photograph courtesy of C.W. McCoy.)

sporidia), Haplosporidia, and Ciliophora (ciliates). For a general description of the life cycle of entomopathogenic protozoa see Chapter 16. For detailed treatments of the biologies of individual groups see Tanada and Kaya (1993). Of these, most forms of interest as biological control agents are microsporidia.

Zoomastigina (Flagellates). The entomopathogenic trypanosomatids are found principally in the genera *Herpetomonas*, *Crithidia*, and *Blastocrithidia*. They occur most frequently in Diptera and Hemiptera and most often infect the Malpighian tubules or the gut. They have not been employed for biological control purposes. Some typanosomes in insects also cause diseases in vertebrates (*Leishmania* spp., *Trypanosoma* spp.).

Rhizopoda (Amoebas). The best known example in this group is *Malamoeba locustae* (King and Taylor) which infects the Malpighian tubules and midgut epithelium of grasshoppers. Infected hosts have lowered reproductive potentials (Henry 1990).

Apicomplexa (Gregarines, Eugregarines, Neogregarines, and Coccidia). An example of this group is the neogregarine *Mattesia trogodermae* Canning which infects the fat body of various stored-product beetles such as *Trogoderma* spp. Infected hosts are sluggish and survive for shorter periods. Brooks and Jackson (1990) provide a review of the eugregarines.

Microspora (Microsporidia). Microsporidia of potential importance to biological control include species of *Nosema* (Fig. 4.7), *Pleistophora*, and *Vairimorpha*. Larsson (1988) provides a guide to the identification of microsporidian genera. Microsporidia, formulated in baits, have been tested for control of locusts and *Ostrinia nubilalis*, a lepidopteran pest of maize (Henry 1990). When present in species such as coccinellids or herbivorous arthropods being imported for weed control, microsporidia and other protozoans constitute a potentially serious contaminate reducing the fitness of the beneficial insects being introduced (Kluge and Caldwell 1992).

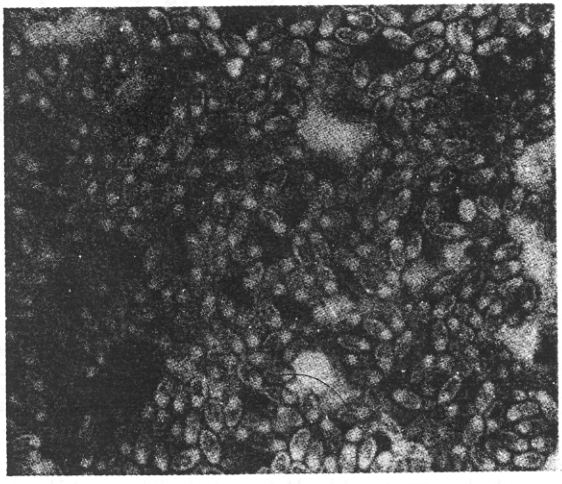

Figure 4.7. Spores of *Nosema* sp. in midgut of the cabbage looper, *Trichoplusia ni* (Hübner). (Photograph courtesy of G.L. Nordin.)

Haplosporidia. Members of this group infect the digestive tract, fat body, oenocytoids, and Malpighian tubules of insects.

Ciliophora (Ciliates). A few species of Ciliophora, such as *Lambornella clarki* Corliss and Coats, are pathogens of mosquito larvae.

Nematodes Attacking Arthropods

The classification of entomopathogenic nematodes at the family level has changed significantly in recent years. Recent treatments are given by Maggenti (1991) and Remillet and Laumond (1991). Reviews of nematodes associated with arthropods are provided by Poinar (1986), Kaya (1993), Kaya and Gaugler (1993), and Tanada and Kaya (1993). Of the 30 or more families of nematodes associated with insects, only nine have members with potential as biological control agents: Tetradonematidae, Mermithidae, Steinernematidae, Heterorhabditidae, Phaenopsitylenchidae, Iotonchiidae, Allantonematidae, Parasitylenchidae, and Sphaerulariidae. Most attention has been focused on two families, the Steinernematidae and Heterorhabditidae. These are associated with pathogenic symbiotic bacteria that enable them to rapidly kill a wide range of hosts. Members of five other families (Tetradonematidae, Mermithidae, Phaenopsitylenchidae, Iotonchiidae, and Sphaerulariidae) also merit discussion. For comments on the life cycles of entomopathogenic nematodes and symptoms of infection, see Chapter 16.

Steinernematidae and Heterorhabditidae. Several species in the genera *Steinernema* and *Heterorhabditis* have been the focus of most efforts to develop commercial uses of nematodes (Fig. 4.8ab) (Gaugler and Kaya 1990; Kaya 1993; Kaya and Gaugler 1993; Tanada and Kaya 1993). Synonomies of species names in these genera complicate the interpretation of literature citations. Ten species are recognized in *Steinernema* (Doucet and Doucet 1990) and three in *Heterorhabditis* (Poinar 1990). Kaya and Gaugler (1993) list these and discuss their nomenclature, and Smith et al. (1992) give a bibliography of research on these families.

These families have been used as commercial pest control agents because they have the following attributes (Poinar 1986):

- a wide host range
- an ability to kill the host within 48 hours
- a capacity for growth on artificial media
- a durable infective stage capable of being stored
- a lack of host resistance
- apparent safety to the environment

These nematodes invade hosts through natural openings (mouth, spiracles, anus) or wounds and penetrate into the hemocoel. Bacteria in the genera *Xenorhabdus* or *Photorhabdus*, symbiotic to the nematode but pathogenic to the host, are released into the hemocoel and quickly kill the host. The nematodes then develop saprophytically on the decomposing host tissues. Gaugler and Kaya (1990) and Kaya and Gaugler (1993) provide information on rearing and using these groups of nematodes for pest control. These nematodes work best in moist environments such as soil. Commercial markets for some strains have been established and large scale production systems developed (Kaya 1985; Gaugler and Kaya 1990).

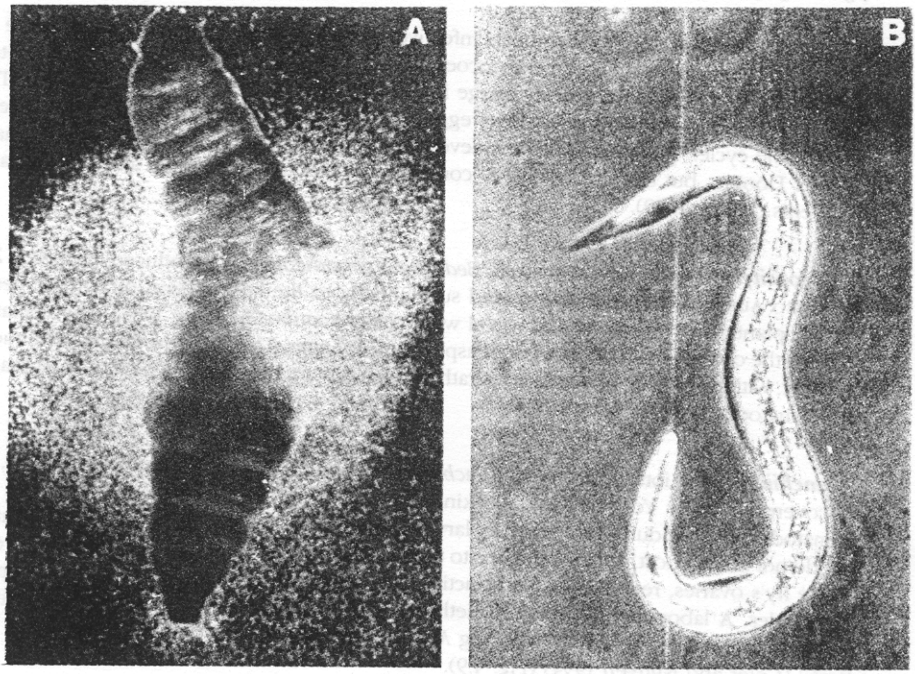

Figure 4.8. (A) Infected host larva (*Galleria mellonella* [Linnaeus]) opened to show juveniles of *Steinernema* sp. nematodes; (B) single infective-stage juvenile nematode. (Photographs courtesy of R. Gaugler.)

In addition to the augmentative use of nematodes in these two families, some species with more narrow host ranges have been imported for the control of immigrant pests; for example, *Steinernema scapterisci* was introduced into Florida in a program to provide biological control of immigrant species of *Scapteriscus* mole crickets (Parkman et al. 1993).

Tetradonematidae. *Tetradonema plicans* is a tetradonematid that infects larvae of *Sciara* spp. (Diptera: Sciaridae), which are pests in glasshouse and mushroom crops. Effective control of these root gnats was obtained by Peloquin and Platzer (1993) with applications of *T. plicans* eggs at a ratio of 10 eggs per host larva. Cultures of the nematode can be maintained on hosts reared on a composted media of sphagnum moss, shredded paper, and commercial rabbit food. Host larvae ingest eggs or, perhaps, young larvae. Nematode larvae then penetrate the gut and enter the hemocoel, where they mature and cause the death of the host. Adult nematodes mate in the host and females either exit and lay eggs outside the host or remain in the host, in which cases eggs are released into the soil as the host and female nematode decay.

Mermithidae. The nematode *Romanomermis culicivorax* Ross and Smith attacks larvae of various mosquitoes. The life cycle is divided between stages that occur within the host and

Haplosporidia. Members of this group infect the digestive tract, fat body, oenocytoids, and Malpighian tubules of insects.

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CHAPTER
6

BIOLOGICAL CONTROL AGENTS FOR PLANT PATHOGENS

INTRODUCTION

Biological control of plant pathogens is fundamentally a matter of ecological management of a community of organisms, as is all biological control. In the case of plant pathogens, however, there are two distinctions from biological control of organisms such as insects and plants. First, the ecological management occurs at the microbial level, typically in microcosms of the ecosystem such as leaf and root surfaces (Andrews 1992). Second, biological control agents include competitors, as well as parasites. While hyperparasites of plant pathogens and natural enemies of nematodes function in much the same way as do natural enemies (parasitoids) in arthropod systems (by destroying the pest organisms), competitors function by occupying and using resources in a nonpathogenic manner and in so doing exclude pathogenic organisms from colonizing plant tissues. Microbes which negatively affect pathogenic organisms are referred to as antagonists.

Diseases of roots, stems, aerial plant surfaces, flowers, and fruit are caused by a wide variety of pathogens. Because of this diversity, the antagonist species which negatively affect plant pathogens and the mechanisms by which they accomplish their beneficial action are also quite varied (definitions and examples of these mechanisms are given in Chapter 12). Their biology and taxonomic diversity is covered in some detail in several texts and reviews, including Cook and Baker (1983), Fokkema and van den Heuvel (1986), Campbell (1989), Adams (1990), and Stirling (1991). This chapter briefly introduces the antagonists of some important plant pathogens as representative of the broad taxa which are important in this field, beginning with agents affecting microbial pathogens of roots, and proceeding through pathogens of stems, leaves, flowers, and fruit. Natural enemies of plant parasitic nematodes are treated in the last section.

ROOT PATHOGENS

Root diseases are caused by a wide variety of fungi, and by some bacteria, in many crops and plant systems. Biological control agents recognized as significant in suppression of these diseases are largely antagonists which can occupy niches similar to the pathogens and either naturally or through manipulation outcompete the pathogens in these niches. Antibiotic production is also important in a few cases, as are mycoparasitism and induced resistance.

Streptomyces scabies, the causative organism of potato scab, is suppressed by naturally-

occurring populations of *Bacillus subtilis* and saprotrophic *Streptomyces* spp. Other microorganisms recognized as suppressing fungal diseases include species of *Pseudomonas* and *Bacillus*. Saprotrophic *Fusarium* fungi are able to suppress populations of pathogenic *Fusarium* spp. through competition for nutrients. There are few well-documented cases of induced resistance for soil-borne pathogens, and these are mostly of wilt diseases. Examples of organisms that induce resistance in plants to pathogens include nonpathogenic strains of *Fusarium* spp., *Verticillium* spp., and *Gaeumannomyces* spp. Mycoparasitic flora such as *Arthrobotrys* spp. (Fig. 6.1), *Coniothyrium minitans* Campbell and *Sporidesmium sclerotivorum* Uecker et al. can be added to soil against fungal diseases. *Bacillus* spp. and especially *Pseudomonas* spp. are among bacteria that have properties particularly suited to effective suppression of root-infecting pathogens in soil, such as antibiotic production and competition for Fe^{3+} ions. Mycetophagous soil amoebae have also been noted feeding on pathogenic fungi (Fig. 6.2). These amoebae generally require moist conditions in which to function, and may be important in natural control of some fungi.

STEM PATHOGENS

Stem diseases produce symptoms which include decay and cankers on forest and orchard trees, and such wilts as Dutch elm disease and chestnut blight (caused by the fungus *Cryphonectria parasitica* (Murrill) Barr of Asian origin infecting the American chestnut, *Casta-*

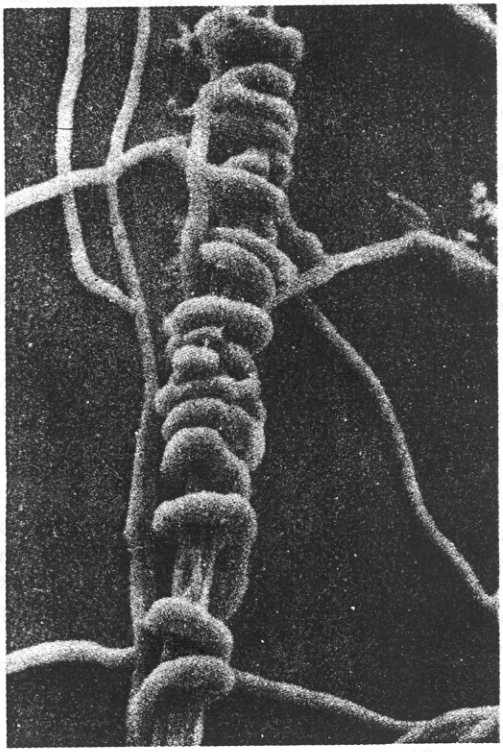

Figure 6.1. Example of a mycoparasitic fungus: hyphae of *Arthrobotrys* sp. coiled around a hypha of *Rhizoctonia* sp. that has died and collapsed. (Photograph courtesy of R. Campbell. *Biological Control of Microbial Plant Pathogens*, Cambridge University Press, with permission.)

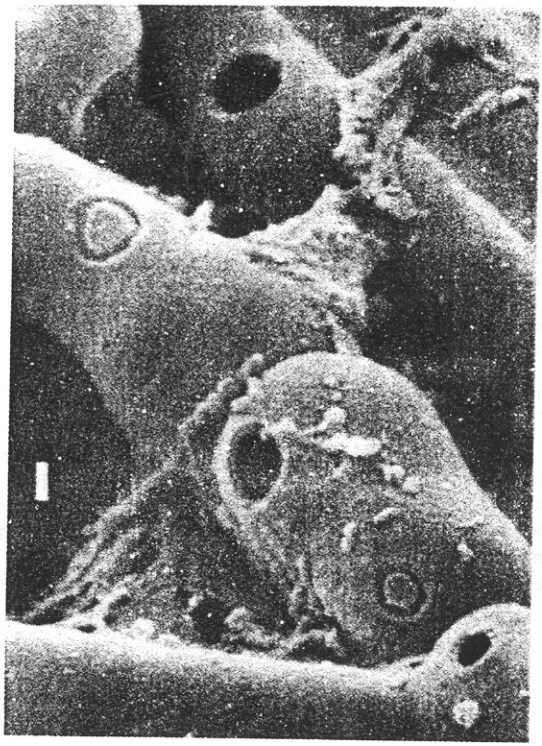

Figure 6.2. Holes in hyphae of the fungal pathogen *Gaeumannomyces graminis* (Saccardo) Arx and Olivier caused by mycetophagous amoebae. Scale bar is 1.0 μm. (Photograph courtesy R. Cook, from Homma et al. 1979. *Phytopathology* 69: 1118–22, with permission.)

nea dentata [Marsham] Borkjauser). Because the etiologies of stem diseases vary, the taxa involved in biological control also vary. In many stem diseases, the pathogen colonizes a part of the host which initially is relatively free of microorganisms, such as a pruning wound. Successful biological control in such circumstances depends on rapidly colonizing this pristine environment with a nonpathogenic antagonistic competitor. Primary among these are competitively antagonistic fungi, including saprotrophic members of the genera *Fusarium*, *Cladosporium*, *Trichoderma*, and *Phanerochaete*, and such antibiotic producing bacteria as *Bacillus subtilis* and *Agrobacterium* spp. In the case of chestnut blight, hypovirulent strains of the pathogen itself are crucial in bringing about biological control. In this case, hypovirulence is transmitted cytoplasmically to virulent strains already infecting trees, and disease symptoms decline and disappear.

LEAF PATHOGENS

The growth of microorganisms on leaves is normally severely restricted by environmental factors (Fig. 6.3). Nutrient levels generally are low on leaf surfaces, and microclimate variables, especially leaf surface moisture, temperature, and irradiation, are often unfavorable for microbial development. In temperate climates and in arid tropical regions, water will be intermittent on leaf surfaces, but may be continually present in humid tropical regions. Temperatures on leaf surfaces exposed to direct radiation may rise to several degrees above ambient. The result

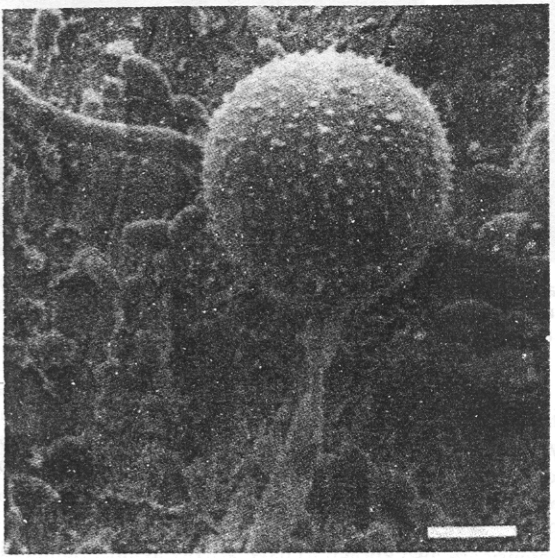

Figure 6.3. A rust spore (*Uromyces vicia-fabae* Persoon: Schröter) together with numerous yeast cells on a leaf surface; this is an unusually dense flora for a temperate leaf surface. (Photographs courtesy of A. Beckett, from Campbell, R. 1989. *Biological Control of Microbial Plant Pathogens*, Cambridge Univeristy Press, with permission.) Scale bar is 10 μm.

of such variation is that microbial floral development on leaf surfaces varies from general scarcity in temperate climates to more extensive microbial films in tropical rain forests (Campbell 1989).

The microbes most frequently recorded as saprotrophs on surfaces of crop plants in temperate conditions and, consequently, the species which are candidates as antagonists of pathogens, include the fungi *Aureobasidium pullulans* (de Bary) Arnaud, *Cladosporium* spp., and such yeasts as *Cryptococcus* spp. and *Sporobolomyces* spp. Beneficial bacteria in the phyllosphere include members of such genera as *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Chromobacterium*, and *Klebsiella*. These lists, based on microbial surveys, usually give no indication of activity of the organisms, but this information can be obtained from experimental studies. For example, early studies on control of botrytis rot in lettuce (Wood 1951) indicated that several organisms were successful in suppressing the disease when sprayed on lettuce (*Lactuca sativa* Linnaeus) plants, among them *Pseudomonas* sp., *Streptomyces* sp., *Trichoderma viride* Persoon: Fries, and *Fusarium* sp. Similar studies show varying degrees of effectiveness in other cropping systems (Peng and Sutton 1991; Sutton and Peng 1993a,b; Zhang et al. 1994). The microbial composition and biological activity of phylloplane microbes can vary with season, position on the top or bottom of the leaf and on location in the plant canopy, depending on the degree of exposure relative to prevailing winds and rain (Campbell 1989).

Biological control of the black-crust pathogen (*Phyllachora huberi* Hennings) on rubber tree (*Hevea brasiliensis* Müller Argoviensis) foliage is accomplished by the hyperparasites *Cylindrosporium concentricum* Greville and *Dicyma pulvinata* (Berkeley and Curtis) Arx (Junqueira and Gasparotto 1991). Botrytis leaf spot in onion (*Allium cepa* Linneaus) was suppressed by *Gliocladium roseum* Link: Bainier (Sutton and Peng 1993a). Other examples include control of powdery mildews, other botrytis rots, and turfgrass diseases (Sutton and Peng 1993a).

Nonpathogenic species of the fungal genus *Colletotrichum* (Kúc 1981; Dean and Kúc 1986)

can be used to induce resistance in cucumbers against pathogenic species of the same genus. Inoculation with a nonpathogenic strain of a virus confers protection to plants from pathogenic strains in many diseases. The bacterium *Bdellovibrio bacteriovorus* Stolp and Starr is a parasite of pathogenic bacteria. Finally, there are numerous parasitic fungi which attack pathogenic fungi (Kranz 1981). Among those which have been studied in detail, principally as agents against leaf rusts and mildews, are *Sphaerellopsis filum* (Bivona-Bernardi ex Fries) Sutton, *Verticillium lecanii* (Zimmerman) Viegas, and *Ampelomyces quisqualis* Cesati ex Schlechtendal.

FLOWER AND FRUIT PATHOGENS

Flowers are ephemeral structures and as such have limited opportunity to become infected. One major disease of flowers which has received attention is fire blight of rosaceous plants, caused by the bacterium *Erwinia amylovora* (Burril) Winslow et al. Biological suppression of the disease has been achieved through use of the nonpathogenic species *Erwinia herbicola* (Lohnis) Dye (Beer et al. 1984; Lindow 1985b), sometimes in combination with *Pseudomonas syringae* van Hall (Fig. 6.4). *Erwinia herbicola* was used successful by spraying aqueous suspensions of it onto the flowers just before the time of potential infection (Campbell 1989). The mode of action is primarily competitive exclusion, with the antagonist competing with the pathogen for a growth-limiting resource and possibly other effects such as induced cessation of nectar secretion or accumulation of a host toxin (Wilson and Lindow 1993a).

The fruit diseases addressable through biological control include diseases of fruit on the plant and post-harvest diseases. One of the first systems developed was against *Botrytis cinerea* Persoon: Fries in vineyards, where sprays with spore suspensions of the antagonist *Tricho-*

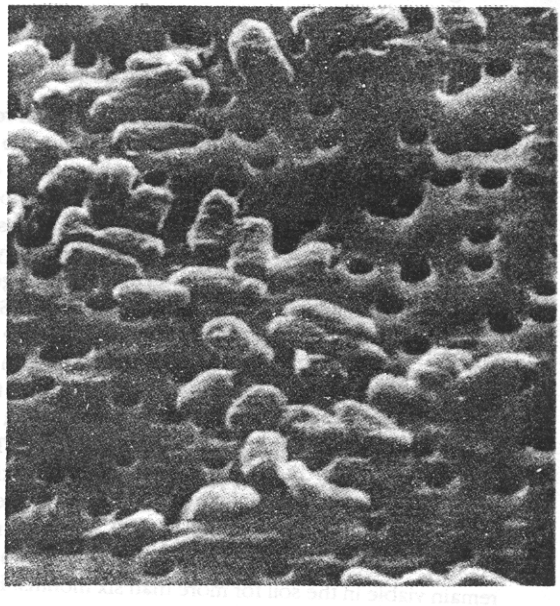

Figure 6.4. The bacterium *Pseudomonas syringae* van Hall, used as a biological control agent of fire blight and other diseases, shown here collected on a millipore filter surface. (Photograph courtesy of S. Lindow.)

derma harzianum Rifai were effective in suppressing disease incidence. Several organisms, including *Gliocladium roseum*, *Penicillium* sp., *Trichoderma viride*, and *Colletotrichum gloeosporioides* were as effective as fungicides in suppressing *B. cinerea* on strawberries (Peng and Sutton 1991). A number of other examples also have been reported (Sutton and Peng 1993a).

Post-harvest diseases, which can be responsible for 10–50% loss of produce (Wilson and Wisniewski 1989; Jeffries and Jeger 1990), have received considerable attention. Numerous reports deal with suppression of post-harvest disease in fruit crops (Campbell 1989; Wilson and Wisniewski 1989; Jeffries and Jeger 1990) by such organisms as species of *Penicillium*, *Bacillus*, *Trichoderma*, *Debaryomyces*, and *Pseudomonas*. The mode of action of many of these is generally antagonism, often through the production of antibiotics which reduce the longevity and germination of spores of pathogens. Others appear to suppress pathogen growth through nutritional competition or induction of host resistance (Wilson and Wisniewski 1989). Post-harvest rots include major diseases caused by *Botrytis cinerea*, *Rhizopus* spp., and other fungi in several crops. Competitive and parasitic fungi, including *Trichoderma* spp., *Cladosporium herbarum* (Persoon: Fries) Link and *Penicillium* spp., give control as good as commercial fungicides. *Enterobacter cloacae* (Jordan) Hormaeche and Eduards reduces rots by *Rhizopus* spp., but there are hesitations about its use on uncooked food products.

PLANT-PARASITIC NEMATODES

Plant-parasitic nematodes inhabit many soils and attack the roots of plants. They are affected by a range of natural enemies, including bacteria, nematophagous fungi, and predacious nematodes and arthropods. There is some limited evidence for virus association with nematodes (Loewenberg et al. 1959), but the etiology of these viruses is not well-known (Stirling 1991). The biologies of natural enemies of nematodes have been reviewed by Sayre and Walter (1991) and Stirling (1991).

Bacteria Affecting Plant-Parasitic Nematodes

Several bacterial diseases of nematodes have been reported (Saxena and Mukerji 1988); other bacteria produce compounds that are detrimental to plant-parasitic nematodes (Stirling 1991). The most widely studied of the bacterial pathogens of nematodes are in the genus *Pasteuria*. Early work was focused on *Pasteuria penetrans* (Thorne) Sayre and Starr *sensu stricto* Starr and Sayre (Fig. 6.5). Recent evidence indicates that this taxon represents an assemblage of numerous pathotypes and morphotypes, and probably represents several taxa (Starr and Sayre 1988). This bacterium has been found infecting a large number of nematode species (more than 200 in about 100 genera, Sayre and Starr 1988; Stirling 1991), does not attack other soil organisms, and is the most specific obligate parasite of nematodes known. Its spores attach to and penetrate the nematode cuticle. Most attention has been centered on populations (*Pasteuria penetrans sensu stricto*, Starr and Sayre 1988) that attack root-knot nematodes (*Meloidogyne* spp.). The spores of *P. penetrans* germinate a few days after a contaminated nematode begins feeding on a root (Sayre and Wergin 1977). The bacterium reproduces throughout the entire female body, and the female may either be killed or may mature but produce no eggs. Bacterial spores (about 2 million from each infected nematode, Mankau 1975) are released when the nematode body decomposes, and they remain free in the soil until contacted by another nematode. They tolerate dry conditions and a wide range of temperatures, and may remain viable in the soil for more than six months. Because it is an obligate parasite, it has not

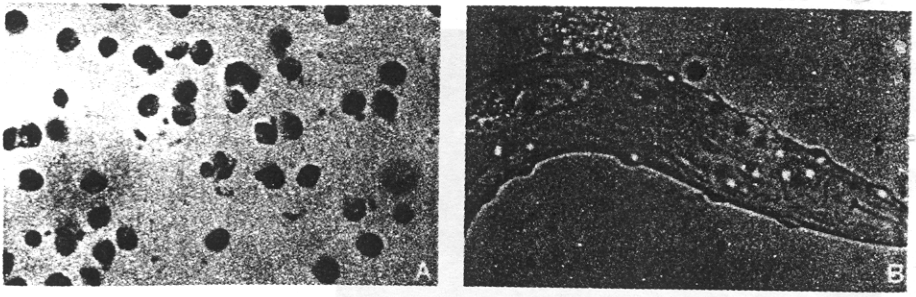

Figure 6.5. The bacterium *Pasteuria penetrans* (Thorne) Sayre and Starr, a pathogen of plant-parasitic nematodes. (A) spores of the bacterium; (B) spores attached to the exterior of a nematode, where they germinate once the nematode enters a plant root. (Photographs courtesy of R. Mankau.)

yet been possible to develop *in vitro* culturing techniques for this bacterium. Different populations of the bacterium show varying degrees of specificity to small numbers of nematode species, but the mechanisms and degree of specificity remain to be elucidated (Stirling 1991). *Pasteuria penetrans* appears responsible for some cases of natural regulation of nematode populations (Sayre and Walter 1991).

A few strains of *Bacillus thuringiensis* are also known to have activity against nematodes, including plant-parasitic species. Zuckerman et al. (1993) report efficacy of a strain against *Meloidogyne incognita* (Kofoid and White) Chitwood, *Rotylenchus reniformis* Linford and Oliveira, and *Pratylenchus penetrans* Cobb in field and glasshouse trials. The body openings of these nematodes are too small to permit the ingestion or other ingress of the bacterium, and Zuckerman et al. (1993) suggest that the mode of action is either a beta exotoxin (Prasad et al. 1972; Ignoffo and Dropkin 1977) or a delta endotoxin released following bacterial cell lysis. A strain of *B. thuringiensis* with a nematotoxic delta endotoxin is the subject of a European Patent Application by Mycogen Corporation of San Diego, California (Zucherman et al. 1993).

Fungi Affecting Plant-Parasitic Nematodes

A large group of fungi attack nematodes in the soil (Barron 1977; Stirling 1991). Numerous species have been reported from all types of soils. The taxonomy of the group has been subject to revision, and we use here the generic names recognized in Stirling (1991).

Some nematophagous fungi are endoparasitic in nematodes. Among these are genera which reproduce through motile zoospores (e.g., *Catenaria anguillulae* Sorokin, *Lagenidium caudatum* Barron, *Aphanomyces* sp.), which generally appear only weakly pathogenic in healthy nematodes (Stirling 1991). Other endoparasitic fungi possess adhesive conidia, and the infection process begins when conidia adhere to a nematode's cuticle (e.g., the genera *Verticillium*, *Drechmeria*, *Hirsutella*, *Nematoctonus*). In *Nematoctonus* spp., the germinating spores secrete a nematotoxic compound which causes rapid immobilization and death of nematodes (Giuma et al. 1973). A few species (*Catenaria auxilaris* [Kuhn] Tribe, *Nematophthora gynophila* Kerry and Crump) parasitize adult females or nematode eggs rather than juveniles.

Other fungi capture nematodes through use of special trapping structures, and have been termed "predatory." Among the more common of these fungi are species in such genera as *Monacrosporium*, *Arthrobotrys*, and *Nematoctonus*. These fungi consist of a sparse mycelium,

modified to form organs capable of capturing nematodes. These organs include adhesive structures, such as adhesive hyphae, branches, knobs, or nets (Stirling 1991). There are also nonadhesive rings, the cells of which expand when touched on their inner surface, constricting the interior of the ring and trapping nematodes. Most of these fungi are not specific and attack a wide range of nematode species. They are widely distributed (Gray 1987, 1988) and most are capable of saprotrophic growth, but often appear limited in this phase in the soil. Many soils suppress the growth of these fungi (a condition called soil fungistasis or mycostasis). This is possibly due to two different causes. Mankau (1962) concluded that a water-diffusible substance was responsible for inhibited germination in tests of soil from southern California (U.S.A.). Other studies have indicated increased activity following soil amendments with nutrients (Olthof and Estey 1966) or organic material (Cooke 1968), which implies fungistasis may be a result of resource limitation. Following saprotrophic growth, formation of trapping structures occurs and is, apparently, stimulated by nematodes (Nordbring-Hertz 1973; Jansson and Nordbring-Hertz 1980). Stirling (1991) suggests that this phase of predacious activity is followed by diversion of resources to reproduction, followed by a relatively dormant phase.

Several species of fungi are facultatively parasitic on nematodes. Of the few of these fungi that are significant pathogens of root-knot and cyst nematodes, *Verticillium* spp. are among the most important. These fungi can parasitize nematode eggs, and *Verticillium chlamydosporium* Goddard plays a major role in limiting multiplication of *Heterodera avenae* Wollenweber in English cereal fields (Kerry et al. 1982a,b). *Paecilomyces lilacinus* (Thom) Samson parasitizes eggs of *Meloidogyne incognita* (Jatala et al. 1979) and *Heterodera zeae* Koshy, Swarup, and Sethi (Dunn 1983; Godoy et al. 1983). *Dactylella oviparasitica* Stirling and Mankau, a parasite of *Meloidogyne* eggs, is thought to be at least partly responsible for natural decline of root-knot nematodes in Californian peach orchards (Stirling et al. 1979).

Predacious Nematodes Affecting Plant-Parasitic Nematodes

Predatory nematodes are found in four main taxonomic groups—Monochilidae, Dorylaimidae, Aphelenchidae and Diplogasteridae—each with a distinct feeding mechanism and food preferences (Stirling 1991). The monochilids have a large buccal cavity that bears a large dorsal tooth; all species are predacious, feeding on protozoa, nematodes, rotifers, and other prey, which may be swallowed whole, or pierced and the body contents removed. The dorylaimids are typically larger than their prey and possess a hollow spear which is used either to pierce the body of the prey or to inject enzymes into the food source and suck out the predigested contents. The group is considered omnivorous, but the feeding habits are known only for a few species (Ferris and Ferris 1989). Almost all the predatory aphelenchids are in the genus *Seinura*. Although small, they can feed on nematodes larger than themselves by injecting the prey with a rapidly-paralyzing toxin through their stylet. The diplogasterids, typically a bacteria-feeding group, have a stoma armed with teeth, and the species with large teeth prey on other nematodes. Species in all these groups are generally omnivorous, feeding on free-living as well as plant-parasitic nematodes. The role of individual species in the population dynamics of plant-parasitic nematodes in the soil has been difficult to quantify, but it is possible that a number of species may act together to produce a significant impact (Stirling 1991).

Insects and Mites

Several microarthropods in the soil, including mites and Collembola, prey on nematodes, and high predation rates have been recorded *in vitro* (Stirling 1991). A few genera are obligate

predators of nematodes, while other genera are more general feeders and consume nematodes as well as other foods (Moore et al. 1988; Walter et al. 1988; Sayre and Walter 1991). The information available suggests that as a group, microarthropods are probably significant predators on nematodes in some soils and habitats. Limited information about predation rates in soil is available, however, and more work will be necessary to assess the impact of this group on nematode populations.

SUMMARY

This overview touched briefly on groups of organisms which are antagonistic to plant pathogens and nematodes. These antagonists vary both in their innate ability to suppress plant pathogens and in their ability to thrive and compete in different environments. Consequently the selection of an organism or organisms for any particular biological control program will be a compromise among these parameters and abilities. In addition, the selection of organisms will depend on the approach taken for their use (inoculative augmentation, inundative augmentation, or natural control through conservation). These approaches are discussed in more detail in Chapter 12.

CHAPTER
11

AUGMENTATION OF PATHOGENS AND NEMATODES

INTRODUCTION

Numerous species of nematodes and microorganisms have potential for use as commercial products (Starnes et al. 1993). Successful commercial development of pathogens for augmentative biological control involves: agent selection (to obtain the best species and strains for the target pest); development of cost-effective methods for mass rearing; effective methods for storage and shipping of the agent; creation of formulations to protect and deliver the agent to the target pest's location; field testing of the product's efficacy and methods for its application; economic factors affecting product development and markets; and demonstration of safety of products to man and the environment.

AGENT SELECTION

Selection of a pathogen for the control of a target pest involves choices at two levels. First, a choice may be possible among different agent groups (viruses, bacteria, fungi, protozoa, and nematodes). Second, within a given group of agents, the particular species, strain, or isolate must be identified that has the best properties for the desired use. Patent and registration requirements may differ among agents, also affecting choice of agent.

Choosing Among Groups of Pathogens

Choice among agent groups, given that candidates exist for a given target pest, can be guided by three factors: ease and cost of production; degree of pathogenicity and host specificity; and environmental or habitat features influencing effectiveness.

Ease and Cost of Rearing. The most basic factor affecting differences in ease and cost of rearing of different organisms is whether or not living hosts are required for pathogen production. The microbial pesticide of greatest commercial application in the United States, *Bacillus thuringiensis*, for example, can be grown on fermentation media. In contrast, *Bacillus popilliae* which requires living hosts for effective production, has not been as successful commercially. Some bacteria, fungi, and nematodes can be grown in nonliving media, a factor promoting their use. Other species within these groups, for example, many species of Entomophthoraceae

fungi, require living hosts. However, all viruses and the protozoa (principally, microsporidia) of biological control interest must be grown in living hosts or host cell cultures, increasing the cost of producing these agents and limiting their use. Other aspects of production, such as use of liquid media in place of solid media, or development of simple systems of on-farm pathogen production by farmers, can also affect the cost of labor and machinery needed for production. Ease of production is a function of the technology available for the task, which is subject to improvement. Development of higher yielding cell lines for virus production, for example, might in the future reduce the cost of virus production enough to make commercial *in vitro* production feasible. Similarly, development of rearing media that employ cheaper ingredients, such as locally produced cereals, in place of chemically-defined but more costly media, can reduce the cost of the production of fungi (Hoti and Balaraman 1990).

Degree of Pathogenicity and Host Specificity. In choosing a pathogen for development as a commercial product, the degree of pathogenicity and level of host specificity of any particular agent are important considerations (Charudattan 1989). The degree of pathogenicity directly affects the cost of pathogen-based pesticides by determining the quantity that must be applied to achieve control. Because production costs of many pathogens are relatively high, selecting a highly pathogenic strain, which is effective in smaller doses, is essential to increasing the cost competitiveness of the pathogen. High levels of pathogenicity may also be important for controlling a range of instars of a pest, as some strains of a pathogen may be more effective than others in killing less susceptible stages. Such characteristics can be important in making the use of a product commercially successful.

The host specificity of a pathogen is important in that it determines the size of the potential market for the product. For highly specific agents to have commercial value, they must attack pests affecting widely grown or high-value crops to support sufficient sales. Many viruses, for example, are relatively host specific. Many are limited to hosts in just one or a few genera, for example, the virus of the brown tailed moth, *Euproctis chrysorrhoea* (Linnaeus), which is limited to a single host (Kelly et al. 1988). Such viruses currently have no commercial potential (unless their host is a major pest in a high-value crop) because their markets are typically too small to permit economies of large-scale production. Viruses with broader host ranges do exist, such as the *Autographa californica* nuclear polyhedrosis virus which attacks at least six species (rigorously confirmed) and perhaps up to 43 species in 11 families of insects (Payne 1986). Genetic engineering can be used to broaden the host spectrum of some types of pathogens. This has been done for the bacterium *Bacillus thuringiensis*. Strains that are specific for certain types of hosts (subsp. *kurstaki* for Lepidoptera, subsp. *israelensis* for Diptera, subsp. *tenebrionis* for Coleoptera) can be genetically manipulated so that the host ranges of several strains are combined in the newly created form (Crickmore et al. 1990; Gelernter 1992).

Environmental or Habitat Features Affecting Effectiveness. The choice between major groups of agents may be dictated in some cases by similarities in environmental conditions in the pest's microhabitat to those favoring pathogenicity or reproduction of the agent. Nematodes, for example, have enjoyed greatest success in moist habitats such as soil and, to a lesser degree, inside plant tissues for control of leafminers or stem borers. While means may be found in the future to make nematodes work in drier environments such as on leaf surfaces, current circumstances dictate that the ecological requirements of the agent be met by developing products targeted at pests in favorable microhabitats. In other cases, formulation methods may be developed that overcome some of the environmental limitations of agents. Viruses, for

example, are destroyed by exposure to ultraviolet light and may be protected by adding chemicals to the product's formulation that absorb radiation of damaging wavelengths. Similarly, fungal requirements for moisture may be overcome by formulation in oil or oil-water invert emulsions. The role of formulations in extending the effectiveness of pathogens is covered in more detail in the section on formulation methods.

Choosing the Best Species or Strain

Discoveries of new microbial agents may be the result of chance discoveries, laboratory screening efforts, or field surveys. Chance discoveries are those in which an agent with important new properties, not specifically being sought, is encountered and its value recognized. The discovery, for example, of a *Bacillus thuringiensis* isolate (later termed *israelensis*) that is pathogenic to dipteran larvae was such an unforeseen event. This discovery demonstrated the possibility of finding isolates of this pathogen effective against important new types of pests and stimulated screening programs to search for other useful isolates. Other chance encounters of useful new organisms include the finding in Texas of a new nematode, *Steinernema riobravis* Cabanillas et al., that is highly effective against pupae of the maize pest *Helicoverpa zea* (Raulston et al. 1992; Cabanillas and Raulston 1994).

Screening programs may also be used to find pathogens effective against a specific pest. Screening may be done by examining the activity of existing laboratory collections of pathogen isolates for activity against pests of concern. Kawakami (1987), for example, screened 61 isolates of *Beauveria brongniartii* (Saccardo) Petch for pathogenicity against the mulberry pest *Psacothea hilaris* (Pascoe).

Field surveys, however, are the basic source of new pathogen isolates. New isolates effective against a specific target pest may be encountered by collecting large numbers of the pest in the field, searching for dead or moribund specimens, and examining them by microbial culturing techniques. Koch's postulates must then be followed to determine which of these isolates are pathogenic in the target organism. Isolation of a pathogen from the actual target pest can be (but isn't always) important because pathogenicity may vary for the same species of pathogen depending on which host the isolate comes from. *Verticillium lecanii* strains, for example, originating from whiteflies, aphids, or thrips, are most virulent to the host from which the isolate was collected (Hirte et al. 1989). New pathogens may also be encountered in broad field surveys of pathogen groups. Efforts to find new nematodes, for example, have been made in Hawaii, Northern Ireland, Italy, and Sweden, among other locations, using wax moth larvae as baits to collect nematodes from randomly selected soils (Burman et al. 1986; Blackshaw 1988; Deseo et al. 1988; Hara et al. 1991). Surveys conducted to detect new natural enemies of adventive pests in their countries of origin may also be a source of new pathogens that are used either as agents of introduction or for augmentative use as commercial products.

Patents and Legal Issues

Sale of microbial pesticides, unlike the sale of arthropod parasitoids, predators, or nematodes, is regulated in the United States and some other countries under laws that govern the use of chemical pesticides. Such regulation affects strain selection because pathogens sold for pest control must be registered as pesticides and, consequently, with the exception of fungi, may gain patent protection. Patent protection gives finders of new pathogen isolates proprietary interests in their discoveries. Availability of patent protection stimulates the search for or laboratory creation of new strains.

PRODUCTION

Pathogens may be reared either in intact living hosts (*in vivo*), or in fermentation media or live host cell lines in media (both approaches termed *in vitro*). From the earliest days, pathologists have recognized that dependency on living hosts limits large scale production. Some groups of pathogens, however, are difficult to rear apart from living hosts. These pathogens include all viruses, microsporidia, many of the Entomophthoraceae fungi, a few bacteria such as *Bacillus popilliae* (which can be reared on fermentation media but does not produce spores efficiently apart from living hosts), and some families of nematodes. Pathogens that must be reared in living hosts require more labor for their production because rearing systems based on live hosts are difficult to automate and often lack economies of scale. Production costs for *in vivo* systems, therefore, are relatively high in countries where labor costs are high (Huber and Miltenburger 1986). *In vitro* production systems can usually take advantage of automation of rearing and economies of scale. Taborsky (1992) provides technical information on small-scale pathogen production for use in tropical areas.

In vivo Rearing Systems

Examples of agents that have been produced commercially in the United States in living hosts include the microsporidium *Nosema locustae* Canning, the bacteria *Bacillus popilliae*, *Bacillus lentimorbus* Dutky, and five nuclear polyhedrosis viruses (Huber and Miltenburger 1986). The process of *in vivo* rearing may be divided into four steps (Huber and Miltenburger 1986): mass-rearing the host insect; propagating the pathogen in the host; processing the pathogen; and controlling pathogen quality.

Mass-Rearing the Host. The insect hosts used for production are normally taken from a laboratory colony. In some cases, the cost of maintaining such a colony can be avoided if the host is easily obtained in the field in sufficient numbers. For example, the bacterium *Bacillus popilliae* is reared in field-collected larvae of *Popillia japonica*, since these are more easily collected than reared (Ignoffo and Hink 1971). Field collections have the limitation that hosts may only be available in certain seasons and may be contaminated by parasitoids or other pathogens. If the virus or other pathogen to be reared will grow in more than one host, it may be possible to rear the pathogen in an alternative host, choosing the most easily reared species from the host range.

Propagating the Pathogen. Production of viruses and microsporidia is typically initiated by contaminating the host's food with pathogen inoculum. If oral inoculation is ineffective, other methods may be used, as in the case of *Bacillus popilliae*, in which each host larva must be individually inoculated by injection. The methods used to rear the host during the growth of the pathogen must be such that dead, infected hosts at the end of the rearing period are easily harvested. Hosts cannot be allowed to burrow deeply into masses of diet. For hosts with such habits, alternative methods of diet presentation must be developed.

Processing the Pathogen. Harvesting and purification of the pathogen must be done inexpensively and must protect the viability of the pathogen. Hosts should be collected after death but before putrefaction (to minimize contamination by other microbes). Hosts can be collected manually or by vacuum suction (in the case of fragile cadavers). If necessary, cadavers may first

be frozen to make them more durable for collection, but caution should be used as some pathogens (certain viruses) experience reduced viability if frozen. For small-scale rearing systems (as for research), pathogens such as viruses may be separated from host remains via centrifugation, but this approach is too expensive for commercial production. In commercial systems, infected host cadavers are dried and ground and the resultant mixture of viruses and host tissue particles is the final product. This has the advantage that presence of host tissue increases the shelf-life of the virus (Huber and Miltenburger 1986). For nematodes and microsporidia, other methods of collecting the reared pathogens from host cadavers are required. Nematodes, for example, may be collected by allowing them to move out of host cadavers into water.

Quality Control. Tests must be performed regularly to ensure that the culture has not become contaminated, especially by microbes pathogenic to humans (Podgwaite and Bruen 1978). In addition, the quantity and virulence of the pathogen being produced must be determined frequently. The quantity of pathogens being produced can be assessed by counting the number of nematodes, bacterial spores, or viral inclusion bodies per host or unit of medium. Virulence can be measured by conducting a bioassay on the target host, with comparison to an appropriate standard such as a properly stored sample of the original isolate of the pathogen. The activity of different batches of the *Anticarsia gemmatalis* baculovirus in Brazil, for example, was monitored and results indicated that 85% of batches had activity equal to the original strain. Partial loss of activity in 15% of the batches was related to excessive drying times, and the production process was modified to correct the problem (Moscardi et al. 1988). Only such tests, conducted regularly, can prevent the unknowing production of an ineffective product should the strain change in virulence or be overtaken by a competing contaminant organism superficially similar to the one being reared. The quality of agents reared by means of several different methods can be measured to determine if differences exist between rearing methods (Gaugler and Georgis 1991). For *Bacillus thuringiensis*, potency of the produced agent can also be indirectly measured by determining the size of the crystal mass of the bacterial toxin (Muratov et al. 1990).

In Vivo *Rearing of Viruses* (Fig. 11.1). Bell (1991) describes an *in vivo* system for the nuclear polyhedrosis virus of *Helicoverpa armigera* (Hübner), reared in larvae of *Helicoverpa virescens*. Host larvae were reared in cups of artificial diet and were exposed to virus through its application as a spray to the diet one week after host eggs were added. At the end of the second week most larvae were dead, at which time they were collected, homogenized, strained through cheese cloth, and the virus particles harvested via centrifugation. Optimal viral inoculation rates were determined by comparing yields from a series of different viral doses per cup. Low doses failed to infect all larvae. High doses killed larvae while still small, reducing viral yield per larva. The cost of rearing was calculated at about $0.02 (U.S.) per host, 80% of which cost was for labor. Shapiro (1986) gives a detailed discussion of procedures for *in vivo* rearing of baculoviruses.

In Vivo *Rearing of Microsporidia.* Microsporidia are reared in living hosts, using procedures similar to those for viruses (Brooks 1980, 1988; Kurtti and Munderloh 1987). Productivity of *in vivo* systems for microsporidia depends strongly on the agent being produced and the host used. Much of the rearing cost is that of rearing the insect host. The next biggest determinant of

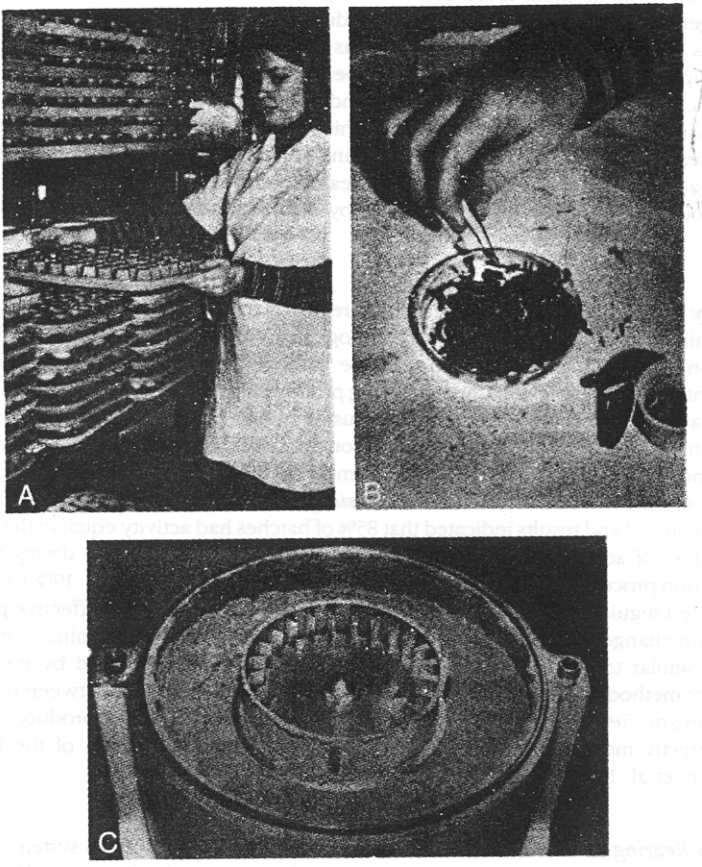

Figure 11.1 (A) *In vivo* rearing of baculovirus using live larvae of spruce budworm, *Choristoneura fumiferana* (Clemens); (B) harvest of virus from host cadavers; (C) centrifugal mill used for grinding lyophilized, virus-infected cadavers. (Photographs courtesy of J. C. Cunningham.)

rearing cost is the quantity of microsporidia produced per infected host, which can vary greatly depending on a variety of factors. For example, some microsporidia produce general systemic infections, whereas others are limited in their attack to specific tissues. Yield per host will be reduced in the latter case because only a portion of the host is actually infected. Henry et al. (1978) describe an *in vivo* production system for *Nosema locustae* for grasshopper control in which sufficient spores to treat one ha could be produced at a cost of less than U.S. $0.25 (1978 dollars) (costing $1.86 once formulated with bait). This was an economically acceptable price for application on range lands producing at least $12.00 per year of forage.

In Vivo *Rearing of Nematodes.* All nematodes can be reared in living hosts. For example, heterorhabditid and steinernematid nematodes, the groups of greatest commercial interest,

may be reared in larvae of the greater wax moth, *Galleria mellonella*. Methods for rearing the insect host on a laboratory medium, initiating nematode infection, harvesting, and storing juvenile nematodes of the Heterorhabditidae and Steinernematidae have been described (Dutky et al. 1964; Woodring and Kaya 1988; Lindegren et al. 1993). Harvesting nematodes is achieved by allowing nematodes to migrate towards a water trap away from host cadavers (Fig. 11.2). This system is relatively expensive with costs of about U.S. $1.00 (1990 dollars) per million infective juveniles. Economies of scale can not be achieved for commercial production levels because procedures cannot be automated. Yields of *Steinernema glaseri* (Steiner) can be increased 3 to 4 times by rearing in dead, rather than live, wax moth larvae (Leite et al. 1990). *In vitro* methods are available for large scale, automated rearing (Friedman 1990). Nematodes in some families such as the Mermithidae (for example, *Romanomermis culicivorax*, a parasitoid of mosquito larvae) must be reared in living hosts (Poinar 1979).

In Vivo *Rearing of Fungi.* Most entomopathogenic fungi are facultative parasites which also exist as saprotrophs and therefore can be grown apart from living hosts. Only a few groups are obligate parasites which must be reared in living hosts. Ignoffo and Hink (1971) recognized eight species of entomopathogenic fungi that had been cultured successfully in living hosts. Among these were several species of *Entomophthora*, including a species which attacks the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in California (U.S.A.) and of *Coelomomyces*, which is grown either in mosquito larvae or copepods (McCoy et al. 1988). Fungi are not, however, generally reared commercially *in vivo* due to the higher cost of such rearing compared to *in vitro* systems.

In Vivo *Rearing of Bacteria.* *Bacillus popilliae* production involves both an *in vitro* and an *in vivo* step. Sporulation cannot be effectively obtained on artificial media (Stahly and Klein 1992). Therefore, the final step in obtaining large quantities of *B. popilliae* spores involves inoculation of scarab larvae with vegetative cells of the pathogen (Fig. 11.3). Dulmage and Rhodes (1971) describe the process of *in vivo* spore production. However, because only one spore is obtained

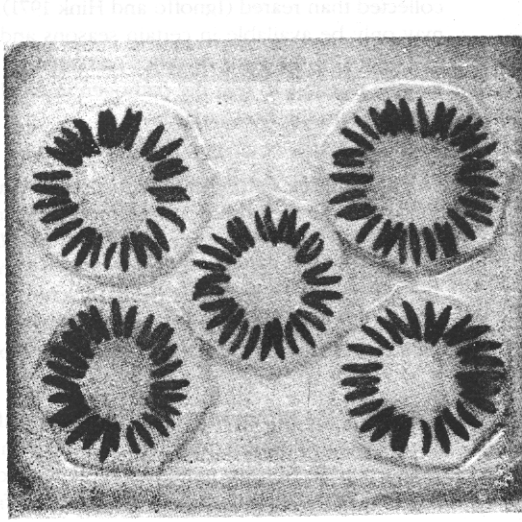

Figure 11.2. Heterorhabditid and steinernematid nematodes may be reared *in vivo* and collected by use of water traps from host cadavers. (Photograph courtesy of R. Gaugler.)

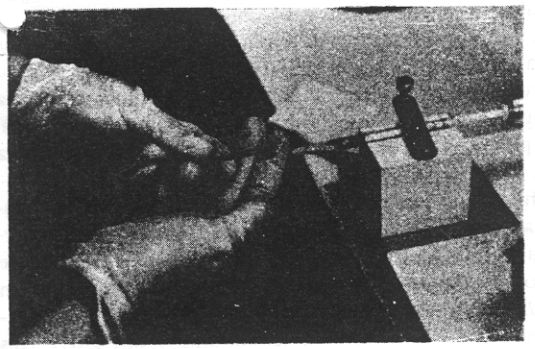

Figure 11.3. Hand inoculation of scarab grubs with vegetative cells of *Bacillus popillae* Dutky to obtain spores through *in vivo* rearing. (Photograph courtesy of S. Roy.)

from each infective vegetative cell, it is necessary to first rear large quantities of vegetative cells on fermentation media. Methods for this are also described by Dulmage and Rhodes (1971).

In Vitro Rearing Systems

In vitro rearing employs either fermentation media or insect cell cultures. Many fungi, bacteria, and nematodes can be grown in fermentation media, which permits large-scale production using relatively inexpensive materials and automated systems. Virus and microsporidia can be produced in insect cell cultures, but commercial *in vitro* production is not yet economical.

In Vitro *Rearing of Viruses.* Insect cell cultures may be used to produce insect viruses. Granados et al. (1987) noted that at least four cytoplasmic polyhedrosis viruses, two entomopoxviruses, four nuclear polyhedrosis baculoviruses, one granulosis virus, and two nonoccluded viruses could be grown in insect cell cultures. Virus production depends on having cell cultures from an appropriate host species and host tissue. The first insect cell cultures were initiated from hemocytes or ovarian cells, whereas most viruses in nature replicate in midgut or fat body cells. Initial attempts to establish cell cultures from these tissues from Lepidoptera were unsuccessful (Granados et al. 1987), but subsequently, cell cultures from such tissues were developed (Lynn et al. 1990). Rearing productivity of a virus can differ greatly in different cell cultures from the same host species and tissue (Lenz et al. 1991). Screening programs are often required to identify the most productive cell cultures in which to rear any particular virus. For example, to find cells of *Cydia pomonella* in which its granulosis virus would reproduce, 81 cell cultures were established and screened and only nine were susceptible to the virus (Naser et al. 1984). Concentrations of polyhedral occlusion bodies produced by two different *Helicoverpa zea* cell cultures differed 85-fold and the number of occlusion bodies produced per cell differed 10-fold (Lenz et al. 1991).

Reducing cost of virus production will require: developing systems suitable for large batch size production, increasing viral productivity per unit of media, and lowering the cost of cell culture media. Some of these problems have been partially solved in recent years.

The potential use of cell cultures for large scale production is discussed by Weiss and Vaughn (1986). Initially, most insect cells were grown only as monolayer cultures on surfaces. This culture form is unsuitable for commercial use because it lacks economy of scale that would

allow for large production volumes. More recently, however, a number of insect cell suspension cultures have been developed in which cells exist free in liquid media without physical support (Huber and Miltenburger 1986). Such suspensions, however, must be oxygenated. Bubbling oxygen through the media was found to be mechanically damaging to the insect cells, but diffusion across the wall of a silicon tube placed inside the fermenter was both safe and effective (Huber and Miltenburger 1986).

The yield of virus particles per unit volume of cell suspension in view of the cost of the rearing media is an economic issue of great importance. Field studies suggest that effective application rates for various viruses are in the range of 10^{11}–10^{12} polyhedral inclusion bodies per ha. Many *in vivo* systems are able to produce this quantity of virus in about 1000 diseased caterpillars, each costing about U.S. $0.02 for a cost of $20 per ha per application. In contrast, the cost of producing the same quantity of virus in insect cell culture in 1986 was prohibitive, about $900. Virus yields differ between cell cultures. Media costs are a major factor in determining the cost of viruses reared in cultures of insect cells. Initial media required the use of fetal bovine serum, and this factor alone accounted for 90% of the medium's cost (Huber and Miltenburger 1986). Alternative media using less expensive ingredients are actively being developed and several defined media exist which do not require bovine serum. Continued improvements in methods for virus production in cell cultures are likely. King et al. (1988) reported a method of culturing the *Autographa californica* virus in cells of *Spodoptera frugiperda* J. E. Smith in multiple-membrane alginate-polylysine capsules. This method increased virus yield tenfold compared to conventional cell suspension cultures. Similarly, production of the nuclear polyhedrosis virus of *Lymantria dispar* is possible in cultures of fat body cells in batches of up to 40 liters (Lynn et al. 1990). These and other improvements may eventually reduce *in vitro* virus production costs sufficiently to be competitive with chemical pesticides.

In Vitro *Rearing of Microsporidia.* At least six species of *Nosema* and two of *Vairimorpha* have been grown successfully in insect cell cultures (Kurtti and Munderloh 1987). However, the use of insect cell cultures to mass produce microsporidia is not yet economically feasible because of limitations on the mass culture of insect cells themselves, and because the yield of microsporidia spores per infected cell is too low. Field application rates for microsporidia have been in the range of 10^{9}–10^{13} spores/ha, while yields of microsporidia from cell cultures have been 10^{6} and 10^{7} spores per ml (Kurtti and Munderloh 1987).

In Vitro *Rearing of Nematodes* (Fig. 11.4). Heterorhabditid and steinernematid nematodes may be grown in media that do not contain any live hosts. Glaser et al. (1940) were the first to establish a successful, large-scale *in vitro* production process for a steinernematid nematode. Three problems must be solved for *in vitro* rearing of nematodes: suppression of microbial contaminates, maintenance of the symbiotic bacterium (*Xenorhabdus* spp., *Photorhabdus* spp.) which actually kills the host insect, and provision in the medium of all key nutrients for nematode growth. The microbial contamination problem can be addressed by developing axenic nematode cultures (ones containing no other live organisms but the nematodes) either by using antibiotics or rigorous sterilization of the media and surface sterilization of the nematodes eggs used to initiate the colony (Lunau et al. 1993). Such axenic cultures can then be inoculated with the microbial symbiont needed by the nematode to kill hosts, resulting in mixed cultures of the desired nematode and symbiotic bacterium (Lunau et al. 1993).

To achieve larger scale nematode production at commercially competitive prices, three factors were important historically: identifying inexpensive nutrients, identifying culturing conditions that promoted high yields, and using liquid rather than solid culture media

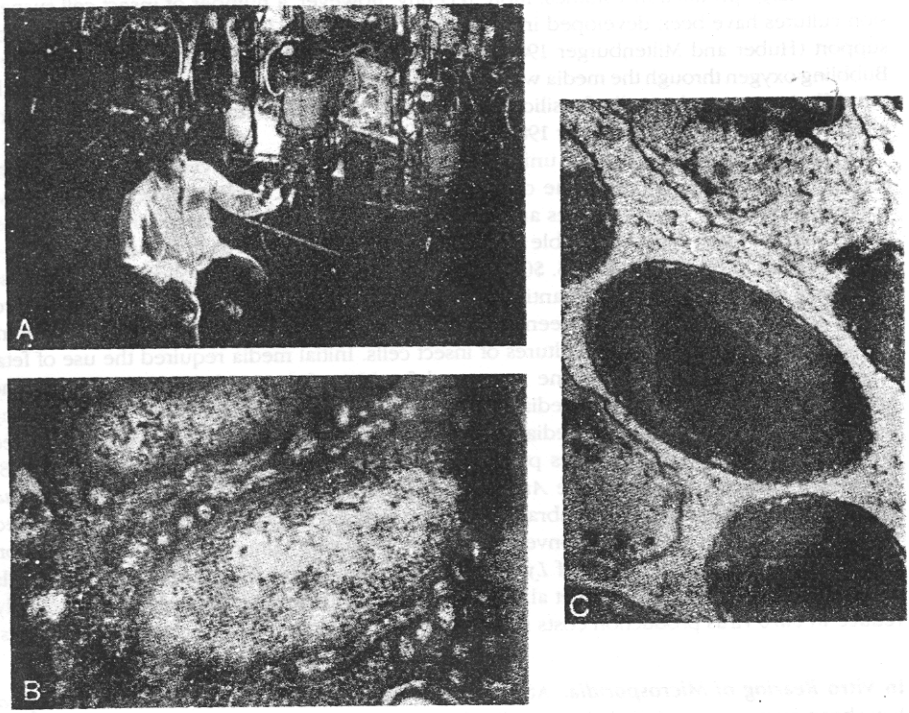

Figure 11.4. Heterorhabditid and steinernematid nematodes can be reared in nutrient broth (A); effectiveness requires retention of symbiotic bacteria (*Xenorhabdus* spp. [B], *Photorhabdus* spp.). Toxin crystal visible in cell (C). (Photographs courtesy of R. Georgis [A]; and R.J. Akhurst, CSIRO [B,C].)

(Friedman 1990). The search for better nutrients (cheaper, yet high yielding) led to the consideration of many materials. Ultimately, however, it was recognized that incorporating the symbiotic bacteria (*Xenorhabdus* spp., *Photorhabdus* spp.) in the rearing media was beneficial because these bacteria produced enzymes that broke down proteins into materials usable by the developing nematodes. With these bacteria in the media, dog food was found to be an acceptable medium. Culture of nematodes together with their symbiotic bacteria is termed monoxenic culture.

The search for optimal rearing conditions for high yield also considered many physical types of rearing systems. Both solid and liquid media can be successfully employed, but liquid culture has better economies of scale and is more amenable to commercial use. In liquid media, however, as the volume of the reaction vessel increases, oxygen may become limiting in some portions of the media. Methods to add oxygen need to take into account susceptibility of nematodes to mechanical damage from shearing caused by stirring the medium (for aeration) or bubbling oxygen into it. Acceptable limits for these procedures have been identified and Biosyst, one of the major commercial producers of nematodes, now uses large-scale liquid monoxenic culture systems employing vats of 15,000 liters or greater (Friedman 1990).

In Vitro *Rearing of Fungi.* Small scale *in vitro* production of entomopathogenic fungi is typically done on solid culture media, with the fungus growing as a mat on the surface of the medium and then producing spores on aerial hyphae (Fig. 11.5A). Media may be either defined agar-based media or natural substances such as rice or bran. McCoy et al. (1988) list media for the production of a number of fungi that are candidate biological control agents. Conidial spores (the stage typically used as the pest control propagule) are harvested from solid-media cultures by washing fungal mats with distilled water.

Surface cultures on solid media, while suitable for small scale production, lack the economies of scale and potential for automated handling that are desirable for large scale commercial production. For large scale production of microbial agents, liquid (submerged) culture systems are desirable (Fig. 11.5B). Entomopathogenic fungi of several types can be grown in liquid cultures. However, only a few (*Beauveria bassiana*, *Hirsutella thompsonii*) will sporulate in submerged culture (Dulmage and Rhodes 1971; van Winkelhoff and McCoy 1984). This problem can be resolved by a two-step culturing process in which submerged cultures are first used to produce large quantities of mycelia, which then are placed onto solid culture media for the production of conidial spores (McCoy et al. 1988). Commercial two step culture systems (termed diphasic) have been developed for a number of fungi, including *Beauveria bassiana* (Miao et al. 1993), *Hirsutella thompsonii*, *Nomuraea rileyi* (Farlow), and *Verticillium lecanii*.

An alternative method for the commercial production and use of entomopathogenic fungi is to develop application methods to use mycelial fragments, rather than conidial spores, as the infective propagule to be applied. This approach has been explored with *Hirsutella thompsonii*, and a patented process has been developed in which mycelia can be produced in submerged culture, dried, and stored under refrigeration until applied (McCoy et al. 1975; McCabe and Soper 1985). Bartlett and Jaronski (1988) give further details on commercial production of entomopathogenic fungi. Methods for rearing *Beauveria bassiana* that require

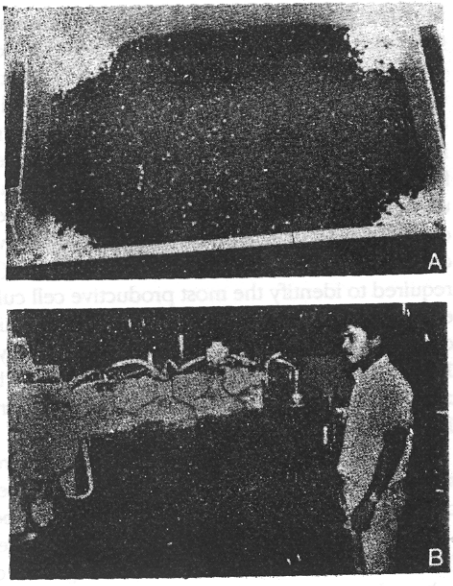

Figure 11.5. Entomopathogenic fungi may be reared *in vitro* using either solid (A) or liquid media (B). (Photographs courtesy of R.M. Pereira [A] and D.W. Roberts [B].)

minimal training and equipment have been developed in Colombia for fungus production on individual farms to control coffee pests (Antía-Londoño et al. 1992). Feng et al. (1994) review the production, formulation, and application of *B. bassiana*.

Methods and issues of concern in the *in vitro* production of plant pathogenic fungi to be used as mycoherbicides are essentially the same as for entomopathogenic fungi. Boyette et al. (1991) review culture techniques for plant pathogenic fungi, and Stowell (1991) discusses large-scale industrial production systems.

In Vitro *Rearing of Bacteria.* The principal bacterium reared *in vitro* for pest control is *Bacillus thuringiensis*. Dulmage and Rhodes (1971) present detailed descriptions of methods for production of *B. thuringiensis*. This species can be reared either on semisolid media or liquid media (Fig. 11.6). Semisolid systems utilize bran for the growth of the organism, and the rearing material is simply dried and ground at the end of the rearing process. Bran, however, swells when wetted and so is unsuitable for the production of wettable powder formulations. Semisolid systems are used primarily to produce material for dust formulations. Material for wettable powder formulations can be produced by liquid culture, based on such ingredients as molasses, corn-steep liquor solids, and cottonseed flour. The reared bacteria and associated toxins may be recovered by a variety of methods including filtration, centrifugation, and precipitation (Dulmage and Rhodes 1971). Methods for *in vitro* production of the vegetative cells of *Bacillus popilliae* are also given in Dulmage and Rhodes (1971). A method for rearing *Bacillus*

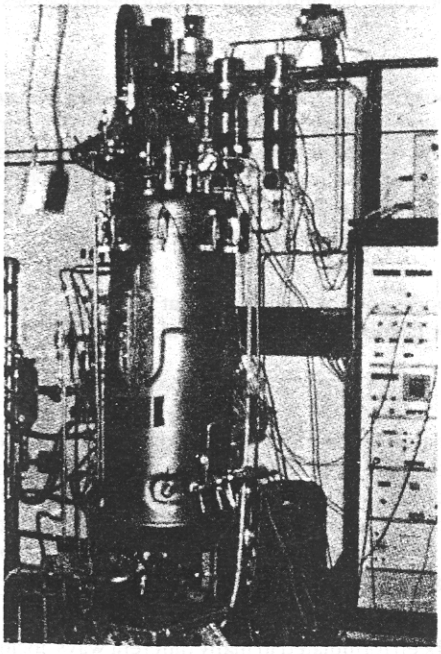

Figure 11.6. Small scale liquid fermenter used for production of *Bacillus thuringiensis* Berliner. (Photograph courtesy of D. Cooper.)

thuringiensis subsp. *israelensis* in coconuts has been developed for use in remote areas of Peru that allows villagers to rear this bacterium to treat ponds for mosquito control (Metcalfe 1991).

Maintaining and Improving Genetic Quality

Preventing Deterioration of Strain Quality. Microbial agents reared on fermentation media can lose infectivity upon repeated *in vitro* reproduction. *Entomophaga maimaiga* Humber, Shimazu and Soper, for example, declined in infectivity to *Lymantria dispar* after 15 to 50 cycles of reproduction on fermentation media (Hajek et al. 1990). Repeated rearing of *Nomuraea rileyi* by conidial transfer led to the loss of virulence to larvae of *Anticarsia gemmatalis* in 16 generations. Loss of virulence was associated with the conidial stage as no loss of virulence in this species was seen in up to 80 passages based on mycellia transfers (Morrow et al. 1989). Attenuation has also been observed in at least seven other fungi species (Hajek et al. 1990 and references therein). Similarly, baculoviruses produced in alternative hosts may have reduced infectivity in the original host. Such a reduction occurred with the nuclear polyhedrosis virus of the silkworm *Bombyx mori* when reared continuously in the Asiatic rice borer, *Chilo suppressalis* (Walker), for 18 or more generations (Aizawa 1987). In other species of pathogens, frequent passage *in vitro* has not been associated with decline in infectivity (Hajek et al. 1990). For cases in which loss of infectivity from continuous rearing in fermentation media does occur, it can be prevented by maintenance of a parent culture of the agent on living hosts. Material from the parent colony can be used to inoculate production batches, and thus the product that is sold is always only one generation away from pathogens reared *in vivo*. If methods are available to store the stock culture, for example, under refrigeration or in liquid nitrogen, then the frequency with which the stock culture must be recycled in living hosts can be reduced, decreasing cost and increasing convenience. Quality of stock cultures may decline with time, however, and the length of time over which storage may be employed for any given natural enemy must be determined on a case-by-case basis.

Genetic Improvement of Nematodes and Pathogens. Nematodes and microbes can potentially be improved in a variety of characteristics, such as infectivity rate to a given host, host range, and pesticide resistance. Gelernter (1992) discusses several examples of possible improvements in *Bacillus thuringiensis* strains through a variety of techniques. Improvements are also possible for characteristics affecting production, such as yield of spores or rate of growth under production conditions. For nematodes (but generally not for microbes), host finding behaviors may also be subject to improvement (Gaugler et al. 1989).

Aizawa (1987) expanded the host range of silkworm nuclear polyhedrosis virus to include the Asiatic rice borer, *Chilo suppressalis*, through repeated rearing in the new host. Using genetic engineering, Crickmore et al. (1990) expanded the host range of an isolate of *Bacillus thuringiensis* by combining genes for toxins allowing for attack of Diptera and Coleoptera into a single strain of the bacterium. Gaugler et al. (1989) reported a 20 to 27 fold improvement in host-finding ability by *Steinernema carpocapsae* (Weiser) through laboratory selection. Thirteen rounds of selection increased the host finding distance of *S. carpocapsae* from 3.5 cm to 20 cm/hr (Gaugler et al. 1991). However, the selected strain did not provide increased field efficacy (Gaugler unpublished, in Kaya and Gaugler 1993). Little else has been done in the area of selection for improvement of nematodes (Hominick and Reid 1990), but the concept appears sound and awaits future development. Plasmids have been used to genetically transform the fungus *Metarhizium anisopliae* to be resistant to the fungicide benomyl (Goettel et al. 1990).

Such fungicide-resistance in entomopathogenic fungi would allow their use to control insects in crops also subject to plant diseases for which fungicides must be applied.

STORAGE AND SHIPPING

Some pathogens can be stored for months or years at room temperature (Fig. 11.7). This is an important advantage for such products because it allows for year-round production, with storage until the season of use. This improved storage ability also allows agents to be shipped by slower (lower cost) methods without concern for deterioration in route. Such microbial products may approximate chemicals in regards limits on their shipping, storage, and shelf life. Other pathogens such as nematodes and some fungi are more delicate and can be stored only for several months and may require refrigeration. Improvement in the storage characteristics of many pathogens is an essential step for large-scale commercial use of these organisms.

Storage of Nematodes. Heterorhabditid and steinernematid nematodes survive well for a number of months if refrigerated and stored in thin, moist, well-aerated layers. Optimal temperatures for nematodes storage vary between species but, in general, steinernematids survive best when stored at 5–10°C and heterorhabditids at 10–15°C (Georgis 1990). Refrigeration, however, is not a strict requirement. The Biosyst product Biosafet, for example, is a formulation of nematodes on a thin layer of gel supported by a mesh screen, and in this form, nematodes could be successfully stored for up to one month at 25°C (Freidman 1990). *Steinernama carpocapsae* can be stored up to five months at room temperature, and 12 months under refrigeration (Georgis 1990; Georgis and Hague 1991).

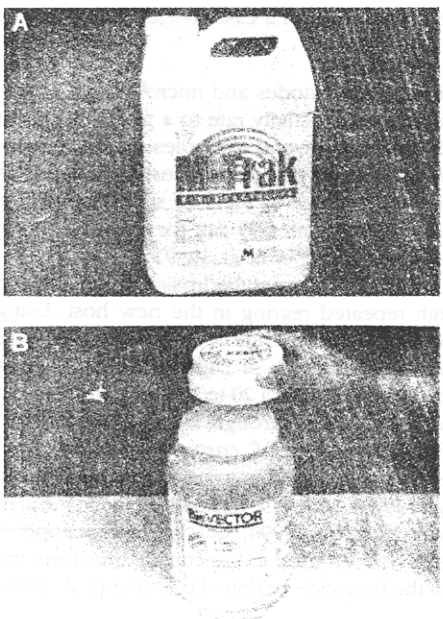

Figure 11.7. Formulation and packaging are essential steps to make pathogens and nematodes available for use as bio-pesticides. (A) *Bacillus thuringiensis* Berliner and (B) heterorhabditid and steinernematid nematodes. (Photographs courtesy of Mycogen [A] and Biosys [B].)

Storage of Fungi. Storage properties of fungi used for insect or weed control vary dependi on the species and what form the infective unit takes. Fungi may be marketed as conidial spores, chlamydospores, blastospores, or mycelial granules or fragments, among other possibilities. The mycoherbicide DeVinet, formerly marketed for control of strangler vine (*Morrenia odorata* Lindle) in citrus in Florida contained chlamydospores of the fungus *Phytophthora palmivora* (Butler) Butler formulated as a liquid concentrate. These had to be held under refrigeration until applied and had a shelf life of only about 6 weeks (Boyette et al. 1991). Commercial use was possible because the product was marketed in a small region to a small set of users (Kenney 1986). McCabe and Soper (1985) describe a process for the production and use of fungal mycelia of those entomophthorans amenable to culture on artificial media. The mycelial product, however, has to be stored at or below 4°C to maintain its viability. The fungal mosquito pathogen *Lagenidium giganteum* produces oospores which can be harvested and stored in dry form for many months, producing infective zoospores when re-wetted (Latgé et al. 1986). Spores of Deuteromycete fungi are often easier to store than those of Entomophthoraceae. Blastospores of the Deuteromycete *Verticillium lecanii*, marketed as Vertalec and Mycotalc, must be stored under refrigeration and are viable only for a few months (Bartlett and Jaronski 1988).

Storage of Microsporidia. Survival of microsporidian spores is highly variable depending on the species, temperature, and nature of the preparation stored. Various species survive from 2 to 18 months at temperatures from 0 to 6°C (Brooks 1988). Spores of some species (*Vairimorpha necatrix* Kramer), when lyophilized (dried slowly and then frozen) in 20–50% sucrose or in host tissues (cadavers), have retained rates of infectivity of 80% for up to two years (Brooks 1988). This method appears to have potential for enhancing the storage properties of microsporidian spores.

Storage of Viruses. Unformulated entomopoxvirus of the grasshopper *Melanoplus sanguinipes* (Fabricius) retained high activity for up to 9 months when stored at 4°C. The same virus, if formulated as a bait in bran, lost activity over time, possibly due to bacterial antagonism. This did not occur if the virus was encapsulated in starch granules in place of bran (McGuire et al. 1991). In general, polyhedra of most nuclear polyhedrosis viruses are stable when frozen or refrigerated, but nonoccluded virus are not (Young and Yearian 1986).

Storage of Bacteria. *Bacillus thuringiensis* spores and toxins are stable at room temperature and do not require refrigeration for storage or distribution (Dulmage and Rhodes 1971).

FORMULATION AND APPLICATION

Formulation and application methods for nematodes and microbial pathogens seek to place the agent in the correct location to maximize contact with the target pest. In addition, they help protect the agent from adverse physical conditions, enhancing the survival of the infective propagule and extending the period over which an application of the agent is effective. Angus and Lüthy (1971) present a general discussion of the various formulations used for microbial pesticides and the functions of the various ingredients.

Formulation of Nematodes. Nematodes have been formulated in many different ways, including being combined with alginate, clay, activated charcoal, gel-forming polyacrylamides,

vermiculite, peat, evaporetardants, ultraviolet protectants, placed on sponges and in baits, and stored in anhydrobiotic form (Georgis 1990). Some of these formulations are aimed at extending nematode survival in storage, enhancing ease of handling, or improving performance after application is made. Development of a flowable concentrate formulation, for example, eliminates the need to dissolve a carrier matrix and suspend nematodes prior to application (Georgis, personal communication in Kaya and Gaugler 1993).

Some nematode formulation practices seek to raise nematode survival under unfavorable physical conditions at the application site, increasing effectiveness. The principal factor limiting nematode performance in many habitats is desiccation. Gel-forming polyacrylamides have been used to retain water at the application site, which improved control of the citrus pest *Diaprepes abbreviatus* (Linnaeus) (Georgis 1990). Glazer et al. (1992) report that addition of the antidesiccant Folicotet enhanced nematode control of the foliar pest *Earias insulana* (Boisduval) on cotton foliage in glasshouse tests in Israel. Ultraviolet light also kills nematodes; *Heterorhabditis bacteriophora* Poinar is more sensitive than *Steinernema carpocapsae* (Gaugler et al. 1992). Such sensitivity to ultraviolet light suggests that nematodes are going to be of greatest value in soil and other protected environments. Some protection against damage from ultraviolet light can be obtained by adding protective materials to the formulation (Gaugler and Boush 1978). Successful use of nematodes for control of foliage pests may be achieved through further development of materials to protect nematodes from desiccation and ultraviolet light. An alternative approach that is also under investigation is the use of desiccated (anhydrobiotic) nematodes. Research has shown that some nematodes can survive slow desiccation, and when rehydrated inside an insect's gut, revive and successfully attack the host (Georgis 1990). Desiccated nematodes are less sensitive to dry conditions at the application site. The approach, however, is not developed well enough for commercial use.

Certain formulations seek to deliver the nematode to a particular target pest or habitat that might not be reached by more common methods such as a broadcast water spray. Baits, for example, attempt to target groups of pests such as cutworms (Noctuidae) and crickets (Gryllidae) that might not be contacted by a simple broadcast spray but which are likely to locate and eat baits. The application method used can also be part of the process of increasing the likelihood of contact with the target pest. Borers in stems of cane berries, for example, can be targets for nematodes because nematodes, applied as a spray to the canes, enter tunnels in canes where pest larvae feed (Miller and Bedding 1982). Nematodes may be directed against insects that attack the roots of seedlings of such plants as cabbage (*Brassica oleracea*) by applying nematodes to the roots of seedlings prior to planting, so that nematodes are in position to protect the plants immediately. In turf, penetration of nematodes through the thatch into the plant root zone is critical for effective control. Nematode movement downward can be enhanced on small acreages such as golf courses by irrigating after the application is made (Shetlar et al. 1988). At a larger scale, such as pastures, irrigation may not be possible because of the cost of applying large quantities of water. Berg et al. (1987) describe a mechanical device that uses drill action to introduce nematodes into the root zone, reducing the water needed from 20,000 to 1520 liters/ha.

Formulation of Fungi. Fungi used for plant or arthropod control can be applied in a variety of life stages, and these stages may differ in their environmental sensitivities and physiological requirements for initial survival and initiation of infection. The effectiveness of fungi is likely to be affected by the degree of spread and adhesion of the applied material to the target substrate. Fungal spores need close contact with the plant surface or arthropod integument to

initiate infection. Stickers are, therefore, likely to be important components of many fungal biopesticides. Surfactants (wetting agents) may, however, reduce spore attachment to hosts and spore viability; each surfactant-fungus combination should be checked for compatibility (Connick et al. 1990).

For mycoherbicides that are intended for application to the soil, alginate granules provide a uniform medium that supports fungal growth well, allowing spore production for an extended period after application, up to 6 weeks in some cases (Connick et al. 1990). Spores produced by such granules on the soil are not likely to redistribute themselves to aerial plant parts if the spores are large. This approach, therefore, works best where spores are intended to contact their target in or on the soil, or when target weeds are still short (less than 2–4 cm). Granular formulations of vegetative cells of entomopathogenic fungi such a *Metarhizium anisopliae* have also been developed (Storey et al. 1990) and appear promising. Nongranular formulations must be used for products intended to deliver fungal spores to aerial parts of tall weeds or to insects feeding on plants.

Some fungal spores germinate rapidly in water, so liquid formulations are not usable because spores germinate prematurely. In such cases, dust or wettable-powder formulations may be used. Other fungi are applied as conidia that germinate and initiate infection after exposure to either very high relative humidities or free water such as dew for a critical period of time. Because natural free water on treated surfaces may be present for an insufficient amount of time, formulations have been examined that retain water around the applied fungal spores or other structures. For fungi which require a specific period of exposure to free water or a carbon source for spore germination, invert emulsions of water in oil can be used (Connick et al. 1991). Use of invert emulsions, under conditions lacking a natural dew, increased control of the weed *Cassia obtusifolia* Linnaeus (sicklepod) by a conidial suspension of *Alternaria cassiae* Jurair and Khan from 0% to 88% (Connick et al. 1990). Commercial use of invert emulsions is limited by the large quantities of liquid that must be applied to obtain the droplet sizes required for adequate water retention (greater than 600 microns) (Egley et al. 1993). Other formulations that have similar water-retention action include the use of vegetable oils, and the ultralow-volume application of such oils. Bateman et al. (1993) found that formulation of *Metarhizium flavoviride* Gams and Rozsypal in cottonseed oil reduced the LD_{50} of the pathogen to the desert locust, *Schistocerca gregaria* Forskal, over 100-fold. Performance of oil formulations was especially enhanced with respect to water formulations in arid environments (relative humidities less than 35%) and preliminary trials in Niger gave satisfactory results under arid conditions (Bateman 1992). Formulation of fungal spores in oils also provides partial protection against destruction caused by ultraviolet light (Moore et al. 1993). In some cases, efficacy of mycoherbicides can be enhanced by the inclusion in the formulation of nutritional materials, such as sucrose or soy flour to support growth of the fungus prior to host invasion (Walker 1981; Weidmann and Templeton 1988).

Entomopathogenic fungi for control of outdoor arthropod pests may be manipulated by farmers by moving infected hosts between sites. Branches bearing cadavers of the whitefly *Dialeurodes citri* (Ashmead) killed by *Aschersonia aleyrodis* Webber were used successfully in China to create fungal epidemics on new trees (Gao et al. 1985). In Japan, polyurethane sheets impregnated with conidia of *Beauveria brongniartii* were used to control the whitespotted longicorn beetle, *Anoplophora malasiaca* (Thomson), by wrapping the trunks of citrus trees (Hashimoto et al. 1989). Electrostatic application devices have been used to increase deposition of the blastospores of *Verticillium lecanii* on the undersides of plant foliage in greenhouses for aphid control (Sopp et al. 1989). Many of the same issues affecting formulations for fungal pathogens of arthropods also are important for fungal pathogens of plants, especially the

need to contact the target host, by virtue of correct placement and good adhesion, and the need for adequate water or high relative humidity for spore germination.

Formulation of Microsporidia. Spores of microsporidia have been applied both as water suspensions and in baits. Control with baits has been better than with water sprays (Brooks 1988). Microsporidia are sensitive to ultraviolet light and ultraviolet light protectants have been added to some water formulations. Bait formulations of *Nosema locustae*, used for control of grasshoppers, have consisted of bran sprayed with spores and thickened with 0.2% hydroxymethyl cellulose as a sticker (Brooks 1988). The neogregarine *Mattesia trogodermae* has been formulated as spores applied to paper disks treated with pheromone of the target pest. *Trogoderma glabrum* (Herbst) becomes inoculated with spores in the process of walking on the pheromone-treated surfaces (Shapas et al. 1977).

Formulation of Viruses. Formulations of viruses seek to create products that have stable physical properties (no caking or clogging) suitable for application with conventional pesticide application machinery. In addition, formulations often contain spreaders, ultraviolet light protectants, and food items intended to stimulate consumption by the pest (Young and Yearian 1986).

Baculovirus formulations consisting of filtrates of crushed host cadavers mixed with water, if stored under refrigeration or frozen, usually perform as well or better than more complicated formulations. However, such a simple approach is not useful for production of a product for commercial use, which must be stored for up to six months and which must have physical characteristics that allow the material to be applied with various types of application machinery. Several methods have been used to formulate commercial products. The first of these is lyophilization of the virus. Clumping may be prevented by mixing the host cadavers with lactose prior to lyophilization. A second approach is mixing attapulgite clay with the virus in a water solution, which is then sprayed and allowed to dry. This process yields a stable wettable powder in which the virus is microencapsulated by a coating of clay. A third approach is to microencapsulate virus occlusion bodies with materials such as methyl cellulose or gelatin (Young and Yearian 1986).

Materials that act as ultraviolet light protectants for viruses include a variety of dyes, especially Congo red (Shapiro and Robertson 1990), starch encapsulation (Ignoffo et al. 1991), and optical brighteners (Shapiro and Robertson 1992). Adding such optical brighteners as Leucophor BSt and Phorwite ARt reduced the LC_{50} concentration for the virus of *Lymantria dispar* 400 to 1800-fold, depending on the material. Such an increase in efficiency, if realized under field conditions, has potential to drastically lower the amount of material needed for control, reducing the cost.

Application methods for viruses other than broadcast foliar treatments have been less often considered, but other methods exist to deliver virus to target pests. Ignoffo et al. (1980) found that if cabbage seedlings were dipped at planting in a solution of *Trichoplusia ni* virus, pathogen activity remained high for up to 84 days. This approach reduces the quantity of virus needed for treatment, and minimizes labor and machinery costs. Jackson et al. (1992) experimented with the placement of *Autographa californica* virus in *Helicoverpa virescens* pheromone traps to determine if male moths would effectively disperse the virus within the population. Although autodissemination did occur, transmission rates and subsequent larval mortality rates were low. Honey bees have been used to disseminate *Heliothis* nuclear polyhedrosis virus, using special pathogen-applicator devices attached to hives (Gross et al. 1994).

Formulation of Bacteria. The majority of bacterial pest-control products that have been marketed have been based on *Bacillus thuringiensis*. Angus and Lüthy (1971) provide summaries of kinds of additives that have been components in formulations of this bacterium. Most products contain both live spores and toxins. Spores are relatively stable and are marketed as both wettable powders and liquids. Most products of *B. thuringiensis* are formulated to be mixed with water and applied as foliar sprays. In the case of *B. thuringiensis* subsp. *israelensis*, used for control of mosquito, blackfly, and other dipterous larvae, formulations exist that are intended to be applied as liquids to aquatic habitats (Mulla et al. 1990). Other formulations exist, such as briquettes, which are intended to dissolve over an extended period and which can be tossed into mosquito breeding areas by hand. Superabsorbent-polymer, controlled-release systems have been developed for both *B. thuringiensis* subsp. *israelensis* and *Bacillus sphaericus* for use in aquatic habitats that extend the effective control period from an application from a few days to over a month (Levy et al. 1990).

Other formulations of *B. thuringiensis* use starch granules to encapsulate spores, together with such additives as stickers, ultraviolet light protectants, or feeding stimulants. McGuire et al. (1990) report that starch encapsulation together with the phagostimulant Coax® enhanced control of the maize borer *Ostrinia nubilalis*.

Formulations of *B. thuringiensis* must be ingested to be effective, and most products are directed against larval stages. A bait formulation combining *B. thuringiensis* spores with liquid sugars was found to control adult moths of some species (Potter et al. 1982). More recently, genes for the toxic proteins of *B. thuringiensis* have been introduced into other organisms, using these as, in effect, novel formulation systems to deliver the *B. thuringiensis* toxins in new locations or ways. A sporefree formulation of *Bacillus thuringiensis* toxins, encapsulated in dead cells of *Pseudomonas* bacteria, has been created by transferring *Bacillus thuringiensis* genes into a *Pseudomonas* bacterium, which produces the toxins and is then killed at the end of the production cycle (Gelernter 1990). Genes from *B. thuringiensis* coding for various toxins have been introduced into a maize root bacterium (*Pseudomonas fluorescens* [Trevisan] Migula) to suppress damage from larvae of root-feeding beetles. Genes of *B. thuringiensis tenebrionis* have also been introduced into potatoes and tobacco (*Nicotiana tabacum* Linnaeus), causing toxins to be produced in plant foliage and protecting plants from foliage-feeding pests (Vaeck et al. 1987; Peferoen et al. 1989). The evolutionary implications of such manipulations on the development of resistance to *Bacillus thuringiensis* need to be considered before widespread use of such cultivars occurs (Gould 1988).

EVALUATION

Because pest control products based on microbial pathogens or nematodes are applied to specific locations at particular times, measuring their effects is like evaluating the efficacy of chemical pesticides in many regards (Fig. 11.8 through 11.12). Differences, however, include the ability of some pathogens to reproduce in the field for additional generations after the initial application and greater variability in efficacy due to sensitivity to abiotic and biotic conditions. Evaluations of microbial pathogens and nematodes address: differences among agents; differences of formulations and application methods; effects of environmental factors on product effectiveness; and persistence of an agent's effect, resulting from agent reproduction. Longer term, use of microbial pathogens or nematodes may change the dynamics of the pest management system in the crop, increasing the potential for additional biological control in the system. Product evaluation should incorporate assessment of such changes as well as the immediate consequences of the product's use.

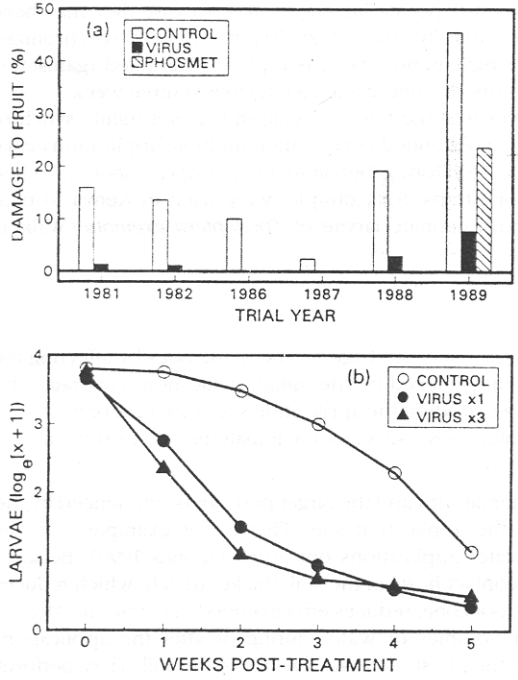

Figure 11.8. (a) Effect of granulosis virus on apple infestation by *Cydia pomonella* (Linnaeus) (after Jaques 1990); (b) effect of granulosis virus on populations of the cabbage pest *Pieris rapae* (Linnaeus) (after Tatchell and Payne 1984).

Comparisons among Agents

When a number of biological control agents are available that will attack a target pest of interest in the laboratory, tests will be needed to identify which might be best suited to control the pest under field conditions. Many heterorhabditid and steinernematid nematodes, for example, are broad enough in their host ranges that for pests in favorable habitats, such as soil, it would be reasonable to include several different nematode species or strains in field trials. Capinera et al. (1988) compared three species of nematodes for control of *Agrotis ipsilon* (Hufnagel) and

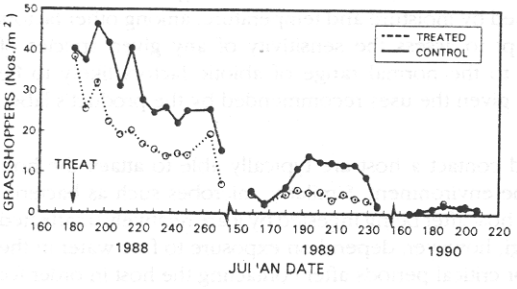

Figure 11.9. Population densities of several grasshopper species in shortgrass prarie following single treatment with bran bait containing *Nosema locustae* Canning (after Bomar et al. 1993).

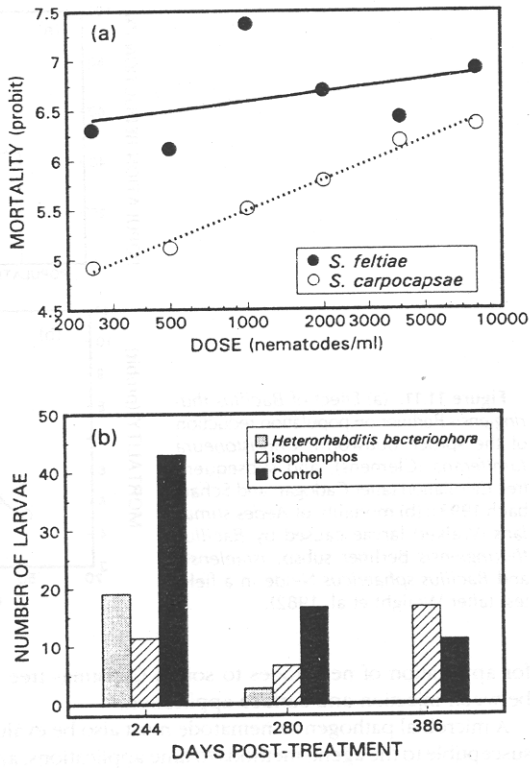

Figure 11.10. (a) Relationship between dose and mortality for the nematodes *Steinernema feltiae* (Filipjev) (= *S. bibionis*) and *Steinernema carpocapsae* (Weiser) when used in treatments against the currant borer *Synanthedon tipuliformis* (Clerck) (after Bedding and Miller 1981); (b) Number of live Japanese beetle (*Popilia japonica* Newman) larvae in control plots and plots treated with isophenphos or the nematode *Heterorhabditis bacteriophora* Poinar (after Klein and Georgis 1992).

Wright et al. (1988) compared four species for control of the turf pests *Popillia japonica* (Japanese beetle) and *Rhizotrogus majalis* (Razoumowsky) (European chaffer).

Comparisons of Variations in Formulation and Application Methods

To take best advantage of whatever biological possibilities a species of nematode or pathogen may have, field research is needed to identify the most effective application rates, formulation, manner and timing of application, and seasonal pattern of applications. Because pathogens are relatively expensive, optimizing these variables is crucial in reducing the amount of inoculum required and thus reducing cost. Such trials are also valuable in determining what formulations and application methods perform consistently so that growers can use them with confidence, thus encouraging their adoption. Wright et al. (1988), for example, in their tests of nematode species considered rates of nematodes spanning an eightfold range. Capinera et al. (1988) compared three methods of delivery of nematodes for cutworm (Lepidoptera: Noctuidae) control: calcium alginate capsules, wheat-bran baits, and aqueous suspensions. While baits might be supposed to be a more efficient method of delivery for nematodes (in view of the common use of baits as means to deliver chemical pesticides to this group of pests), no improvement was noted compared to water sprays. Kard et al. (1988) compared two methods

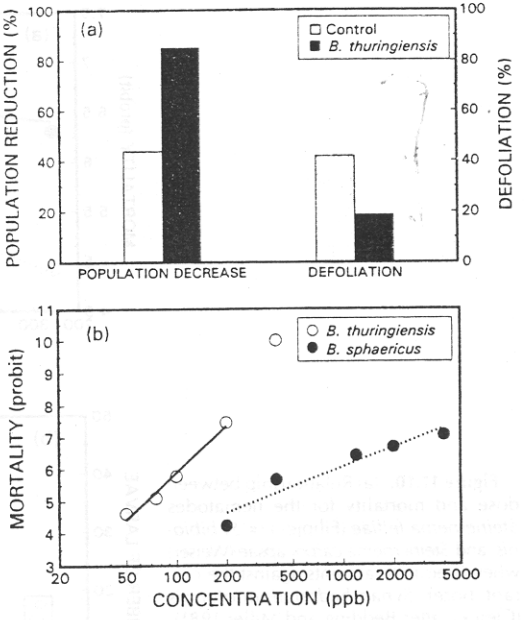

Figure 11.11. (a) Effect of *Bacillus thuringiensis* Berliner on population reduction of the spruce budworm, *Choristoneura fumiferana* (Clemens), and subsequent tree defoliation (after Cadogan and Scharbach 1993); (b) mortality of *Aedes stimulans* (Walker) larvae caused by *Bacillus thuringiensis* Berliner subsp. *israelensis* and *Bacillus sphaericus* Neide in a field test (after Wraight et al. 1982).

for application of nematodes to soil in Christmas tree plantations, but found no differences between injection and surface application.

A microbial pathogen or nematode must also be evaluated to determine the host stage most susceptible to the agent, methods to time applications, and to assess the number of applications needed to protect the crop for its entire period of risk from the pest. Bari and Kaya (1984), for example, found that older larvae of the artichoke (*Cynara scolymus* [Linnaeus]) pest *Platyptilia carduidactyla* (Riley) (artichoke plume moth) were more susceptible to *Steinernema carpocapsae* than were first and second instar larvae. Webb and Shelton (1990) found that late second and early third instar larvae of the cabbage pest *Pieris rapae* were more susceptible to granulosis virus infection than were either first, fourth, or fifth instar larvae. Microbial products,

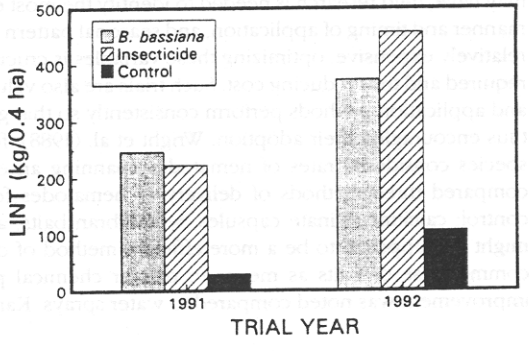

Figure 11.12. Cotton lint yield in trials of the fungus *Beauveria bassiana* (Balsamo) Vuillemin against boll weevil, *Anthonomus grandis* Boheman (after Wright 1993).

because they are often effective only against specific life stages and because they may have short residual times in the field, must be carefully timed. *Pieris rapae*, in tightly synchronized populations, was well controlled with a single granulosis virus application applied against first instar larvae, and under these conditions one treatment was as effective as three weekly applications (Tatchell and Payne 1984). However, if the field population was not tightly synchronized, so that oviposition occurred over an extended period, then multiple applications gave better control than a single application. Monitoring becomes increasingly important when microbial pesticides are used. Pheromone traps, for example, were used in Kenya to time applications of *B. thuringiensis* to control neonate larvae of *Spodoptera exempta* (Walker) (Broza et al. 1991).

Effects of Environmental Factors

Environmental factors can strongly influence microbial agents and nematodes by affecting the degree of contact between pest and agent achieved by the initial application (coverage), by reducing the life span of the microbe or nematode at the application site (survival), or by reducing the rate at which pathogens that contact hosts successfully initiate infections (infectivity).

Coverage. Degree of contact between nematodes and the target pest can be influenced by the nature of the soil and plant cover at the application site. Thatch, for example, reduces penetration of nematodes applied as water applications onto turf (Georgis 1990). Because nematodes must reach the root zone to contact hosts in the soil, thicker thatch, which reduces the numbers of nematodes reaching the root zone, reduces effectiveness. Penetration of thatch may be improved by applying greater volumes of water during or after the application. Nematodes are also strongly affected by the physical characteristics of the soil. They perform best in sandy loam soils that retain water and which have adequate spaces between soil particles to permit movement by nematodes (Kaya 1990). For nematodes to be used reliably, effects of these factors must be tested and product labeling and use recommendations written to list appropriate application sites, rates, amounts of irrigation, or other factors as needed.

Foliar applications of microbial products directed against such pests as whiteflies, aphids, and some lepidopteran larvae such as *Plutella xylostella* (Linnaeus) must adequately cover the undersides of leaves to provide effective control. Dense canopies or hairy leaf surfaces may reduce deposition rates on undersurfaces of leaves and thus reduce effectiveness in some crops.

Survival. Many microbial agents are killed by exposure to ultraviolet light or other environmental conditions such as excessive dryness. The granulosis virus of *Pieris rapae* was more than 67% deactivated in one day in trials on cabbage in the United Kingdom (Tatchell and Payne 1984). Nematode survival is affected by moisture and temperature, among other factors (Kaya 1990). Efficacy trials must attempt to assess the sensitivity of any given species of pathogen and its various formulations to the normal range of abiotic factors likely to be encountered at typical application sites, given the uses recommended by the product's label.

Infectivity. Nematodes that survive and contact a host are typically able to attack the host without being strongly influenced by the environment. Similarly, microbes such as bacteria, viruses, and microsporidia that infect the host after being ingested by it are relatively unaffected by the external environment. Some fungi, however, depend on exposure to free water in the form of dew, or very high humidities, for critical periods after contacting the host in order for spores to germinate and penetrate the host's integument (Connick et al. 1990). The effect of

this environmental limitation can be reduced by modifications of product formulation, such as the use of invert emulsions or oils as diluents (see section on Formulation and Application). Product evaluation, however, would require that such formulations be fully tested under field conditions under the full range of normal moisture levels in the crops and countries for which the product is being sold.

Persistence of Agent Impact Due to Agent Reproduction

Discussion of factors affecting the evaluation of nematodes and microbial pathogens has been based on the view that control will result solely from the nematodes or microbes actually applied. In some cases, however, pathogens or nematodes may successfully reproduce at the field application site causing subsequent cycles of attack over some period of time (termed "recycling of the pathogen"). For example, Allard et al. (1990) found that levels of infection by the fungus *Metarhizium anisopliae* in the sugarcane froghopper, *Aeneolamia varia* Fabricius var. *saccharina*, remained higher in treated plots than in control plots for up to six months after a single application. In sugarcane in Australia, a single application of the same fungus provided commercial levels of control of the pest *Antitrogus* sp. for more than 30 months (Samuels et al. 1990). Young and Yearian (1989) showed that following death of larvae of *Anticarsia gemmatalis* from nuclear polyhedrosis virus, viral particles persisted on soybean plants for several weeks at levels effective in initiating epizootics. The increase in persistence of virus in this test, as compared to short residuals often observed from water applications of viral particles to tops of plants, was attributed to protection from ultraviolet light provided by the host cadaver and later to position on the plant, often lower and in cracks or crevices protected from direct light. Jackson and Wouts (1987) found that the degree of control of the grass grub *Costelytra zealandica* provided by applications of the nematode *Heterorhabditis* sp. in New Zealand increased from 9 to 56% over an eighteen-month period, indicating an increase in the numbers of nematodes at the site over time through reproduction. Kaya (1990) discusses nematode recycling and suggests that in cases in which hosts are present throughout the year, are moderately susceptible to nematodes, occur on a crop which can tolerate a significant pest load, and occur at sites where the soil characteristics are favorable to nematodes, nematodes could be used in an inoculative rather than an inundative manner.

While manufacturers of nematode and microbial pathogen products of necessity must focus on measurement of the immediate impact of use of their materials, evaluations should also include study of longer term effects. Persistence, where it occurs at high levels, will affect both the economics of the use of the product (by reducing frequency of application) and the product's role in the pest management system, because long-term conservation of the agent may be possible in the crop. An economic analysis of the potential to control damage to pastures in Tasmania caused by *Adoryphorus couloni* (Burmeister) showed recycling (with control from single treatments persisting 5–10 years) to be a critical factor in making the use of the fungus economical in view of the annual costs of renovation of damaged pastures, and in view of the competing cost of chemical control (Rath et al. 1990). Persistence of pathogens and nematodes may also have implications for protection of nontarget invertebrates, especially if the pathogens are not native to the country where they are applied (see Safety in this chapter).

Changes to the Pest Management Dynamics of the Crop System

Using arthropod pathogens or nematodes in place of chemical insecticides for key pests in a crop can significantly increase the possibility of conserving native parasitoids and predators of

other pests in a crop and the ability to import and establish new beneficial species. Evaluation of augmentative uses of nematodes and pathogens should, therefore, include an assessment of how the overall dynamics of the pest management system for a crop will be changed.

In potatoes in Massachusetts, for example, substituting *B. thuringienis* subsp. *tenebrionis* to control *Leptinotarsa decemlineata* in place of broad spectrum contact insecticides greatly expanded the possibility of conserving natural enemies of pest arthropods in the crop. The use of this microbial product, which affects no other insects in the crop except the pest, conserved the beneficial coccinellid *Coleomegilla maculata*, a predator of aphids and of the eggs of *L. decemlineata*, and several tachinid parasitoids of the pest in the genus *Myiopharus*. In addition, it made possible the conservation of existing braconid parasitoids of the aphids in the crop and the opened the possibility of importation of new species of aphid parasitoids. In Europe, use of granulosis virus to control the apple pest *Cydia pomonella* reduced outbreaks of European red mite, *Panonychus ulmi*, by better conserving predacious mites (Huber 1986). The effect of such substitutions on the dynamics of a pest management system, however, depends on the structure of the entire pest and natural enemy complex in the crop. The substitution of steinernematid nematodes for chemical pesticides to control the black vine weevil, *Otiorhynchus sulcatus* (Fabricius), in Massachusetts cranberries had little effect on the control of other pests in the system, because applications for the weevil were not suppressing important natural enemies of other pests. Therefore, control of most other pests in the system remained based on the use of chemicals (Shanks and Agudelo-Silva 1990).

PRODUCT DEVELOPMENT

A variety of factors affect whether or not an agent that is biologically successful can also be commercially successful. Market potential (potential for a producer to make a profit) determines the amount of research on the commercial development of a given microbial agent that is likely to occur (Falcon 1985). Many factors affect the market potential of agents over and above the degree to which they are biologically effective. These include the volume of potential sales of the product and the likelihood that growers will choose the product instead of chemical pesticides that may already be in use. In addition, legal factors affecting use, such as requirements for product registration and availability of patent protection for new products, also affect the economic viability of developing an agent for commercial use. The influence of such forces on product development is illustrated by Huber (1990) who recounts the twists and turns between the 1963 discovery of the granulosis virus of the codling moth, *Cydia pomonella*, in Mexico, and the marketing of it decades later in Germany as Granupomt. Cunningham (1988) lists 19 species of target pests against which baculoviruses have been produced for use as microbial insecticides.

In some locations, local production of microbial pesticides is likely to increase their use, compared to imported products, by reducing costs and need for foreign currency (Bhumiratana 1990). Implementing a program to rear and use the *Anticarsia gemmatalis* virus in Brazil to control soybean pests has increased the area treated with this virus from 2000 ha in 1982–1983 to over 1,000,000 ha in 1989–1990 (Moscardi 1990).

Size of the Market

Perhaps the biggest factor influencing investment in development of microbial pathogens or nematodes as pest control products is the potential volume of sales, given that an effective product can be produced and that it is widely adopted. Products with narrow activity ranges

must be directed at important pests and be relatively inexpensive to produce. The manufacturer of an effective mycoherbicide (DeVine®) ceased production because the market was too specialized to be profitable (Heiny and Templeton 1993). (For more on the economics of mycoherbicides, see Heiny and Templeton [1993].) In contrast, *Bacillus thuringiensis* subsp. *tenebrionis*, directed against the potato pest *Leptinotarsa decemlineata*, has been commercially successful because the pest was of considerable importance on a widely grown crop and because competing control options (chemical pesticides) were failing rapidly in many areas due to resistance. An opening into the market existed because growers were ready to try something new when existing methods failed. In contrast, *Bacillus popilliae*, marketed for control of the turf pest *Popillia japonica*, was commercially unsuccessful because it was relatively expensive (having to be reared in live insects), worked well only under certain, poorly defined soil and temperature conditions, and was in competition with a variety of pesticides that still provided effective control of the pest. Currently in North America, there is commercial interest in pathogens for control of domestic cockroaches because the economic value of this market is very large.

For highly specific agents, such as viruses, commercial development is likely only for key pests of crops grown in large acreage such as cotton, maize, and soybeans (Huber 1986). Development of viruses for smaller markets, such as codling moth (*Cydia pomonella*) on apples, will require a reduction in the cost of producing and registering viruses. Products for specialty crops grown on limited numbers of ha have little or no chance for commercial development unless a product exists which is already being produced for another, larger market. The use of *Bacillus thuringiensis* subsp. *israelensis* for control of flies in mushroom houses and sewage plants, for example, is feasible only because this agent is already being produced for mosquito control, a much larger market. Products for public sector uses, such as for the control of defoliators of public forests or grasshoppers on public grazing lands, may be feasible if public funds can be used to support the development, registration, and perhaps production of the product as, for example, the viral product Gyp-Check® developed by the U.S. Forest Service to control the gypsy moth, *Lymantria dispar* (Podgwaite and Mazzone 1981) and baits containing *Nosema locustae* to control grasshoppers in the North American prairie region. Development of broad spectrum pathogens is feasible to some degree by genetic modification of pathogens (Gelernter 1992), but if carried too far would eliminate one of the major ecological advantages offered by use of microbial pesticides (their relative selectivity) and could raise important concerns about safety to nontarget invertebrates. Some nematodes are relatively broad in their host range and one or a few species may be produced to use against a variety of pests.

Competition with Pesticides

If chemical pesticides did not exist, development of microbial pesticides would be given a higher priority and would occur more rapidly. However, except for markets that are legally closed to pesticide competition (for example public forest lands in many provinces of Canada), microbial products must compete with existing chemical pesticides, with which growers may be more familiar. Opportunities for microbial pesticides to take over markets from competing chemical products exist if: chemical use is prohibited by government; chemicals in existence fail to provide control due to resistance; a microbial pesticide is highly effective and cheaper than existing chemical pesticides; or pesticide-caused problems, such as secondary pest outbreaks, become severe and are correctly linked to pesticide use in the minds of growers, who then are directed to use microbial products that do not cause secondary pest

outbreaks. The out-of-crop environmental harm sometimes caused by chemical pesticides (bird kills, fish kills, water contamination) is likely to slowly create societal interest in alternative pest control methods. This interest, combined with government restrictions on pesticide use that make pesticides more expensive (increased registration costs, taxes on pesticides, applicator insurance) or less convenient to use (regulations requiring posting of treated areas, reentry times for treated areas, applicator training, pesticide application record keeping), in the long term may create demand for efficacious, nonchemical products. Conversion of such generalized interest in alternatives into actual new microbial products, however, is an uncertain process.

Issues that strongly affect growers' views of the relative merits of chemical versus microbial pesticides are level of pest control achieved, speed of control, and predictability of control after application. Chemicals are believed to be, and often are, able to act rapidly, kill a high percentage of the pests, and do so reliably every time they are used. In practice, of course, chemicals vary in these characteristics and often are not as effective or reliable as commonly believed. Microbial pesticides are believed to act slowly, be less effective, be somewhat erratic in performance, and be strongly influenced by circumstances that vary from application to application. Development of microbial pesticides should attempt to address these problems in several ways. First, the variability of microbial pesticide performance should be reduced by developing, through research, a detailed understanding of the factors that affect efficacy and then adjusting either the formulation or the directions for use to eliminate variation in results as completely as possible. Second, extension agents must educate growers to understand that neither extremely high levels of kill nor rapid kill are truly necessary for effective pest control. Extension agents should stress, rather, the importance of rapid cessation of pest feeding, long-term reduction in pest reproduction rates, sustained mortality at more moderate levels, and leaving some pests to be killed by parasitoids and predators so as to sustain the populations of these beneficial organisms in the crop.

Another issue affecting the adoption of formulated pathogens as pesticides is the occurrence of multiple pests in a crop in cases where only some of the pests may be controlled by a pathogen but many of the pests are susceptible to a single pesticide. While pathogens and chemical pesticides are often compatible, the use of two products (a pathogen and a chemical) when one (the chemical) will satisfactorily accomplish the task is unlikely to be adopted.

Legal Factors

Legal factors can also affect the feasibility of commercial success of a microbial pesticide or nematode product. These factors include the requirements and costs of registering pest control products with governments, the availability of patent protection, and taxes on or subsidies for pesticides.

Chemical pesticides are subject, in many countries, to government requirements for registration as a condition of sale. Such registration typically includes the obligation of the company selling the product to submit a body of scientific evidence on the identity, efficacy, safety, and environmental fate of the product. In the United States and many other countries, microbial pesticides, but not nematode products (Hominick and Reid 1990), are also subject to product registration. The exact body of information that must be submitted is, however, different and in general less costly to produce (Environmental Protection Agency 1983; Aizawa 1990; Betz et al. 1990; Kandybin and Smirnov 1990; Quinlan 1990). Podgwaite and Mazzone (1981) discuss the process required for the registration of the virus of *Lymantria dispar* as a pest control product in the United States.

Chemicals, when registered for use as pesticides, are able to obtain patent protection in many countries, which prohibits other companies from selling, for a set number of years, the same product in competition with the company that did the developmental work. Microbial pathogens when developed as pesticides are also able to seek patent protection. Patent protection has generally not been available in the case of multicellular pathogens such as fungi and nematodes. However, patents have been granted covering technology for rearing, formulating, or applying such organisms. In addition, in a few cases patents have been granted for novel use patterns, such as the use of the nematode *Steinernema scapterisci* for control of mole crickets.

Taxes and subsidies, by affecting directly the costs of pest control products, can significantly affect the balance between microbial and chemical pesticides. In some countries, for example, pesticides may be taxed. In such countries, if microbial pesticides are exempt from such taxes, their competitive standing against chemical pesticides improves. Similarly, in some countries, governments subsidize pesticides to make pesticides more affordable to farmers. If such subsidies were to be allowed for microbial pesticides, but denied for chemical pesticides, this would encourage the use of microbial pesticides. For more on such issues, see Chapter 20 on the effects of government policies on biological control.

SAFETY

Safety of microbes used as insecticides has been covered in depth by Laird et al. (1990), including registration requirements in various parts of the world, safety of bacteria, viruses, nematodes, and fungi to man and other vertebrates and to nontarget invertebrates. The environmental risks of genetically engineered agents are considered as well. Most microbes and nematodes used as biological control agents occur naturally in many environments, often in large quantities when epizootics occur. The general absence in the medical literature of cases of these agents infecting man is important evidence that these agents do not pose a significant health risk. Further evidence on safety is gathered on each specific microbial agent in the course of its commercial development. For safety testing procedures for microbial agents see the following reviews for various taxa: plants (Campbell and Sands 1992), fish and crustaceans (Spacie 1992), birds (Kerwin 1992), mammals (Siegel and Shadduck 1992), and nontarget insects and acari (Fisher and Briggs 1992).

Safety of Nematodes

Commercial production and application of nematodes for pest control is viewed by government regulators in most countries (except France and Japan) as being equivalent to the augmentative use of parasitoids or predacious arthropods. Nematodes are considered safe to man and other vertebrates. Rats exposed by mouth or intraperitoneal injection to *Steinernema carpocapsae* showed no signs of pathogenicity, toxicity, or infection (Gaugler and Boush 1979). Effects of nematodes on nontarget invertebrates have been reviewed by Akhurst (1990). Based on data from laboratory tests, species of nematodes in the families Steinernematidae and Heterorhabditidae appear to have broad host ranges within the Insecta. However, in such tests high dosages of nematodes are presented with candidate hosts under unnatural physical conditions that are moist and otherwise favorable to the nematodes, which is likely to increase the host range beyond that typically seen in nature. Field data suggest that risks to nontarget species from nematode applications are low because nematodes have limited motility and are restricted to specific environments due to intolerance of dryness and other unfavorable physi-

cal conditions (Georgis et al. 1991). *Steinernema carpocapsae*, for example, has been shown to have no effect on intact earthworms (*Aporrectodea* sp.) (Capinera et al. 1982). Georgis et al. (1991) did not observe any harm to nontarget soil arthropods in golf course turf, maize or cabbage fields, or cranberry (*Vaccinium macrocarpon* Aiton) bogs from applications of steinernematid or heterorhabditid nematodes. Nematodes do kill immature stages of parasitic wasps if parasitized hosts ingest nematodes (Kaya and Hotchkin 1981; Akhurst 1990). Jansson (1993) provides a broad discussion of potential effects of non-native nematode species used for augmentative biological control.

Safety of Microbial Pathogens

In the United States, Europe, Russia, and Japan, pest control preparations that are sold commercially and which contain pathogens or other living microbes are treated as pesticides. These products must be registered with the appropriate government agency that regulates chemical pesticides and must demonstrate their safety to that agency before being offered for sale. This process generates information to demonstrate that a microbial product, as actually manufactured and offered for sale, is safe for use as recommended on the label. The information required for registration of microbial products, due to the nature of the products, differs in some regards from the information required for the registration of chemical pesticides. At a minimum, data are needed to: define the active agent taxonomically; define the methods of culture of the active agent; demonstrate that the commercial product is free from contamination by other, potentially dangerous, microbes; and demonstrate that the pathogen is not infectious in man or domestic animals. In addition, studies on the fate of the pathogen in the environment or of its effect on nontarget organisms may be needed as, for example, the assessment of the effect of *Bacillus thuringiensis* subsp. *israelenis* on non-target aquatic organisms (Merritt et al. 1989; Welton and Ladle 1993). Countries with industries based on culture of invertebrates, such as Japan's silkworm industry, may require that preparations such as *Bacillus thuringiensis* do not contain live spores, but only pathogen-derived toxins (Aizawa 1990).

In countries which do not treat microbial pesticides as products requiring governmental registration, local systems for pathogen production may be developed (Metcalfe 1991; Antía-Londoño et al. 1992). Local pathogen production systems, at the village or farm level, or by national in-country producers, should be monitored by government health agencies to ensure that systems, as operated, produce high quality preparations of the intended pathogen, free of other microbial agents.

Requirements for registration of microbial pesticides have been summarized for the United States (Environmental Protection Agency 1983; Betz et al. 1990), western Europe (Quinlan 1990), eastern Europe and Russia (Kandybin and Smirnov 1990), and Japan (Aizawa 1990). While each country's requirements differ somewhat, the broad theme is to treat microbial pesticides under the same laws as pertain to chemical pesticides and to vary the data requirements to allow for differences between chemicals and infectious agents. The United Kingdom and Germany have more extensive regulations; other European countries less so. Only Denmark exempts infectious agents from regulation as pesticides (defining them as biological control agents).

The rationale used to develop regulations in the United States is discussed by Rogoff (1982). Currently some 45 products, representing 26 species of microbes, have been registered for pest control in the United States. The majority of these (18) are targeted against insects, and two are targeted against weeds (Table 11.1; others are targeted against plant pathogens, Table 12.4). The system employed by the U.S. Environmental Protection Agency is to organize testing require-

TABLE 11.1 Microbial Pesticides Registered in the United States Against Insects and Weeds (as of 1993)

Species of Microbe	Pest Controlled
Bacteria	
1. *Bacillus popilliae* Dutky 1 *Bacillus lentimorbus* Dutky	Japanese beetle larvae
2. *Bacillus thuringiensis* Berliner subsp. *kurstaki*	Lepidopteran larvae
3. *Bacillus thuringiensis* subsp. *israelensis*	Dipteran larvae
4. *B. thuringiensis* var. *san diego*	Coleopteran larvae
5. *B. thuringiensis* subsp. *tenebrionis*	Coleopteran larvae
6. *B. thuringiensis* subsp. *kurstaki* strain EG2348	Lepidopteran larvae
7. *B. thuringiensis* subsp. *kurstaki* strain EG2424	Lepidopteran/Coleopteran larvae
8. *B. thuringiensis* subsp. *kurstaki* strain EG2371	Lepidopteran larvae
9. *Bacillus sphaericus* Neide	Dipteran larvae
10. *B. thuringiensis* subsp. *aizawai* strain GC-91	Lepidopteran larvae
11. *B. thuringiensis* subsp. *aizawai*	Lepidopteran larvae
Fungi	
12. *Phytophthora citrophthora* (Smith and Smith) Leonian, Strangler vine race	Citrus strangler vine
13. *Colletotrichum gloeosporioides* (Penzig) and Saccardo in Penzig f. sp. *aeschynomene* ATCC 20358	Northern joint vetch
14. *Lagenidium giganteum* Couch	Mosquito larvae
15. *Metarhizium anisopliae* (Metchnikoff) Sorokin strain ESF1	Cockroach and fly control
Protozoa	
16. *Nosema locustae* Canning	Grasshoppers
Viruses	
17. Polyhedral inclusion bodies of *Heliothis* nucleopolyhedrosis virus (NPV)	Cotton bollworm, budworm
18. Polyhedral inclusion bodies of Douglas-fir tussock moth NPV	Douglas-fir tussock moth larvae
19. Polyhedral inclusion bodies of gypsy moth NPV	Gypsy moth larvae
20. Polyhedral inclusion bodies of pine sawfly NPV	Pine sawfly larvae

ments into tiers, with all agents being to subjected to Tier I tests, and only those agents which give some indications of survival, replication, infectivity, toxicity, or persistence in Tier I tests being required to undergo Tier II or III tests. Tier I tests include a series of acute tests in various laboratory animals by oral, dermal, and inhalation routes. In addition, intravenous infectivity tests are required for bacteria and viruses and an intracerebral test for viruses and protozoa. Intraperitoneal infectivity tests are required for fungi and protozoa. Primary dermal and eye tests are required for all agents. Also, agents must be tested for ability to cause hypersensitivity reactions and immune responses. Tissue-culture tests for infectivity to mammalian cells are required for viruses. Nontarget tests are done for birds, wild mammals, plants, insects, and honey bees. All Tier I tests must be completed before permits are issued for outdoor tests of the agent (Podgwaite 1986). In general, microbial agents studied so far pose no threat to man or vertebrates, with the exception of occasional allergenic responses and, for some fungi, infections. Invertebrates may be killed by some microbial products under some circumstances. More detailed comments are summarized below.

Safety of Bacteria. *Bacillus thuringiensis* strains that produce beta-endotoxin are variably pathogenic in mice and chickens, but all strains currently used for pest control are deficient in ability to produce this particular toxin (Podgwaite 1986). Strains in commercial use do not infect man or other vertebrates under normal circumstances. Two instances have been reported of

apparent *B. thuringiensis* infection in man, one finger infection (caused by a needle injury to a laboratory worker manipulating *B. thuringiensis* cultures) and one eye infection. In both cases, infections were not serious and responded to antibiotics. Some possibility exists that these cases, particularly that of the eye infection, represented persistence of the bacterium, but not infection and that another unidentified infectious agent was present and was the actual cause of the infection. This is possible because the diagnosis was based only on occurrence of the *B thuringiensis* bacterium in the eye, not demonstration of infection. Persistence of *B. thuringiensis* cells for a few weeks in exposed tissues is known to occur (Siegel and Shadduck 1990a).

Laboratory tests on rabbits and other vertebrates for *Bacillus sphaericus* and *Bacillus thuringiensis* subsp. *israelensis* (Shadduck et al. 1980; Seigel and Shadduck 1990a) and for *Clostridium bifermentans* Weinberg and Séguin serovar *malaysia* (Thiery et al. 1992) all indicated that, while these bacteria may persist for varying lengths of time in exposed vertebrate tissues, they do not cause any pathogenic effects and are not harmful. The literature on *B. sphaericus* and *B. thuringiensis* are reviewed in detail by Siegel and Shadduck (1990b,c). Based on these and other tests, these bacteria have been recognized as safe for use as pest control agents in circumstances involving human exposure. However, some concern has been raised about potential *Bacillus thuringiensis* contamination of drinking water supplies in Germany because *B. thuringiensis* is closely related to *Bacillus cereus* Frankland and Frankland, a bacterium implicated in food poisoning in humans (Helmuth 1988). Epidemiological studies of human populations exposed to pathogens used in pest control operations can be conducted to help assess whether or not human populations are at risk, as was done, for example in Oregon (U.S.A.) for rural populations exposed to *B. thuringiensis* subsp. *kurstaki* used in attempts to eradicate *Lymantria dispar* from forested areas (Green et al. 1990).

Nontarget invertebrates may or may not be at risk from these bacteria. Neither *B. sphaericus* nor *B. thuringiensis* affects honey bees under field conditions (Vandenberg 1990). Other invertebrates, such as the silkworm, *Bombyx mori*, nontarget forest Lepidoptera, and immature parasitoids inside target pests, are likely to be killed if exposed to *B. thuringiensis* (Podgwaite 1986). Data on such effects are summarized by Flexner et al. (1986). Concern over potential harm to beneficial insects such as silkworms has led some countries, such as India, to prohibit the use of *B. thuringiensis* products, a position that might now logically be revised, given the diversity of available *B. thuringiensis* strains, some of which do not affect Lepidoptera (Padidam 1991). Miller (1990) assessed the effect of *B. thuringiensis* subsp. *kurstaki* applications on nontarget forest Lepidoptera in Oregon; some species found in control areas were absent from treated areas, but the degree of impact was viewed as lower than that which would have occurred with chemical pesticide applications.

B. thuringiensis subsp. *israelensis*, when applied to aquatic systems, shows little effect on nontarget invertebrates, with the exceptions of those in the families Chironomidae, Dixidae, and Certopogonidae, which may be moderately to severely affected (Flexner et al. 1986). Merritt et al. (1989) provide an example of an evaluation of application of *B. thuringiensis* subsp. *israelensis* to river systems for control of simuliid larvae. Zgomba et al. (1986) recorded some effect of this bacterium on Ephemeroptera and Odonata, but much less than that caused by applications of chemical pesticides in the same circumstances.

Safety of Viruses. The majority of viruses which have been considered for use in pest control are either nuclear polyhedrosis or granulosis viruses, both of which are in the family Baculoviridae, whose members are only known to infect arthropods. Several nuclear polyhedrosis viruses have been extensively tested in over 24 vertebrate species, including a range of mammalian, avian, and fish species to determine if they have the ability to infect vertebrate

hosts. None has shown any ability to infect vertebrates (Burges et al. 1980a; Podgwaite 1986). Granulosis viruses have been less extensively tested, but available data suggest that this group, which is only known to infect Lepidoptera, is unable to infect vertebrates. Other families of viruses such as the Parvoviridae, which are not currently being considered for use as pest control agents, are capable of infecting vertebrates and potentially pose risks to man or domestic animals. Saik et al. (1990) summarizes information on non-Baculoviridae effects on domestic animals.

Guidelines for safety tests for baculoviruses of Lepidoptera and sawflies (Hymenoptera) are given by Burges et al. (1980b). Required information includes:

(1) identity and information on the formulated product (name, formulation, composition statement)

(2) identity of agent (name of virus, diagnostic tests for agent, list of known impurities)

(3) biological properties of the active agent (host spectrum, natural geographic occurrence, natural infectivity, stability)

(4) manufacture, formulation, and quality control (production methods, assays to ensure standardization, tests to show freedom from specific human pathogens, assays for general microbial contaminant level)

(5) application (pests controlled, crops to be treated, rates, numbers and timing of applications, method of application, use pattern if nonagricultural)

(6) experimental data on efficacy (laboratory and field tests on efficacy and resistance)

(7) residues (identification method for virus residues, quantification of residues on crop at harvest, residues in the environment)

(8) infectivity and toxicity in mammals (single dose, nonocular by various routes; ocular, single dose; repeated dermal and respiratory exposure to test allergenicity; cell culture studies to test for infectivity to mammalian cells; carcinogenicity and teratogenicity tests)

(9) effects on humans (in manufacturing facilities, applicators)

(10) information on environmental and wildlife hazards (honey bees, parasitoids and predators of target pest, earthworms, two fish, two birds).

Viruses tested so far do not directly infect or harm adult parasitoids of pest species because viruses are infective only if ingested. Immature parasitoids in infected hosts may die, but not as the result of virus infection, but rather of premature loss of the host (through its death from virus) or alteration of the quality of the host (Flexner et al. 1986).

Viruses of arthropods are typically limited in their host ranges such that only species in one genus or in related genera in one family are infected. Therefore, distantly related types of invertebrates are not at risk from virus applications (Podgwaite 1986). The nuclear polyhedrosis virus with the widest known host range is the *Autographa californica* virus, which infects up to 43 species of Lepidoptera (variation in count reflects different levels of proof as to the virus's identification). Cytoplasmic viruses, one of which is being considered for commercial development in Japan, have broader host ranges than most nuclear polyhedrosis viruses. The virus from the caterpillar of the lasiocampid moth *Dendrolimus spectabilis* Butler, for example, also infects caterpillars in several other genera of Lepidoptera (Podgwaite 1986).

Safety of Fungi. Of the various fungi that have been developed for commercial use as pest control agents, most have shown no infectivity to man or other vertebrates (Podgwaite 1986).

Negative findings were made for mice fed or exposed to *Nomuraea rileyi* (Ignoffo et al. 1979), for rats, rabbits, and guinea pigs fed or exposed to *Hirsutella thompsonii* (McCoy and Heimpel 1980), for mice injected with *Verticillium lecanii* (Podgwaite 1986), and for mice injected with *Lagenidium giganteum* (Kerwin et al. 1990).

Health concerns for man have been recognized for a few fungi of potential economic use. *Beauveria bassiana*, for example, has been reported to cause allergies in humans (York 1958) and is at least an opportunistic pathogen in man and other mammals (Burges 1981b). Two species of *Conidiobolus* in the Entomophthorales have been reported to be pathogenic in man (Wolf 1988).

Fungi infect and kill invertebrates which contact or ingest fungal spores. However, mortality of nontarget invertebrates from spore contact is typically less than 10% (Flexner et al. 1986). Higher mortality appears to occur if fungal spores are ingested. Larvae of *Cryptolaemus montrouzieri* suffered 50% mortality when fed Boverint, a commercial preparation of spores of *Beauveria bassiana*. Adult ladybird beetles, however, were not affected (Flexner et al. 1986). Honey bee workers experienced 29% mortality when fed spores of *Hirsutella thompsonii* (Cantwell and Lehnert 1979). Both *Beauveria bassiana* and *Metarbizium anisopliae* infect *Bombyx mori* and, also, have been associated with honey bee kills following field applications (Podgwaite 1986). Some evidence also exists suggesting that parasitized hosts of some species experience increased susceptibility to fungi and that populations of some overwintering carabids and other invertebrates, normally subject to mortality from fungi, may be at increased risk if large amounts of fungal inoculum are added to soils as a consequence of agricultural pest control (Flexner et al. 1986). Granular mycelial formulations of fungi appear relatively safe to nontarget organisms. Furthermore, what effects may occur are likely to be small compared to the relatively large reductions in populations of invertebrates currently caused in agricultural fields by chemical pesticides.

Safety of Microsporidia. Relatively few microsporidia have been studied for commercial development as pest control products. Consequently less information is available on this group of organisms. Studies on *Nosema locustae* showed that this pathogen did not irritate, replicate, or accumulate in rats, guinea pigs, rabbits, rainbow trout, or bluegill sunfish following administration by appropriate routes (Brooks 1988). Existing evidence suggests that *Nosema* and related entomopathogenic protozoa pose no risk to man (Siegel and Shadduck 1992) and little or no risk to other vertebrates (Saik et al. 1990a). Microsporidia are known to infect various invertebrates, reducing longevity and fecundity. Host ranges of individual species of microsporidia may span several orders of insects (Brooks 1988). When parasitoids oviposit in hosts with microsporidial infections, the resulting adult parasitoids reared from infected hosts carry microsporidial infections (Vinson 1990a). Such infections reduce longevity and lower fecundity. Microsporidia have been shown to adversely affect laboratory cultures of various insects, including parasitoids (Flexner et al. 1986). *Nosema locustae* has been shown not to infect honeybees (Podgwaite 1986). Studies of *Nosema furnacalis* Wenn showed that none of nine species of predacious arthropods fed infected prey developed active *N. furnacalis* infections (Oien and Ragsdale 1993). The parasitoid *Macrocentrus grandii* Goidanich, however, reared in infected hosts did become infected.

Genetically Altered Microbes. Genetic engineering and cell fusion methods are being used to alter microbial pathogens for improved characteristics for biological control. In the forefront of such activities are efforts to alter the virulence or host ranges of baculoviruses (Betz 1986; Wood

and Granados 1991) and of such bacteria as *Bacillus thuringiensis* (Gelernter 1992). More rapid cessation of feeding by baculovirus-infected hosts has been achieved by incorporating genes in the *Autographa californica* nuclear polyhedrosis virus that code for production of an insect-specific neurotoxin derived from a scorpion (Stewart et al. 1991).

Products arising from new genetic combinations will require registration as microbial pesticides and new regulations may be needed to assess whether these new organisms pose risks to man or the environment. Because these modified organisms may differ very little from their unmodified sources, information available on spread and persistence of natural viruses or other microbes in the environment is useful to determine the likely persistence of such organisms. Fuxa (1989) has summarized existing information on microbial agents from this point of view. Persistence varies greatly depending on the microhabitat, ranging from mere days on surfaces, such as foliage, which are exposed to ultraviolet light, to years in soil.

General concepts stressed in evaluating the risk of genetically modified organisms are persistence, dispersal, host range, and recombination with other microbes (Maramorosch 1987; Cory and Entwistle 1990; Fuxa 1990b). One proposed principle is that genetically modified organisms should be judged based on what they are (the characteristics they have been altered to possess) rather than on the technology used to make the modification (e.g., genetic or classical breeding). Speculations about potential risks have included: that new gene combinations from different species or genera might result in totally unforeseen properties in the modified organism; that modified organisms might become pests by virtue of excessive abilities to survive and spread; and that genetically modified organisms might interact with other species in ways leading to the transfer of genetic material to these other species, with unknown consequences (Fuxa 1990b). Some authors have considered that infectious agents with overly broad invertebrate host ranges might put native invertebrate populations at some risk. Williamson (1991), for example, estimates that 5–10% of Britain's Lepidoptera would be susceptible to a strain of the *Autographa californica* virus which has been modified to increase its host range. He recommends further genetic modifications, such as removal of the polyhedral gene, to render the virus incapable of sustained persistence in the wild. Field trials of modified *Autographa californica* viruses indicate that removing genes for production of polyhedron protein production is successful in making this virus nonpersistent (Possee et al. 1990). Because such nonoccluded viruses are rapidly inactivated, efficacy under field conditions is reduced. Co-occlusion (in which modified viruses and wild type viruses are used to simultaenously infect hosts to produce viruses of both strains in shared occlusion bodies) has been proposed as a strategy for formulating such nonoccluded viruses to permit their effective use (Wood et al. 1994). Wood and Granados (1991) give an overview of the potential uses of genetically modified baculoviruses. Charudattan (1990) summarizes information concerning use and release of genetically modified fungal pathogens of plants and concludes that modified fungi tend to decline after mycoherbicidal use and appear safe.

CHAPTER
12

METHODS FOR BIOLOGICAL CONTROL OF PLANT PATHOGENS

INTRODUCTION

Organisms for biological control of plant disease can be used in various ways, but most attention has been given to their conservation and augmentation in a particular environment, rather than to the importation and addition of new species as is often done for insect or weed control. The choice of these approaches is in part because there is usually a diverse set of microbes already associated with plants. These microbes provide substantial opportunity for development of resident species as competitors or antagonists to pathogenic organisms.

Both conservation and augmentation have some application in each of the main groups of plant diseases. The use of microbes for control of plant pathogens is covered in more detail in several texts, including Cook and Baker (1983), Parker et al. (1983), Fokkema and van den Heuvel (1986), Lynch (1987), Campbell (1989), and Stirling (1991) and in other review articles (Wilson and Wisniewski 1989; Adams 1990; Jeffries and Jeger 1990; Sayre and Walter 1991; Andrews 1992; Cook 1993; Sutton and Peng 1993a).

The first section of this chapter briefly reviews how the paradigms of conservation and augmentation apply to biological control of plant pathogens. The next section describes the general environment in which biological control of plant pathogens takes place. The third section discusses the principal mechanisms by which antagonists of plant pathogens achieve biological control. The two subsequent sections deal with conservation and augmentation of antagonists, respectively. Both of these sections examine the uses of biological control against root, stem, leaf, flower and fruit diseases, and also discuss biological control of plant-parasitic nematodes. The final section deals with some technological issues facing the development of antagonists in agricultural and social systems.

PARADIGMS OF BIOLOGICAL CONTROL OF PLANT PATHOGENS

Biological control of plant pathogens through conservation is accomplished either by preserving existing microbes which attack or compete with pathogens or by enhancing conditions for their survival and reproduction at the expense of pathogenic organisms. Conservation is applicable in situations where microorganisms important in limiting disease-causing organisms already occur, primarily in the soil and plant residues but in some cases also on leaf surfaces. They may be conserved by avoiding practices which negatively affect them (such as soil

treatments with fungicides). The soil environment may be enhanced for some beneficial organisms through adding organic matter (soil amendments).

Biological control of plant pathogens through augmentation is based on mass-culturing antagonistic species and adding them to the cropping system. In the context of the paradigms discussed in this text, this is augmentation of natural enemy populations, because the organisms used are usually present in the system, but at lower numbers or in locations different than desired. The purpose of augmentation is to increase the numbers or modify the distribution of the antagonists in the system. In some cases, such organisms are taken from one habitat (for example the soil) and augmented in another (for example the phyllosphere). The activity of augmenting microbial agents is sometimes termed "introduction" in the plant pathology literature, in the sense of "adding" them to the system (Andrews 1992; Cook 1993). The organisms introduced, however, are usually found in a local ecosystem and are not introduced from another region of the world (in the sense of Chapter 8).

Augmentation of antagonists falls naturally into two approaches. The first is direct augmentation, at potential infection sites or zones, with organisms antagonistic or parasitic to the pathogens themselves. In this approach, the antagonist population is directly responsible for disease suppression. A second approach is to inoculate plants with nonpathogenic organisms that prompt general plant defenses against infection by pathogens (induced resistance). Disease control is then achieved through greater plant resistance to infection.

Substantial work has been done in characterizing the role of microorganisms in biological control of plant diseases. The biological mechanisms underlying the success of these antagonists in such settings may include initial competition for occupancy of inoculation sites, competition for limiting nutrients or minerals, antibiotic production, and parasitism.

HABITAT CHARACTERISTICS

To understand the principles that apply to biological control of plant pathogens, we must first consider the ecology of the system at the level of the pathogens and the agents used to control them. Aerial plant surfaces, by and large, present hostile environments to colonizing microbes, in many cases consisting of surfaces protected by cuticular waxes, with very small amounts of nutrients available on these surfaces. Further, surfaces of the above-ground portions of plants may be dry. Consequently, pathogenic microbes attempting to colonize these surfaces may face a number of difficulties, including competition with other, nonpathogenic, microbes.

The rhizosphere (the roots and the region immediately adjacent) is a somewhat richer environment than the phyllosphere because of simple sugars, amino acids, and other materials exuded by the roots, but in the remainder of the soil the growth of microbes is often carbon-limited (Campbell 1989). Moisture in the rhizosphere may be more continuous in time and space than on the above-ground surfaces of plants (the phylloplane), but the rhizosphere may be subject to periodic drying.

Various forms of competition in these environments are important in the ability of any particular organism to increase in numbers and consequently reduce the numbers or activity of other organisms, including plant pathogens (Campbell 1989; Andrews 1992). Microbial competition can be important at two main stages of growth of pathogen populations. First, there may be competition during initial establishment on a fresh resource that was not previously colonized by microorganisms. Second, after initial establishment, there is further competition to secure enough of the limited resources present to permit survival and eventual reproduction. Microorganisms show many traits which may characterize them as particularly adept at either the colonization phase or subsequent phases of competition. Species referred to as *r*-strategists (ruderal species) have a high reproductive capacity. These species produce so many spores or

reproductive bodies that there is a high likelihood that some will be found near any newly available resource. These species are effectively dispersed and establish readily in disturbed habitats or in the presence of noncolonized resources. They are found in disturbed settings where easily decomposable organic matter or root exudates are found, and where initial resource capture is crucial for survival. In contrast to these *r*-strategists, species found in more stable situations face competition for space and limited resources (Begon et al. 1986). These organisms, termed *K*-strategists, become more dominant as a community matures and becomes more crowded. These concepts form the endpoints of a continuum, and there are varying degrees of *r*- and *K*-related characteristics in different microbes in various habitats (see Andrews and Harris [1985] for further discussion on these concepts in microbial ecology).

Plant pathogens are spread across this *r*-*K* range of characteristics and vary in other important biological characteristics. There are opportunistic pathogens that are able to attack young, weakened, or predisposed plants, but may be poor competitors (*Botrytis*, *Pythium*, *Rhizoctonia*). There are pathogens that tolerate environmental stresses. These organisms often live in situations with few competitors, because few species are able to exist in such environments. Some pathogens, such as the *Penicillium* species that cause postharvest rots, produce antibiotics that inhibit competitors. Other species (such as *Fusarium culmorum* [Smith] Saccardo) have a very high competitive ability. It is important to understand the ecology of a target pathogen before one can effectively consider what biological control strategy might be most effective. Stress-tolerant and competitive species, for example, would require different biological control strategies and agents than would ruderal ones.

In the same way that antagonists of plant pathogens vary in *r*-*K* and other characteristics, the properties of an effective biological control agent will depend on the setting in which it is intended to function. In many agricultural settings, disturbance makes new resources available to microbes through crop residue burial, cultivation, or planting. A frequent need, therefore, is a control agent that has the characteristics of an *r*-strategist (Campbell 1989), which can grow quickly and colonize new resources rapidly, with minimal nutrient and environmental restrictions. It should function well in disturbed environments and have some means (such as spores) of surviving in the soil or on the plant near to the pathogen inoculum or the source or site of infection. Biological control agents that are *r*-strategists are an approximate equivalent of a protectant fungicide, being in place before the pathogen infection cycle can begin. In other programs, such as those directed against a pathogen which has already invaded the plant host, a more competitive species will be required. Finally, a biological control agent may have to be tolerant of abiotic stresses, particularly for use in dry climates or on leaves.

Before discussing some of the microbes and other agents used in biological control of plant diseases, we will discuss briefly some of the important ecological properties of plants and their environments. These properties affect the ways in which biological control is implemented, as well as what kind of properties might be important in an antagonist of a plant disease.

Soil and the Rhizosphere

Although there is much variation in soil types in different locations, soils are typically rich in microflora, with propagules numbering in the hundreds of thousands per gram of soil (Campbell 1989). In most soils, growth of microorganisms is carbon-limited, either because what carbon is available is not physically accessible or because the microbes do not possess the enzymes necessary to degrade the carbon-containing molecules that are present. An exception to this general limitation is the region immediately surrounding plant roots. This region, the rhizosphere, contains easily metabolized carbon and nitrogen sources such as amino acids,

simple sugars, and other compounds exuded by the roots. Consequently, this region is more favorable than surrounding soil for the support of microflora. Root pathogens and plant-parasitic nematodes may be found growing on or in roots, but many microbes in the soil will be dormant because of resource limitations. Because there are many dormant organisms in the soil prepared to take advantage of any favorable period or opportunity, competition for resources in the soil may be significant and may limit the ability to augment beneficial organisms and have them flourish, unless soils are first sterilized to eliminate potential competitors. Therefore, much research surrounding biological control of root diseases and nematodes has centered around identifying soils which are naturally suppressive to particular disease organisms and investigating the microbial components of the soil responsible for the suppression. Management of such antagonistic organisms for biological control can range from treatment of soil to favor the desirable organisms (conservation) through inoculation of soils or plants with specific beneficial microorganisms (augmentation).

Phyliosphere

The phyllosphere is significantly different from the rhizosphere in its structure, ecology, nutrient availability, and exposure to climatic factors (Andrews 1992). Leaves are relatively hostile to microorganisms. They are generally hydrophobic and covered with cutin and wax, which limits the amount of exudate (and hence nutrients) that reaches the leaf surface. These and other factors impose severe environmental restrictions to microbial growth on leaf surfaces. Fungal pathogens of leaves often enter the leaf tissue very shortly after germination of the pathogen and, consequently, are protected inside the plant for much of their growth. Bacterial pathogens may multiply on the leaf surface before invading leaf tissues. Biological control of disease can take place either through general inhibition and competition on the leaf surface prior to invasion of leaf tissues or through suppression of the disease after the pathogen has invaded. Biological control within leaf tissues can occur through one of several mechanisms, including induced resistance in the plant and hyperparasitism of the pathogen.

Woody stems are habitats low in nutrients and often difficult for pathogens to penetrate. Because the wood itself supports very few saprotrophic microorganisms, pathogens colonizing the wood through wounds, dead branches, or roots find very few competitors. Because there are few organisms present to conserve, protection of the wood from these decay organisms can be achieved by protecting the relatively small, well-defined wound or branch stub through inoculation (augmentation) with specific microorganisms. These wounds are initially very low in sugars or other nonstructural carbohydrates, and antagonists such as *Trichoderma* spp. can successfully compete for these limited resources. Many of the organisms used in the biological control of stem diseases are employed by applying them directly to stem wounds, where they colonize resources and subsequently exclude pathogenic forms. This initial occupancy by antagonists subsequently limits infection by decay-causing organisms, and hence controls the succession of microorganisms in the wood. Of the successful, commercially-available biological control products for plant diseases, several are for diseases of woody stems (Campbell 1989).

MECHANISMS OF BIOLOGICAL CONTROL OF PLANT PATHOGENS

There are several different ways in which a microbial biological control agent can operate against a targeted plant pathogen (Elad 1986). Among these are competition, induction of plant defenses, and parasitism.

Some agents act through competition for limited resources, and through this competition th growth of the pathogen population is suppressed, reducing the incidence or severity of disease. One important component of competition can be competition for Fe^{3+} ions. These ions are sequestered by chemicals called siderophores, which are produced by many species of plants and microbes. Highly efficient siderophores from nonpathogenic microbes can remove Fe^{3+} ions from the soil, outcompeting siderophores from pathogens and thereby limiting the growth of pathogen populations. Some biological control agents compete through the production of antimicrobial substances such as antibiotics which inhibit the growth of pathogens directly, rather than by preemptive consumption of limiting resources.

Another important mechanism limiting infection is the induction of plant defenses against pathogens by nonpathogenic organisms. Cross-protection and induced resistance are mechanisms in which plants are intentionally exposed to certain (nonpathogenic or mildly pathogenic) microbes, thereby conferring in the treated plants some resistance to infection by pathogens. Induced plant defenses may include lignification of cell walls through the addition of chemical cross-linkages in cell wall peptides which makes the establishment of infection through lysis more difficult, suberification of tissues (where plant cell walls are infiltrated with the fatty substance suberin, making them more corklike), and other general defenses, including production of chitinases and β 1,3-glucanases. These plant defenses then limit later infection by pathogens. The biological control agent employed may be an avirulent strain of the pathogen, a different forma specialis, or even a different species of microorganism.

Parasitism is a third mechanism by which beneficial microorganisms suppress plant pathogens. Some species of *Trichoderma*, for example, attack pathogenic fungi, leading to the lysis of the pathogen (Fig. 2.6). Natural enemies of plant-parasitic nematodes include bacterial diseases and nematophagous and nematopathogenic fungi.

CONSERVATION

Root Diseases

Just as in the case of conservation of natural enemies of pest arthropods and weedy plants, conservation activities for the suppression of plant pathogens consist of either avoiding practices which reduce desirable antagonists or actively modifying the environment to favor or selectively enhance the growth of such species. In the case of soil microflora, species employed for biological control of plant pathogens are often competitive antagonists. Adding amendments to soil is one way in which soil microorganisms may be managed to enhance populations of these beneficial organisms. Addition of organic matter to soils for control of *Streptomyces scabies*, the causative organism of potato scab, is one example. Addition of carbon sources to soil increases general microbial activity which leads to reductions in *S. scabies*. Specifically, *Bacillus subtilis* and saprotrophic species of *Streptomyces* were encouraged by barley, alfalfa, or soy meal (Campbell 1989). Soy meal was also a substrate for antibiotic production against *S. scabies*. A general rise in soil organic matter also gave control of *Phytophthora cinnamomi* Rands in avocado in Australia (Manajczuk 1979). The addition of more than ten tons of organic matter per hectare per year led to general increases in numbers of bacteria. Lysis of the hyphae and sporangia of the pathogen were attributed to species of *Pseudomonas*, *Bacillus*, and *Streptomyces*.

Some soils appear to suppress disease naturally and may contain antagonistic or antibiotic flora which flourish without the need for amendments. One example of such suppressive soils is the *Fusarium*-suppressive soil in the Chateaurenard District of the Rhone Valley in France

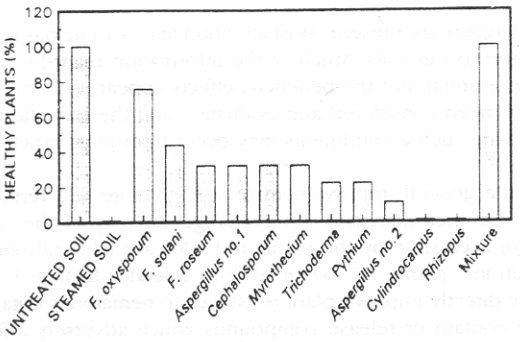

Figure 12.1. The suppressive nature of soil from the Chateaurenard District (Rhone Valley, France) against *Fusarium oxysporum* f.sp. *melonis* Snyder and Hansen is caused by the presence of other nonpathogenic fungi in the soil. Untreated soil and steamed soil with a mixture of the fungi present completely suppress the disease. The species of fungi present vary in their individual ability to suppress the disease, with a non-pathogenic form of *Fusarium oxysporum* Schlechtendal playing an important role (after Alabouvette et al. 1979).

(Fig. 12.1). Here, *Fusarium oxysporum* f.sp. *melonis* Snyder and Hansen is present, but no disease develops when susceptible melon varieties are grown. These soils are suppressive for several other types of *F. oxysporum*, but not to other species or genera of pathogens. The suppressive nature of the soils is clearly biotic, because the soils lose their suppressive ability when steam-sterilized, and the suppressive ability can be transfered to other soils. The antagonists principally responsible for this suppression are nonpathogenic strains of *F. oxysporum* and *Fusarium solani* (Martius) Saccardo. The suppression appears to be due to fungistasis induced by nutrient limitation. The competing fungi appear to have nearly the same ecological niche as the pathogenic forms, and the saprotrophic forms outcompete the pathogens for limiting resources so that dormant chlamydospores of the pathogen do not germinate in the presence of host root exudates. It may be possible to develop systems for other areas using the antagonists from the Chateaurenard area (Campbell 1989), although additional research may be necessary to permit their effective operation in different soils. Other soils suppressive to *Fusarium* wilts are also known. There are numerous other examples of suppressive soils, although some soils or combinations appear to give somewhat variable results.

Leaf Diseases

Conservation of existing flora may be important in limiting the extent of a number of leaf diseases (Campbell 1989). These effects are often revealed through the use of fungicides which deplete extant fungi, permitting the development of previously unimportant diseases. Fokkema and de Nooij (1981), for example, evaluated the effects of various fungicides on leaf surface saprotrophs that have been used in biological control. Wide-spectrum fungicides permitted almost no growth of saprotrophs, while more selective agents permitted some growth of several genera of saprotrophs. In cases where these saprotroph populations play an important role in limiting disease organisms, the application of fungicides would eliminate their contribution to pathogen suppression. One such case is illustrated by Fokkema and de Nooij (1981). Plants treated with benomyl (a systemic fungicide) had fewer saprotrophs and developed more necrotic leaf area when inoculated with *Cochliobolus sativus* (Ito and Kuribayashi) Drechsler ex Dastur than nontreated plants (*C. sativus* is insensitive to benomyl). Another example (Mulinge and Griffiths 1974) is leaf rust of coffee (*Coffea arabica* Linnaeus), caused by *Hemileia vastatrix* Berkeley and Broome. The disease can be controlled by proper application of fungicide. However, if fungicides are applied in one year and not in the next, the disease is

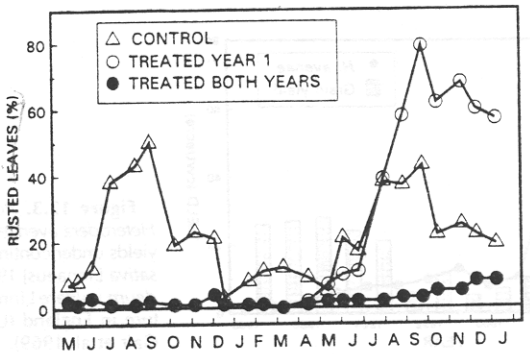

Figure 12.2. The importance of conserving fungi as biological control agents can be demonstrated by the use of fungicides, as in the case of fungicide-treatment effects on incidence of rust (*Hemileia vastatrix* Berkeley and Broome) on coffee (*Coffea arabica* Linnaeus) leaves. The untreated control has seasonally variable incidence with maxima of approximately 50%, while plots treated in both years have very low incidence. Plots treated only in the first year have low incidence in that year, but subsequently have incidences much higher than the untreated control, indicating the presence of microorganisms important in limiting the disease (after Mulinge and Griffiths 1974).

worse on the treated plants than on those which did not receive treatments either year (Fig. 12.2). The elimination of the saprotrophic flora by the fungicide removes their natural suppressing influence on the disease organisms, permitting the disease to be worse. Here, careful use of selective fungicides will be crucial to conserving the important antagonistic flora and permitting their beneficial action.

Plant-Parasitic Nematodes

There are several reports of substantial natural control (control by natural enemies without intentional manipulation) of plant-parasitic nematodes. Stirling (1991) and Sayre and Walter (1991) review several of these; one example is that of the natural suppression of the cereal cyst nematode *Heterodera avenae* in cereal cultivation in the United Kingdom (Gair et al. 1969). In this case, populations of the nematode initially increased for the first 2–3 years of cultivations, and then declined continually during 13 years of continuous cultivation of both oats and barley (a more susceptible crop) (Fig. 12.3). Four species of nematophagous fungi were present in the soil. The two species principally responsible for nematode suppression were *Nematophthora gynophila* and *Verticillium chlamydosporium*. Both fungi attacked female nematodes, either destroying them or reducing their fecundity. The activity of both fungi was greatest in wet soils during laboratory trials (Kerry et al. 1980). Although natural suppression of the nematode population takes some time to develop in these soils, once established it maintains the population below the economic threshold (Stirling 1991).

Conserving nematode antagonists in soils (as opposed to directly enhancing their numbers), is a matter that has received relatively little attention. The application of toxins (insecticides, fungicides) to aerial portions of crops or directly to soils often leads to pesticide activity in the soil. All nematicides are nonselective in their action and, hence, will kill predatory nematodes (Stirling 1991). In addition, herbicides have well-documented effects on soil microorganisms (Anderson 1978) and may well exert some influence on microbial antagonists of nematodes,

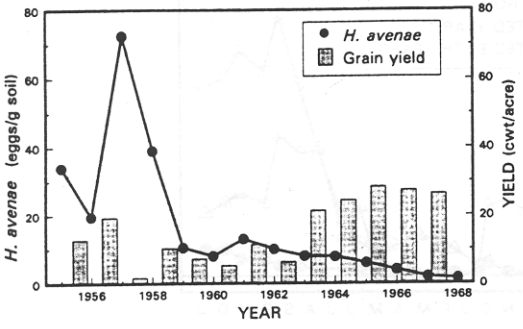

Figure 12.3. Post-cropping levels of *Heterodera avenae* Wollenweber and crop yields under continuous grain (oats [*Avena sativa* Linnaeus] 1955–1962, barley [*Hordeum vulgare* Linnaeus] 1963–1968) culture in England (United Kingdom) (after Gair et al. 1969).

and insecticides may negatively affect soil microarthropods. Many fungicides are known to be detrimental to nematophagous fungi (Mankau 1968; Canto-Saenz and Kaltenbach 1984; Jaffee and McInnis 1990), but at levels higher than would be expected under normal field practice. Among the fumigant nematicides, ethylene dibromide (EDB) and dibromo-chloro-propene (DBCP) appear nontoxic to the nematode-trapping fungi (Mankau 1968), and several herbicides were shown to be not harmful to *Arthrobotrys* sp. (Cayrol 1983). Despite these potentially significant effects on beneficial microflora and fauna and the possibility of conserving these organisms by appropriate choice of material, little has emerged to integrate these ideas into normal farming practice. Perhaps because there has been no serious emergence of nematode problems associated with the use of these materials, this *status quo* is justified. Nonetheless, the opportunities for conserving biologically important agents should be considered in the development of future integrated management programs for plant-parasitic nematodes. Similarly, cultivation practices may be selected to favor natural enemies of nematodes. Among these are minimum or conservation tillage, which reduced the number of cysts of *Heterodera avenae* on roots and the amount of damage caused by the nematode on wheat in Australia (Roget and Rovira 1987). Other practices which may affect populations of natural enemies include normal tillage (which adds crop residue to the soil and thus may favor certain beneficial organisms) and crop rotation sequences (Stirling 1991).

The knowledge that some soils are naturally suppressive to nematodes prompts the question of whether or not the features of these soils can be used to improve biological control. In all documented instances where they have been studied, the suppressive properties of these soils appear to result primarily from the action of one or two specific biological control agents (Stirling 1991). The suppressiveness requires substantial time to develop, and considerable crop loss might be incurred during such an initial phase. Some risk is involved also, because the suppressive nature of the soil may not develop to suitable levels. Careful management of crop varieties, particularly using varieties resistant or tolerant to nematode damage during the initial phases of land use for cropping, is an important part of taking advantage of the potential of these resident natural enemies. Farmers have large amounts of capital invested in land, equipment, and cropping costs, and consequently require a certain degree of reliability in pest control measures. Because of the variable nature of natural suppressiveness of nematodes, any natural control of nematodes in the foreseeable future is most likely to arise fortuitously rather than result from any deliberate actions by scientists or farmers (Stirling 1991).

Where soils are not naturally suppressive to nematode populations, they may be manipu-

lated to enhance what natural control agents are present. Most attention in this arena has been given to the addition of organic matter to the soils. Much of the information regarding the effects of these amendments is circumstantial, but the beneficial effects appear widespread. Many different soil amendments have been considered and evaluated, and the reduction of plant damage from nematodes following such amendments may occur through a variety of mechanisms (Stirling 1991).

One such mechanism is through the general improvement of soil structure and fertility. Addition of crop residue or animal manures increases ion exchange capacity of the soil, chelates micronutrients to make them accessible by the plant, and adds available nitrogen. Grown under such improved conditions, plants are better able to tolerate damage from nematodes. Certain amendments may directly improve plant resistance to nematodes (Sitaramaiah and Singh 1974). Others may contain or release compounds which adversely affect nematodes. Among amendments containing such compounds are those of neem (*Azidirachta indica* A. Jussien) seeds or leaves and of castorbean (*Ricinus communis* Linnaeus) (Stirling 1991 and references therein). Other amendments release nematicidal compounds during decomposition. The most widely studied of these compounds is ammonia. Because nitrogen is a constituent of nearly all soil amendments, ammonia is usually produced during decomposition. A careful balance must be maintained in the carbon:nitrogen ratio, together with sufficient concentrations of ammonia, to provide optimal effect without phytotoxicity (Stirling 1991).

Finally, there is the direct stimulation of nematophagous or antagonistic organisms. Spores of many nematophagous fungi fail to germinate in otherwise suitable but nonamended soils (Dobbs and Hinson 1953), and this soil mycostasis can affect both spores and mycelia (Duddington et al. 1956ab, 1961; Cooke and Satchuthananthavale 1968). Before predation of nematodes can take place, mycelial growth and trap formation must occur. The addition of organic matter provides a substrate which may stimulate spore germination. Organic amendments stimulate a broad range of soil microorganisms, so the effects of amendments on populations of these organisms is complex. Microbial population growth generally increases immediately following the addition of organic matter and, subsequently, as part of the community succession, there is an increase in populations of nematode-trapping fungi. The general hypotheses regarding the beneficial effects of organic amendments center around the stimulation of the saprotrophic growth phase of nematophagous fungi, and stimulation of other general microorganisms which may be detrimental to nematodes, such as antibiotic-producing bacteria. A general rise in enzymatic levels also occurs following soil amendment, and the enzymes may attack the structural proteins in nematode cuticle or egg shell. Chitin amendments in particular have received attention, and addition of chitin to soil is followed by a relatively long-term (4–10 weeks) rise in chitinase activity in the soil. Chitin is the principal structural component of nematode egg shells, and the increase in chitinase activity may be accompanied by decreased survival of nematode eggs. However, the decomposition of chitin also releases ammonia, which may contribute to its beneficial effects. Speigel et al. (1988, 1989) concluded that the beneficial effects of chitin amendments resulted from the action of specialized microorganisms.

A present limitation of the implementation of amendments for nematode control is that such amendments must be applied in large amounts, between 1–10 t/ha to be effective. The use of local resources for such amendments will keep transport costs minimal. One product, the chitin-based Clandosant (derived from crab shells), has been marketed commercially. There is some evidence that the effectiveness of certain amendments may be enhanced by inoculating them with degradative microorganisms (Galper et al. 1991), and Stirling (1991) suggests consideration of systems in which amendments can be inoculated with a specific microorganism as they are applied to the soil.

AUGMENTATION

Augmentation of antagonists of plant disease organisms can generally be of two types, inoculation and inundation (also see Chapter 10). Inoculative releases consist of small amounts of inoculum, with the intention that the organisms in this inoculum will establish populations of the antagonist which will then increase and limit the pathogen population. In inundative releases, a large amount of inoculum is applied, with the expectation that control will result directly from this large initial population with limited reliance on subsequent population growth. Biological control of plant pathogens may also rely on a hybrid of these two concepts. A large amount of inoculum may be applied, both to increase the population of the antagonist and to improve its distribution to favor biological control, and antagonism can result from both these applied organisms and the increased population of antagonists resulting from their reproduction. Biological control of blackcrust (*Phyllachora huberi*) on rubber tree foliage by the hyperparasites *Cylindrosporium concentricum* and *Dicyma pulvinata* (Junqueira and Gasparotto 1991) is one example of long-term control of a plant pathogen by a single augmentation in an agricultural system (Cook 1993). In this case, rubber trees were treated with spore suspensions of the antagonists (inundatively), which resulted in control over more than one season. More generally, beneficial microorganisms are added seasonally or more frequently.

Where the beneficial organisms involved are being placed into a habitat or environment other than where they originated, the organisms are often referred to as "introduced" in plant pathology (Andrews 1992; Cook 1993). There are several examples where such organisms, when moved to a new habitat (for instance, from the soil to the above-ground part of a plant) colonize and serve as successful agents of biological control (Andrews 1992; Cook 1993).

Competitive Antagonists and Antibiotic Production

Root Diseases. One way in which flora may be manipulated to protect against disease is to intentionally inoculate soils or seeds with microbial antagonists. Such antagonists, to be successful in their task, must be able to colonize plant surfaces and survive in the competitive environment of the soil. Flora with demonstrated ability to achieve this under field conditions include fungi, principally *Trichoderma* spp., and, among the bacteria, *Bacillus* spp. and *Pseudomonas* spp.

Among the bacteria, species of *Bacillus* are regularly used for biological control of root diseases. Members of the genus have advantages, particularly that they form spores which permit simple storage and long shelf life, and they are relatively easy to inoculate into the soil. The consequence of this biology is, however, that although the inoculant may be present in the soil, it may be in dormant or resting stages. Nonetheless, species of *Bacillus* have provided good control on some occasions. Capper and Campbell (1986) showed a doubling of wheat yield over wheat plants naturally infected with take-all by those also inoculated with *Bacillus pumilus* Meyer and Gottheil. *Bacillus pumilus* and *B. subtilis* were also used to protect wheat from diseases caused by species of *Rhizoctonia* (Merriman et al. 1974). A major difficulty with the use of *Bacillus* spp. is that the control provided is often variable, with different results in different locations, or even in different parts of a season in the same location (Campbell 1989). *Bacillus subtilis* is used as a seed inoculant on cotton and peanut (*Arachis hypogaea* Linnaeus), with nearly 2 million ha treated in 1994 (Blackman et al. 1994). Treatment promotes increased root mass, nodulation, and early emergence, and suppresses diseases caused by species of *Rhizoctonia* and *Fusarium*.

Of substantially more promise as antagonists of root diseases are species of *Pseudomonas*,

particularly the *Pseudomonas fluorescens* and *Pseudomonas putida* (Trevisan) Migula groups (Campbell 1989). These bacteria are easy to grow in the laboratory, are normal inhabitants of the soil, and colonize and grow well when inoculated artificially. They produce a number of antibiotics as well as siderophores. Several have received patents and are marketed commercially for control of root rot in cotton (Campbell 1989). An isolate of another species of *Pseudomonas* has been used as an antagonist of take-all disease of wheat (Weller 1983). Isolates of *Ps. fluorescens* from soils showing some control of take-all can be applied as seed coats and inoculated into fields suffering from the disease. Such treatments give 10–27% yield increases compared with untreated, infected control groups. Evidence points to both siderophore and antibiotic production as important.

Species of the fungal genus *Trichoderma* can be saprotrophic and mycoparasitic and have been used against wilt diseases of tomato, melon, cotton, wheat, and chrysanthemums. The antagonists were applied to seeds or through a bran mixture incorporated into the planting mix at transplanting. Although disease did develop, it did so much more slowly than in untreated soils, resulting in a 60–83% reduction in disease (Siven and Chet 1986). The mode of action against *Verticillium albo-atrum* Reinke and Berthier wilt of tomatoes appeared to be antibiosis.

Stem Diseases. The control of *Heterobasidion annosum*, the causative agent of butt rot in conifer stumps, by *Phanerochaete gigantea* was one of the first commercially available agents for biological control of a plant pathogen (Campbell 1989). The disease caused by *H. annosum* is primarily a disease of managed plantations. The fungus colonizes freshly cut stumps, invades the dying root system, and can then infect nearby trees through natural root grafts, causing death of the trees. *Heterobasidion annosum*, however, is a poor competitor, and when a stump is intentionally inoculated with *Ph. gigantea* (and usually with chemical nitrogen sources which encourage growth of the antagonist) the antagonist rapidly colonizes the resource, excluding future attack by the pathogen and even eliminating existing pathogen infection (Table 12.1). Very little inoculum is needed on a freshly-cut stump, and the shelf life of the pellet formulation is about two months at 22°C. The antagonist is able to outcompete *H. annosum* even when the initial inoculum favors the pathogen by as much as 15:1 (Rishbeth 1963).

The ascomycete fungi *Eutypa armeniaceae* Hansford and Carter and *Nectria galligena*

TABLE 12.1 Colonization of Scots Pine Stumps (*Pinus sylvestris* Linnaeus) After Inoculation with Various Fungi as Antagonists to *Heterobasidion annosum* (Fries) Brefeld (Which Was Inoculated into All Stumps) (After Rishbeth 1963)

	% of Mean Area of Stump Section Colonized After:					
	10 Weeks			6 Months		
Inoculated Antagonist	Species Inoculated	Pg[a]	Ha[b]	Species Inoculated	Pg	Ha
None	—	28	38	—	80	7
Botrytis cinerea Persoon: Fries	5	5	55	0	0	25
Trichoderma viride Persoon: Fries	0	10	65	0	43	40
Leptographium lundbergii Lagerberg and Melin	95	9	5	37	47	0
Phanerochaete gigantea (Fries: Fries) Rattan et al.	80	80	Trace	75	75	0

[a] *Phanerochaete gigantea* (Fries: Fries) Rattan et al.
[b] *Heterobasidion annosum* (Fries) Brefeld

Bresadola in Strass infect apricots and apples, respectively, and cause stem cankers and eventual death of the trees. Pruning wounds in apricots are treated with *Fusarium laterium* Nees: Fries through specially adapted pruning cutters. *Fusarium laterium* produces an antibiotic which inhibits germination and growth of *E. armeniaceae*. When applied, the concentration of the antagonist must be greater than 10^6 conidia/ml. Integrated application which includes a benzimidazole fungicide gives better control than either fungicide or antagonist alone. *Nectria galligena* infection can be reduced through sprays of suspensions of *Bacillus subtilis* or of *Cladosporium cladosporioides* (Fresenius) de Vries. These antagonists are not in commercial use because apples are treated for *Venturia inaequalis* (Cooke) G. Winter (apple scab) so frequently that *N. galligena* is controlled by those sprays.

Crown gall is a stem disease caused by the bacterium *Agrobacterium tumefaciens* (Smith and Townsen) Conn. It affects both woody and herbaceous plants in 93 families. Infection is typically from the soil, rhizosphere, or pruning tools. Control can be effected by treating plants with a suspension of a related saprotrophic bacterium *Agrobacterium radiobacter* (Beijerink and van Delden) Conn strain K-84. This strain of the bacterium produces an antibiotic which is taken up by a specific transport system in the pathogen bacterium, which is then killed. The commercially-available formulations of this agent are effective primarily against pathogen strains which attack stone fruits, but other bacteria are under investigation for use against strains pathogenic in other crops. This agent has been altered by gene-modifying technology to produce a new strain (strain 1024) which lacks the ability to transfer antibiotic resistance to the target bacterium.

The fungus *Chondrostereum purpureum* (Persoon: Fries) Pouzar infects stems of fruit trees and produces a toxin which leads to a condition known as silverleaf disease. Stems can be inoculated with a species of *Trichoderma* grown on wooden dowels or prepared as pellets which are inserted into holes bored in the affected stem. Treated stems recover from the disease more rapidly than untreated stems. The *Trichoderma* sp. can be applied to pruning wounds to prevent initial establishment of *C. purpureum*.

Leaf Diseases. Control of leaf diseases at the time of pathogen germination has been shown in the laboratory. This control occurs in the presence of competitive organisms, which may include fungi, yeast, or bacteria. The mode of action in some cases is competition for nutrients which, together with water, are necessary for successful germination and invasion of many pathogens. The germination of *Botrytis* sp., for example, is inhibited by certain bacteria and yeasts (Blakeman and Brodie 1977). This inhibition is less pronounced if additional nutrients are supplied, indicating that the mechanism is, at least in part, resource competition. Studies on control of *Botrytis* rot in lettuce (Wood 1951) indicated that several organisms were successful in suppressing the disease when sprayed on lettuce plants, among them species of *Pseudomonas*, *Streptomyces*, *Trichoderma viride*, and *Fusarium*. Peng and Sutton (1991) evaluated 230 isolates of mycelial fungi, yeasts, and bacteria and tested them as antagonists of *B. cinerea* in strawberry in both laboratory and field trials. Several organisms (including members of each taxonomic group tested) were effective, some as effective as captan (a commercial fungicide) (Table 12.2). Sutton and Peng (1993b) further evaluated *Gliocladium roseum* and determined that the suppression of *B. cinera* by this antagonist was probably a result of competition for leaf substrate. The fungi *Gliocladium roseum* and *Myrothecium verrucaria* (Albertini and Schweinitz) Ditmar were also effective in suppressing *B. cinerea* in black spruce (*Picea mariana* [Miller] Britton Stearns Poggenburg) seedlings (Zhang et al. 1994).

Bacteria may also be used to limit frost damage to leaves and blossoms of plants. Certain

TABLE 12.2 Effects of Various Microorganisms and Captan on Incidence of *Botrytis cinerea* Persoon: Fries in Strawberry. All Plots Were Treated with *B. cinerea* Conidia. Several Antagonists Were as Effective as Captan in Reducing Disease Levels. (After Peng and Sutton 1991)

Treatment	Incidence of *B. cinerea* (%)[a]			
	Cambridge Plots		Arkell Plots	
	Stamen	Fruits	Stamens	Fruits
Water check	4 b	35 c	2 b	59 b
Botrytis cinerea Fries: Persoon check	19 a	59 a	16 a	71 a
Captan	5 b	40 b	3 b	56 b
Bacillus sp.	15 a	56 a	12 a	74 a
Cryptococcus laurentii (Kufferath) Skinner	17 a	43 b	11 a	72 a
Rhodotorula glutinis (Fresenius) Harrison	6 b	33 c	12 a	48 c
Alternaria alternata (Fries) Kessler	9 b	51 a	3 b	52 c
Myrothecium verrucaria (Albertini and Schweinitz) Ditmar	15 a	48 b	13 a	60 b
Fusarium graminearum Schwabe	18 a	47 b	16 a	65 a
Fusarium sp.	17 a	46 b	16 a	64 a
Drechslera sp.	16 a	26 c	9 a	56 b
Trichoderma roseum (Persoon) Link	12 a	27 c	13 a	41 c
Epicoccum purpurascens Ehrenberg ex Schlechtendal	6 b	43 b	4 b	51 c
Colletotrichum gloeosporioides Penzig	4 b	44 b	6 b	41 c
Trichoderma viride Persoon: Fries	5 b	38 b	2 b	37 c
Penicillium sp.	4 b	14 d	1 b	38 c
Gliocladium roseum Link: Bainer	4 b	25 c	3 b	43 c

[a]Numbers in a column with same letters were not significantly different (P = 0.05)

bacterial species such as *Pseudomonas syringae* and *Erwinia herbicola* serve as nucleation sites on leaves for the formation of ice, and, in their presence, ice forms soon after temperatures fall below freezing. If these ice-nucleating bacteria are replaced by competitive antagonists (such as certain strains of *Ps. syringae*) that lack the protein that causes ice-nucleation, frost is prevented even at temperatures from −2 to −5°C (Lindow 1985b). The protective bacteria, after being applied to the leaves, colonize them for up to two months, an interval suitable to protect from frost during the limited season that low temperatures are likely. A naturally-occurring, non-ice nucleating strain of *Ps. fluorescens* is registered in the United States as a commercial product (Frostban B®) for suppression of frost damage (Wilson and Lindow 1994).

Spraying suspensions of propagules, generally at high concentrations, is the principal method for applying biological control agents to foliage (and to flowers), and dusts (such as lyophilized bacterial preparations) are also used. Spray methodology has yet to be refined in terms of sprayer characteristics, droplet size, and pressures, and other methods of application with greater efficiency may be necessary to effectively target certain plant parts (Sutton and Peng 1993a).

Flower Diseases. One of the principal diseases of flowers which has received attention is fire blight of rosaceous plants, which is particularly severe on pear (Campbell 1989). The causal bacterium, *Erwinia amylovora*, also occurs on leaves and may cause stem cankers. The bacterium is transferred by insects to flowers in the spring from overwintering sites on stem cankers, and subsequently from flower to flower. Infection enters the pedicel and from there the stem. Infected flowers and small stems die, and cankers form on other stems. Chemical

control is difficult and expensive, and sometimes is ineffective because of resistance to copper compounds and streptomycin. Biological control has been effective using *Erwinia herbicola*, sometimes in combination with *Pseudomonas syringae* (Wilson and Lindow 1993). Suspensions of *E. herbicola* are sprayed onto the flowers just before the period of potential infection. The antagonist occupies the same niche as the pathogen, reducing the numbers of *E. amylovora* by competition, and there is also evidence for the production of bacteriocins (chemicals which suppress population growth of related bacteria) by some strains. Control can be good, comparable to that achieved by commercial bactericides, though repeated application of the bacterium was necessary (Isenbeck and Schultz 1986). Another approach to control is to reduce secondary infections on leaves, which leads to reductions in the overwintering population of the pathogen. This control is achieved by treatment with the antagonists *Ps. syringae* and other bacteria (Lindow 1985b). A novel approach to dissemination of the antagonistic bacteria has been evaluated by Thomson et al. (1992). These workers mixed *E. herbicola* and *Ps. fluorescens* with pollen in a special apparatus at the entrance to honey bee (*Apis mellifera*) hives. Bees emerging from these hives through the mixtures transmitted the antagonists to the flowers efficiently, although disease control was not evaluated because of absence of disease in the test orchards.

Fruit Diseases. Fruits are subject to attack both by general pathogens (*Botrytis, Rhizopus, Penicillium*) and by a few specialist pathogens such as the coffee berry disease fungus *Colletotrichum coffeanum* Noack and *Monilinia* spp., which cause brown rots of rosaceous fruits. While many of these are controlled by fungicides, *Trichoderma viride* has been shown to limit disease from *Monilinia* spp. Various *Bacillus* spp. also are antagonistic to these fungi through production of antibiotics and by reducing the longevity and germination of spores. Both the bacteria and culture filtrates have been used with some success against these pathogenic fungi, but there has been no commercial development, probably because fungicides used routinely in orchards for control of other diseases give some control of brown rot (Campbell 1989).

Among the most serious diseases of soft fruits are postharvest rots (Dennis 1983), especially that caused by *Botrytis cinerea*. Potential for biological control of postharvest diseases has been reviewed by Wilson and Wisniewski (1989) and Jeffries and Jeger (1990) (also see Wilson and Wisniewski 1994). In strawberries, *B. cinerea* grows saprotrophically on crop debris and from there infects flowers or fruit. Various species of *Trichoderma* have been evaluated and gave control as good as standard fungicides (Tronsmo and Dennis 1977). The antagonists *Cladosporium herbarum* and *Penicillium* sp. gave excellent results in controlling *Botrytis* rot on tomato (Newhook 1957). Honey bees have been used to distribute *Gliocladium roseum* to strawberry flowers (Peng et al. 1992) and raspberry flowers (Sutton and Peng 1993a) to suppress *Botrytis* rot.

Induced Resistance

Root Diseases. Induced resistance is a form of biological control in which the natural defense responses of the plant, which may include production of phytoalexins, additional lignification of cells, and other mechanisms (Horsfall and Cowling 1980; Bailey 1985), are promoted in the plant prior to exposure to the pathogen. These resistance mechanisms are induced by challenging the plant with a nonpathogenic organism. The induced plant defenses then limit later

infection by the pathogen. The organism employed may be an avirulent strain of the pathogen, or a different forma specialis, or even a different species. There are few well-documented cases of induced resistance for soil-borne pathogens, and these are mostly of wilt diseases. Dipping tomato roots in a suspension of *Fusarium oxysporum* f.sp. *dianthi* a few days before likely exposure to the pathogen *F. oxysporum* f.sp. *lycopersici* (Saccardo) Snyder and Hansen conferred protection that lasted a few weeks. Cotton may be protected for three months or longer by spraying the roots at transplanting with a mildly pathogenic strain of the disease-causing pathogen *Verticillium albo-atrum* (Fig. 12.4). The role of some fungi against take-all of wheat includes some elements of induced resistance. *Gaeumannomyces graminis* var. *graminis* grows on grass roots and also has been found on wheat, where it occupies a niche similar to that of the pathogen *G. graminis* var. *tritici* Walker. The antagonist invades the root cortex but not the stele, and is halted by the lignification and suberization of the cortex and stele. Root cells with these chemically-changed walls are less susceptible to invasion by the pathogen. Although this interaction produced yield increases in Europe, the strains or species present in the United States did not appear to confer resistance, and in Australia there were only slight yield increases (Campbell 1989). These variable results, while somewhat common for biological control of soil-borne pathogens, do not reduce the value of the antagonists where they do work, but rather indicates some potential challenges in defining the taxonomy, biology, and host-plant relationships important to biological control in this group of organisms.

Leaf and Stem Diseases. Induced resistance can control anthracnose diseases caused by *Colletotrichum* spp. (Kúc 1981; Dean and Kúc 1986). *Colletotrichum lindemuthianum* (Saccardo and Magnus) Lamson-Scribner causes anthracnose of beans, *Colletotrichum lagenarium* (Passerine) Saccardo causes cucumber anthracnose, and *Cladosporium cucumerinum* Ellis and Arthur causes scab in cucumbers. Inoculation of cucumbers with *Colletotrichum lindemuthianum* (which does not cause disease in cucumbers) made plants resistant to both *Colletotrichum lagenarium* and *Cladosporium cucumerinum*. Treatment applied to an early leaf resulted in protection of later leaves, even when the initially inoculated leaf was removed. The factor causing resistance travels systemically through the plant. Variations on this approach include inoculating an early leaf with a pathogen, inducing resistance throughout the plant, and then removing the infected leaf. Induced resistance also occurs in some virus diseases (Thomson 1958) and may last for years, as in the case of healthy citrus seedlings being inoculated with an avirulent strain of citrus tristeza virus.

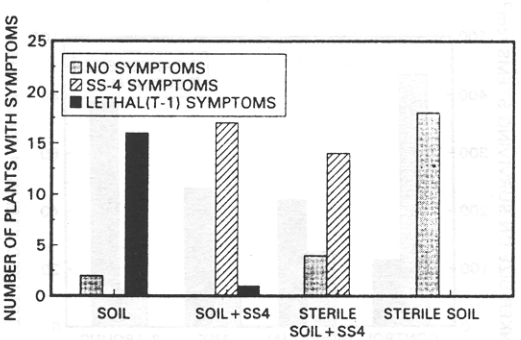

Figure 12.4. Induced resistance with a reduced virulence strain (SS-4) of *Verticilium albo-atrum* Reinke and Berthier in a field of cotton (*Gossipium hirsutum* Linnaeus) infested with a virulent strain (T-1) of the pathogen (after Schnathorst and Mathre 1966).

Stem rot in carnations, caused by *Fusarium roseum* Link: Fries '*Avenaceum*,' can be prevented by inoculating wounds inflicted during propagation with the nonpathogenic *F. roseum* '*Gibbosum*.' This inoculation produced a germination inhibitor and also reduced the time needed for the stems to develop resistance to the pathogen. This hastening of resistance was caused by activation of the host's defense mechanisms, and is another example of induced resistance.

Hypovirulence

Chestnut blight, caused by *Cryphonectria parasitica*, is controlled by employing hypovirulent strains of the disease pathogen. A number of hypovirulent strains are known, and inoculating infected trees with a hypovirulent strain leads to reduced canker size and greater stem survival (Fig. 12.5). In the field, hypovirulent strains are inoculated into infected trees at the rate of 10 inoculated trees/ha. The hypovirulent strain spreads from these locations and, on contacting more virulent strains, fuses with these strains and exchanges a viral element infecting the pathogen. The hypovirus, which causes hypovirulence, is transferred to the virulent strains, attenuating their effects. Active cankers are eliminated in ten years (van Alfen 1982).

Parasitism of Pathogens and Nematodes

Root Diseases. The mycoparasites *Trichoderma* spp. have been used successfully against diseases caused by *Rhizoctonia* and *Sclerotium* pathogens. One example is the pathogen *Sclerotium rolfsii* Saccardo, which attacks many crop plants and survives unfavorable periods by forming sclerotia in the soil. Strains of *T. harzanium* that have β 1–3 glucanases, chitinases, and proteases have been isolated. These enzymes permit *T. harzanium* to parasitize the hyphae and sclerotia of the pathogen, invading and causing lysis of the cells. *Trichoderma harzanium* is grown on autoclaved bran or seed, and this material is then mixed with the surface soil (Chet and Henis 1985). Two other fungi known to parasitize sclerotia are *Coniothyrium minitans* and *Sporidesmium sclerotivorum* (Ayers and Adams 1981).

Sporidesmium sclerotivorum is a hyphomycete that in nature behaves as an obligate parasite of sclerotia of *Botrytis cinerea* and several species of *Sclerotium* (Adams 1990). It has been studied as an agent against botrytis rot in lettuce, where it shows considerable potential. It

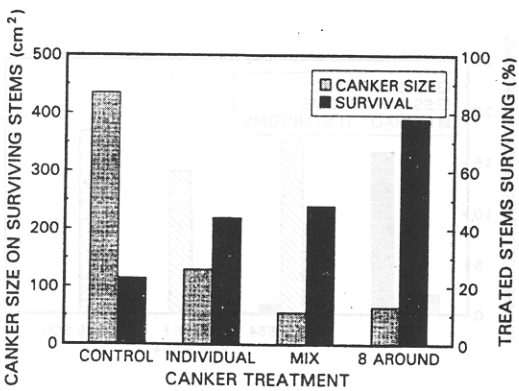

Figure 12.5. Example of hypovirulence in American chestnut (*Castanea dentata* [Marsham] Borkjauser) inoculated with normal (control) and hypovirulent strains of *Cryphonectria parasitica* (Murril) Barr. "8 around" is 8 individual strains placed around a canker (after Jaynes and Elliston 1980).

can be grown *in vitro* on various carbon sources and is efficient in converting glucose into mycelium. Spores produced in mass culture are collected, processed, and applied to infected soil, and field tests are promising (Adams 1990).

Leaf Diseases. Some plant pathogens, including fungi and some bacteria, are known to be attacked by other pathogens. *Bdellovibrio bacteriovorus* is a bacterium that can attack other bacteria by penetrating the cell wall and lysing the host bacterium, subsequently reproducing inside its host. Different strains of *Bd. bacteriovorus* have been examined for virulence against *Pseudomonas syringae* pv. *glycinae* (Coeper) Young, Dye and Wilkie, the cause of soybean blight. By applying *Bd. bacteriovorus* at sufficiently high rates, disease symptoms were reduced more then 95% (Scherff 1973) (Fig. 12.6). Parasites of fungi pathogenic on leaves are numerous (Kranz 1981), but only a few have been studied in much detail, such as *Sphaerellopsis filum*, *Verticillium lecanii*, and *Ampelomyces quisqualis*. The mycoparasite typically penetrates the host hypha or spore and kills it. Some of the control may be from the pathogen overgrowing the sporulating pustules of the pathogen and preventing spore release and thus reducing inoculum in the environment, even if the spores are not killed. A typical problem with implementation of these mycoparasitic fungi is that they often do not affect a large proportion of the pathogens unless humidity and temperature are high. Consequently, although much reduction of spore production may take place, there is still sufficient inoculum of the pathogen remaining to cause disease. These mycoparasites often are seen only at high incidences of disease, which is unsuitable for general control of the target pathogens. They may have some use in particular systems, either in the tropics or in greenhouses, where environmental conditions are more favorable.

Plant-Parasitic Nematodes. The bacterial pathogen of nematodes most studied is *Pasteuria penetrans sensu stricto* Starr and Sayre (Starr and Sayre 1988), which is an obligate parasite of root-knot nematodes (*Meloidogyne* spp.) and has not been successfully cultured in vitro. This restriction in mass culturing has limited attempts to test the bacterium's effectiveness (Stirling 1991). In experimental trials, it has shown potential for controlling root-knot nematodes (*Meloidogyne* spp.) (Mankau 1972; Stirling et al. 1990) (Fig. 12.7), infesting a high proportion of nematodes in soil to which bacterial spores had been added, and in other trials (U.S. Depart-

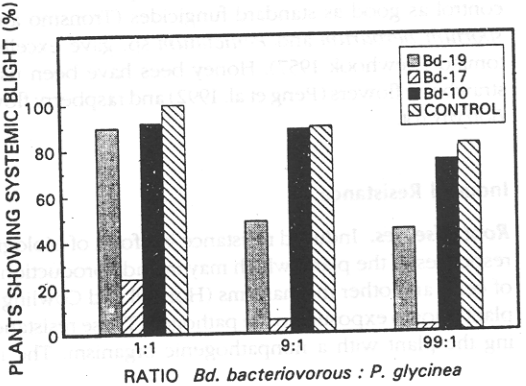

Figure 12.6. Percentage of soybean (*Glycine max* [Linnaeus]) plants showing symptoms of systemic blight after leaves were inoculated with different strains of *Bdellovibrio bacteriovorus* Stolp and Starr mixed in varying ratios with the pathogen *Pseudomonas syringae* pv. *glycinea* (Coeper) Young, Dye and Wilkie (after Scherff 1973).

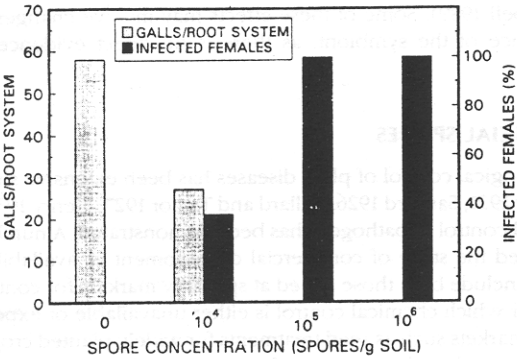

Figure 12.7. Number of galls caused by *Meloidogyne javanica* (Treub) Chitwood (and percentage of females infected with *Pasteuria penetrans* (Thorne) Sayre and Starr *sensu stricto* Starr and Sayre) after movement as juveniles through 4 cm of soil infested at various densities with *P. penetrans* spores (after Stirling et al. 1990).

ment of Agriculture 1978) reducing damage to plants in plots containing the bacterium. Observations by Mankau (1975) indicated that populations of the bacterium did not increase rapidly in field soil. The development of a mass production method in which roots containing large numbers of infected *Meloidogyne* spp. females were air-dried and finely ground to produce an easily handled powder enabled more extensive testing (Stirling and Watchel 1980). When dried root preparations laden with bacterial spores were incorporated into field soil at rates of 212–600 mg/kg of soil, the number of juvenile *Meloidogyne javanica* (Treub) Chitwood in the soil and the degree of galling was substantially reduced (Stirling 1984) (Fig. 12.8); other authors have reported similar results (Stirling 1991). Effective use of this bacterium through such inundative release would require concentrations on the order of 10^5 spores/g soil (Stirling et al. 1990). Such quantities could only be produced on a large scale with an efficient *in vitro* culturing method, a problem which has received attention but has not yet yielded a solution (Stirling 1991). Use in inoculative releases, where smaller numbers of spores are applied and a crop tolerant of nematode damage is grown to permit the increase of both nematode and bacterial populations, has been suggested (Stirling 1991). Conserving the bacterium in the presence of nematicides appears possible. Of seven tested nematicides, only one showed slight toxicity to the bacterium (U.S. Department of Agriculture 1978).

The use of *Bacillus thuringiensis* strains with activity against nematodes is also possible. As

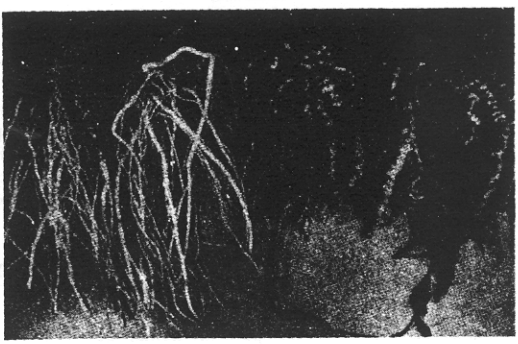

Figure 12.8. Roots of tomato grown in field soil infested with *Meloidogyne javanica* (Treub) Chitwood and treated with 600 mg of a dried root preparation of *Pasteuria penetrans* (Thorne) Sayre and Starr per kilogram of dry soil (left) and grown in untreated soil (right). (Photograph courtesy of G. Stirling, from Stirling, G. 1984, *Phytopathology* 74:55–60, with permission.)

these bacteria may be cultured in fermentation media, their mass culture is simpler than for *P. penetrans*. Suppression of nematodes was possible through drench applications and through incorporating the bacterium into a methyl cellulose seed coat (Zuckerman et al. 1993).

Considerable attention has been given to the nematode-trapping fungi as possible augmentative agents. Mass culture on nutrient media is possible for these fungi. Two cultures of nematophagous *Arthrobotrys* fungi have been developed and tested for addition to soil for specific target environments. Cayrol et al. (1978) reported the successful use of *Arthrobotrys robusta* Cooke and Ellis var. *antipolis*, commercially formulated as Royal 300® against the mycetophagous nematode *Ditylenchus myceliophagus* Goodey in commercial production of the mushroom *Agaricus bisporus* (Lange) Singer. The nematophagous fungus was seeded simultaneously with *A. bisporus* into mushroom compost, which led to 28% increases in harvest and reduced nematode populations by 40%. The results justified the commercial use of the fungus for nematode control in mushroom culture. Cayrol and Frankowski (1979) reported the use of *Arthrobotrys superba* Corda (Royal 350®) in tomato fields, applied to the soil at a rate of 140 g/m², resulting in protection of the tomatoes and colonization of the soil by the fungus. Other reports have indicated little efficacy of fungal preparations when added alone to soil (Barron 1977; Sayre 1980; Rhoades 1985). In general, there has been limited success in the use of these agents (see Stirling [1991] for a summary). The fungistatic nature of soil (Mankau 1962; Cooke and Satchuthananthavale 1968) may limit the ability of these fungi to grow even when added in substantial numbers to soil. Additional work is needed, perhaps in the areas of colonization and soil amendments together (for example, Table 12.3), for the use of nematophagous fungi to become suitably reliable for general use as a control method.

Many predacious fungi may be unsuited for control of root-knot nematodes, *Meloidogyne* spp. Stirling (1991) suggested that *Monacrosporium lysipagum* (Drechsler) Subramanian and *Monacrosporium ellipsosporum* (Grove) Cooke and Dickinson, which can invade egg masses, may warrant further investigation. The nematode-trapping fungi are likely to be more effective against ectoparasitic nematodes and such species as *Tylenchus semipenetrans* Cobb, where juvenile stages migrate through the rhizosphere. Little attention has been given to testing predacious fungi against such nematodes.

Fungi which are internal parasites of nematodes have proven difficult to culture on nutrient media, and consequently there have been few attempts to use them for augmentative control of nematodes. Alternative mass-culturing techniques may hold some promise (Stirling 1991). In the few experiments reported, the fungistatic effects of soil often limited fungal growth and the effectiveness of the antagonists. Lackey et al. (1993) report the production and formulation of

TABLE 12.3 Results of a Field Microplot Experiment with *Heterodera schachtii* Schmidt on Sugarbeet Showing the Effects of Soil Amendments and Nematode-Trapping Fungi on Nematode Populations and Yield (After Duddington et al. 1956a)

Treatment	Final nematode population/100 g soil		Yield of roots + tops (t/ha)
	Cysts	Eggs	
1. Untreated	467	114	45.4
2. Bran (20 t/ha)	383	103	56.3
3. *Monacrosporium thaumasia* (Drechsler) de Hoog & van Oorschot mycelium (6.8 t/ha) at planting	488	144	56.3
4. Treatment 2 + treatment 3	333	94	59.2
5. Treatment 4 + *M. thaumasia* mycelium (6.8 t/ha) in mid season	363	128	60.1

Hirsutella rhossiliensis Minter et Brady on alginate pellets (see also Fravel et al. 1985) which, when added to soil, led to transmission of the fungus to the nematode *Heterodera schachtii* Schmidt and suppressed nematode invasion of roots.

Among the facultatively parasitic fungi which attack nematodes, *Paecilomyces lilacinus* and *Verticillium chlamydosporium* have received the most attention as possible augmentative agents. The results of studies on *P. lilacinus* have been variable, with some studies showing some positive effect of the fungus, while others show little or no effect (Stirling 1991). The mechanisms leading to the beneficial effect have not been clearly elucidated, but may be from metabolic products or effects other than direct parasitism of eggs. Studies have generally involved the addition of fungal preparations to the soil at the rate of 1-20 t/ha, which is likely too great for widespread commercial use. Additions at lower rates (0.4 t/ha) in a variety of carriers (alginate pellets, diatomaceous earth, wheat granules) have also shown limited beneficial effects (Cabanillas et al. 1989; Stirling 1991). Tribe (1980) suggested the direct addition of *V. chlamydosporium* to the soil. Kerry (1988) added hyphae and conidia, formulated in sodium alginate pellets or in wheat bran, to soil, and the fungus proliferated in the soil only from granules containing bran. When chlamydospores were used as inoculum, the fungus was able to establish without a food base (De Leij and Kerry 1991). Of three isolates studied, only one successfully colonized tomato root surfaces. This species apparently has considerable promise, but screening programs will be necessary to identify isolates with characteristics suitable for biological control (Stirling 1991).

Predacious microarthropods and nematodes have evoked considerable interest. Most work has been done in simple microcosms, and there have been no attempts to evaluate augmentative release of these organisms in a field setting. In one experiment, Sharma (1971) found nematode numbers reduced by 50% or more in glass jars inoculated with mites and springtails compared with similar jars containing no predators, but the author pointed out possible causes for the reduction other than simple predation. Experiments with predacious nematodes have in general failed to demonstrate a measurable impact of the predator (Stirling 1991). One exception was the reduction of galling by *Meloidogyne incognita* on tomato by predacious nematodes (Small 1979). The general suitability of these groups of organisms for inundative release is questionable, because of the potential difficulties in developing technologies for their rearing, packaging, transport, and delivery beneath the soil in a viable state (Stirling 1991).

Mycorrhizae

Mycorrhizae are nonpathogenic fungi associated with roots in some temperate forest trees. Ectomycorrhizae are mostly basidiomycetes which form a sheath over the root, and hyphae spread out into the soil. These fungi have been studied in relation to nutrient uptake, but they also affect root disease. Because they completely enclose the root, they change the quantity and quality of exudates reaching the soil; consequently, roots with mycorrhizae have a different rhizosphere flora than uninfected roots (Campbell 1985). In at least one case, the mycorrhizal fungus *Pisolithus tinctorius* (Persoon) Coker and Couch, the thick symbiont sheath forms a barrier to infection by such pathogens as *Phytophthora cinnamomi* attacking eucalyptus trees. Other mycorrhizal fungi produce antibiotics effective against *P. cinnamomi* in plate tests. The intentional manipulation of mycorrhizal fungi for disease control has not been widely implemented, but opportunities for selected uses may be possible (Campbell 1989).

Another group of fungi are the vesicular arbuscular mycorrhizae (VAM), which are phycomycete fungi associated with the roots of many plant species including many crops. These fungi do not form a sheath surrounding the root, and their effects on disease are complicated,

but are in general beneficial (Campbell 1989). Some of these effects may involve changes in host plant physiology in the presence of the symbiont, as there is no direct evidence of pathogen inhibition by these fungi.

DEVELOPING AND USING BENEFICIAL SPECIES

Growth of our knowledge about biological control of plant diseases has been extensive since the first experimental reports (Hartley 1921; Sanford 1926; Millard and Taylor 1927; Henry 1931), and substantial potential for microbial control of pathogens has been demonstrated. A number of products or programs have reached the stage of commercial development or availability (Table 12.4). Products in current use include both those aimed at specialty markets for control of certain stem or flower diseases (for which chemical control is either unavailable or expensive) and those aimed at larger scale markets such as seed treatments for widely planted crops.

The cycle for research, development, and implementation of antagonists of plant pathogens

Table 12.4 Some Commercially Available Antagonists or Products for Plant Pathogens and Plant-Parasitic Nematodes

Organism	Trade Name	Target
(a) Targeted against plant pathogens		
Agrobacterium radiobacter (Beijerink and van Delden) Conn strain K-84	Agtrol; Galltrol; Norbac 84-C	*Agrobacterium tumefaciens* (Smith and Townsend) Conn (crown gall)
Bacillis subtilis (Ehrenberg) Cohn	Kodiak, Epic	Seed treatment against *Rhizoctonia* spp., *Pythium* spp. and *Fusarium* spp. root diseases
Pseudomonas cepacia (Burkholder) Palleroni and Holmes	Blue Circle; Intercept	*Rhizoctonia* spp. *Pythium* spp., and *Fusarium* spp. diseases of seedlings
Pseudomonas fluorescens (Trevisan) Migula	Dagger G	*Rhizoctonia* spp. and *Phythium* spp. diseases of seedlings
Coniothyrium minitans Campbell	Coniothyrin	*Sclerotinia sclerotiorum* (Libert) de Bary sunflower
Fusarium oxysporum Schlechtendal (nonpathogenic strains)	Fusaclean; Biofox C	Diseases from pathogenic strains of *Fusarium oxysporum*
Gliocladium virens Millers, Giddens and Foster	GlioGard	*Rhizoctonia* spp. and *Phythium* spp. diseases of seedings and bedding plan
Mycorrhyzae	Vaminoc	*Botrytis* spp. and *Phythium* sp. diseases
Phanerochaete gigantea (Fries: Fries) Rattan et al.	—	*Heterobasidion annosum* (Fries) Brefeld (butt rot)
Pythium oligandrum Drechsler	Polygandron	*Pythium ultimum* Trow in sugar beet
Streptomyces griseoviridis Anderson et al.	Mycostop	*Alternaria* sp. and *Fusarium* spp. disease
Trichoderma harzanium/polysporum (Link) Rifai	BINAB	Wood-rot fungi; *Verticillium malthousei* Ware in mushrooms
Trichoderma harzianum Rifai	F-Stop; Trichodex; Supravit; T-35; TY	*Heterobasidion annosum*; diseases cause by *Rhizoctonia* spp.,, *Pythium* spp., *Fusarium* spp. *Botrytis cinerea* Persoo Fries, and *Sclerotium rolfsii* Saccardo
Trichoderma lignorum (Tode) Harz	Trichodermin-3	*Rhizoctonia* spp. and *Fusarium* spp. diseases
(b) Targeted against plant-parasitic nematodes		
Arthrobotrys robusta Cooke and Ellis var *antipolis*	Royal 300®	*Ditylenchus myceliophagus* Goodey
Arthrobotrys superba Corda	Royal 350®	*Meloidogyne* spp.
Chitin-based amendment	Clandosan®	Plant-parasitic nematodes

is composed of several steps. These include initial discovery of candidate agents, refinement of knowledge of their biology, ecology, and mode of action, microcosm and field trials of their efficacy, and large-scale development for commercial production.

The first challenge in the development of a biological control program is the discovery process. Many microorganisms show potential as antagonists of particular pathogens. Protocols have been proposed to make the process of screening these candidates more efficient (Andrews 1992; Cook 1993). The principal difficulties are screening out candidates that are effective only during *in vitro* (agar plate) trials but are not effective in natural settings, and in selecting candidates that can be successfully cultured in large quantities. Following discovery of suitable candidates, research focuses on their mode of action and on factors which may enhance or limit their efficacy in targeted settings (glasshouses, field plots). In addition, experimental fermentation and formulations must be developed for production of materials suitable for use in agricultural settings. Finally, issues of large-scale production and delivery must be addressed. Products for use must be effective on an economical basis, and economies of scale may play an important role in the eventual availability of any organism or product. Each must have a satisfactory shelf life, and safe and effective methods for application must be discovered or developed (Cook 1993; Sutton and Peng 1993a). Such application methods might include sprays of suspensions or dusts, contact application, bee vectoring, and production of antagonists in a crop environment (Sutton and Peng 1993a).

The adoption of any biological control agent in commercial agriculture is dependent on its reliability and its availability. Limitations to the process of eventual adoption, therefore, include cost of development and size of potential market. Many pesticides for control of plant diseases have a broad spectrum of activity, are applicable in a variety of crops and settings, and may act either prophylactically, therapeutically, or both. Biological controls, in contrast, often have narrow ranges of activity and may work in only a few crops or soil types, and while they can often act both prophylactically and therapeutically, their action may take some time to develop. Therefore, they may have a narrower market than a chemical pesticide and be unattractive for development by major corporations (Andrews 1992). In this context, it may be appropriate for public institutions such as government experiment stations to undertake the development of such biological controls, in the same way that they take the responsibility for development of new plant varieties (Cook 1993).

Microorganisms intended for use as biological control agents must be viewed in a biological rather than a chemical paradigm (Cook 1993). Where an effective pesticide may work in many places, each place may have unique soil, edaphic, and biological features which limit or enhance the effectiveness of microbial antagonists of pathogens. Consequently, each microbial biological control system may have to make use of locally adapted strains, taking advantage of resident antagonistic flora and fauna and augmenting their effectiveness with additional species or strains, or enhancing resident populations through soil amendments. Although the different strains may use common mechanisms to achieve biological control (such as production of antibiotics), competitive abilities adapted to local conditions may be vital to permit the organisms to compete for resources and effectively control pathogens.

CHAPTER
16

BIOLOGY AND DYNAMICS OF PATHOGENS

INTRODUCTION

Pathogens of arthropods include bacteria, viruses, fungi, protozoa, and nematodes. Many phyla, families, and species are involved and collectively display an enormous diversity in their biologies. These have been summarized recently by Tanada and Kaya (1993), from whom much of the information presented here has been drawn. Concepts presented here on epizootiology of disease in arthropod populations have been drawn from Fuxa and Tanada (1987).

BASIC PROCESSES IN PATHOGEN BIOLOGY

To complete their life cycles successfully, most pathogens must contact a host, gain entrance to the host's body, reproduce within one or more host tissues, and emit propagules which subsequently contact and infect new hosts. This chapter examines these components of pathogen biology, first as concepts, and then as components of the biologies of selected pathogen groups.

Host Contact

Unlike predators and parasitoids, many arthropod pathogens lack a motile stage. Therefore, host contact typically results not from active search processes but rather from chance contact following the passive dispersal of some stage of the pathogen such as fungal spores by wind, rain, or other organisms (such as parasitoids). The amount of contact between a pathogen population and its hosts is determined by the spatial pattern of the pathogen's propagules relative to the spatial distribution of the host, and the survival of these propagules over time. The occlusion bodies of nuclear polyhedrosis viruses from the cadavers of diseased gypsy moth larvae (*Lymantria dispar*), for example, are released when host cadavers rupture (Fig. 16.1). Virus occlusion bodies are initially concentrated near the site of host death, but later become distributed over nearby foliage (especially foliage directly beneath the initial point) by rain (Woods and Elkinton 1987). In agricultural systems, pathogen dispersal may also occur as the result of some irrigation practices (Young 1990). Similarly, wind acts to redistribute fungal conidia, which are produced on the cadavers of hosts, to new locations in the habitat (Fig. 16.2). Factors which affect disease transmission at the population level are discussed further in the following section section on epizootiology.

While contact between propagules of many types of pathogens and new hosts is largely a

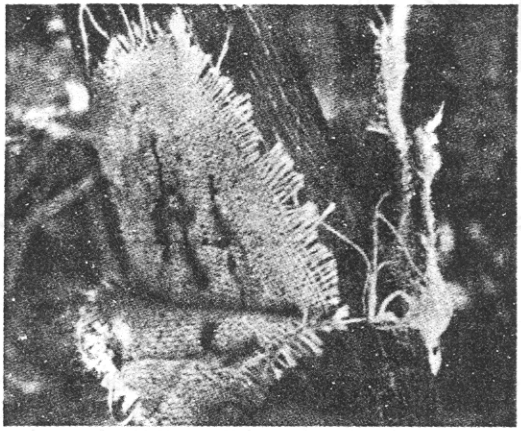

Figure 16.1. Cadavers of virus-killed gypsy moth larvae (*Lymantria dispar* [Linnaeus]) rupture and release virus particles which contaminate bark and foliage. (Photograph courtesy of J. Cunningham, Forestry Canada.)

random process mediated by abiotic factors (horizontal transmission) (Fig. 16.3), some pathogens are transmitted between generations of hosts from mother to offspring (vertical transmission), a process that eliminates the need to contact new hosts randomly (Fig. 16.4). Additionally, a few types of pathogens, such as some nematodes and aquatic fungi, are able to move towards hosts. Some nematodes move in water between soil particles and use chemical cues such as CO_2 and host feces to aggregate near hosts (Ishibashi and Kondo 1990). Similarly, the motile spores of such aquatic fungi as *Coelomomyces* spp. and *Lagenidium* spp. move toward hosts in response to chemical cues emitted by hosts (Carruthers and Soper 1987).

Host Penetration

Once propagules of pathogens have contacted a host, the body of the host must be penetrated to reach tissues susceptible to attack. The arthropod cuticle provides protection from many

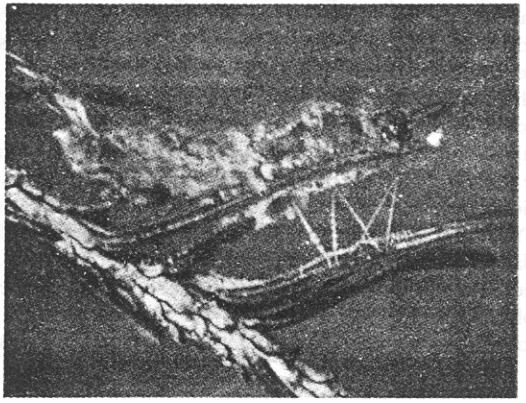

Figure 16.2. Conidia of *Zoophthora radicans* (Brefeld) Batko are produced by conidiophores which emerge from fungus-killed *Choristoneura fumiferana* (Clemens) larvae. These condia are dispersed by wind and rain. (Photograph courtesy of D. MacLeod, Forestry Canada.)

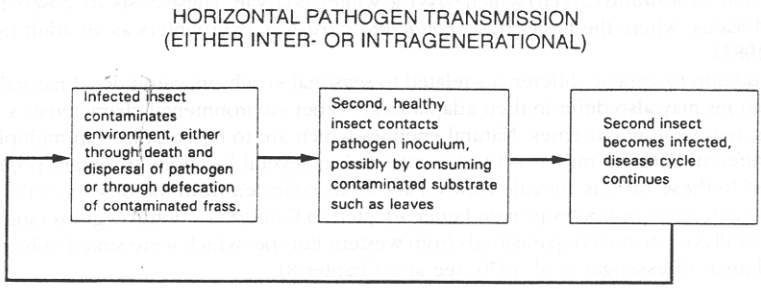

Figure 16.3. Horizontal transmission of pathogens in insect populations occurs among members of the same general life stage and usually among members of the same generation.

types of pathogens. Most bacteria, viruses, and protozoa enter arthropods through the thin (nonchitinized) wall of the midgut after being ingested. Consumption of food that is contaminated with pathogen stages is a major mechanism of contagion for chewing arthropods. Sucking arthropods, in contrast, escape exposure to such contamination by feeding on plant fluids, which are relatively free of microbial contaminants. As a consequence, sucking insects such as aphids are little affected by such pathogens as bacteria, viruses, and protozoa that normally enter hosts by ingestion. Nematodes and fungi are able to penetrate arthropod hosts through routes other than the midgut. Nematodes may enter hosts either through the midgut (after being ingested or actively entering the mouth), or may enter hosts directly through wounds or spiracles, or may mechanically penetrate intact cuticle using stylets or spears as cutting devices. The characteristic route of entry for fungi is through the cuticle, a process that is achieved by special penetration hyphae that produce enzymes capable of digesting insect cuticle (Fig. 16.5).

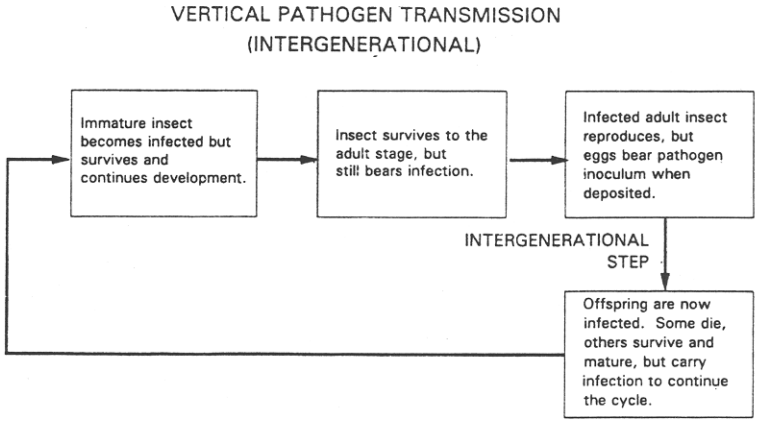

Figure 16.4. Vertical transmission of pathogens in insect populations occurs between members of two succeeding generations and involves passing the inoculum from reproducing parents to offspring.

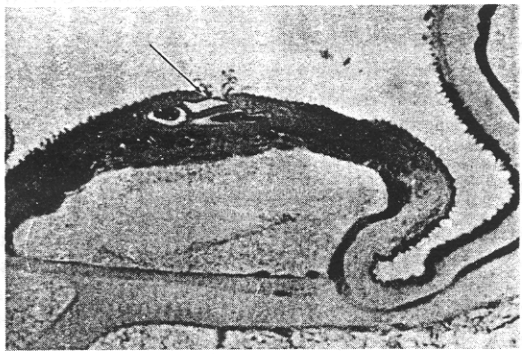

Figure 16.5. Host penetration by fungi is usually through the cuticle. Penetration hyphae are used by an *Entomophthora* species to enter (arrow) its host, the pine sawfly larva, *Diprion similus* (Hartig). (Photograph courtesy of M.G. Klein, from Klein, M. G. and H. C. Coppel. 1973, *Annals of the Entomological Society of America* 66:1178–1180, with permission.)

Reproduction in Host Tissues

Once a pathogen has penetrated its host, the pathogen typically proceeds to reproduce in one or several tissues (Fig. 4.3b,d). In some pathogen groups, reproduction may be limited to specific tissues as, for example, the nonoccluded *Oryctes* virus that reproduces principally in fat body and midgut epthelium. Others reproduce in virtually all tissues in their hosts. The range of tissues infected affects the number of pathogen propagules produced per unit weight of the host, such that pathogens producing systemic infections in all tissues may be more economical to rear than ones limited to only certain of their host's tissues. Steinernematid and heterorhabditid nematodes continue to reproduce after hosts have been killed by symbiotic bacteria. Consequently, most host tissue is available for nematode reproduction.

Exit of Pathogen Propagules from Host or Cadaver

Following reproduction of a pathogen inside the body of its host, the offspring of the pathogen must in some fashion contact other hosts to continue the transmission cycle. In groups in which vertical transmission from parent to offspring occurs, contact is achieved by contamination of eggs which are then deposited in some part of the general environment. In most cases, however, pathogen propagules are released independently back into the environment and later contact new hosts. If pathogens kill the host, release may take place as a consequence of the physical disintegration of the host cadaver, as in the case of virus diseases in which host cadavers liquify and are broken apart by abiotic factors. For fungi, release of spores may be by way of the growth of special spore-producing hyphae which reach through the cuticle to the outside of the host cadaver. Release from such structures may be passive or may be assisted by active mechanical discharge of spores. Nematodes leave hosts in several ways. In some groups, juveniles or adults may actively disperse from host cadavers in soil or water (Fig. 16.6). In other groups, nematodes may be dispersed by way of the host's reproductive tract during oviposition attempts by infected hosts. Dispersal of protozoans and bacteria from infected hosts may occur in the form of contaminated feces while the host lives, or later through breakup or consumption (by other hosts) of the host cadaver.

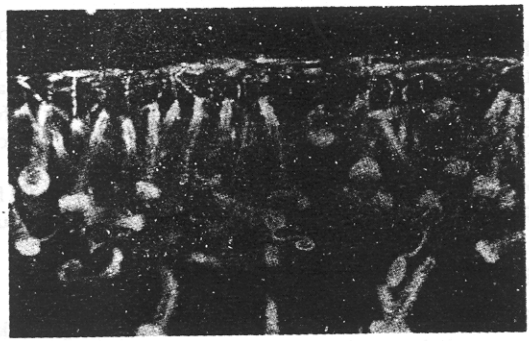

Figure 16.6. Emergence of the post-parasitic stage of *Romanomermis culicivorax* Ross and Smith from its mosquito host. (Photograph courtesy of J.J. Petersen.)

Spread and Persistence of Pathogen Propagules in the Environment

Following the release of infectious pathogen particles into the environment, the continuity of the pathogen's population depends on contacting new hosts. Because the host's physical and temporal occurrence may be patchy and unpredictable, pathogens require adaptations both for dispersal and persistence. Dispersal in the environment is largely accomplished, as mentioned above, by physical factors such as wind and rain, with host contact being largely a matter of chance. Transmission to new hosts by such dispersal is most likely when hosts are highly aggregated. Insects such as whiteflies, aphids, Lepidoptera, or other insects undergoing high-density population outbreaks are especially favorable for disease transmission. Insects being reared in colonies in laboratory or commercial settings, unless reared individually, are also especially susceptible to the propagation of disease.

The likelihood of a pathogen contacting a host is enhanced by a variety of life history characteristics. Some groups of pathogens are transmitted vertically from parent to offspring, such as the microsporidium *Nosema pyrausta* (Paillot) which is transmitted primarily through transovarial infection in the first generation of its host noctuid *Ostrinia nubilalis* (Siegel et al. 1988). Other pathogens are transmitted by infected hosts to the specific habitat where additional hosts are likely to be present. Some nematodes in the families Iotonchiidae and Phaenopsitylenchidae, for example, are deposited in the habitat of their hosts' larvae by infected adults (muscoid flies and wood wasps) whose ovaries are infected by the nematodes (Fig. 4.9). Because at times hosts are likely to be scarce or environmental conditions unfavorable for host infection, many bacteria, protozoa, and fungi produce resting stages (of various types) that are able to persist for long periods. The production of such structures aids host contact by permitting pathogen propagules to be conserved until conditions improve.

At the population level, two general routes of transmission can be defined. Vertical transmission occurs when pathogens are passed directly from parents to offspring (Fig. 16.3). There are two types of vertical transmission: transovarial and transovum. In transovarial transmission, the pathogens are present inside the eggs at the time they are laid due to invasion by the pathogen during egg development inside the mother. This manner of transmission predominates, for example, in the nonoccluded iridescent viruses and the microsporidia. Transovum transmission occurs when offspring acquire disease as a consequence of external contamination of their surfaces at the time of oviposition by pathogens from within the mother. Such transmission occurs, for example, in some baculoviruses and cytoplasmic polyhedrosis viruses of Lepidoptera and Hymenoptera (Andreadis 1987).

All other routes of transmission are considered to be horizontal (Fig. 16.4). One form of horizontal transmission occurs when pathogen particles emitted by an infected individual enter the environment and subsequently contact and infect another host. For example, horizontal transmission occurs when an infected caterpillar defecates pathogen-contaminated feces on foliage that is later eaten by a healthy caterpillar, which then contracts the disease. Horizontal transmission is common in baculoviruses and fungi, among other groups. Transmission of venereal diseases during copulation is also considered a form of horizontal transmission. In this process, pathogens pass directly between hosts without entering the nonhost environment.

EPIZOOTIOLOGY OF ARTHROPOD PATHOGENS

The prevalence of a disease in a system may change markedly over time. Periods when disease is particularly prevalent are termed epizootic outbreaks of a disease. Some groups of pathogens are more frequently involved in arthropod epizootics (viruses, fungi, protozoa) than others (bacteria). A number of factors can contribute to the outbreak of a disease in a particular insect population at a particular time and place. These include characteristics of both the host and the pathogen, host population density and distribution, and environmental conditions such as temperature, rainfall and humidity. The study of how such factors determine the course of disease outbreaks is termed epizootiology; Fuxa and Tanada (1987) discuss epizootiology of insect diseases.

Host Features That Influence Disease Prevalence

Among the host factors that can affect the development of an epizootic are host density, spatial distribution, health of hosts, age, moulting status, and behavior.

Because pathogen propagule density will be diluted as the cube of the distance to the next nearest host, contact rates will be highest when hosts are closest together. Disease transmission is facilitated in insect-rearing colonies with artificially high densities, or in kinds of hosts that occur in nature in colonies or groups, or have significantly aggregated spatial distributions. For chewing insects such as caterpillars, horizontal transmission is facilitated when hosts are dense because contact with feces or fragments of host cadavers is more likely to occur. For sucking insects, aggregation enhances transmission by proximity to sources of pathogens such as fungal spores released from diseased aphids within aphid colonies.

The health of hosts is also a factor affecting the rate of transmission of a pathogen because hosts that are stressed by other pathogens, poor nutrition, or adverse physical conditions are often less resistant to pathogen invasion. Similarly, age and moulting status affect susceptibility to infection. Young caterpillars are often more susceptible to the bacterium *Bacillus thuringiensis* and to viruses. Newly moulted insects, in which the cuticle is still rather thin, are more susceptible to fungal invasion than insects whose cuticle is fully hardened. When large numbers of individuals in a population are stressed, or in a susceptible age class, or moulting status, an epizootic of a disease becomes more likely because hosts are more easily infected.

A variety of host behaviors can affect the rate of transmission of a disease in an insect population. Diseased individuals may exhibit abnormal behaviors that increase the dispersal of the pathogen propagules released from the individual. In some diseases, infected individuals frequently die in relatively high positions on their food plant or habitat. Some caterpillars infected with virus, for example, migrate upward and die at the tips of branches, a behavior that positions the cadaver to contaminate foliage lower down as the cadaver disintegrates. Similarly,

some flies infected with various fungal pathogens die at the tips of grass stems, such that wind dispersal of spores produced on the cadaver is facilitated. In other instances, normal behaviors such as grooming of nestmates in termites or cannibalism (in which moribund individuals may be consumed by other members of the group) may increase the spread of a disease.

Pathogen Features That Influence Disease Prevalence

Many pathogen characteristics influence the rate of disease in a host population. These include: infectivity, virulence, production of toxins, nature of the pathogen life cycle, and inoculum density, distribution, and persistence. Understanding pathogen biology and constraints on pathogen transmission in the environment is important in the development of pathogens as pest control products.

The genotype of a particular pathogen strain will strongly influence its infectivity and virulence to a particular host. Infectivity is the ability of the pathogen to gain entrance to the host's body and virulence is the ability, once inside the host, to cause disease. Pathotypes within a pathogen species vary significantly with regard to what host species can be successfully attacked. In fungi, strains may vary in the level of enzymes produced by penetration hyphae, changing their infectivity to the host. In *Bacillus thuringiensis*, isolates differ in the kinds and quantities of the toxins they produce. These differences in toxins determine which groups of hosts will be affected by different strains of the bacterium.

Pathogen life cycles vary from simple to highly complex, with the need in some cases for alternate hosts. Highly complex life cycles may place constraints on pathogen transmission if alternate hosts or special conditions are available in only some habitats or periods. The requirement for copepods or ostracods as alternate hosts by fungal pathogens in the genus *Coelomomyces*, for example, means that continuous reproduction of this pathogen following artificial application is only possible if these hosts are present (Tanada and Kaya 1993).

The density, distribution, and persistence of the inoculum of a pathogen is important in determining the normal rate of the disease in the host population and the frequency and intensity of epizootics. Inoculum alone in the absence of favorable environmental conditions is insufficient to cause epizootics. However, abundant, persistent sources of inoculum in the habitat favor the occurrence of epizootics by ensuring the presence of inoculum during periods when environmental conditions are favorable for disease initiation. Factors that influence the amount of inoculum in the local habitat include those that affect the level of production of the inoculum, dispersal in the habitat, and persistence of the inoculum in various specific parts of the habitat. Quantities of inoculum produced per host will vary greatly between types of pathogens for a variety of reasons. Some pathogens, for example, multiply in the entire host, while others may be restricted to specific tissues. Pathogens killing hosts at a early stage are likely to produce less inoculum per host than ones killing older, larger hosts.

Spread of a given pathogen in the habitat will depend on the nature of the release mechanism from the host. Wind-blown fungal spores are likely to be more widely dispersed than viruses liberated by liquification of host cadavers with local contamination of foliage in the drip zone below the cadavers. Persistence of pathogen inoculum will be strongly influenced by physical factors that destroy inoculum, particularly ultraviolet light, high temperatures, and dryness. The production of pathogen stages resistant to such degradation will be important in determining how well a given pathogen can persist at a site. Some microhabitats, especially soil and protected spaces such as bark crevices, provide physical conditions that are more favorable for the survival of pathogen inoculum. Host contact with these zones, or movement

of material from these areas to areas where hosts feed, such as the movement of soil onto crop foliage by the splashing of rain droplets, can be important mechanisms for bringing inoculum back into contact with hosts.

Environmental Factors That Influence Disease Prevalence

Many abiotic and biotic factors affect the level of disease in an arthropod population. Among these are temperature, humidity, desiccation, light, soil characteristics, and other organisms that aid in pathogen transmission. A more detailed discussion of the effects of these factors on insect epizootics is given by Benz (1987).

Effects of temperature on disease rates are complex. Temperature differences may affect either the pathogen or host directly, but the effect on the disease rate can only be understood by also considering the impact on behavior, growth, movement, and other factors that taken together define the effect of temperature on the pathogen-host interaction. The route of entry of the pathogen, for example, can play an important role in this process. For organisms in which ingestion of contaminated food is the principal route of entrance, infections can only be acquired at temperatures at which hosts feed. For fungi, which enter hosts primarily through the integument rather than the mouth, infections may be acquired at temperatures below those at which the host feeds, if temperatures are favorable for fungal spore germination and hyphal growth.

Humidity, free water, and desiccating conditions are important in some situations. High humidity generally favors outbreaks of fungi, promoting both the germination of existing spores and formation of new spores on cadavers. High humidities and soil moisture levels are also important in favoring nematode epizootics. Bacterial, viral, and protozoan disease prevalences seem less influenced by these factors. Rain appears to have relatively little direct effect on disease rates and does not wash significant amounts of pathogen inoculum from plant surfaces. Desiccation, in contrast, is an important, lethal factor for many types of pathogens, including nematodes, bacteria, and protozoa. Many pathogens have special stages that can resist desiccation. These include the occlusion bodies of baculoviruses, the spores of some bacteria (*Bacillus* spp.), the cysts of amoeba and other protozoans, the resting spores of fungi, and the eggs and juvenile resting stages of some nematodes.

The deleterious effect of sunlight, especially ultraviolet light, on baculoviruses is well known. Baculoviruses deposited on upper leaf surfaces exposed to sunlight are typically inactivated in a short period, ranging from a few hours to a few days. Fungal spores are also sensitive to light, but many are protected by light-absorbing pigments. Few data are available concerning the effect of light on protozoa and nematodes.

Soil is a complex habitat with many physical and biotic components. Because soils are often moist and dark, they are a favorable location for the survival of resting stages of such pathogens as bacteria, baculoviruses, and fungi. Soil pH and organic content influence the rate of degradation of pathogens, as does the species composition and abundance of soil microorganisms.

Contamination of predators and parasitoids with viruses or other pathogens may occur when these feed on or emerge from diseased hosts. Pathogens may then be spread to new hosts by the movement and behaviors of such agents. Fuxa et al. (1993) demonstrated this process by tracking the movement of an introduced virus (the *Anticarsia gemmatalis* virus from Brazil) by such agents in a soybean (*Glycine max*) field in Louisiana (U.S.A.) following its release. Because the virus was not native to the region, all virus detected was known to have originated from the released material.

In addition to these environmental and biotic factors, several features of interacting host-pathogen systems have been identified that can cause periodic or aperiodic cycles of disease prevalence. These features are part of the natural cycling of densities between a host and its pathogen independent of changes in host susceptibility, environmental suitability, or pathogen virulence. These issues have received considerable attention in the literature on dynamics of interacting host-pathogen systems. An introduction to this topic is given in Chapter 18.

BACTERIAL PATHOGENS OF ARTHROPODS

Bacteria are unicellular organisms that have rigid cell walls. Their shapes include rods, spheres, spirals, and forms that have no fixed shape. A description of the families and genera in which arthropod bacterial pathogens are found is given in Chapter 4. The biologies of species causing disease in arthropods are discussed in detail by Tanada and Kaya (1993).

A generalized arthropod bacterial infection may be described as follows. Most pathogenic bacteria enter arthropod hosts through the mouth when contaminated food is ingested. They multiply in the gut, producing enzymes (such as lecithinase and proteinases) and toxins that damage midgut cells, aiding the bacteria to invade the hemocoel. Once they have successfully invaded the hemocoel, bacteria cause a general septicemia, during which more toxins are produced. Host death results from bacterial multiplication in the hemocoel and poisoning by bacterial toxins. Before death, hosts lose their appetite and cease feeding. Diseased hosts become diarrhetic and discharge watery feces. Hosts may vomit. Both of these processes distribute infective particles into the environment, promoting horizontal transmission to new hosts. Insects killed by bacteria darken and become soft. Tissues become viscous and have a putrid odor. The integument remains intact, while cadavers shrivel, become dry and harden.

While most arthropod bacterial pathogens enter hosts through the mouth, a few are able to penetrate the integument directly, as is the case of *Micrococcus nigrofaciens*, a pathogen of scarab beetles in the genus *Lachnosterna* (Northrup 1914). Some bacteria are transmitted vertically from parent to offspring, for example, *Serratia marcescens* Bizio in the brown locust, *Locustana pardalina* (Walker) (Prinsloo 1960). In some cases, bacteria can be introduced into the host's hemocoel directly on the ovipositors of parasitic Hymenoptera. However, such cases are of minor importance. The dominant route of host entry for arthropod bacterial pathogens is by mouth when contaminated foliage or other food is eaten.

Because the hemocoel is the characteristic site for most bacterial infections in arthropods, mechanisms to gain entrance to this region are important determinants of which groups of bacteria are effective pathogens of arthropods. Several mechanisms exist that permit bacteria to reach the hemocoel. Some species in the genus *Bacillus* produce crystalline toxic proteins. These aid the bacteria in penetrating the midgut epithelial cells. Bacteria enter the hemocoel by causing pores to open in midgut membrane cells, altering their permeability (Honée and Visser 1993). Many other groups of bacteria, however, lack such toxins. These bacteria are usually unable to gain entrance to the hemocoel and normally exist as saprophytes in the insect gut and exteriorly in other habitats. Such groups (*Proteus*, *Serratia*, *Pseudomonas*) are pathogenic if they penetrate into the hemocoel, but usually do not do so unless the host is stressed by some other factor. When the host is stressed, these bacteria are able to multiply in the gut and more effectively penetrate into the hemocoel, aided by enzymes such as chitinase. Bacteria in the genera *Photorhabdus* and *Xenorhabdus* are symbiotic with nematodes in the families Heterorhabditidae and Steinernematidae, respectively. The nematodes serve as vectors that penetrate mechanically into the insect hemocoel and deposit these bacteria there directly.

Some species of bacterial pathogens of pest arthropods, such as *Bacillus thuringiensis*,

do not cause significant epizootics in nature, due to low spore production and ineffective horizontal transmission. Indeed, some evidence suggests this species may be primarily a saprophyte in nature, and only incidentally an insect pathogen (Martin 1994). Nevertheless, such species may be of importance to biological control as augmentative biological control agents (see Chapter 11). Death of hosts from *Bacillus thuringiensis* products requires delta endotoxins (for bacteria to gain access to hemolymph), but LC_{50} values of *B. thuringiensis* products are greatly reduced by the presence of live spores, demonstrating the importance of live cells in the disease process (Miyasono et al. 1994). Physiological modes of action of *B. thuringiensis* endotoxins in insects are reviewed by Gill et al. (1992). Other species, such as *Bacillus sphaericus* and *Bacillus popillae*, are more effective in horizontal transmission and do maintain continuous disease cycles in affected arthropod populations for many years.

VIRAL PATHOGENS OF ARTHROPODS

Viruses are obligate intracellular parasites consisting of a genome (either DNA or RNA) enclosed in a protective protein coat (**capsid**) and further enclosed in additional layers termed **envelopes**. The capsid plus the DNA or RNA it encloses is termed **nucleocapsid** or **virion**. Virions may be further embedded (in some viruses) in protective protein matrices termed **occlusion bodies**. Viruses replicate inside host cells using the host's protein-synthesizing metabolism and materials in the host cell (Matthews 1991). Some features of virus biology important to biological control are: virus susceptibility to abiotic factors such as ultraviolet light and high temperatures; need for a mechanism to penetrate into host cells; and the occurrence of significant virus epizootics in nature, especially those of members of the Baculoviridae.

Susceptibility to abiotic factors is reduced in the Baculoviridae, Entomopoxviridae, and Reoviridae by protein matrices (occlusion bodies) which surround and protect the virions. Occlusion bodies increase virion stability and persistence during periods spent outside the host. Other viruses, such as Rhabdoviridae, are not exposed to such abiotic factors because they are closely associated with hosts at all times, being transmitted vertically between host generations (Tanada and Kaya 1993).

Initial entrance into hosts by the Baculoviridae is by mouth when contaminated food is consumed. The high pH found in the insect midgut dissolves the protein occlusion bodies of baculoviruses, liberating virions. Virion envelopes merge with the cell membranes of gut microvilli, and nucleocapsids enter host cells. Alternatively, an enveloped virion may be envaginated in a vacuole of cell membrane and later be liberated in the cell after enzymes have dissolved the vacuole and the virion envelope. This process is call viroplexis. Once in the hemocoel, nonoccluded forms of the virus (plasma-enveloped virions) are the infective structure. Details of cytopathology and viral replication may be found in Tanada and Kaya (1993). In most Lepidoptera, baculoviruses in the nuclear polyhedrosis subgroup infect many host tissues (fat body, hypodermis, trachea, blood cells). In sawflies (Symphyta, Hymenoptera), nuclear polyhedrosis viruses infect only the midgut tissue. Such differences influence greatly the number of virions produced per infected host, affecting both the dynamics of horizontal transmission in nature and the economics of commercial virus production.

Arthropod larvae (such as those of many Lepidoptera) infected with baculoviruses lose their appetite but continue to feed at lower rates up until a few days prior to death, which may occur 5–21 days after infection. Before death, some species of infected larvae move to positions high in the plant canopy, a behavior that facilitates the horizontal transmission of viruses in nature through food contamination. Dead hosts often become flaccid and the integument ruptures, liberating occlusion bodies containing virions into the environment. Consumption of foliage

contaminated by occluded virions by new hosts completes the virus transmission cycle commonly leading to epizootics. Transmission of *Oryctes* virus (a nonoccluded virus) in rhinoceros beetle, *Oryctes rhinoceros*, occurs by means of contact with virus-contaminated feces produced by infected adult beetles that may live as long as 30 days. Adult feeding and defecation in sites also used for oviposition and larval development promote horizontal transmission of this virus.

FUNGAL PATHOGENS OF ARTHROPODS

Morphologically, fungi may occur as single cells (such as yeasts), or branched filaments (hyphae) which form mats (mycelia). Hyphae may be uninucleate, or multinucleate segments which have numerous nuclei not separated by transverse walls. Fungi may reproduce sexually, asexually, or both. Sexual reproduction involves fusion between two structures. These may be motile gametes, or one motile and one stationary gamete, or two sexually differentiated hyphae, or two nondifferentiated hyphae. Fungi may be either homothallic or heterothallic, with one fungal body producing both sexes in the former and only one sex in the latter.

Infective propagules in fungi are of several distinct types, including spores, conidia, zoospores, planonts, and ascospores, among others. While these differ in their etiology, most (with the exception of the zoospores of aquatic fungi) are functionally similar in the way in which they contact and penetrate hosts. Host entry is most often through the integument, less often through natural body openings. Transovarial transmission is extremely rare. Unlike the bacteria and viruses, most fungi do not invade hosts through the gut after being ingested. Arthropods such as aphids that feed by sucking plant fluids are often subject to fungal infections (though the integument) but relatively unaffected by bacterial or viral infections. Host ranges of fungi vary from narrow to broad, and species with broad host ranges may consist of a series of pathotypes that are relatively specific to different hosts within the overall host range.

Fungal infections (termed mycoses) begin with contact between susceptible hosts and infective particles (such as spores or conidia). Zoospores of some aquatic fungi such as the Oomycota (*Lagenidium* spp.) are mobile (by flagellae) and able to detect chemical clues from hosts and actively orient towards them. In contrast, spores of most terrestrial fungi lack this active mobility and contact hosts through random movement caused by wind and other forces.

Following host contact, the first step in fungal infection is adhesion and germination of the spore on the host's cuticle. The physical and chemical properties of a potential host's cuticle contribute to the specificity of fungal pathogens by influencing the success of adhesion and spore germination. The degree of adhesion of fungal spores is influenced by the presence of mucous materials and physical structures (appressoria). Penetration of the host's integument is accomplished by mechanical entrance of a penetration hypha (germination tube). Penetration involves both physical pressure from the penetrating hypha and degradation of the components of the cuticle by proteinases and other enzymes. Fully hardened cuticle presents a greater barrier to fungal penetration than does new cuticle following a moult. For this reason, insects are more susceptible to fungal invasion after a moult. Differences in infectivity of fungal strains may relate to variation in levels of chitin-degrading enyzmes (Gupta et al. 1994). Fungal penetration through the digestive tract (buccal cavity, esophagus, gut) has been observed in some instances.

Fungi such as the Deuteromycotina, once they have entered the hemocoel, reproduce quickly and generally kill the host. Fungal growth can be through various structures including yeast-like hyphal bodies, hyphal strands, or wall-less protoplasts. Protoplasts assist fungi in

overcoming host defenses because they are not recognized by host immune systems. These yeast-like cells develop rapidly and produce toxins that help suppress the host's immune reactions. After the host dies, further fungal growth is saprotrophic. This growth leads to the development of a mycelium, which becomes a sclerotium (a durable resting structure), from which sexual reproductive spores are later produced. In addition, in many groups non-sexual hyphae emerge from the cadaver and under favorable humidities produce asexual spores. These spores disperse and are often important in horizontal transmission to new hosts, leading to epizootics of the pathogen in the host population. Dispersal of such spores may sometimes occur through movement of insects, but most often is passive by means of wind or water. Initial local dispersal may be actively facilitated by the forceful discharge of spores from spore-producing structures on the host cadaver.

Temperatures most favorable for the development of fungal infections are 20–30°C. High humidities (above 90%) are often required for spore germination and for spore production outside of hosts. Films of free water are necessary for conidial germination of some Deuteromycotina but are unfavorable for most Entomophthorales. Lower humidities (below 50%), darkness, and vibration may be needed for spore release for some groups, such as *Beauveria* and *Metarhizium*.

As with many pathogens, reproductive effort of pathogenic fungi is divided into the production of spores of different types. Under certain conditions, conidial (or other type) spores are produced in large numbers, facilitating short-term horizontal transmission, often resulting in epizootics. Under less favorable conditions, thicker-walled resting spores are produced that are more resistant to adverse environmental conditions. These spores aid the pathogen in surviving periods of environmental stress or periods when suitable hosts are unavailable.

Fungal biology also strongly influences the degree to which any given group may be used in pest control through augmentation (based on commercial production of infective particles) (Chapter 11). Some fungi are able to grow and sporulate on nonliving media. Other groups, for example, many members of the Entomophthorales and the water molds in the Chytridiomycota (*Coelomomyces*), have an obligate need for live hosts to complete their life cycles and, therefore, are poorly suited for commercial production. Detailed treatments of the biologies of various groups of fungi are found in Tanada and Kaya (1993).

PROTOZOAN PATHOGENS OF ARTHROPODS

Protozoans are minute, single-celled organisms found in most habitats, except perhaps the air. They vary widely in shape, color, and morphology. They exhibit both sexual and asexual reproduction. Of the 15,000 described species about 1200 are associated with insects, some of which are pathogenic (Tanada and Kaya 1993).

Protozoan infections typically begin when infective forms are ingested and enter the host gut. A few forms such as the ciliates in the genus *Lambornella* are able to attach to and penetrate the integument. The infective stage is most often a cyst or spore, but may be an actively-living stage. Cannibalism may be an important route of horizontal transmission, as may be the consumption of infected prey of other species (for example in flagellate infections). Most of the protozoans associated with insects remain in the gut and groups such as ciliates, flagellates, and gregarines often cause little pathology. Some forms, however, such as the apicomplexans and microsporidia, are more likely to invade the hemocoel where they are pathogenic. The microsporidia are the most important group of protozoan pathogens affecting insects. Epizootics of microsporidia occur in nature. These are all obligate parasites and multiply only in living cells. Tissues affected by protozoan infections vary but may involve the gut epithelium, the Malpighian tubules, or the fat body, among others.

In addition to entering hosts through ingestion, microsporidia may be introduced into hosts on the ovipositors of Hymenoptera. Microsporidian infections can reduce the effectiveness of rearing insects for research or for commercial mass production of beneficial insects for augmentative control. Vertical transmission from parent to offspring of the host occurs in many groups of protozoa, especially the microsporidia.

Most protozoan infections are chronic infections that persist for extended periods but do not kill their hosts. Infected hosts often show few or no external signs or symptoms of infection. Toxins have not been detected in protozoan infections. Infections may be either systemic or limited to one or more tissues. Complex sexual or asexual patterns of multiplication may occur. Details concerning the reproduction cycles of individual groups are given by Tanada and Kaya (1993).

NEMATODES PATHOGENIC IN ARTHROPODS

Nematodes represent a single phylum, the Nematoda, within which about nine families occur that are parasitic on insects and have potential for use as biological control agents (see Chapter 4). Nematodes are translucent, usually elongate, and cylindrical in form. The body is covered with an elastic, noncellular cuticle, but is not segmented. Unlike bacteria, viruses, and protozoa, nematodes are multicellular animals that possess well-developed excretory, nervous, digestive, muscular, and reproductive systems. They do not have circulatory or respiratory systems. The digestive system consists of a mouth, buccal cavity, intestine, rectum, and anus. Nematode taxonomy is based largely on sexual characters of adults; consequently, immature stages are difficult or impossible to identify.

Nematodes are diverse and are found in nearly all habitats. Nematodes may be free- living or parasitic on either plants or animals. Nematode associations with insects range from phoresy to parasitism. Some nematodes, such as *Beddingia siricidicola*, have complex life histories with both parasitic and free-living cycles that may continue indefinitely.

Many nematodes have relatively simple life cycles with three life stages: eggs, juveniles, and adults. Mated female nematodes deposit eggs in the environment; the first stage juvenile usually moults inside the egg and emerges as a second stage juvenile. Most nematodes moult four times. In many groups, the third stage juvenile remains ensheathed in the cuticle of the second stage, which provides it with increased resistance to adverse conditions. This third stage form is called a dauer juvenile, dauer being the German word for durability. Moulting to the adult stage may occur inside the host or free in the environment. All nematodes stages, except the egg, are mobile. Most nematodes have separate, single-sexed individuals and mating is required.

Nematode infections usually occur in the hemocoel, but in some groups such as the Phaenopsitylenchidae (e.g., *Beddingia*) and Iotonchiidae (e.g., *Paraiotonchium*) nematodes may invade the sexual organs. Nematode infections may severely affect the host, causing debilitation, castration, or death. Most of the obligately parasitic nematodes are relatively host-specific and are associated with one or a small group of hosts. Some groups, however, such as the steinernematids and heterorhabditids, often have broad host ranges under laboratory conditions. However, such laboratory host ranges are typically broader than actual host ranges in nature because of the absence in such tests of ecological factors that restrict host contacts to species found only in certain habitats.

In nematodes, unlike most of the other pathogens discussed in this chapter, host finding may be an active process in which nematodes move towards and recognize hosts using cues such as bacterial gradients, host fecal components, or carbon dioxide (Grewal et al. 1993a). Nematode species vary in their host searching strategies, with some being ambush predators

and others actively moving in search of hosts (Kaya et al. 1993). Host entrance may be a passive process, as when nematode eggs or juveniles of Tetradonematidae are ingested by larvae of sciarid flies. In most instances, however, host penetration is an active process in which juvenile nematodes penetrate hosts through the integument or natural openings (mouth, anus, spiracles). In the cases of natural openings, nematodes seeking entrance have only to move through the opening, avoiding efforts of the host to brush them aside (in the case of the mouth). Once inside the gut, nematodes use mechanical devices (stylets, spears) to puncture the gut wall and enter the hemocoel, the site of nematode infection. Stylets and spears may also be used externally to perforate the cuticle to penetrate directly to the hemocoel in some groups of nematodes. Other kinds of nematodes, such as the Sphaerulariidae, may use adhesive materials which attach the nematode to the host's cuticle to assist in perforating the cuticle with stylets.

Nematode infections produce relatively few external signs other than, in some cases, distended abdomens or changes in color. One exception to this is the formation of intercaste or intersex individuals infected by mermithids. Internal effects of infection may be profound. Sterility is induced by several groups of nematodes, including Mermithidae, Phaenopsitylenchidae, and Iotonchiidae. Moulting may be inhibited in some cases. Behaviors of nematode-infected individuals may be abnormal. Infected individuals may have difficulty walking or flying normally, or may exhibit unnatural phototropisms.

Mermithids differ from other nematodes because they leave their hosts before reaching the adult stage. Postparasitic juveniles exit from hosts and then moult to adults that mate and produce progeny as free-living stages.

Steinernematids and heterorhabditids, the groups of nematodes used most extensively in augmentative biological control, kill their hosts in 2–3 days, a much shorter time than for other groups of nematodes. This occurs because these families of nematodes have mutualistic bacteria in their guts (*Xenorhabdus* spp., *Photorhabdis* spp.) that kill hosts by septicemia. Juvenile nematodes reach the hemocoel by penetrating the midgut wall after being ingested by the host, or by penetrating the host integument. *Xenorhabdus* spp. or *Photorhabdis* spp. bacteria are then released into the host hemocoel by defecation of the juvenile nematodes. Juveniles feed saprophytically on the dead host's tissues and then mature to adults which reproduce. When a new generation of the dauer stage is attained, they leave the host cadaver.

Nematodes in the families Phaenopsitylenchidae and Iotonchiidae include both facultative and obligate parasites. The phaenopsitylenchid *Beddingia siricidicola* has two life cycles. One is free living and feeds on a fungus that is mutualistic with the insect host. This fungus is spread by the host (a siricid wood wasp) and grows in the cambium of the host tree attacked by the wasp. In the free-living cycle, juvenile nematodes feed on fungus, become adults, mate, and lay eggs. In the parasitic life cycle, adult female nematodes penetrate the cuticle of wood wasp larvae which themselves feed on the fungus. After the host insect has pupated, nematodes develop in the hemocoel and produce offspring that invade the developing eggs of the wood wasp. When the wood wasp oviposits in new fungal patches, it deposits nematode-infected eggs rather than healthy ones. The eggs are killed by the nematodes, which emerge and continue their development, either through the fungus-based life cycle or the insect-based life cycle depending on the presence or absence of insect hosts on the patch. In a similar manner, the iotonchiid *Paraiotonchium autumnale*, a parasite of *Musca autumnalis*, invades ovaries of its host and is dispersed in the habitat by the fly's oviposition attempts, as does *Paraiotonchium muscadomesticae* Coler and Nguyen, a parasitoid of *Musca domestica* (Coler and Nguyen 1994).

Further details on the biologies of specific groups of nematodes are given in Gaugler and Kaya (1990), Kaya (1993), and Tanada and Kaya (1993).

CASE HISTORIES I: TWO VIRUSES

Gypsy Moth Nuclear Polyhedrosis Virus

The gypsy moth, *Lymantria dispar*, is a Eurasian forest lepidopteran that undergoes occasional outbreaks in North America, an area to which it was introduced. Outbreaks are ended by epizootics of a nuclear polyhedrosis virus. This virus is amplified within years by horizontal transmission from young to old larvae and maintained between years in field populations by surface contamination of eggs. Egg masses become contaminated with occlusion bodies at the time of oviposition by being deposited on surfaces that have been contaminated by virus from cadavers of older larvae which died of virus. Viruses mixed into the egg mass, which is covered with setae from the moth, are protected from environmental degradation and are able to persist from late summer of one year until early spring of the following year. Infection occurs in young larvae (first and second instars) when they ingest chorion from contaminated egg masses. Cadavers of young larvae killed by virus disintegrate, contaminating adjacent foliage and causing a new set of infections in older larvae that consume this foliage (Elkinton and Liebhold 1990). Because this type of horizontal transmission becomes more efficient as host densities increase, epizootics become more likely and more intense as larval densities increase over a series of years, culminating in an epizootic that depresses the pest population to extremely low levels. This in turn reduces the effectiveness of disease transmission, reducing the prevalence of disease in the population.

Oryctes Non-Occluded Virus

In contrast to the gypsy moth virus which is able, once incorporated into a host egg mass, to survive in the habitat for an eight-month period (August-April), the nonoccluded *Oryctes* virus degrades in less than a week if left unprotected in the environment (Hochberg and Waage 1991). This virus infects the rhinoceros beetle, *Oryctes rhinoceros*, a pest of coconut palms in the southwest Pacific and southeast Asia through to east Africa. The virus has been introduced into several locations with subsequent reduction in the level of damage from the beetle (Zelazny et al. 1990). Virus persistence in the host population is the result of infected adult beetles surviving for 2–4 weeks, during which time their feces are contaminated with virus particles. During this period, beetles are capable of normal flight and feeding behaviors and visit feeding sites in the crown of palms and oviposition sites in decayed palm logs. Feces from infected beetles dropped at such sites are ingested by larvae and other adults which then acquire the disease. The disease prevalence is augmented by releases of laboratory-infected adult beetles. The dynamics of this system have been modeled by Hochberg and Waage (1991).

CASE HISTORIES II: TWO BACTERIA

Most bacterial pathogens have not been found to be important regulators of insect populations in nature. However, the biologies of several species are such that they, or their chemical products, can be used in augmentative biological control. The greatest commercial success with the augmentative use of bacteria for insect control has been with members of the genus *Bacillus*. Two species, *B. thuringiensis* and *B. popilliae*, illustrate how biological features of individual species can constrain the augmentative uses of microbes.

Bacillus thuringiensis

Bacillus thuringiensis is a bacterium that does not persist in sufficient numbers in nature in most habitats to cause epizootics, but which produces stable toxins that can be harvested and used as pest control chemicals. Commercial production of the bacterium is feasible because it will grow and produce spores and toxins in fermentation media, making inexpensive, large-scale production systems possible. Several strains which produce toxins effective against various insect groups are known. Genetically-modified strains have been constructed that combine several toxins into one bacterial strain, making it feasible to

produce products that are effective against pests in three insect orders: Lepidoptera, Coleoptera, and Diptera.

Bacillus popilliae

In contrast, in North America, *B. popilliae* is able to persist in soil and affect the larval populations of its host, the immigrant scarabaeid *Popillia japonica*, for decades (Ladd and McCabe 1967) and cause a degree of population suppression. While interest also exists in using this organism for augmentative biological control, differences between its biology and that of *B. thuringiensis* have made this unsuccessful to date. Toxins play little or no role in the infection process or host death for *B. popilliae*. Rather, spores must be ingested and disease induced through bacterial cell multiplication for death to occur. While abundant vegetative growth of this pathogen can be induced on artificial media, formation of spores (the obligate infective stage) is limited and difficult to obtain apart from living hosts (Tanada and Kaya 1993). Because of the lack of efficient systems for producing spores, commercial production of spores of this bacterium is limited to the small-scale, expensive process of rearing the bacterium in live host larvae which must be collected in the field and inoculated individually by injection (Fig. 11.3). Consequently, this organism, while useful in nature for biological control through introduction, has been less successful than *B. thuringiensis* as a commercial product for augmentative control.

CASE HISTORIES III: TWO NEMATODES

Recent improvements in large scale production of nematodes in the Steinernematidae and Heterorhabditidae have led to an increasing augmentative use of several species of nematodes in these families for control of soil-dwelling pests (see Chapter 11). The role of nematodes in biological control, however, also includes the introduction of specialized nematodes for the permanent suppression of immigrant pest species. This use of nematodes is illustrated by the release of the phaenopsitylenchid *Beddingia siricidicola* in Australia for the control of a wood wasp and of the steinernematid *Steinernema scapterisci* for control of immigrant mole crickets in Florida.

Beddingia siricidicola

The siricid wood wasp *Sirex noctilio* is a pest of European origin that attacks Monterey pine (*Pinus radiata* D. Don) in Australia. The nematode *Beddingia siricidicola* was imported and released in Tasmania by placing it and an associated fungus (*Amylostereum chailletii* [Fr.] Boidin) into holes bored in trees (Bedding 1968). The nematode has two possible life histories. In one, it develops and feeds on the fungus that is vectored by the wood wasp. (The fungus spread by the wood wasp is the agent that kills the tree, making the infected tree suitable for reproduction by the wood wasp.) Also, the nematode can infect larvae of wood wasps. When it does, it defers development until the wood wasp has reached the adult stage. At this time the nematodes reproduce in the host's hemocoel and the resulting juveniles migrate into the wasp's ovaries, where they remain until the wood wasp attempts to oviposit in new breeding locations. At this point nematode-infected, rather than healthy, eggs are deposited. Nematodes emerge (the wasp egg dies), placing nematodes in locations where both new fungus and new wood wasp larval hosts are present. The nematodes then either feed on fungus or infect other wasp larvae. Using inoculations of this nematode, Bedding (1974) was able to reduce the number of trees killed per year by wood wasps in a 1000 acre test area from 200 to none over a four-year period.

Steinernema scapterisci

Scapteriscus spp. mole crickets are turf and pasture pests in Florida; all species of *Scapteriscus* in Florida are immigrant species from South America. Surveys of natural enemies of mole crickets in Uruguay revealed a species of steinernematid, subsequently described as *Steinernema scapterisci* (Nguyen and

Smart 1990). Host specificity studies of this species have shown that it is specific to certain mole crickets at moderate inoculum levels (Nguyen and Smart 1991), although a broader set of species can be attacked if higher inoculum levels are administered in laboratory petri dish assays (Grewal et al. 1993b). *Steinernema scapterisci* has persisted at release sites for up to five years and continues to infect a substantial proportion of hosts (Parkman et al. 1993). Natural spread of the nematode to additional locations has been observed and potential for control of the target pests appears promising.

These projects indicate that importing and establishing new species of nematodes is a valuable approach for control of immigrant pests.