Chapter 1
Scope and Basic Principles of Insect Pathology

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SUMMARY
Insects are the dominant animals in the world, with more than one million described species. The vast majority of insects are innocuous or beneficial to humans, but a small percentage are pests that require a significant amount of our time, effort, and funds to reduce their negative effects on food production and our health and welfare. One environmentally acceptable method to control these insect pests is to use pathogens. The study of pathogens infecting insects is referred to as “insect pathology”. Insect pathology is the study of anything that goes wrong with an insect and, therefore, includes non-pathogenic and pathogenic causes. The present focus is on pathogens that can be used as microbial control agents of insects. Here, the basic principles in insect pathology including the microorganisms that cause diseases, their classification and phylogeny, portal of entry, infectivity, pathogenicity and virulence, course of disease, Koch’s postulates, and diagnosis are covered.

1.1. INTRODUCTION
Insects represent three-quarters of all animal species in the world, with the vast majority of them being terrestrial and/or occurring in freshwater systems (Daly et al., 1998). More than 99% of the approximately one million described insect species (Grimaldi and Engel, 2005) are either innocuous or beneficial to humans, such as the silkworm (Bombyx mori), cochineal scale (Dactylopius coccus), pollinators, and parasites and predators (Gullan and Cranston, 2005; Pedigo and Rice, 2009). To place this in another context, only 1% of the known insect species are our competitors that vie for our crops and stored products, damage our belongings, serve as vectors for plant pathogens, or are of medical or veterinarian importance by feeding on us or our livestock and pets and, in some cases, serving as vectors of disease agents to humans and other vertebrates (Pedigo and Rice, 2009). Even though the number of insect pest species is small compared to the number of described insect species, they require a significant amount of our time, effort, and funds to reduce their negative effects on food production and our health and welfare.

One of the main tactics to control insect pests is the use of chemical pesticides. Unfortunately, the application of chemical pesticides can (1) have a negative effect on human health and the environment; (2) result in resistance of the pest...
species to pesticides; and (3) kill or negatively affect non-target organisms. An alternative to chemical control is biological control (or biocontrol), which is the study and use of living organisms for the suppression of population densities of pest insects (Eilenberg et al., 2001). The living organisms are predators, parasitoids and entomopathogens (meaning microorganisms capable of causing diseases in insects; from the Greek entoma = insect, pathos = suffering, gennaein = to produce, synonymous with “insect pathogens”).

The use of microorganisms for biological control is commonly referred to as microbial control, an approach that includes four strategies: classical, inoculation, inundation, and conservation (Eilenberg et al., 2001). Classical biological control involves the intentional importation, release and establishment of entomopathogens into a new environment. Inoculation biological control deals with the release of an entomopathogen with the expectation that it will multiply and will provide temporary control. This approach is sometimes referred to as augmentation biological control. Inundative biological control is dependent upon the release of significant amounts of inoculum of an entomopathogen to provide immediate control of the pest; control ensues from the release inoculum and not from its progeny. Conservation biological control involves the modification of the environment to protect and enhance an established entomopathogen. An entomopathogen can also be judiciously used with chemical pesticides in an integrated pest management program (Tanada and Kaya, 1993) or combined with a chemical substance(s) that enhances its effectiveness as a microbial control agent (Koppenhöfer et al., 2002; Koppenhöfer and Fuzy, 2008).

Before the development of microbial control agents as management tools for insect pests, maladies had been recorded from beneficial insects for a long time. Thus, the discipline of insect pathology dates back to over 2000 years ago when the Chinese recorded diseases in the silkworm and the Greeks noted diseases in the honey bee, Apis mellifera (see Chapter 2). Through the ages, many diseases have been discovered and described from both beneficial and pestiferous insects. This early development of the discipline can be attributed to (1) the curiosity of scientists in describing and ascertaining the cause of pathological conditions in insects; (2) the need to find cures for diseases that afflicted beneficial insects; and (3) the potential use of pathogens to control insect pests.

What is insect pathology? Broadly defined, insect pathology is the study of anything that goes wrong [i.e., disease (“lack of ease”)] with an insect. Disease is a process that represents the response of the insect’s body to insult or injury (Steinhaus, 1949, 1963c). Often, it is not easy to separate a healthy insect from one that is diseased owing to the absence of symptoms. Steinhaus (1963c) differentiates a healthy insect from a diseased one as follows: “A healthy insect is one so well adjusted in its internal environment and to its external environment that is capable of carrying on the functions necessary for its maintenance, growth, and multiplication with the least expenditure of energy. A diseased insect is simply one that is not healthy; it is an insect that can no longer tolerate an injury or hardship without having an abnormal strain placed upon it.”

The scope of insect pathology encompasses many subdisciplines in entomology (Fig. 1.1). In ecology, for example, epizootics of viral, bacterial, fungal,
microsporidian, and nematode diseases can cause significant mortality to devastate insect populations. In physiology, biochemistry, and toxicology, diseased insects will affect the results of the experiments with differences in enzyme, lipid, or protein profiles compared to healthy insects, and diseased insects will be more susceptible than healthy insects to pesticides. Moreover, our knowledge of insect pathology has made significant contributions in (1) the control and eradication of diseases in laboratory insect colonies reared for research, sterile insect technique programs, pet food sales, educational purposes, exhibits (e.g., insect zoos), and for the sale of beneficial insects such as the silkworm and honey bee (see Chapter 12) and parasites and predators (Inglis and Sikorowski, 2009a, b); (2) the investigation of the intracellular (Chapter 9) and extracellular symbiosis including the disruption of mutualistic relationships between insects and microbes (Douglas, 2007, 2010); (3) the development of Bacillus thuringiensis (Bt) and other bacteria for microbial control (Chapter 8); and (4) the diagnosis or identification of etiological agents that cause insect diseases in the laboratory and field (Hukuhara, 1987; Inglis and Sikorowski, 2009b). In human medicine, baculoviruses in tissue culture systems have been used for the production of papilloma-virus, influenza vaccines (Safdar and Cox, 2007; Einstein et al., 2009) and research for malaria vaccines (Blagborough et al., 2010). Steinhaus (1963c) indicated that the basic elements of insect pathology embrace etiology, pathogenesis, symptomatology, morphopathology, physiopathology, and epizootiology. Thus, insect pathology contributes to many other disciplines beyond entomology such as microbiology, veterinary and human medicine, agriculture, and basic biology.

1.2. CATEGORIES OF DISEASE

Insects are exposed to a wide array of non-living (abiotic) or living (biotic) factors (i.e., causal agents) that can result in disease. The factors leading to a disease state can be referred to as non-infectious or infectious (Steinhaus, 1963c), making it clear that both types of maladies are an integral part of insect pathology. Furthermore, Steinhaus (1963c) separated the non-infectious diseases using the following categories: (1) mechanical injuries; (2) injuries caused by physical agents; (3) injuries caused by poisons or chemical agents; (4) diseases caused by nutritional disturbances or deficiencies of proper nutriments; (5) diseases caused by deranged physiology and metabolism; (6) genetic diseases or inherited abnormal conditions; (7) congenital anomalies and malformations, non-generic teratologies; (8) certain tumors and neoplasms (i.e., those not associated with microbes); (9) disturbances in development and in regenerative capacity of tissues; and (10) injuries caused by parasitization or infestation by other insects or arachnids or by predation. For more detailed information on diseases caused by non-infectious agents, the reader is referred to Steinhaus (1963a, b), Cantwell (1974), and Tanada and Kaya (1993).

Tanada and Kaya (1993) summarized the categories of insect diseases following the classification scheme used by Steinhaus (1949) that included both non-infectious and infectious diseases. These were: (1) the presence or absence of an infectious microorganism (i.e., diseases caused by infectious and non-infectious agents); (2) the extent of the disease (i.e., local, focal, or systemic disease); (3) the location or site of the disease (i.e., midgut, fat body, nerve, hemocyte, hypodermis, etc.); (4) the course of the disease (i.e., chronic, subacute, acute); (5) the source of the infectious agent (i.e., exogenous, endogenous, or idiopathic); (6) the etiological or causal agent (virus, bacterium, fungus, protist, or nematode); (7) the distribution or prevalence of the disease in an insect population (i.e., sporadic, enzootic, or epizootic); (8) the method of transmission (i.e., direct contact, vector, per os, transovum, or transovarial); and (9) the basis of sequence (i.e., primary, secondary, attenuated, progressive, mixed, or multiple). Although these categories are useful, Tanada and Kaya (1993) used the broader categories of diseases as those caused by amicrobial (non-infectious) agents and those caused by microbial (infectious) agents.

The focus of this book is on microbes that cause diseases in insects, with emphasis on their use as microbial control agents. In line with this focus, the book includes the historical development of insect pathology and microbial control (see Chapter 2), the principles of microbial control and epizootiology (Chapter 3), the various pathogen groups infecting insects (Chapters 4–8, 10, and 11), and resistance to entomopathogens (Chapter 13). It also covers Wolbachia, a genus of obligate bacteria, to control arthropods using transinfection into novel hosts (Chapter 9), and pathogens of beneficial insects, especially of silkworms and bees (Chapter 12).

1.3. BASIC PRINCIPLES IN INSECT PATHOLOGY

Basic principles in insect pathology include: (1) the microorganisms that cause diseases (entomopathogens); (2) understanding the classification and phylogeny of entomopathogens; (3) how the microorganisms invade an insect host (portal of entry); (4) whether toxins are involved in the disease process (microbial toxins); (5) infectivity of the microorganisms (infectivity); (6) the disease-producing power of the microorganisms (pathogenicity and virulence); (7) the number of microorganisms needed to cause an infection (dosage); (8) the manifestation of disease...
(signs, symptoms, and syndromes); (9) the progress of the infection (course of disease); (10) types of infection (acute, chronic, and latent); (11) the proof that a given microorganism is the cause of the disease (Koch’s postulates); and (12) how to determine and/or identify the causal agent (diagnosis).

The definition of a pathogen as used in this book is “A microorganism capable of producing disease under normal conditions of host resistance and rarely living in close association with the host without producing disease” (Steinhaus and Martignoni, 1970; also see Martignoni et al., 1984 and Onstad et al., 2006). Even though the term parasite is often used interchangeably or synonymously with pathogen, the term parasite is used in this book as defined by Onstad et al. (2006) as “an organism that lives at its host’s expense, obtaining nutrient from the living substance of the latter, depriving it of useful substance, or exerting other harmful influence upon the host”. The term parasitic is functionally distinct from pathogenic in that the parasite usually does not cause the mortality of the host, whereas the pathogen is routinely lethal to the host (Steinhaus and Martignoni, 1970; Onstad et al., 2006). Thus, there is a distinction between parasites and pathogens. The term “entomopathogen” is used in the context as defined earlier as a microorganism capable of producing a disease in insects, and will be used interchangeably with “insect pathogen” and “pathogen.” Therefore, entomopathogenic bacteria would refer to bacteria that produce diseases in their insect hosts and have the capability of being lethal to them. Yet, in the case of many entomopathogenic protists, they will infect their insect hosts but are not lethal to them. Thus, use of the term entomopathogenic can be paradoxical, and the reader should be aware of this situation. Furthermore, for nematodes (see Chapter 11), some (i.e., mermithids) are referred to as parasites as the second stage juveniles enter a host and no reproduction takes place, whereas others (i.e., steiner nematids and heterorhabditids) are referred to as entomopathogens because they kill their host and reproduce (see Section 1.3.1).

1.3.1. Entomopathogens

The infectious agents, entomopathogens, are microorganisms that invade and reproduce in an insect and spread to infect other insects. These entomopathogens include noncellular agents (viruses), prokaryotes (bacteria), eukaryotes (fungi and protists), and multicellular animals (nematodes). In the latter group, nematodes differ from the other entomopathogens by having digestive, reproductive, nervous, and excretory systems, and characteristics of parasitoids and predators. However, they have no functional response and often produce pathologies similar to other entomopathogens, and many, especially steiner nematids and heterorhabditid nematodes, can invade and reproduce in an insect, and can spread to infect other insects (Kaya and Gaugler, 1993; Grewal et al., 2005).

Not all microorganisms cause infection even after they enter the insect’s hemocoel. The lack of an infection may be due to the resistant characteristics of the host or to the inability of the microbe to survive and reproduce in the host. Many entomopathogens show a high degree of specificity and will infect only one or several insect species, whereas others are generalists and infect a number of insect species in different orders and may infect species in different phyla. These infectious microorganisms can be separated into four broad categories of opportunistic, potential, facultative, and obligate pathogens, keeping in mind that they do not infect all insects. The following definitions are from Onstad et al. (2006):

- **Opportunistic pathogen**: “A microorganism which does not ordinarily cause disease but which, under certain conditions (e.g., impaired host immunity), becomes pathogenic” (e.g., *Aspergillus flavus*).
- **Potential pathogen**: “1) A microorganism that has no method of invading or infecting a host but can multiply and cause disease if it gains entrance, for example, through a wound; potential pathogens generally grow readily in culture and do not cause specific diseases in specific hosts. 2) a secondary invader” (e.g., *Serratia marcescens*).
- **Facultative pathogen**: “A pathogen that can infect and multiply in host animals but is also capable of multiplying in the environment; facultative pathogens generally are readily cultivated *in vitro* (e.g., *Bacillus thuringiensis, Beauveria bassiana*).
- **Obligate pathogen**: “A pathogen that can multiply in nature only within the bodies of specific hosts in which it causes specific diseases. Obligate pathogens usually have a narrow host range and can be cultured *in vitro* only with difficulty, if at all; therefore, some mechanism must exist for their transmission from one host generation to another” (e.g., *Paenibacillus popilliae*, microsporidia, baculoviruses).

1.3.2. Some Major Classification and Taxonomic Changes

Major changes in the classification of some entomopathogens have occurred since the publication of the first edition of “Insect Pathology” by Tanada and Kaya (1993). Some of these changes include the exclusion of Oomycota from the Kingdom Fungi (see Chapter 6), the Microsporidia were in the Phylum Protozoa but are now in the Kingdom Fungi (Chapter 7), and the Protozoa were in the Kingdom Animalia and have been reclassified to the Kingdom Protista (Chapter 10). Several major reclassifications have also occurred at various levels within most of
the pathogen groups (see “Classification and Phylogeny” in each of the pathogen chapters).

At the generic and species level, recent taxonomic revisions within various entomopathogen groups can confuse the reader not familiar with the older literature. With viruses, for example, the International Committee for the Taxonomy of Viruses (ICTV) (http://www.ictvonline.org/index.asp?bhcp=1) has adopted the following guidelines for taxonomic nomenclature of viruses. Italicization occurs in formal taxonomic usage, when the writer is explicitly referring to a taxon (e.g., family Baculoviridae or Reoviridae and genus Alphabaculovirus or Cypovirus), but no italicization is used when the viruses are referred in the vernacular (e.g., baculovirus infection, alphabaculoviruses, or cypoviruses) (see Chapters 4 and 5). A name referring to any virus species (not just the type species of a genus or family) is fully italicized. For example, references to the species Autographa californica multiple nucleopolyhedrovirus are printed in italics. In some cases, the type species uses a common or descriptive name such as Yellow fever virus (family: Flaviviridae; genus: Flavivirus) or Deformed wing virus (family: Iflaviridae; genus: Iflavirus), which is in italics (Chapters 5 and 12).

Examples from fungi include Paecilomyces fumosoroseus and P. farinosus, which have been reclassified as Isaria fumosorosea and I. farinosa, respectively; and Verticillium lecanii was reclassified into several species in the new genus Lecanicillium, which includes L. lecanii (see Chapter 6). In addition, Metarhizium (Bischoff et al., 2009) and Beauveria (Rehner et al., 2011) have undergone major changes based on phylogenetic analyses. An example of a generic reclassification within the microsporidia is the new genus Endoreticulatus replacing Pleistophora. Although Nosema is still a valid genus, a well-known species, N. locustae, used as a microbial control agent of locusts, is now Paranosema locustae.

With bacteria, some Bacillus species that are obligate pathogens of insects have been reclassified into the genus Paenibacillus. In the older literature, Bacillus popilliae and B. larvae, pathogens of scarab larvae and honey bee larvae, respectively, are now called Paenibacillus popilliae (see Chapter 8) and P. larvae (Chapter 12). In another example, the genus of the facultative mosquito pathogen, Bacillus sphaericus, has been reclassified to the genus Lysinibacillus. In this case, the older name has been retained in Chapter 8 because it is still commonly used even after the taxonomic change.

1.3.3. Portal of Entry

The portals of entry are the sites through which an entomopathogen invades or gains entry into an insect host (Fig. 1.2). The most likely portals of entry into the insect host are through the mouth (per os) or integument. The entomopathogens, especially nematodes, may also invade through the anus (per anal) or spiracles. Other routes of invasion for pathogens include through wounds or injuries to the integument, congenital passage within the ova (transovarial transmission) or on the ova (transovum transmission), and the contaminated ovipositor of parasitoids.

The usual portal of entry for most entomopathogens (viruses, bacteria, protists, microsporidia, some fungi, and some nematodes), especially for insects with chewing, chewing/lapping, and sponging mouthparts, is per os, or for most entomopathogenic fungi and many nematodes, it is through the integument. For per os entry, once the entomopathogen gets into the gut, it may reproduce in the digestive lumen (e.g., bacteria) or it may penetrate through the peritrophic membrane, infect the midgut cells (e.g., viruses, bacteria, microsporidia, some fungi, and protists) and/or invade directly into the hemocoel (e.g., nematodes). Those entomopathogens that invade into the midgut cells and then into the hemocoel can infect and reproduce in specific tissues (e.g., some granuloviruses only infect the fat body), reproduce in the hemolymph (e.g., Paenibacillus popilliae), or cause a systemic infection of many different tissues (e.g., nucleopolyhedroviruses, many microsporidia, and protists). For entry through the integument, fungi use enzymes, specialized structures, and pressure to penetrate through the cuticle into the hemocoel (see Chapter 6), and nematodes use a stylet or tooth to penetrate directly into the hemocoel (Chapter 11).

Once the entomopathogen becomes established and reproduces in the insect, it usually produces a resistant stage that can survive in the environment for varying periods. The resistant stages include occlusion bodies for baculoviruses, cypoviruses, and entomopoxviruses, spores for bacteria and protistans, environmental spores for microsporidia, resting spores or sclerotia for fungi, and infective juveniles for steinernematid and heterorhabditid nematodes. These resistant stages are usually the infective stages that will infect a new host. Some non-resistant stages such as non-occluded virions, vegetative bacterial rods and fungal conidia will infect insects but do not survive long in nature. In other cases, the entomopathogen may survive in an alternate host, require an alternate host for successful completion of its life cycle, or remain in its usual host through the adult stage when it is transmitted to the next generation. Thus, a pathogen may have one or more mechanisms of survival. When a susceptible host is encountered and the pathogen is in its infectious state, it can initiate a new infection in a healthy host (Fig. 1.2).

1.3.4. Microbial Toxins

In some cases, the entomopathogen (e.g., some bacteria) does not need to infect cells or invade into the hemocoel to
cause disease. It is confined to the digestive tract, produces dysentery, and causes the insect to shrink in size (brachyosis), resulting in death (Bucher, 1961; Jackson et al., 2001). In such cases, the insects appear to be affected by toxins that are produced by the bacterium. Hence, a disease can be brought about in a susceptible insect host by the pathogen through the actions of chemical or toxic substances.

Two types of toxins, catabolic and anabolic, can be produced by entomopathogens (Tanada and Kaya, 1993). Catabolic toxins result from decomposition brought about by the activity of the pathogen, whereas anabolic toxins are substances synthesized by the pathogen. The breakdown of proteins, carbohydrates, and lipids by the pathogen may produce toxic alcohols, acids, mercaptans, alkaloids, etc. In the case of anabolic toxins, the substances synthesized by the pathogen are:

- Catabolic toxins result from the decomposition of the host's tissues by the pathogen.
- Anabolic toxins are substances synthesized by the pathogen and can cause disease by disrupting the host's metabolism.

**FIGURE 1.2** The primary portal of entry for most entomopathogens (viruses, bacteria, protists, microsporidia, and some nematodes) is primarily through the mouth (per os), whereas for fungi and some nematodes, the primary portal of entry is through the cuticle. The entomopathogens that enter through the mouth can infect the midgut cells and/or penetrate into the hemocoel to cause a systemic infection. Similarly, the entomopathogens that penetrate through the integument may initiate the infection on the cuticle (fungi) with subsequent penetration into the hemocoel or penetrate directly into the hemocoel (some protists and nematodes). In some cases, a few fungal species may enter a host through the mouth or nematodes may enter the anus or spiracles (not shown in figure). Once the entomopathogens reproduce in the first host, the infective propagules are released or leave from a dead or living host and can initiate a new infection in another susceptible host. (Modified after Tanada and Kaya, 1993.)
the pathogen can be classified as exotoxins and endotoxins. Exotoxins are excreted or passed out of the cells of the pathogen during reproduction and have been isolated from entomopathogens, especially bacteria and fungi. Endotoxins produced by the pathogen are not excreted but confined within the cell. These endotoxins are liberated when the pathogen forms a resistant stage, dies, or degenerates. The best known endotoxin in insect pathology is produced by Bt (see Chapter 8). In this case, as the bacterium initiates the sporulation process, excess proteins are produced, resulting in the formation of a proteinaceous crystal (δ-endotoxin) within the sporangial wall adjacent to the spore. The sporangial wall is easily ruptured, releasing the δ-endotoxin and spore into the environment. The δ-endotoxin is a protoxin, and when it is placed in a high pH solution as occurs in the insect gut, the toxic component becomes activated and adversely affects the midgut cells. Thus, “intoxication” is brought about by the activity of the pathogen in the form of toxins.

Not all toxins produced by microorganisms are in the realm of insect pathology. For example, at least two bacteria (actinomycetes), Streptomyces avermitilis and Saccharopolyspora spinosa, produce exotoxins that have been developed into the insecticides, avermectins and spinosad, respectively. In contrast to Bt, these actinomycetes are not covered in this book because the toxins alone are used with no living pathogenic microorganisms involved. However, Bt spore or vegetative rod does not need to be present to cause the disease because the δ-endotoxin alone can kill a susceptible insect host. Although this statement appears contradictory with what was stated above for the actinomycetes, the application of Bt is with the δ-endotoxin and the spore. Transgenic plants containing the Bt δ-endotoxin, on the other hand, are not considered to be part of insect pathology per se, but are aspects of host plant resistance. Yet, with the frequent application of Bt as an organism in agricultural systems and the planting of transgenic plants with the Bt toxin genes in major agricultural crops (e.g., corn and cotton), the resistance to Bt can and has occurred even before transgenic crops were available. Accordingly, insect resistance to pathogens including managing resistance in Bt transgenic crops is covered in Chapter 13.

### 1.3.5. Infectivity

Infectivity is the ability of a microorganism to enter the body of a susceptible insect and produce an infection. However, many insect species harbor microorganisms that are beneficial rather than harmful. These mutualistic microorganisms infect and reproduce in the insects but are contained within specific cells (mycetocytes or bacteriocytes) or tissues (mycetomes or bacteriome) and are beneficial by producing some useful product for their insect hosts. The relationship is mutualistic because the microorganisms also benefit by obtaining nutrients and protection from their insect hosts. Thus, an infection may result in a non-diseased condition. The focus here is on the diseased condition, but the reader should be aware that many microorganisms are mutualistically associated with insects (Bourtzis and Miller, 2003, 2006, 2009). This book does include Wolbachia (Chapter 9) because they have the potential to be manipulated and used as control agents of some insect pest species.

When infection results in disease, it generally causes detectable pathological effects such as injuries or dysfunctions (i.e., impairments in function, especially of a bodily system or organ). There are two main factors associated with a disease: invasiveness and pathologies resulting in abnormalities or dysfunctions. In some cases where toxins are involved, invasiveness of the pathogen into cells and tissues or into the hemocoel need not occur.

When an infectious agent is transmitted naturally by direct contact to an insect, the resultant disease is called “contagious” or “communicable”. Contagious diseases are common in insects and occur with all major pathogen groups (i.e., viruses, bacteria, microsporidia, fungi, protists, and nematodes). Infection and “contamination” are not the same, as a susceptible insect may be contaminated or be harboring a pathogen without being infected. A non-susceptible insect, other organisms or objects may also be contaminated with a pathogen. In both situations, the pathogen is a potential source to infect a susceptible host.

### 1.3.6. Pathogenicity and Virulence

Pathogenicity and virulence are two terms used regularly in insect pathology. These two terms have spurred some debate among scientists (see Thomas and Elkinton, 2004; Shapiro-Ilan et al., 2005), but the definitions by Onstad et al. (2006) and Tanada and Kaya (1993) and recommended by Shapiro-Ilan et al. (2005) are used in this book. Pathogenicity is defined as “the quality or state or being pathogenic, the potential or ability to produce disease”, whereas virulence is defined as “the disease producing power of an organism, the degree of pathogenicity within a group or species” (Shapiro-Ilan et al., 2005). Thus, pathogenicity is a qualitative term and for a given host and pathogen, it is absolute, whereas virulence quantifies pathogenicity and is variable owing to the strain of the pathogen or to environmental effects. Furthermore, pathogenicity can be considered as an all-or-none response; that is, the microorganism is either pathogenic to a host or not and is applied to groups or species (Shapiro-Ilan et al., 2005). Virulence is a measurable characteristic of the ability of the microorganism to cause disease and is intended for within-group or within-species comparisons.
Perhaps the best way to illustrate the difference between these two terms is to provide an example of each. The nematode—bacterium complex of *Steinernema carpocapsae—Xenorhabdus nematophila* shows pathogenicity to some non-insect arthropods (e.g., ticks) (Samish *et al.*, 2000) but not to vertebrates (Akhurst and Smith, 2002). The fungus *Beauveria bassiana* GA strain is pathogenic to the pecan weevil, *Curculio caryae*, but *B. bassiana* MS1 strain is not as virulent to this host (Shapiro-Ilan *et al.*, 2003). Finally, the *Agrotis ipsilon* nucleopolyhedrovirus (NPV) is more virulent to its original host, the black cutworm (*Agrotis ipsilon*) from which it was isolated, compared to *Autographa californica* NPV, which has a wide lepidopterous host range but is less virulent to *Agrotis ipsilon* (Boughton *et al.*, 1999).

### 1.3.7. Dosage

One pathogen can infect and kill a host, as has been demonstrated with the infectious juvenile of the nematode—bacterium complex (*Steinernema—Xenorhabdus* or *Heterorhabditis—Photorhabdus*) (Kaya and Gaugler, 1993). In general, however, with other entomopathogens, a minimal number of infective propagules is needed to pass through the portal of entry for infection to occur. This number is referred to as a dose that can be defined as the quantity of an active agent (i.e., entomopathogen) to which an insect is exposed at any one time. Dosage can be expressed quantitatively depending upon the host susceptibility in terms of mortality, infection, or time to death as lethal dose (LD), effective dose (ED), or lethal time to death (LT). The 50% or 90% level of response is assigned as LD50 or LD90, ED50 or ED90, or LT50 or LT90. The 50% level of response is referred to as the median lethal dose, median effective dose, or median lethal time. To obtain this type of information, a bioassay is conducted with a minimum of five dosage levels of the infective stage (i.e., occlusion bodies, spores, conidia, infective juveniles) plus a control treatment administered to a given stage of the host, and the response (i.e., mortality, infection, or time to death) is plotted with dosage on the x-axis and the response on the y-axis. If host mortality occurs in the control, Abbott’s formula can be used to correct for the mortality in the treatments (Abbott, 1925). Ideally, the range of the dosages should provide a response level between 10% and 90%. The lowest and highest dosages should not give a 0% or 100% response, respectively. The range of dosages obtained from the bioassay when plotted against the host response (i.e., mortality, infection, or time to death) should give an S-shaped curve which is transformed into a straight line by converting the response to a probit scale, and the dosage to the log scale. The level of response can be obtained by going up the probit scale on the y-axis and reading across to where the slope of the line intersects the dosage scale on the x-axis, which then provides the dosage for that response. The bioassay should be conducted at least twice with a different cohort of insects and entomopathogens to demonstrate that the data are reproducible. For further information on conducting bioassays with entomopathogens, the reader should refer to Burges and Thomson (1971) and Navon and Ascher (2000).

Dose is a precise number of the infective stage to which the bioassay insects are subjected to and usually attaining this level of precision is not possible. That is, it is not possible to determine the dosages quantitatively because of the size of the insect, the bioassay system (e.g., placing the infective stage on the diet surface and not knowing the amount of entomopathogen acquired), or because the insects live in an aqueous habitat where the dose cannot be calculated. In such cases, median concentration of the entomopathogen which produces a response in half of the test insects is used because the experimental method is not sufficiently accurate to determine the precise dose to which the test insects were exposed. For example, the median lethal concentration would then be expressed as LC50 or the median effective concentration would be EC50.

### 1.3.8. Signs, Symptoms, and Syndromes

Diseased insects exhibit characteristic aberrations or dysfunctions that are designated as signs and symptoms. When there is a physical or structural abnormality, the term sign is used, whereas when there is a functional or behavioral aberration, the term symptom is used. A sign is indicated by abnormalities in the morphology or structure such as color, malformed appendages or body segments, fragility of the integument, etc., whereas a symptom may be expressed by abnormal movement, abnormal response to stimuli, digestive disturbances (vomiting or diarrhea), inability to mate, etc.

A particular disease has a group of characteristics signs and symptoms which is called a syndrome. The syndrome refers to a system complex or a particular combination or sequence of signs and symptoms. Sometimes, the syndrome is very characteristic and specific for a disease caused by an entomopathogen. More often, the same syndrome occurs with many different diseases. Vomiting and diarrhea may develop from ingestion of chemical pesticides, bacterial toxins, or entomopathogenic bacteria, viruses or protistans. A distinct syndrome occurs when a silkworm (*B. mori*) larva ingests Bt subspecies *sotto* spores and δ-endotoxin. The larva stops feeding in a few minutes, becomes sluggish in about 10 min, the pH of the blood increases and the pH of the midgut decreases, and within an hour, the larva becomes moribund and dies.

### 1.3.9. Course of Infection

After an entomopathogen invades a healthy, susceptible insect, it starts to reproduce and the course of infection can
be partitioned into various phases or stages: the incubation period, the beginning of disease with the appearance of the first signs and/or symptoms, and peak of disease (Fig. 1.3). The incubation period is the time from when the entomopathogen infects a host until the development of signs and/or symptoms. The appearance of the signs and/or symptoms indicates that there is a patent or frank infection including the production of toxins with some entomopathogens marking the beginning of disease. As the entomopathogen reproduces, the disease manifests itself to the fullest extent in the host reaching the peak of disease. The peak of the disease is when the signs and symptoms are most severe and either start to abate or attain a steady state. Also at this time, the entomopathogen has usually reached its highest level of reproduction, and if toxins are produced, they are present in the greatest amount. However, many entomopathogens, especially bacteria, fungi, and steinernematid and heterorhabditid nematodes, continue to develop and reproduce after the death of the host. When the peak of disease is reached, the insect may recover as the result of an immune response or die because of the absence of an immune response or the presence of only a weak immune response. In the case where the entomopathogen has low virulence, the insect may have a chronic infection and survive to adulthood and reproduce.

The time from when the insect enters and infects the host until the insect dies is referred to as the period of lethal infection, or if the insect recovers as the period of infection. A short period of lethal infection indicates that the entomopathogen has high virulence, whereas a long period of lethal infection indicates that it has low virulence. An entomopathogen with high virulence is not necessarily the most infectious. For example, Bt subspecies *kurstaki* has high virulence to cabbage butterfly (*Pieris rapae*) larvae with a period of lethal infection of 48 h. But high virulence does not mean that the entomopathogen is highly contagious, and Bt subspecies *kurstaki* is not easily transmitted from one insect to another. Conversely, an entomopathogen with low virulence (i.e., period of lethal infection of several weeks as occurs with some microsporidian infections) may be highly contagious, with the pathogen being easily transmitted from one insect to another.

1.3.10. Acute, Chronic, and Latent Infections

Infections in insects may vary from latent to chronic to acute. Acute infections are the most apparent because of the distinct and characteristic response of the insect to the pathogen causing the disease. Acute infections are of short duration and usually result in the death of the host.
The contributions of molecular biology have opened up the concept of “molecular Koch’s postulates” to help characterize whether a particular microbial gene is an essential constituent of the ability of a microorganism to infect and cause disease in a given host (Falkow, 1988). Recently, Falkow (2004) summarized the application of the molecular Koch’s postulates to bacterial pathogenicity in animal systems. Table 1.1 shows Koch’s original three postulates with the proposed molecular Koch’s postulates (Falkow, 2004). Koch’s original three postulates are essentially the same four steps as stated by Tanada and Kaya (1993). The molecular Koch’s postulates are used to demonstrate that a particular gene or set of genes is the virulence factor and inactivation of this gene(s) results in the loss of pathogenicity. Restoration of pathogenicity should occur with the reintroduction of the gene into the microorganism. To the authors’ knowledge, the molecular Koch’s postulates as proposed by Falkow (2004) are not intentionally used in insect pathology. However, the insecticidal protein genes of Bt have been cloned into other Bt subspecies or other bacterial species and have proven to be efficacious against the target insects (Park and Federici, 2009).

### TABLE 1.1 Comparison of Koch’s Original Postulates and the Molecular Koch’s Postulates

<table>
<thead>
<tr>
<th>Koch’s Original Three Postulates</th>
<th>Molecular Koch’s Postulates</th>
</tr>
</thead>
<tbody>
<tr>
<td>The microorganism occurs in every case of the disease and can account for the pathological changes and course of the disease</td>
<td>The phenotype or property under investigation should be associated with a pathogenic microorganism of a genus or strain of a species</td>
</tr>
<tr>
<td>The microorganism occurs in no other disease as a fortuitous and non-pathogenic microbe</td>
<td>Specific inactivation or deletion of the gene(s) from the microorganism should lead to the loss of function in the clone</td>
</tr>
<tr>
<td>After being fully isolated from the host’s body and grown in pure culture, the microorganism can induce the same disease anew</td>
<td>Restoration of pathogenicity should occur with the reintroduction of the wild-type gene</td>
</tr>
</tbody>
</table>

*Koch’s original three postulates state essentially the same four steps as used by Tanada and Kaya (1993).*  
*Source: Modified after Falkow (2004)*
1.3.12. Diagnosis

Diagnosis is a fundamental branch of insect pathology which involves the process by which one disease is distinguished from another. The identification of the etiological or causal agent alone is not diagnosis, but only one of a series of steps in the operation to determine the cause of the disease. To conduct a proper diagnosis, a study has to be made of the etiology, symptomatology, pathogenesis, pathologies, and epizootiology of the disease. Steinhaus (1963d) stressed that “The importance of diagnosis in insect pathology lies in the fact that one must know the nature of the disease and what ails or has killed an insect before the disease can be properly studied, controlled, or suppressed, used as a microbial control measure, its potential for natural spread determined, or its role in the suppressing, used as a microbial control measure, its potential for natural spread determined, or its role in the ecological life of an insect species ascertained”. Steinhaus (1963d), Tanada and Kaya (1993) and Inglis and Sikorowski (2009b) provide detailed procedures on how to submit diseased insect specimens and how to perform a disease diagnosis properly.


REFERENCES


Principles of Epizootiology and Microbial Control

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Thus, epizootiology deals with disease on a population level rather than an individual basis. The subject concerns both infectious and non-infectious diseases (though this chapter will focus primarily on the former) and affects all pathogen groups. Epizootiology deals with epizootic and enzootic levels of animal disease. An epizootic is defined as an outbreak of disease in which there is an unusually large number of cases, whereas an enzootic refers to a low level of disease that is constantly present in a population (Steinhaus, 1967; Onstad et al., 2006).

The study of epizootiology crosses multiple interests within insect pathology. Epizootiology is the foundation for studying disease fluctuation in natural insect populations from an ecological perspective. However, the primary motivation of many insect pathologists is not the study of natural disease levels but the use of diseases to suppress insect pest populations, i.e., microbial control. Microbial control can be viewed as applied epizootiology with the goal of inducing an epizootic in the targeted insect population through manipulation. In contrast, many insect pathologists are motivated by reducing or eliminating the prevalence of disease in insect populations, e.g., in beneficial insects (e.g., pollinators) or insects in culture (see Chapter 12). In either case, understanding factors that cause epizootics is critical to the successful implementation of microbial control and equally important in developing methods to guard against epizootics.

Steinhaus (1949) first introduced terminology regarding epizootiology in relation to insect pathology, and made the connection between the principles of epizootiology and microbial control. Despite that introduction more than six decades ago and significant reviews on the principles of epizootiology since then (Tanada, 1963; Tanada and Kaya, 1993), including an entire book on the subject (Fuxa and Tanada, 1987), the importance of epizootiology in insect pathology and its application to microbial control has arguably not received the attention it deserves. It is hoped that this chapter will offer additional support for incorporating concepts of epizootiology into studies of insect pathology and microbial control.

Specifically, this chapter offers an updated examination of the principles of epizootiology and microbial control. Four primary areas that influence epizootiology (Tanada, 1963; Fuxa and Tanada, 1987) are discussed: (1) the pathogen population; (2) the host population; (3) transmission; and (4) the environment. The chapter then examines microbial control as applied epizootiology and discusses factors that influence success in microbial control as well as methods to enhance efficacy. Case studies and other specific examples are offered to illustrate points, and the chapter concludes with a synthesis of topics and suggestions for future research.

3.2. EPIZOOTIOLOGY: BASIC PRINCIPLES

A clear understanding of the basic principles of epizootiology is necessary to understand fully the science of causes and forms of disease. Given that the basic definition of an epizootic entails an unusually large number of cases of a disease in a host population, the question arises: what constitutes an unusually large number (Fuxa and Tanada, 1987)? To answer this question, one must establish what the long-term prevalence of the disease is in a space—time framework. Prevalence is defined as the total number of cases of a particular disease at a given time, in a given population (Onstad et al., 2006). The prevalence of a causal agent at any point in time from any location can then be subjected to statistical analysis to establish when prevalence is outside the norm. While epizootics are sporadic in their occurrence and marked by a sudden change in prevalence, enzootics are present over very long periods and their prevalence in the host population varies little. With proper environmental conditions, host densities and a suitable entomopathogen (= insect pathogen) population, an enzootic disease can become epizootic. Steinhaus (1949) described this transition in disease prevalence in time from enzootic to epizootic levels and back again as an epizootic wave (Fig. 3.1). Steinhaus further divided the epizootic wave into the preepizootic, epizootic and post-epizootic phases.

Two epidemiological terms often misused in insect pathogen epizootiology are prevalence and incidence. Prevalence, as defined above, is dependent on both the proportion of hosts afflicted with a disease and the duration of the infection. When one determines the prevalence of disease at a single point in time, infected individuals early and late in the progress of the infection are counted equally. This is a key point that differentiates disease prevalence from incidence. The incidence of a disease is the number of new cases of infection over a defined period (Tanada and Kaya, 1993). Because prevalence refers to all incidences of disease, new and old, at a given point in time, whereas incidence is specifically the new cases of infection over a defined duration, these terms must not be used interchangeably.

To further avoid confusion, the present discussion will rely on the following definitions of terms commonly used in insect pathology. The definitions of pathogenicity and virulence used are those proposed by Steinhaus and Martignoni (1970) and reiterated by Shapiro-Ilan et al. (2005a) and Onstad et al. (2006), which in large part have been adhered to in the insect pathology literature. To restate, pathogenicity is the quality or state of being pathogenic, and virulence is the disease-producing power of an organism, i.e., the degree of pathogenicity within a group or species. Pathogenicity is absolute, a disease-causing...
organism is pathogenic to a host or it is not, whereas virulence is a quantitative measure of the ability of a given amount of an agent to cause disease. Infectivity is the ability of an organism to cause an infection (Tanada and Kaya, 1993).

The four basic components of an epizootic — the pathogen population, the host population, transmission, and the environment — work alone or in combination in a manner that is conducive for the development of an epizootic or precludes an epizootic from taking place. This text will discuss how these components affect epizootic development individually and in combination. In the field, it is the sum effect of all of these factors that determines whether or not an epizootic takes place.

### 3.2.1. The Pathogen Population

Numerous properties associated with the pathogen population (e.g., pathogen density, dispersal, infectivity and latency, virulence, and genetics) play a large role in determining whether or not conditions are in place for an epizootic wave to be initiated. Foremost, the pathogen population density must be sufficient to initiate an epizootic. In addition, within the vicinity of the host, the pathogen population must also be able to disperse sufficiently, actively or passively, to reach the host, and once making contact, the pathogen must be able to infect; therefore, dispersal and infectivity are among the key factors of importance. Once reaching the host, the pathogen’s level of virulence is of utmost importance. Furthermore, there are various genetic factors associated with pathogen strains that may influence pathogenicity and virulence. Each of the properties associated with the pathogen population alone or in combination with one or more of the others may have a large impact on whether or not disease occurs within a host and subsequently spreads to other individuals in the population resulting in an epizootic. These pathogen properties, which vary widely among pathogen groups, are discussed below.

**Pathogen Density**

Of all of the factors of the pathogen population that influence an epizootic, none is more important than pathogen density, which includes the spatial distribution of the pathogen (Tanada and Fuxa, 1987; Onstad, 1993; D’Amico et al., 1996). Although pathogen density is fundamentally related to other intrinsic characteristics (e.g., pathogen reproductive rate and capacity to survive), it is an intrinsic character of a pathogen in and of itself. A pathogen with a high density and widespread spatial distribution in the field is inherently well suited to cause an epizootic. Susceptible hosts encountering a high pathogen density are more likely to come into contact with and become infected than those in an environment of low pathogen density and limited spatial distribution. However, pathogens with low densities in the field or limited spatial distribution can result in localized enzootics, and under favorable biological and environmental conditions can result in epizootics (Dwyer and Elkinton, 1993). High pathogen densities often occur after a widespread epizootic in which local host populations are depleted and propagule numbers maximized. This phenomenon is often seen with lepidopteran pests infected with a virus (Fuxa, 2004). After a severe epizootic, insect populations often fail to develop in the subsequent year, as neonate larvae are highly susceptible to infection and die before causing severe damage. The implementation of a microbial control program most often attempts to inundate an entire crop with high levels of a pathogen and

![FIGURE 3.1 A curve showing the epizootic wave. The epizootic cycle is divided into the enzootic, preepizootic, epizootic, and postepizootic phases. (Modified after Steinhaus, 1949, and Tanada and Kaya, 1993.)](image)
Insect Pathology

initiate an epizootic. Timing of the pathogen’s application in microbial control programs is critical so that the pathogen and the susceptible stage of the insect occur simultaneously in the environment.

**Dispersal**

The ability of a pathogen to disperse, either under its own power or passively, has implications on pathogen spatial distribution. Most pathogens have limited or no capacity to disperse on their own. In the aquatic environment, Oomycte (kingdom Chromista) and Chytridiomycete pathogens of mosquitoites and other aquatic Diptera produce zoospores which actively seek out and penetrate the cuticle of their host (Donnas, 1981; Sweeney, 1981; Andreadis, 1987). Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae (see Chapter 11) are the best example of terrestrial entomopathogens capable of dispersing under their own power. Under proper soil conditions, nematodes are able to move through the soil to locate a host. Foraging strategies exhibited by entomopathogenic nematodes exist along a continuum from ambushers to cruisers. Ambushers use a sit-and-wait strategy; they usually stand on their tails (nictating) and wait until a host comes close before infecting. Cruisers actively seek out their hosts and cue into certain target volatiles (e.g., carbon dioxide) before contacting the host. Examples of nematodes that exhibit foraging behavior characteristic of ambushers include *Steinernema carpocapsae* and *S. scapterisci*. Those exhibiting behavior typical of cruisers include *Heterorhabditis bacteriophora*, *H. megidis*, and *S. glaseri*, and those with intermediate search behavior include *S. feltiae*, and *S. riobrave* (Campbell and Gaugler, 1997; Lewis, 2002; E. E. Lewis et al., 2006). Other pathogens that use the soil as a reservoir (viruses, fungi, bacteria) have no capacity to disperse on their own. Rather, these pathogens rely on biotic and abiotic factors to distribute them in the environment (see Sections 3.2.2 and 3.2.3).

**Infectivity and Latency**

Infectivity is closely related to transmission, as it is the method of entry into a potential host. Routes of entry include oral ingestion (*per os*) and entry via the digestive tract, direct cuticular penetration, tracheal entry, entry via the reproduction system, or entry via the action of parasitoids. Pathogens with multiple routes of entry into their host are expected to result more easily in epizootics as disease transmission is facilitated (Tanada and Fuxa, 1987). Fungi and nematodes commonly use multiple routes of entry. Fungal entomopathogens most commonly gain entry to their host directly through the cuticle, but entry can also be gained through the alimentary canal (Wright et al., 2007). Entomopathogenic nematodes in the Steinernematidae primarily penetrate their host through the mouth, anus, and spiracles, while members of the Heterorhabditidae use these routes of entry in addition to direct penetration of the cuticle. However, Köppenhöfer et al. (2007) demonstrated that *S. glaseri* and *S. scarabaei* directly penetrated scarab cuticles, albeit at lower rates than *H. zealandica* and *H. bacteriophora*, which demonstrated excellent cuticular penetration. *Steinernema feltiae* is also reported to penetrate directly the cuticle of crane flies (Peters and Ehlers, 1994). Furthermore, microsporidia (see Chapter 7) most often cause chronic infections and use multiple routes of entry via the egg, digestive tract, or passive transfer via the ovipositor of parasitoids (Reardon and Podgwaite, 1976; Solter and Becnel, 2007). Bacterial and viral infections are most often transmitted orally (Szewczyk et al., 2006; Cory and Evans, 2007; Garczynski and Siegel, 2007) or are passively transferred via the ovipositor of parasitoids (Reardon and Podgwaite, 1976; Cossentine, 2009).

Some pathogen infections may be latent (i.e., a non-infective and non-replicative state) until triggered by some environmental stressor. However, the development of a visible infection after an environmental stressor is not proof in and of itself that a disease is latent, as would be the case for pathogens causing chronic infections (Tanada and Fuxa, 1987). Latency occurs in most pathogen groups. Molecular tools now allow for detailed studies of latency of nucleopolyhedrovirus (NPV) infections of lepidopterans that had been suspected for several decades. Persistent latent NPV infections occur in wild populations of cabbage moth, *Mamestra brassicae* (Burden et al., 2003), and African armyworm, *Spodoptera exempta* (Cory and Evans, 2007; Vilaplana et al., 2010). Covert baculoviral infections are present, and potentially frequent, in wild lepidopteran populations in which the virus can persist in the latent state (Kukan, 1999; Burden et al., 2003). Overwintering European corn borer (*Ostrinia nubilalis*) larvae also harbor latent *Beauveria bassiana* infections that become active with the breaking of larval diapauses, resulting in fungal spores being produced and released into the environment in spring when insect hosts are abundant (Bruck and Lewis, 1999).

**Virulence**

Of the key pathogen properties discussed, the concepts of pathogenicity and virulence have been debated recently in the literature (Thomas and Elkinton, 2004; Shapiro-Ilan et al., 2005a). The median lethal dose of a pathogen needed to kill 50% of the tested insects (LD<sub>50</sub>) is a typical measure of virulence (Shapiro-Ilan et al., 2005a). A more virulent pathogen requires fewer infective propagules to cause disease relative to a pathogen that is less virulent. The length of time from infection to 50% death of the host (median lethal time or LT<sub>50</sub>) is also a common measure of virulence (Tanada and Kaya, 1993). A pathogen that infects
and kills its host quickly is more virulent than those that are slower acting or result in long-term non-lethal chronic infection. Speed of replication in the host is also an important factor. Pathogens that replicate quickly are necessarily more virulent that those that replicate slowly. Bioassays can also be performed in the laboratory using a distinguishing dosage (based on prior work) of a pathogen with sufficient replication to indicate relative virulence of unique isolates (Shapiro-Ilan et al., 2003a; Bruck, 2004; Fisher et al., 2011). Pathogen virulence can be manipulated in the laboratory by a variety of approaches such as: (1) repeated passages through susceptible hosts; (2) identification of strains within a species of pathogens with differential virulence; and (3) introducing pathogens with other substances to enhance their invasive ability or virulence. These approaches are discussed in detail later in this chapter, in the context of microbial control programs (see Section 3.3.3).

The production of toxins and secondary metabolites is a common biological property of entomopathogens that is tied to pathogenicity and virulence. The production of toxins often results in reduced LD$_{50}$ and LT$_{50}$ values. Toxins and secondary metabolites may function in one or more of the following ways: direct toxicity to the host (thereby directly aiding or causing disease and death, immunosuppression (aiding in overcoming host defense; antibiotic activity), or to ward off competition from other pathogens or saprobes (Vey et al., 2001; Charnley, 2003). Secondary metabolites are commonly produced by bacteria and fungi to aid the pathogen before or in the course of invasion (Schnepf et al., 1998; Vey et al., 2001; Bravo et al., 2007).

*Bacillus thuringiensis* (Bt) is the most well-studied example of a pathogen that produces both exotoxin and endotoxins during the infection process (Schnepf et al., 1998). Bt produces a wide variety of toxins, many of which form the basis of transgenic plants producing insecticidal toxins (James, 2006; Sanchis and Bourguet, 2008). Fungal entomopathogens produce enzymes including chitinases, lipases, and proteases to aid in invasion of the integument (da Silva et al., 2005; Silva et al., 2005; Boldo et al., 2009). Overproduction of the cuticle-degrading protease (Pr1) in *Metarhizium anisopliae* increased virulence against the tobacco hornworm, *Manduca sexta* (St. Leger et al., 1996). In addition, fungal entomopathogens produce secondary metabolites that can have a wide range of effects (Charnley, 2003) (see Chapter 6). Destruxins are secondary metabolites produced by *M. anisopliae* (and other fungi) that could increase the rate of mortality of infected insects (Schrank and Vainstein, 2010). *Metarhizium anisopliae* isolates that produce higher quantities of destruxins are more virulent (Sree and Padmaja, 2008). The symbiotic bacteria *Xenorhabdus* and *Photorhabdus*, of *Steinernema* and *Heterorhabditis*, respectively, also produce toxins that benefit the nematodes in the infection process (Forst et al., 1997; Bowen et al., 1998; ffrench-Constant and Bowen, 1999). An analysis of the *Photorhabdus luminescens* genome identified more predicted toxin genes than any other bacteria in prior studies (Duchaud et al., 2003), which has led to increased interest in these symbiotic bacteria as a new source of genetic material for second generation transgenic plants. A large toxin gene from *P. luminescens* has been expressed in transgenic *Arabidopsis* plants and has been shown to convey resistance to *M. sexta* (Liu et al., 2003). Larvae of the beet armyworm (*Spodoptera exigua*), diamondback moth (*Plutella xylostella*), and black vine weevil (*Otiorhynchus sulcatus*), as well as nymphs of the desert locust, *Schistocerca gregaria*, were killed by both *Xenorhabdus nematophila* cells and cell secretions, indicating that toxic secretions were responsible for the observed lethal effects (Mahar et al., 2008) and suggesting alternative modes for insect pest management with these toxins. The genetics of pathogenicity in *Photorhabdus* appears to be very similar to that described for other enteric bacterial pathogens, indicating the universal nature of pathogenicity (Clarke, 2008).

**Strain Effects and Genetics**

A strain can be defined as a pure culture of a microorganism with relatively constant properties (Onstad et al., 2006). A pathogen isolate from a particular time, place, or substrate may constitute a strain if the population exhibits differences in some measurable biological property relative to other populations (or strains) of that species. The properties that differ among strains can affect the potential for epizootics. Pathogenicity is perhaps the most important biological trait used to differentiate strains and is generally quantified in the laboratory. Strains pathogenic to a variety of hosts are intuitively expected to result in more frequent and severe epizootics in the field as the number of potential hosts, and hence the amount of inoculum produced, is greater than that of an isolate with a limited host range. However, it is clearly desirable in many situations to have a pathogen with a host range limited to the target pest in order to minimize infections in non-target hosts (Goettel, 1995).

Virulence is the other most commonly characterized property between pathogen strains. Reduced time to kill, exit the host, and become available in the environment to infect subsequent hosts no doubt enhances the ability of a strain to induce an epizootic, at least in the short term. The long-term effects of releasing a more virulent strain in the field and its ability to produce subsequent epizootics are less clear as the host may respond with increased resistance, resulting in less severe epizootics and therefore reduced amounts of inoculum in the environment (Tanada and Fuxa, 1987).
Foreign pathogen strains that are introduced into a naïve host population can range from being non-infective to being more virulent than native strains, which have co-evolved with their host (Hajek et al., 1995; Solter et al., 1997). *Microsporidium* sp. from European populations of the gypsy moth, *Lymantria dispar*, exposed to non-target lepidopteran species indigenous to North America did not support optimal reproduction and it is unlikely that horizontal transmission within non-target populations in the field would result (Solter et al., 1997). The reciprocal scenario can also occur. For example, the exotic ladybirds *Harmonia axyridis* and *Coccinella septempunctata* were not susceptible to endemic isolates of *B. bassiana* (Cottrell and Shapiro-Ilan, 2003, 2008).

Recent advances in molecular biology have shed considerable light on the genetics of insect pathogens and strain differentiation. Until recently, morphological characters were most often used to distinguish among species, which did not allow for differentiation of strains within a group of morphologically identical species. However, it is now possible not only to differentiate morphologically identical sister taxa (Rehner and Buckley, 2005; Bischoff et al., 2006, 2009) but to also track individual pathogen strains in the field based on their unique genetic profile (Coates et al., 2002a, b; Enkerli et al., 2004; Wang et al., 2004). Whole genomes of various insect pathogens have been sequenced, e.g., *Autographa californica* NPV (Ayres et al., 1994), *P. luminescens* (Duchaud et al., 2003), *Ascosphaera apis* (Qin et al., 2006), *Pseudomonas entomophila* (Vodavar et al., 2006), *B. thuringiensis* (Challacombe et al., 2007), *M. robertsi* (as *M. anisopliae*), and *M. acridum* (Gao et al., 2011). Genome sequencing allows for a better understanding of phylogenetic relationships and biological traits, and will invariably lead to an improved understanding of the multitude of factors unique to each pathogen.

### 3.2.2. The Host Population

The susceptibility of a host population to pathogen infection plays a critical role in determining whether or not an epizootic takes place. Inherent susceptibility of an insect to infection by a pathogen is genetically based. The more susceptible a host is to infection, the lower the pathogen dose necessary and subsequently the easier it is for infections to spread from one host to another. The level of host susceptibility is most commonly measured with an estimate of LD50. The lower the LD50, the fewer propagules are necessary to cause mortality.

Epizootics generally occur at high host densities (Andreadis, 1987; Watanabe, 1987; Onstad and Carruthers, 1990). High host density facilitates transmission of pathogens between infected and healthy hosts and results in large numbers of infective propagules being produced and released into the environment. Insects that are stressed are also generally more susceptible to pathogen infection. For example, nutritional stress due to host plant senescence increases the susceptibility of insects to NPV (Richter et al., 1987). Crowding and environmental stressors such as high humidity also contribute to making hosts more susceptible to pathogen infection (Fuxa et al., 1999). As a result, insect pathogens primarily act as density-dependent mortality factors infecting more hosts as the host population increases. Yet, epizootics can occur in situations where pathogens are widely distributed and host populations are low (Dwyer and Elkinton, 1993). Interactions between trophic levels can aid in pathogen transmission at low host population densities (Sait et al., 1994; Cory, 2003).

#### Genetic Resistance

Host resistance (see Chapter 13) is one mechanism that can cause a substantial reduction in host susceptibility. The first example of the development of resistance to a pathogen outside the laboratory occurred in the Indian meal moth, *Plodia interpunctella*, exhibiting resistance to Bt (McGaughey, 1985). Moths isolated from treated grain bins were more resistant than those isolated from untreated bins, indicating that resistance developed quickly in the field. Rapid development of resistance to Bt in the laboratory demonstrates that many pests harbor natural genetic variation in susceptibility and have the potential to evolve resistance (Tabashnik et al., 2003, 2006; Gould, 1998; US EPA, 1998).

The first reported resistance to Bt sprays in the field was observed in *P. xylostella* (Tabashnik et al., 1990). The large-scale planting of crops engineered to produce the protein toxins (Bt crops) represents intense selection pressure for development of resistance to the gene product of an insect pathogen. First generation transgenic crops with only one Bt toxin were introduced in the mid-1990s targeting Lepidoptera. Bt cotton and Bt corn have been grown on more than 165 million ha worldwide (James, 2006). The frequency of resistance alleles has increased in some populations of *Helicoverpa zea*, but not in five other major Lepidoptera pests where large-scale Bt crops are grown successfully on a wide scale, suggesting that current refuge strategy has helped to delay resistance (Tabashnik et al., 2008).

Insects develop resistance to pathogens as a result of repeated prolonged exposure through a process of selection. The most detailed work on the mode of resistance inheritance and mechanisms of resistance has been developed for prolonging the effectiveness of transgenic plants expressing Bt protein toxin. A simple model of resistance evolution is conferred by one gene with two alleles, *r* (resistance) and *s* (susceptibility), yielding three possible genotypes (Gould, 1998; Tabashnik and Carrière, 2007). Transgenic crops are designed to produce sufficient toxin to
kill all heterozygous individuals, rendering resistance as functionally recessive (Gould et al., 1995; Liu et al., 2001; Tabashnik et al., 2004). Because the r alleles are rare in the host populations (Gould et al., 1997; Burd et al., 2003; Wenes et al., 2006), the extremely rare rr individuals surviving in a Bt crop are likely to mate with the abundant ss individuals from refuge areas resulting in rs progeny that are susceptible to the Bt crop (Gassmann et al., 2009).

**Behavioral Resistance**

Hosts exhibit a wide range of behavioral responses to the presence of pathogen propagules in the environment before infection, in addition to behavioral responses after infection. The type of behavior response can vary widely. Many responses help the host either to avoid or to mitigate infection, while others aid the pathogen in subsequent dissemination of propagules in the environment.

There are many reports of host-mediated behavior reducing pathogen transmission among social insect colonies. Behavior observed includes grooming, nest cleaning, secretion of antibiotics, avoidance, removal of infected individuals, and colony relocation (Roy et al., 2006). Grooming behavior has been documented among solitary and social insects (Siebeneicher et al., 1992; Oi and Pereira, 1993). Grooming is common among termites and other social insects and can result in the increased spread of a pathogen within a colony (Kramm et al., 1982) or conversely serve as an effective means of actively removing pathogen propagules attached to the cuticle (Oi and Pereira, 1993). Termites reared in groups physically removed 80% of M. anisopliae conidia from their nest mates and eliminated the conidia through the alimentary tract, while individually reared termites did not reduce their surface contamination (Yanagawa and Shimizu, 2007). Hygienic behavior goes beyond grooming to include nest cleaning, i.e., detecting and removing diseased and parasitized individuals from the nest (Wilson-Rich et al., 2009), and enrichment of nest material with antimicrobial substances from the environment (Christe et al., 2003). Synergy among termite defense mechanisms is apparently responsible for protecting colonies against epizoootics (Chouvenec and Su, 2010). Red imported fire ants, Solenopsis invicta, bury nest mates infected with B. bassiana to reduce transmission (Pereira and Stimac, 1992). Ants also spray antimicrobial secretions from the gaster over the brood to reduce B. bassiana transmission (Oi and Pereira, 1993). Reduced genetic diversity of the ant Cardiocondyla obscirior reduces the colony’s collective disease response by reducing the colony’s ability to detect and react to the presence of fungal spores (Ugelvig et al., 2010). Insects can also avoid areas of high pathogen densities when establishing new nest sites (Oi and Pereira, 1993). Mole crickets modify their behavior in response to M. anisopliae and B. bassiana in a way that reduces their exposure to these fungi (Villani et al., 2002; Thompson and Brandenburg, 2005).

Insects can also avoid or reduce the impact of infection by altering their physiology. For example, the brassy willow leaf beetle, Phratora vitellinae, constitutively releases volatile glandular secretions to inhibit a range of pathogens in its microclimate (Gross et al., 2008). Thermoregulation is also a behavioral response in adaptation to pathogen infection. In the field, many locusts and grasshoppers are active thermoregulators capable of maintaining a preferred body temperature in excess of ambient in sunny conditions (Chappel and Whitam, 1990). A further increase above preferred body temperature following fungal infection, termed “behavioral fever”, has been observed in a number of acridids (Blanford and Thomas, 1999) and has been shown to reduce fungal-induced mortality (Inglis et al., 1997; Blanford et al., 1998; Blanford and Thomas, 1999). Although thermoregulation can reduce the impact of M. acridum (as M. anisopliae var. acridum) infection, the insects have been reported to become more active and thus more susceptible to predation (Arthurs and Thomas, 2001).

**Host Associations**

Pathogens have different capacities for surviving in primary or secondary hosts. As such, pathogens capable of infecting more than one host in the environment are often better able to survive and persist (Goettel, 1995). The transmission and spread of a pathogen through the environment may rely not only on the infection of the primary host, but also on associations with alternative hosts, scavengers, parasitoids, predators, or other organisms. While in many cases these associations do not directly result in increased numbers of propagules in the environment, they do play a role in enhancing pathogen spread and persistence. To a large degree, the success, persistence, and spread of a pathogen in the environment are due to associations with organisms other than the primary host.

Primary pathogen survival and replication in the environment take place on primary hosts. Pathogens with long periods of infection that do not kill the host quickly are better suited to persist in the environment as they do not destroy the host population too quickly. For instance, infection of the chronic pathogen Nosema pyrausta in O. nubilalis populations is cyclic, with alternating enzootic and epizoootic patterns over a period of several decades (Lewis et al., 2009). To aid further in maintaining the cycle, prior to death, pathogen propagules are deposited and persist in fecal matter until they are fed on by additional host insects (Lewis and Cossentine, 1986; Vasconcelos, 1996). In another mechanism that enhances pathogen maintenance after the death of the primary host, pathogens are able to persist in the decomposing cadaver until environmental conditions are favorable for subsequent infection.
Secondary hosts (also called intermediate or alternate hosts) are host species in which immature, intermediate, or asexual stages of a pathogen occur (Onstad et al., 2006). Secondary hosts can play an important role in pathogen population dynamics and survival in the field. A secondary host can serve an obligatory role in the host’s life cycle, or it may be non-obligatory and be used solely to prolong the pathogen’s survival until the primary host or target is infected. An early example of an entomopathogen using an obligatory secondary host for completion of its life cycle involves the transmission of the microsporidium Amblyospora sp. infecting mosquitoes to alternate copepod hosts (Andreadis, 1985; Sweeney et al., 1985). It has since been shown that many species require two host generations to complete their life cycle, and members of at least four genera, Amblyospora, Duboscaja, Hyalinocysta, and Parathelohania, require obligatory development in a secondary copepod (Becnel and Andreadis, 1999).

The ability to infect a wide range of hosts beyond the primary target, none of which is obligatory, provides additional resources for pathogen survival. Fungal entomopathogens with wide host ranges are generally facultative pathogens, ubiquitous soil saprophytes and enzootic (Goettel, 1995). Beauveria bassiana is a well-studied example of a facultative pathogen with a broad host range, infecting over 700 species of arthropods from different orders (Li, 1988; Meyling et al., 2009). The ability of B. bassiana and other facultative fungal entomopathogens to utilize various resources in the environment no doubt plays a large role in the persistence of these fungi in nearly all areas on Earth. In addition to secondary arthropod hosts, B. bassiana and M. anisopliae persist as endophytes (Wagner and Lewis, 2000; Vega, 2008) or in the rhizosphere of a wide variety of plants (Fisher et al., 2011).

Carriers, or phoretic hosts, are insects or other organisms that are not susceptible to the pathogen, but play a role in dispersing the pathogen in the environment. Local dispersal of viruses by biotic agents has been reviewed by Fuxa (1989, 1991). Viral particles are dispersed via hemipterans (e.g., Podisus maculiventris), coleopterans (e.g., Calleida decora), hymenopterans (e.g., Brachymeria ovata), dipterans (e.g., Archytas apicifer), spiders (e.g., Misumenops sp.), and bird droppings (Fuxa et al., 1993; Fuxa and Richter, 1994). Phoretic relationships have been reported between entomopathogenic nematodes and earthworms (Shapiro et al., 1995; Campos-Herrera et al., 2006), mites (Epsky et al., 1988), and isopods (Eng et al., 2005). The sap beetle, Carophillus freemani, vectored B. bassiana mechanically and via their fecal material, resulting in O. nubilalis infection in the laboratory (Bruck and Lewis, 2002a). In the soil environment, collembolans vector fungal entomopathogens mechanically and via their gut contents in numbers sufficient to cause host infection (Dromph, 2001, 2003). The vectoring of fungal spores by these small soil arthropods is believed to have an important impact on the epizootiology of soil-borne fungal entomopathogens in the field (Dromph, 2003).

### 3.2.3. Transmission

Transmission is the process by which a pathogen or parasite is passed from a source of infection to a new host (Anderson and May, 1981; Andreadis, 1987; Onstad and Carruthers, 1990). Insect pathogens have evolved a wide array of mechanisms to ensure their long-term survival and transmission to new hosts. A fundamental knowledge of the methods of transmission is key to understanding the dynamics of disease and the occurrence of disease epizootics. Direct transmission of a pathogen occurs when it is transmitted from an infected to a susceptible new host without the aid of another living organism, whereas indirect transmission relies on one or more secondary hosts or vectors to facilitate transmission (Tanada and Kaya, 1993). Insect pathogens are primarily transmitted either directly host to host, or from the infected host into the environment, where they are subsequently acquired by a susceptible host.

### Methods of Transmission

Transmission can be vertical or horizontal. Horizontal transmission is the transfer of a pathogen from individual to individual, either host to host, or host to environment to host (Canning, 1982). Vertical transmission, also referred to as congenital, parental, or hereditary, is the direct transmission of a pathogen from parent to progeny (Fine, 1975; Andreadis, 1987). Horizontal transmission occurs widely in all pathogen groups and is the primary mode of transmission that leads to epizootics, particularly when host population densities are high. Infective propagules in the environment are predominantly transmitted directly to uninfected hosts leading to an epizootic. An increased pathogen titer within an individual may be caused by additional consumption of infected propagules (e.g., bacteria, viruses, protists) or by acquiring propagules externally (fungi), and by initial propagules completing a reproductive cycle and generating new ones.

Fungal pathogens are readily transmitted either host to host, or host to environment to host. Horizontal transmission of M. acridum (as M. flavoviride) is a key biological factor responsible for long-term persistence of the fungus in the environment (Thomas et al., 1995). Host-to-host horizontal transmission of B. bassiana and M. anisopliae between tsetse flies, Glossina morsitans (Kaaya and Okech, 1990), the Mexican fruit fly, Anastrepha ludens (Toledo et al., 2007), and the German cockroach, Blattella germanica (Quesada-Moraga et al., 2004), among others, has been demonstrated. Fungal-treated and control tsetse
flies mixed in cages for 32 days resulted in significant mortality due to fungal infection of untreated flies. In another example, direct and indirect transmission of *M. anisopliae* resulted in 40% infection of mites (*Psoroptes* spp.) responsible for mange in a wide range of vertebrate hosts (Brooks and Wall, 2005).

Horizontal transmission of bacterial and viral pathogens primarily occurs via the host-to-environment-to-host pathway. However, direct horizontal transfer from host to host does occur in the case of cannibalism in which an insect consumes infected conspecifics (Polis, 1981; Boots, 1998; Chapman et al., 1999). Horizontal transmission of bacterial and viral pathogens plays an important role in determining the rate of spatial spread of a pathogen (Dwyer, 1992, 1994).

Microsporidia are a well-studied pathogen group for their proclivity to transmit horizontally within a generation of hosts. The rate of horizontal transmission is governed by the percentage of a population infected, time in an instar, virulence, and the tendency of the microsporidium to transmit vertically. Most investigations on the dynamics of horizontal transmission of *N. pyrausta* to *O. nubilalis* report very little to no horizontal transmission during the first generation of *O. nubilalis* (Siegel et al., 1988; Onstad and Maddox, 1989; Solter et al., 1990). Andreadis (1986) suggested that horizontal transmission is greater in the second generation owing to greater larval density and interplant movement of larvae. Lewis (1978) demonstrated interplant movement of second generation larvae and horizontal transmission of spores. Experimentally, *N. pyrausta* can be transferred from first to second generation larvae when spores deposited by first generation larvae are consumed by second generation larvae feeding on the same plants (Lewis and Cossentine, 1986). In an experiment conducted in a single location for six consecutive growing seasons, researchers found that horizontal and vertical transmission influenced both the intensity of the *N. pyrausta* infection in the *O. nubilalis* population and the percentage of the population infected (L. C. Lewis et al., 2006). In a field study conducted over a 16-year period in two Nebraska counties, an increase in the percentage of the *O. nubilalis* population infected with *N. pyrausta* always followed periods of *O. nubilalis* population build-up, implicating insect density as a basis for horizontal transmission (Hill and Gary, 1979).

Entomopathogenic nematodes are exclusively horizontally transmitted. Infective juveniles (IJs) exit their host at the conclusion of the infection cycle, enter the environment (most often soil) and begin their quest in search of a new host. Recycling of nematodes as infections spread from host to host in the field decreases host populations and aids in nematode performance (Koppenhöfer, 2007). Nematode recycling is highly desirable in pest management programs as it not only provides additional control, but may also allow the nematodes to persist to the next host generation. While recycling is common, the factors that influence its occurrence are not clearly understood, and often the level of recycling is low (Shapiro-Ilan et al., 2006; Koppenhöfer, 2007). Most of the factors that influence nematode persistence, infectivity, and mobility are expected to play a role in nematode recycling.

Vertical transmission of a pathogen from parent to offspring is a key epizootiological factor for viruses and protists. In some instances, vertical transmission is the primary means by which a viral or microsporidian pathogen persists from one host generation to the next. In other cases, vertical transmission simply augments horizontal transmission, particularly when host populations are low. Vertical transmission occurs primarily through the female. The pathogen can be transmitted on the surface of the egg (transovarial transmission) or within the egg via the ovary (transovum transmission). The pathogen gains entry to the egg when the female reproductive organs (ovaries and accessory glands) are infected.

The mechanisms and epizootiological implications of vertical transmission of microsporidia are well studied. However, the intricate relationship between horizontal and vertical infection must be kept in mind. Horizontal infection of *O. nubilalis* larvae with *N. pyrausta* during the first, second, or third instar results in microsporidian spores being evident in ovarian tissue seven days after exposure. If exposed as a fourth or fifth instar, ovarian tissue is not infected until the pupal stage (Sajap and Lewis, 1988). The intensity of the infection during ovarian development governs whether or not there is destruction of germ cells and a subsequent reduction in oogenesis. This results in a fine line between reduction in fecundity because of damage to the ovary and that caused by transovarial transmission of the spore (Sajap and Lewis, 1988).

Many insect viruses are vertically transmitted in the environment. Understanding the relative contribution of vertical transmission and the conditions that maintain the pathogen is critical to understanding their ecology (Cory and Evans, 2007). Vertical transmission has been best studied in baculoviruses, but even within this group, the nature and role of vertical transmission on epizootiology are poorly understood (Cory and Meyers, 2003). Overt disease in the next generation can result from transovum and transovarial transmission (Kukan, 1999). Vertical transmission of NPV through the adult host is an adaptation for long-range environmental transport to new locations or for difficult-to-reach areas within a location (e.g., trees) (Fuxa, 2004). There is strong evidence of vertical transmission of NPV (Smirnoff, 1972; Olofson, 1989) and cytoplasmic polyhedrosis viruses (= cypoviruses) of Lepidoptera (Sikorowski et al., 1973), indicating continuation of gut infections initiated in the larval stage. In these cases, transovum transmission as an external contaminant...
of the deposited eggs appears to be the most likely route of entry.

**Modes of Dissemination**

Dissemination is the ability of the pathogen to spread within a host population and throughout the environment (Tanada, 1963). There are four primary routes of pathogen dissemination in the field (Tanada, 1964; Andreadis, 1987), based on: (1) the pathogen’s motility; (2) the behavior and movements of the primary host; (3) the behavior and movement of secondary hosts and non-host carriers; and (4) the actions of physical environmental factors.

Pathogen motility in general is very limited in terms of both the ability of pathogens to move under their own power and the distance they are able to travel in the environment. As such, their importance in the initiation or continuance of an epizootic is more profound when host densities are high and these mobile propagules are able to contact nearby hosts. Movement and activities of the primary host in the environment are important factors in pathogen dissemination. The direct mechanisms of pathogen dissemination (host to host) by the primary host include vertical transmission, cannibalism, and grooming. Indirect pathogen dissemination (host to environment to host) occurs via horizontal transmission by excretion of infective stages in feces and anal discharge (Fuxa et al., 1998) and meconial discharges, disintegration of cadavers with infective pathogen propagules (Fuxa, 2004), and elimination of infected exuvia during molting. Perhaps the most well-cited example of primary host behavior that aids in the dissemination of disease is the treetop disease (also known as summit disease) of many lepidopterous and hymenopterous larvae infected with NPV (Andreadis, 1987), whereby infected larvae migrate to the tops of the plant or tree on which they are feeding and subsequently die attached to leaves by their prolegs (Fig. 3.2). Being positioned in this way ensures that the foliage below is showered with inoculum as the cadaver breaks down. This facilitates pathogen transmission to healthy larvae on the plant (Harper, 1958; Fuxa, 2004). A similar phenomenon occurs in aquatic systems when mosquito larvae and pupae killed by *Erynia aquatic* are buoyant and the conidia-phores grow from the cadaver above water and are discharged onto mosquitoes nearby (Steinkraus and Kramer, 1989).

Wind and rain are the most important abiotic environmental factors responsible for pathogen dispersal. Their effect on the dispersal and subsequent epizoology of plant pathogens has been well studied (Gregory et al., 1959; Hunter and Kunimoto, 1974; Aylor, 1990). Wind was suspected in the dissemination up to 30 m of *Hyphantria cunea* NPV and *Neodiprion sertifer* NPV (Hukuhara, 1973). Rainfall distributes *L. dispar* NPV from branch to branch in a downward direction, increasing virus spread and the likelihood of horizontal transmission (D’Amico and Elkinton, 1995). Rainfall is an efficient means of transfer from the fungal entomopathogen reservoir in the soil to the surface of whorl-stage corn plants (Bruck and Lewis, 2002b). Plants receiving rainfall had a mean of 8.8 colony-forming units (cfu) of *B. bassiana* per plant, while those not receiving rainfall had a mean of 0.03 cfu per plant. *Beauveria bassiana* conidia splash from the soil to the plant surface via rain splash (Fuxa and Richter, 2001).

**3.2.4. The Environment**

The role of environmental factors in disease dynamics in nature cannot be overstated. Epizootics of insect pathogens are heavily influenced by environmental factors (biotic and abiotic) and in many cases environmental factors are the most relevant in the epizootiological process (Ignoffo, 1992). The three primary factors contributing to the epizootics of disease — the host population, the pathogen population, and transmission — have already been discussed. Environmental factors may have direct or indirect

![Figure 3.2 Soybean looper, *Thysanoplusia orichalcea*, infected with *Thysanoplusia orichalcea* nucleopolyhedrovirus. The image illustrates what is known as treetop disease or summit disease, whereby the host climbs to an exposed position (e.g., on vegetation) before death to facilitate dispersal of the pathogen. (Courtesy of M. Shepard, G. R. Carner, and P. A. C. Ooi, *Insects and their Natural Enemies Associated with Vegetables and Soybean in Southeast Asia*, Bugwood.org, with permission.)](image-url)
impacts on any or all of these primary factors in the environment in which the host and pathogen occur (Benz, 1987). The result is a complex interaction between primary disease factors and the environment that impact all stages and processes of the disease cycle and cannot be separated.

Pathogens have varying levels of capacity to survive both abiotic and biotic factors in the environment. Many pathogens produce resistant forms designed to persist in the environment for extended periods in the absence of a host. The role of abiotic factors on pathogen persistence has been well studied in insect pathology. Because of the long-standing desire to develop microbial programs for a variety of soil, foliar, and aquatic pests, understanding and enhancing the ability of microbial agents to persist in the environment are key components to improving microbial control (see Section 3.3.3). The following text discusses environmental influences on epizootiology in aerial and aquatic environments, edaphic environments, and interactions among trophic levels.

**Aerial and Aquatic Environments**

Abiotic factors or the physical environment are the most well-studied factors affecting the ability of a pathogen to survive. Abiotic factors that are of particular importance for terrestrial applications are ultraviolet (UV) radiation, temperature, humidity, and moisture (Ignoffo, 1992). Of these, UV radiation affects all pathogen groups and is the most damaging (Fuxa, 1987). The exposure of Bt spores to UV radiation is believed to be largely responsible for its inactivation and low persistence in nature (Myasnik et al., 2001). Conidial exposure of multiple isolates of *Metarhizium* and *Beauveria* spp. indicated that the viability of all isolates dropped markedly with increasing exposure to UV radiation (Morley-Davies et al., 1995; Fargues et al., 1996; Braga et al., 2001a). Despite this intrinsic susceptibility, fungal species and strains differ significantly in their tolerance to UV radiation (Morley-Davies et al., 1995; Braga et al., 2001a).

Ambient temperature, humidity, and moisture can affect both pathogen persistence and infectivity (Reyes et al., 2004; Ebssa et al., 2004; Lacey et al., 2006a). In aquatic environments, extreme temperatures can inactivate microsporidian spores or cause them to germinate prematurely (Undeen and Vávra, 1997). Temperature and moisture extremes can be highly detrimental to nematode survival (Smits, 1996; Glazer, 2002; Koppenhöfer, 2007). Occluded viruses are tolerant to fairly wide temperature extremes, surviving cold storage at −70°C and rapid heat inactivation at temperatures above 40°C (Cory and Evans, 2007; Lacey et al., 2008a). Fungal entomopathogens, in general, have a wide range of temperature tolerances, but optimal conditions for infection, growth and sporulation range from 20 to 30°C (Vidal and Fargues, 2007; Wraight et al., 2007). Most studies on the impact of temperature on fungal pathogen efficacy to date focus on constant temperatures and not fluctuating temperatures as would be observed in the field (Inglis et al., 1997; Bruck, 2007). Temperatures below 15°C significantly slowed mycelial growth of *M. anisopliae* in vitro and *O. sulcatus* larval infection in soilless potting media (Bruck, 2007). *Otiorhynchus sulcatus* larval mortality due to *M. anisopliae* occurred at temperatures as low as 10°C; however, the progression of the infection was significantly retarded (Bruck, 2007). The effect of temperature and moisture on fungal spore survival varies considerably among fungal species and strains (Hong et al., 1997). In addition to temperature, humidity, and moisture, pH, dissolved minerals, and inhibitors are important abiotic factors affecting microsporidium survival (Undeen, 1990). Shifts in pH cause spores of *N. algerae* to germinate (Undeen and Avery, 1988), and ammonia and calcium can inhibit spore germination of this microsporidian species as well (Undeen, 1978).

Plants, and more often than not particular areas of the plant architecture, provide a physical barrier from the adverse effects of abiotic factors and enhance the ability of pathogens to survive. The leaf collar of corn plants is a favorable habitat for *B. bassiana* and Bt, providing an environment with adequate moisture and protection from UV radiation (McGuire et al., 1994; Bruck and Lewis, 2002c; Lewis et al., 2002). Inglis et al. (1993) also found improved fungal survival in the protected environment of the alfalfa canopy. Plant leaf surface topography and leaf surface wax can influence conidial acquisition (Inyang et al., 1998; Duetting et al., 2003; Ugine et al., 2007) and leaf microclimate can influence conidial germination (Baverstock et al., 2005). Plant chemistry can also be detrimental to insect pathogens. For example, plant secondary chemicals sequestered by the brassy willow leaf beetle, *Phratora vitellinae*, reduce LD50 and LT50 values of *B. bassiana* and *M. anisopliae* (Gross et al., 2008) as well as germination and conidial production of *B. bassiana* infecting the whiteflies *Bemisia tabaci* and *B. argentifolii* (also known as *Bemisia tabaci* biotype B) (Poprawski and Jones, 2000; Santiago-Álvarez et al., 2006).

Insect pathogens have evolved a variety of adaptations to overcome environmental barriers. For example, there is clear evidence that the presence of an occlusion body increases virus survival in the field over free virions in the environment (Wood et al., 1994; Cory and Evans, 2007). In another example, various fungal entomopathogens have evolved the ability to grow endophytically with a wide variety of plants (Vega, 2008). By doing so, these fungi persist within the plant protected from sunlight.

**Edaphic Environment**

The soil is a natural pathogen reservoir. Pathogens are well suited to persist and survive in the soil, where they are protected from UV radiation and buffered from desiccation
and rapid variations in moisture and temperature. *Bacillus thuringiensis* (Ohba and Aizawa, 1986; Martin and Travers, 1989; Meadows, 1993), fungal entomopathogens (Bidochka et al., 1998; Klingen et al., 2002; Bruck, 2004), nematodes (Chandler et al., 1997; Rosa et al., 2000; Hominick, 2002), and NPVs (Thompson et al., 1981; Fuxa, 2004) are all well established as ubiquitous soil organisms.

The moisture content of soil can affect pathogen persistence and performance. *Invertebrate iridescent virus* 6 loses activity in dry soil (6% moisture) in less than 24 h, while soil moistures between 17 and 37% did not influence persistence (Reyes et al., 2004). Moisture is the most important factor influencing nematode performance, as IJs require a water film on the soil particles for effective movement. If the water layer becomes too thin or the interspaces between soil particles are too thick, nematode movement is restricted (Koppenhöfer et al., 1995). Inactive IJs may persist for longer in dry soil, but infection will be impeded (Kaya, 1990).

Soil protects pathogens from the damaging effects of UV radiation once the pathogen enters the soil profile. However, nematode IJs applied to the soil surface are acutely sensitive to UV radiation. Exposure to short UV radiation (254 nm) and natural sunlight inhibited 95% of *Neoaplectana* (Steinernema) carpopcapsae (Gaugler and Boush, 1978). Exposure of *S. kushidai* IJs to the UV-C portion of the spectrum resulted in 62 and 100% mortality after 20 s and more than 60 s exposure, respectively (Azua and Tomoko, 1999). Exposure to simulated sunlight in the laboratory for a few hours, particularly to the UV-B portion of the spectrum, fully inactivates *Metarhizium* conidia (Fargues et al., 1996; Braga et al., 2001b, c) and delays the germination of surviving conidia (Braga et al., 2001d).

The chemical and structural make-up of the soil affects the persistence and performance of pathogens. Soil parameters such as texture, organic matter, and electrical conductivity can be significant factors in entomopathogenic nematode persistence or efficacy (Kaya, 1990; Sturhan, 1999; Kaspi et al., 2010). Entomopathogenic nematode survival and pathogenicity are generally greatest in soils with larger soil particles (sandy-loam), decreasing as soils transition to smaller soil particles (clay) (Kung et al., 1990; Barbercheck and Kaya, 1991). Fertilization, which is reflected in conductivity and the concentration of soluble nutrients in the soil, has been shown to decrease the persistence of fungal entomopathogens, presumably owing to increased activity of antagonistic microbes (Lingg and Donaldson, 1981; Rosin et al., 1996, 1997). Groden and Lockwood (1991) found that fungistasis levels for *B. bassiana* in soils increased exponentially with increases in soil pH from 6.1 to 7. *Beauveria bassiana* persisted best in peat that also had the highest pH (Vänninen et al., 2000). Conversely, Rath et al. (1992) found no effect of electrical conductivity or pH on the natural occurrence of *M. anisopliae* in Tasmania.

Biotic agents in the soil also play an important role in enhancing, distributing, synergizing, and competing with entomopathogens. The presence of plants and their roots in the soil is proving to be important in the overall biology and ecology of fungal entomopathogens. The rhizosphere has been shown to provide a favorable microhabitat for fungal survival in the soil (Hu and St. Leger, 2002; Bruck, 2010; Fisher et al., 2011). During the six months following fungal application, the *M. anisopliae* titer in the bulk soil decreased from $10^5$ propagules/g in the top 3 cm of soil to $10^3$ propagules/g. However, fungal titers in the rhizosphere remained at $10^5$ propagules/g six months after application, resulting in a 100:1 ratio in fungal densities between the rhizosphere and bulk soil (Hu and St. Leger, 2002). The *M. anisopliae* population in the rhizosphere of *Picea abies* was significantly higher than the population in the surrounding bulk soil (Bruck, 2005). Subsequent studies demonstrated variability within *M. anisopliae* isolates in rhizosphere competence between plants (Bruck, 2010).

Macroorganisms within the soil environment interact with pathogens on multiple levels. Earthworms can enhance the dispersal of *S. carpopcapsae* (Shapiro et al., 1993, 1995). *Lumbricus terrestris* increased the upward dispersal of *S. carpopcapsae* and *S. feltiae* but had no impact on *S. glaseri* upward dispersal. Collembolans vector fungal entomopathogens (mechanically and via their gut contents) in numbers sufficient to cause host infection (Dromph, 2001, 2003). The vectoring of fungal spores by these small soil arthropods is believed to have an important impact on the epizootiology of soil-borne fungal entomopathogens in the field (Dromph, 2003).

In addition to positive biotic associations (e.g., phoresy), there are antagonistic biotic interactions that are fostered natural enemies of entomopathogens. For example, nematophagous mites and collembolans feed on *Steinernema* and *Heterorhabditis* spp. with some mite species able to complete their development on a diet consisting entirely of IJs (Epsky et al., 1988; Cakmak et al., 2010). Besides predators, nematodes (or their bacterial symbionts) have a suite of natural enemies as they are susceptible to infection by microorganisms including phages (Poinar et al., 1989; Boemare et al., 1993), protists (Poinar and Hess, 1988), and nematophagous fungi (Koppenhöfer et al., 1996).

**Interactions among Trophic Levels**

Interactions between trophic levels (i.e., plants and insect pathogens) have been studied extensively (Cory and Ericsson, 2010). Some of the more interesting examples of tri-trophic interactions between pathogens and their host involve the production of herbivore-induced plant volatiles, the cas-sava green mite, *Mononychellus tanajoa*, and its fungal
pathogen Neozygites tanajoae. Green leaf volatiles inhibit the germination of N. tanajoae conidia, while herbivore-induced plant volatiles enhance conidiation (Hountondji et al., 2005). Tritrophic interactions involving host plant volatiles have also been shown to occur below ground in experiments examining entomopathogenic nematodes and their attraction to the conifer Thuja occidentalis after root herbivory by O. sulcatus larvae (van Tol et al., 2001). The induction of natural enemy attractants in response to root herbivores has also been identified in turnips (Neveu et al., 2002), tulips (Aratchige et al., 2004), and corn (Rasmann et al., 2005). In addition, O. sulcatus larvae are attracted to the roots of P. abies grown in the presence of M. anisopliae conidia, indicating the operation of a previously undescribed tritrophic interaction (Kepler and Bruck, 2006).

3.2.5. Modeling Epizootics

A model is an idealized representation of reality that is used to understand a particular defined system. Given the complexity of factors that contribute to epizootiology, mathematical models are required for understanding and predicting the dynamics of insect disease in natural populations. Furthermore, models may be highly useful in understanding and predicting parameters required for success in microbial control applications. Similar to many aspects in epizootiology of insect diseases, a significant amount of the conceptual basis for modeling arises from epidemiology of human diseases or diseases of other non-insect animals (Onstad and Carruthers, 1990). According to Hethcote (2000), deterministic epidemiological modeling started early in the twentieth century for studying the dynamics of measles epidemics (Hamer, 1906). Although much of today’s focus on epidemiological modeling is on non-infectious disease (Hethcote, 2000), the continued importance of infectious diseases in developing countries, as well as emerging or reemerging diseases in developed countries, has sustained a substantial need for and interest in mathematical modeling of infectious diseases (Hethcote, 2000; Maines et al., 2008; Temime et al., 2008). Some basic concepts in epidemiology (as reviewed by Hethcote, 2000) that are carried in epizootiology include basic classes and flow patterns in epidemiological models such as SEIR, where S is susceptible individuals, E is those exposed, I those infected, and R recovered (immune individuals), as well as the threshold concept, i.e., that the density of susceptible individuals must exceed a critical value for an epidemic outbreak to occur.

General Concepts in Epizootiological Modeling

The basis for modeling epizootiology of insect diseases, and the ecological theory behind it, is derived primarily from Anderson and May’s work in the early 1980s (1980, 1981, 1982), with substantial review and analyses by Brown (1987), Onstad and Carruthers (1990), Hesketh et al. (2010), and others. With particular relevance to applied microbial control, Brown (1987) discusses epizootiological modeling using an integrated pest management (IPM) system approach, i.e., taking a defined and delineated (host—pathogen) system and breaking it into mathematical components that adequately represent the whole. Typically, a systems model is generated by building a conceptual framework based on traditional empirical research, developing a mathematical representation of that framework, and finally devising a computer program that will implement the mathematical representation (Brown, 1987).

Fundamentally, host—pathogen models are based on the concept that the number of susceptible individuals in a population at a given time depends on the number of contacts between susceptible and infected individuals, and the transmission rate. A simple equation for this concept may be written as:

\[ S_{t+1} = S_t - pSIt \]

where S is the number of susceptible individuals, t is time, p is transmission efficiency (e.g., if \( p = 0.05 \) then 5% of possible contacts result in transmission), and I is the number of infected individuals.

Similarly, the number of infected individuals at a given time can be represented as:

\[ I_{t+1} = I_t + pSIt - mIt \]

where m is the mortality rate, e.g., if the incubation period is four days then \( m = 0.25 \) (25% of infected individuals die each day); m can be considered virulence (power to cause disease/mortality).

The equations can be rewritten as simple differential equations, e.g., for the change in number of susceptible individuals over time with the intrinsic rate of increase (r) incorporated:

\[ \frac{dS}{dt} = rS - pSI \]

Another basic concept in epizootiological models that can be derived from the above equations is that of the threshold (Brown, 1987; Onstad and Carruthers, 1990). Anderson and May (1980, 1981) defined the threshold as the host density required for the pathogen to persist within the population; the threshold is directly proportional to virulence and indirectly proportional to transmissibility. The threshold occurs when the number of susceptible individuals is equal to the mortality rate divided by transmission (\( St = ml/p \)). If the susceptible population is greater than the threshold then disease prevalence can increase and if the susceptible population is less than the threshold then disease
prevalence can only decrease. In other terms, Hesketh et al. (2010) discusses $R_0$ as the basic rate of pathogen increase; $R_0$ must be $> 1$ for the pathogen to persist and spread, and $H_T$ is a critical threshold below which prevalence will decline and above which it will rise, i.e., when $R_0 = 1$.

Beyond the basic concepts described above, a systems model increases in its complexity as it incorporates additional factors in order to predict prevalence, e.g., impact of the pathogen on reproductive potential, vertical transmission, and impact of non-infective stages. The factors that can be considered for incorporation may be divided into three groups: (1) pathogen stages outside the host (dispersal, survival, and transmission mode); (2) factors at the host–pathogen interface (host stress, inoculum loads, etc.); and (3) pathogen stages inside the host (incubation period; host feeding and aging rate). Certainly, not all potential factors can be incorporated into the model.

**Examples of Epizootiological Models**

Examples of epizootiological models may be found across pathogen groups, e.g., viruses (Bianchi et al., 2002; Sun et al., 2006), bacteria (Zahiri et al., 2004), and with more emphasis in recent years on modeling resistance to Bt crops, e.g., Tabashnik et al., 2008; Onstad and Meinke, 2010), protists (Otterstatter and Thomson, 2008), fungi (Hesketh et al., 2010), microsporidia (Onstad and Maddox, 1990; Kelly et al., 2001), and nematodes (Stuart et al., 2006; Ram et al., 2008). Epizootiological models for insect pathogens have ranged from simple to complex and covered both natural and introduced pathogen populations.

In some cases, relatively simple models have provided benefits toward understanding and predicting epizootiology. For example, Milks et al. (2008) used multiple regression analysis to describe the prevalence of the microsporidium Thelohania solenopsae in populations of S. invicta. Several factors that appeared to impact T. solenopsae prevalence were defined, including the number of colonies at a site, precipitation, proximity to waterways, and habitat type. In another example, Edelstein et al. (2005) used a non-linear model to study the influence of temperature on the developmental rate of the fungus Nomuraea rileyi in larvae of the velvetbean caterpillar, Anticarsia gemmatalis. The estimated lower and upper thresholds of fungal vegetative development were observed to coincide with conditions during the natural epizootics in central Argentina. Although the model was developed under controlled conditions in the laboratory, some understanding of field dynamics and potential for microbial control was gained and opportunities for additional research and further model development were obtained.

Some more complex or comprehensive models than those described above have addressed inundative or inoculative approaches to microbial control of specific target pests. An example is depicted in the work of Bianchi et al. (2002), in which a model was constructed when applying Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV) in the greenhouse. Parameters included crop growth, insect development (growth rate and reproductive potential), spatial distribution of plants, virus inactivation within occlusion bodies (based on expected decay due to UV radiation), rate of infection by ingestion, rate of horizontal transmission, and environmental variables (e.g., temperature). The models were generally in close agreement with experimental data (Bianchi et al., 2002).

In another example directed toward predicting efficacy of microbial control applications, a model was developed to predict the potential of fungal entomopathogens to control mosquitoes and suppress malaria (Hancock et al., 2009). In reference to the mosquito–plasmodium dynamics, the concept was based on a simple model considering susceptible hosts, exposed individuals (those that carry malaria but cannot yet transmit the disease), and infected individuals. Exposure to a fungal entomopathogen and the probability of fungal infection upon exposure were also incorporated. When realistic assumptions were made about mosquito, fungus, and malaria biology and with moderate to low daily fungal infection probabilities, the model indicated the potential for substantial reductions in malaria transmission with fungal applications (Hancock et al., 2009).

In addition to models geared toward optimizing microbial control applications, several models have been developed for predicting the epizootiology of endemic pathogen populations. For example, substantial research has been directed toward the ecology and epizootiology of Entomophaga maimaiga in relation to a mortality factor of L. dispar (Weseloh et al., 1993; Hajek, 1999; Weseloh, 2002). Models to simulate fungal prevalence based on parameters including temperature, humidity, precipitation, fungal reservoirs, and L. dispar density have been developed and tested for validity (Weseloh, 2004). Models that sufficiently account for dispersal of conidia can provide a good fit to infection prevalence in forests (Weseloh, 2004).

Malakar et al. (1999a) demonstrated that models might also be used to simulate interactions between two pathogens. Specifically, a model to determine the impact of E. maimaiga on Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV) in L. dispar populations was developed. Pathogen activity was determined in plots with and without artificial rain (the latter inducing higher fungal mortality in L. dispar). Despite the negative impact of dual
infection on production of LdMNPV in a separate laboratory study (Malakar et al., 1999b), the model for field interactions indicated that in moderate host populations, the fungus would not substantially affect virus infection, owing to temporal separation of the two pathogens’ activities (Malakar et al., 1999a). The fungus remained at low levels until the later instars, at which time L. dispar is less susceptible to LdMNPV infection (Malakar et al., 1999a).

Models have also been used for predictive value in simulating the epizootiology of genetically modified pathogens. For example, Sun et al. (2006) developed a comprehensive model to simulate epizootics in wild-type or genetically modified, *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HaSNPV) for the control of cotton bollworm, *Helicoverpa armigera*, in cotton. With multiple applications, both wild-type and genetically modified viruses were found to be capable of keeping the target pest under its economic injury level (EIL). The recombinant virus, which kills the host more quickly, provided better short-term protection than the wild-type virus. However, limited persistence of the recombinant virus in the host population resulted in reduced horizontal transmission and higher pupal survival at the end of the growing season. Thus, the simulation indicated that the fast acting genetically modified viruses may be more efficacious for short-term (inundative) control, whereas the wild-type may be superior for longer term (inoculative) approaches.

The modeling examples described above are generally directed at representation of whole insect–pathogen systems. There have also been some models that focus on simulating a specific portion of the epizootiological process. For example, models have been developed to explain group infection behavior in entomopathogenic nematodes (Fenton and Rands, 2004; Fushing et al., 2008). Fushing et al.’s (2008) model infection was based on the concept of risk-sensitive foraging and a follow-the-leader behavior, i.e., individuals that are more prone to taking risk infect the host first, and subsequently the remaining infective population follows. In their analytical review articles, Onstad and Carruthers (1990) and Hesketh et al. (2010) recommend advancing the discipline of epizootiological modeling within insect pathology through better incorporation of spatial and temporal heterogeneity and their associated variables, improved merging of empirical and theoretical data, and greater cooperation among the empiricists and theorists. Additional models that focus on the components of epizootiology (such as infectivity, success within the host, and survival outside the host) may lead to superior models of the whole system once the components are coupled together.

### 3.3. MICROBIAL CONTROL (APPLIED EPIZOOTIOLOGY)

#### 3.3.1. Basic Concepts in Microbial Control

Microbial control can be defined as the use of insect pathogens for pest suppression. Some uses include byproducts of the pathogenic organisms as well (e.g., toxins). Furthermore, some consider natural suppression of pests (i.e., without any human intervention) to be included in microbial control, but this chapter will just consider microbial control in relation to intentional manipulation of the targeted system. The goal of microbial control programs is to eliminate or reduce a pest population below an economically damaging level. The goal is achieved by causing disease prevalence in a targeted population that is sufficient to keep the pest below the EIL. The principles that underlie the ability to cause disease in targeted populations are guided by the principles that cause epizootics in natural insect populations. Therefore, microbial control can be considered applied epizootiology.

Like other insect pest management strategies, the decision to implement a microbial control tactic for pest suppression should be weighed against other options, including not taking any action. In most systems, chemical insecticides still predominate as the primary pest control tactic. Therefore, microbial control is often compared with the use of chemical insecticides. In general, relative to chemical insecticides, there are several disadvantages that vary in degree among the pathogen groups, including cost of production, lack of a wider host range, susceptibility to environmental degradation, and a longer time to kill the host (Fuxa, 1987; Tanada and Kaya, 1993; Lacey and Shapiro-Ilan, 2008). There are also a number of general advantages that are associated with microbial control relative to the use of chemical insecticides. Although not all pathogen groups hold these advantages (or the levels at which they hold them vary), the advantages of microbial control include a reduced potential for development of resistance in the target pest and safety to humans, other non-target organisms, and the environment; relative safety to non-targets also leads to a reduced chance of secondary pest outbreaks through conservation of natural enemies (Fuxa, 1987; Tanada and Kaya, 1993; Lacey and Shapiro-Ilan, 2008).

The safety of microbial control agents is largely based on their narrow host ranges, which allows for safe application with reduced potential for impact on beneficial organisms and secondary pest outbreaks. A narrow host range, however, can also be considered a disadvantage; for example, if the user wants to target several unrelated pests at once, then a broad-spectrum tactic may be more attractive. The host ranges of some pathogen groups are
narrower than others, e.g., certain NPVs and granuloviruses (GVs) may infect within only one genus or species, whereas some, such as Autographica californica NPV, infect numerous species in different lepidopteran families (Cory and Evans, 2007). Microsporidia and various protists also tend to have narrow host ranges, whereas entomopathogenic nematodes and hypocrealean fungi have wider host ranges. Yet even within these groups, exceptions exist. For example, S. scarabaei and S. scapterisci are specific to white grubs and orthopterans (especially mole crickets), respectively (Shapiro-Ilan et al., 2002a). Some bacteria have intermediate host ranges, e.g., Bt strains to various Lepidoptera, whereas Paenibacillus popilliae is specific to several subfamilies within Scarabaeidae (Garczynski and Siegel, 2007). Bacillus sphaericus is specific for several, but not all species of the Culicidae (Lacey, 2007).

A primary concern in pest management is protection of human health. In general, microbial control agents are safe to humans and other vertebrates, yet some exceptions exist, e.g., the bacterium Serratia marcescens can be an opportunistic pathogen in humans causing sepsis, formulations of B. bassiana can cause allergic reactions in humans (Westwood et al., 2006), and in rare cases Beauveria sp. has been reported to be capable of causing infection in immunosuppressed humans (Henke et al., 2002). Nonetheless, microbial control agents are overall considerably safer to humans than chemical insecticides, which have been documented to cause approximately 220,000 human deaths per year (Pimentel, 2008).

In addition to safety to humans, microbial control agents are generally considered to have little or no effect on other non-targets including beneficial insects. Even so, exceptions, in which entomopathogens have a negative impact on insect biocontrol agents, have been documented, e.g., a negative association between the microsporidium N. pyrausta and the parasitoid Macrocentrus grandii (Bruck and Lewis, 1999). In another example, entomopathogenic nematodes were observed to infect several hymenopteran parasitoids (Shannag and Capinera, 2000; Lacey et al., 2003; Mbata and Shapiro-Ilan, 2010) or coleopteran predators (Shapiro-Ilan and Cottrell, 2005). However, several studies or analyses have indicated that the impact of entomopathogenic nematodes on field populations of insect natural enemies is negligible owing to various factors such as spatial or temporal dynamics that prevent negative impact between the control agents (Georgis et al., 1991; Bathon, 1996; Koppenhöfer and Grewal, 2005).

Another primary advantage in the use of microbial control agents is the reduced potential for resistance development in the target pest. Resistance to entomopathogens, however, has been demonstrated in the laboratory, e.g., resistance to M. anisopliae in termites (Rosengaus et al., 1998; Traniello et al., 2002). Resistance to certain entomopathogens has also been reported under field conditions, including field resistance to GV (Asser-Kaiser et al., 2007), NPV (Fuxa et al., 1988), Bt (McGaughey, 1985; Tabashnik et al., 1990), and Bt crops (Tabashnik et al., 2008). Host resistance is discussed in Chapter 13.

3.3.2. Factors Affecting Efficacy in Microbial Control

Beyond safety and possessing a low potential for resistance, some other factors affect the efficacy of microbial control agents. An ideal microbial control agent is likely to possess the following attributes: (1) a high level of virulence to the target pest; (2) ease of production and storage; and (3) the ability to persist in the environment. Factors that affect efficacy are discussed below and summarized by pathogen group in Table 3.1.

Production technology is critical to cost competitiveness and meeting market demands for a target pest. Production of some pathogen groups cannot be achieved outside an insect host (e.g., microsporidia) and thus relies on in vivo culture methods, which limit or prevent economy of scale. Insect virus production also relies on in vivo methods, and although culture methods have been streamlined by mechanization, attempts to advance mass production in cell culture have thus far been unsuccessful (Vail et al., 1999; Szewczyk et al., 2006).

Mass production has been greatly facilitated in entomopathogens that can be cultured in liquid or solid fermentation. Among bacteria, the successful bioinsecticides Bt and B. sphaericus are produced via submerged in vitro culture, whereas P. popilliae must be grown in vivo (Garczynski and Siegel, 2007). Hypocrealean conidia can be produced using a solid fermentation or a diphasic approach, whereas liquid culture can be used for production of blastospores, sclerotia, or in some cases, conidia (Feng et al., 1994; Leland et al., 2005; Jackson et al., 2010) (see Chapter 6). Depending on the microbial control approach and level of persistence required, some culture methods for fungi may be more appropriate than others (Jackson et al., 2010). Entomopathogenic nematodes are produced using solid or liquid fermentation as well as in vitro approaches. Most entomopathogenic nematodes are produced using in vitro liquid culture, which offers the greatest economy of scale relative to other approaches (Shapiro-Ilan and Gaugler, 2002; Ehlers and Shapiro-Ilan, 2005). In some studies or for certain species, the efficacy or quantity of nematodes produced in liquid culture was found to be inferior to in vivo produced products (Gaugler and Georgis, 1991; Abu Hatab and Gaugler, 1999; Cottrell et al., 2011), whereas in other cases no differences due to culture method were detected (Gaugler and Georgis, 1991;
Despite advances in the production of microbial control agents, they are still generally more expensive than chemical insecticides, yet with the advent of certain “soft” chemicals (with a narrower host range) the gap in price has decreased.

Virulence clearly has a direct effect on microbial control efficacy. Pathogens, even at the strain level, can vary greatly in their ability to cause high levels of mortality in a specific host. Speed of kill or disease onset can also vary greatly among pathogens. Entomopathogenic nematodes are relatively fast acting, with the ability to kill the host within 24–48 h postinfection. In contrast, hypocrealean fungi and insect viruses (e.g., NPVs) are slower acting and can take a week or more to kill the host (Fuxa, 1987; Shapiro-Ilan et al., 2004a). Some bacterial pathogens such as Bt are fast acting, killing their hosts within hours to a few days, whereas Paenibacillus popilliae is slow, taking 20 days or more to kill their white grub hosts (Dutky, 1963; Fuxa, 1987). Some pathogens do not cause mortality directly; for example, although there are a few exceptions (e.g., Vairimorpha necatrix, which can rapidly cause mortality), most microsporidia cause chronic infections that may affect various aspects of host fitness such as reproductive capacity, longevity, or development without causing direct mortality (Solter and Becnel, 2007). A virulent and fast-acting pathogen is generally considered desirable and is required to suppress pests with a low EIL; however, slower, less virulent pathogens may control pests with higher EILs (Fuxa, 1987). In addition, a low level of virulence and long infection process can enhance the pathogen’s persistence in the host population (Anderson, 1982; Fuxa, 1987). Fast-acting pathogens can be disadvantageous when targeting social insects, which may impede or suppress pests through avoidance and removal of infected individuals (Rath, 2000; Wilson-Rich et al., 2007, 2009). In contrast, chronic pathogens can have the advantage of infiltrating social insect colonies, including accessing the queen (Oi, 2006; Milks et al., 2008).

Pathogen population density affects microbial control efficacy. A higher pathogen population density (as well as

<table>
<thead>
<tr>
<th>Pathogen Group</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus (e.g., baculovirus)</td>
<td>Specificity, High level of virulence, Capable of persisting in the environment (soil), Can store at room temperature</td>
<td>Specificity, Environmental sensitivity (UV), Slow acting for most baculoviruses, Cost of production (in vivo only)</td>
</tr>
<tr>
<td>Bacteria (e.g., Bacillus thuringiensis, B. sphaericus)</td>
<td>Wide host range, Ease of mass production, Speed of kill, Can store at room temperature</td>
<td>Environmental sensitivity (UV), For Paenibacillus popilliae: cost of production (in vivo) and slow acting</td>
</tr>
<tr>
<td>Microsporidia</td>
<td>Persistent stages, Specificity</td>
<td>Cost of production (in vivo), Most cause chronic infection and are slow acting, Environmental sensitivity (UV)</td>
</tr>
<tr>
<td>Fungi (e.g., Hypocreales)</td>
<td>Wide host range, Ease of mass production (in vitro), High virulence, Can penetrate cuticle (infection through contact), Can store at room temperature</td>
<td>Environmental sensitivity (UV, relative humidity), Slow acting</td>
</tr>
<tr>
<td>Nematodes (Steinernema and Heterorhabditis)</td>
<td>Wide host range, Speed of kill, Ease of mass production (in vivo or in vitro), Mobility, can actively detect and seek or ambush host, Little or no registration required</td>
<td>Cost of production (despite in vitro methodology), Environmental sensitivity (UV, desiccation), Storage requires refrigeration</td>
</tr>
</tbody>
</table>

UV = ultraviolet.

The properties described pertain to a generalized view of each pathogen group; exceptions may exist in each category.

Although microsporidia are classified as fungi they are treated separately here.

Shapiro and McCoy, 2000a).
a higher host density) leads to increased host—pathogen contact. Thus, in susceptible hosts, it is assumed that a certain pathogen density is required to suppress the target pest below an EIL, and that lower densities will fail. There are many examples that demonstrate this dose—response relationship between pathogen and host in the laboratory (Lacey, 1997), e.g., using fungi (Hesketh et al., 2008; Wraight et al., 2010), nematodes (Power et al., 2009), or viruses (Figueiredo et al., 2009). Effects of pathogen application rate in field studies have been reported (Lacey and Kaya, 2007; Lacey and Shapiro-Ilan, 2008), e.g., using nematodes (McCoy et al., 2002; Arthurs et al., 2005; Chambers et al., 2010) or fungi (Wraight and Ramos, 2002), whereas in other studies the effects of field application rates varied, were not detected, or were not deemed important relevant to other factors (e.g., strain or species effect) (Cappaert and Koppenhöfer, 2003; Grewal et al., 2004; Dillon et al., 2007). Nonetheless, although specific pest requirements may differ, minimum baseline application rates have been established that are expected to result in pathogen population densities that produce pest suppression, e.g., \(10^{13} - 10^{14}\) conidia/ha for certain hypocrealean fungi (Wraight and Carruthers, 1999; Jaronski, 2010), \(10^{11} - 10^{12}\) occlusion bodies/ha for NPVs (e.g., when applied for control of various lepidopteran pests) (Vail et al., 1999), and 2.5 \(\times 10^9\) IJs/ha for entomopathogenic nematodes (Shapiro-Ilan et al., 2006).

At a minimum, a pathogen must persist in the environment long enough to infect the target host. A longer term persistence can have a positive impact on efficacy, e.g., by allowing the pathogen to persist when host population density is low. In some cases, superior environmental persistence may compensate for lower virulence (Shields et al., 1999; Shapiro-Ilan et al., 2002a). A variety of factors may limit pathogen persistence. As indicated in Section 3.2.4, all pathogen groups are susceptible to degradation by UV radiation (Fuxa, 1987). Thus, achieving persistence of above-ground entomopathogen applications is more challenging than in applications to the soil or other environments (e.g., greenhouses) that are more protected. Nonetheless, some microbial control agents such as Bt and certain viruses have been successful in suppressing pests above ground in the field (Lacey et al., 2001).

In addition to susceptibility to UV radiation, entomopathogenic nematodes are highly sensitive to desiccation (Kaya and Gaugler, 1993; Shapiro-Ilan et al., 2006) and fungal entomopathogens generally require high levels of relative humidity for germination. Yet, exceptions exist for the fungal requirement of high humidity (Wraight et al., 2007); e.g., low humidity was reported to be beneficial for control of the lesser grain borer, *Rhizopertha dominica*, with *B. bassiana* (Lord, 2005). Furthermore, high levels of moisture in the soil can be detrimental to fungi by enhancing environmental degradation, e.g., through increased antagonists in soil (Shapiro-Ilan et al., 2004a; Jaronski, 2007; Wraight et al., 2007) (see Section 3.2.4 for more details on environmental impact).

In nature and in field applications, persistence of entomopathogens in the soil tends to be greater than above ground. Some viruses such as NPVs can persist for several years or even decades in soil (Thompson et al., 1981; England et al., 1998). In contrast, the duration of pest control resulting from most entomopathogenic nematode applications is limited to two to eight weeks (Shapiro-Ilan et al., 2006). Persistence of efficacy, however, can depend on host density, e.g., multiseason persistence of entomopathogenic nematodes was observed for suppression of white grubs with high population densities (Klein and Georgis, 1992). A number of other factors can affect entomopathogen persistence in soil, including pH, texture, aeration, antagonists, temperature, and use of amendments (fertilizers or pesticides) (Lacey et al., 2001; Shapiro-Ilan et al., 2006; Meyling and Eilenberg, 2007).

### 3.3.3. Improving Efficacy in Microbial Control

Based on the factors affecting microbial control efficacy described above, a number of avenues may be considered for improving the use of entomopathogens. Approaches to improving microbial control efficacy may be divided into several categories: (1) improving the entomopathogen; (2) improving production and application methods; and (3) improving the environment.

**Improving the Entomopathogen**

Achieving or improving efficacy in microbial control can rely on choosing the best entomopathogen for a particular system. The most suitable entomopathogen from a variety of candidates can be selected simply by screening existing species and strains that possess superior desired traits such as virulence and environmental tolerance. New entomopathogens can be discovered through surveys and screened in parallel to existing strains; such surveys have been conducted extensively for entomopathogenic nematodes (Shapiro-Ilan et al., 2003a, 2008a; Campos-Herrera et al., 2008) and fungi (Leland et al., 2005; McGuire et al., 2005; Lubeck et al., 2008). The screening process is often accomplished by first narrowing down the number of candidates in laboratory comparisons; such comparisons have been made to find superior entomopathogen strains for numerous target pests such as the emerald ash borer, *Agrilus planipennis* (Castrillo et al., 2010), aphids (Shapiro-Ilan et al., 2008a), cowpea weevils, *Callosobruchus maculatus* (Cherry et al., 2005), plum curculio, *Conotrachelus nenuphar* (Shapiro-Ilan et al., 2008b), *Lygus* spp. (Liu et al., 2002; Leland et al., 2005),
**Epizootiology and Microbial Control**

The importance of verifying laboratory efficacy in the field cannot be overemphasized. An entomopathogen that shows high virulence in the controlled environment of a laboratory could fail to suppress the target pest in the field owing to various biotic or abiotic factors that render the organism incompatible. A lack of understanding of the biological and ecological constraints required for pathogen persistence and proliferation in the environment is likely to lead to a discrepancy between laboratory and field efficacy (Hu and St. Leger, 2002; Bruck, 2005, 2010). Some examples of laboratory screening studies that selected entomopathogen strains or species that later proved successful in the field include *S. riobrave* and *H. indica* for control of the citrus weevil, *Diapreps abbreviatus* (Duncan and McCoy, 1996; Shapiro et al., 1999a; Shapiro and McCoy, 2000b), *S. riobrave* for control of *C. nenuphar* (Shapiro-Ilan et al., 2002b, 2004b; Pereault et al., 2009), hypocrealean fungi for suppression of the brown citrus aphid, *Toxoptera citricida* (Poprawski et al., 1999), and *M. anisopliae* for control of *A. ludens* (Lezama-Gutiérrez et al., 2000).

In contrast, in some cases a high level of laboratory virulence or efficacy has not been corroborated under field conditions. For instance, *S. feltiae* was highly virulent to *C. nenuphar* in the laboratory, but failed to control the pest in Georgia peach orchards, possibly because of unsuitable soil temperatures (Shapiro-Ilan et al., 2004b). In another example, Leland et al. (2005) screened strains of *B. bassiana* isolated from *Lygus* spp. populations for *in vitro* conidia production, temperature growth optima, tolerance to UV radiation, and production of beauvericin and compared these to a commercial *B. bassiana* isolate (GHA). *Lygus* spp. isolates were orders of magnitude more virulent than the commercial isolate based on LC50 values. However, field-collected isolates that were superior to GHA in the laboratory (Leland et al., 2005) did not provide significantly higher levels of control of *L. hesperus* infesting alfalfa (McGuire et al., 2006). Indeed, strain selection based primarily on pathogenicity or mass production that ignores habitat preferences of the pathogen has often been unsuccessful (Hu and St. Leger, 2002; Bruck, 2005, 2010). A recent focus on pathogen ecology and habitat preferences when selecting strains for microbial control is expected to enhance the pathogen performance in the field and the frequency and magnitude of epizootics (Jaronski, 2007, 2010; Vega et al., 2009).

If existing or newly discovered entomopathogen strains or species cannot achieve desired levels of microbial control efficacy, another option is to improve selected candidates through genetic approaches. Genetic improvement is directed toward enhancement of single or various beneficial traits, e.g., virulence, reproductive capacity, or environmental tolerance. Approaches may include molecular or non-molecular methods. One non-molecular method entails selection for desired traits. Selection for improved virulence can be obtained by passing the pathogen through a susceptible host (Steinhaus, 1949; Daoust and Roberts, 1982). Some examples of genetic selection for other traits include improvements in entomopathogenic nematode host-finding (Gaugler et al., 1989a) and nematicide resistance (Glazer et al., 1997). Directed selection can, however, have the shortcoming of inadvertently selecting for an inferior level of one trait while selecting for the targeted trait (Gaugler, 1987). For example, Gaugler et al. (1990) reported a loss in storage capacity in entomopathogenic nematodes that had been selected for improved host finding. In addition, *B. bassiana* and *M. brunneum* selected for fungicide resistance exhibited tradeoffs with other traits, e.g., reproductive capacity (Shapiro-Ilan et al., 2011).

Another non-molecular approach to strain improvement is hybridization, e.g., the transfer of beneficial traits from one strain to another. Examples of hybridization for improved biocontrol include the development of superior environmental tolerance and or virulence in entomopathogenic nematodes (Shapiro et al., 1997; Shapiro-Ilan et al., 2005c) and improved virulence in protoplast fusion hybrids of *B. bassiana* (Couteaudier et al., 1996). The two non-molecular approaches (selection and hybridization) have also been combined for the development of superior entomopathogenic nematode strains (Mukaka et al., 2010).

Substantial progress has been made in using transgenic or other molecular approaches for improving microbial control agents. Thus, transgenic approaches have been used to increase the virulence in NPVs, e.g., through the addition of scorpion toxin (Stewart et al., 1991; Harrison and Bonning, 2000) or insect hormones (Maeda, 1989; Chen et al., 2000). *Beauveria bassiana* has been transformed for benomyl resistance (Sandhu et al., 2001), and *H. bacteriophora* has been transformed for increased heat tolerance (Gaugler et al., 1997). *Metarhizium anisopliae* has been genetically engineered to express the 70 amino acid *Androctonus australis* neurotoxin AaIT, a toxic insect-selective peptide (Zlotkin et al., 2000), resulting in increased virulence against *M. sexta* larvae and *Aedes aegypti* adults (Wang and St. Leger, 2007). Genetic modification for improvement of natural Bt strains has been examined (Sansinena et al., 2010), and as an extension of microbial control research on Bt, various crops have been genetically modified with Bt toxins, resulting in widespread implementation (Tabashnik et al., 2003, 2008).

One issue that can jeopardize strains with beneficial traits is repeated subculturing, resulting in attenuation due to genetic factors (e.g., inbreeding, drift, inadvertent selection) or non-genetic factors (e.g., disease) (Tanada and Kaya, 1993; Hopper et al., 1993; Chaston et al., 2011).
Serial in vitro transfer of *N. rileyi* quickly caused attenuation and reduced virulence to *A. gemmatalis* (Morrow et al., 1989). Trait deterioration has also been observed during laboratory culturing of entomopathogenic nematodes (Shapiro et al., 1996; Wang and Grewal, 2002; Bilgrami et al., 2006). Commercial manufacturers of entomopathogens pay special attention to ensuring that repeated subculturing is avoided, to reduce the likelihood of attenuation of the microbial control agents that they produce. Nonetheless, even with these efforts detrimental trait changes have been observed; e.g., Bt from lepidopteran cadavers produced by DiPel (a commercial Bt formulation) infection was significantly less infective than Bt from cadavers produced by a strain recently isolated from the field, suggesting that the wild-type strain was more efficient at producing spores in the host (Naryanan, 2006). For some organisms (e.g., entomopathogenic nematodes), trait loss can be reduced through the development of selected inbred lines (Bai et al., 2005; Chaston et al., 2011).

The effectiveness of entomopathogen species or strain improvement for implementation in microbial control may also be hindered by the extremely high cost of commercial development and registration. For example, registration of new fungal entomopathogen strains requires the generation of human and environmental safety data, and in some countries, replicated verification of efficacy is also needed; a process that can require approximately 1–1.5 million US dollars (Jaronski, 2010). Registration of modified organisms can be even more costly. Thus, in many cases, private sector development of a new entomopathogen may only be considered if improvements in virulence, production efficiency or other attributes in the new organism are extreme relative to the existing products. Therefore, screening processes should generally include strains and species that are already commercially registered and available. The cost of developing new entomopathogen strains or species, however, is less for some entomopathogen groups than for others, e.g., in the USA endemic entomopathogenic nematodes are not regulated.

**Improving Production and Application Methods**

Improvements in entomopathogen production methods can lead to improved quality, improved fitness, and reduced costs. Lower costs of production can allow for increased application rates and cost competitiveness with other pest management strategies. In vivo production of entomopathogenic nematodes or viruses can be accomplished through enhanced mechanization, thereby reducing labor requirements (Gaugler et al., 2002; Shapiro-Ilan et al., 2002c), or through improved host diets, resulting in lower costs or improved pathogen quality (Shapiro-Ilan et al., 2008c; Elvira et al., 2010).

In vitro solid and liquid culture can be enhanced for entomopathogen production. Similar to in vivo approaches, solid fermentation of entomopathogenic nematodes can be enhanced through improved host nutrition and process automation (Gaugler and Han, 2002). Liquid production of entomopathogenic nematodes has been substantially improved through superior media development and elucidation of bioreactor conditions needed for optimum recovery and fecundity (Shapiro-Ilan and Gaugler, 2002; Ehlers and Shapiro-Ilan, 2005). In vitro approaches to fungal production can also be improved through media enhancement (Gao and Liu, 2010; Jaronski, 2010). Bt has been produced in submerged culture for over 40 years (Couch and Ross, 1980). One of the factors that has played a role in making Bt the most successful microbial control agent for insect pests (Lacey et al., 2001) is the efficient and relatively inexpensive production methods that have been developed. Nonetheless, research on improving Bt production media continues, e.g., using waste materials instead of raw materials (Brar et al., 2009; Zhuang et al., 2011).

Application and delivery methods offer another opportunity to improve microbial control efficacy. Most entomopathogens can be applied using common agricultural equipment including various spray and irrigation systems. Standard application techniques and equipment for microbial control agents are reviewed in Lacey and Kaya (2007) and will not be covered in this chapter. Despite well-established procedures, equipment used for entomopathogen application can be improved, such as optimizing spray systems for enhanced pathogen survival and dispersion (Fife et al., 2006; Shapiro-Ilan et al., 2006). Other application parameters can also be further optimized for many host-pathogen systems, e.g., rate and timing of application.

In addition to optimization of application equipment or parameters, improved application techniques can be sought. For example, application of entomopathogenic nematodes in nematode-killed hosts has been considered (Jansson et al., 1993; Shapiro and Glazer, 1996; Dolinski and Lacey, 2007). Advantages to the cadaver application approach relative to standard application in aqueous suspension have been reported, including superior nematode dispersal (Shapiro and Glazer, 1996), infectivity (Shapiro and Lewis, 1999), survival (Perez et al., 2003), and efficacy (Shapiro-Ilan et al., 2003b), whereas other studies did not detect a benefit in the cadaver approach (Bruck et al., 2005). Methods to facilitate application of cadavers through formulation have been developed to protect cadavers from rupture and improving ease of handling (Fig. 3.3) (Shapiro-Ilan et al., 2001, 2010a; Del Valle et al., 2009). Yet commercial application of host cadavers with nematodes has been minimal, possibly because of costs and a need to develop mass-application
methods. Recently, nematodes applied as within the host cadavers were demonstrated to be effective and persistent when added to bags of potting media for subsequent distribution to target pest sites (Deol et al., 2011).

Another application approach that may offer advantages in microbial control is autodissemination, i.e., the use of insects as natural dispersal organisms to spread pathogens (Vega et al., 2007). This approach can be enhanced with a device that promotes contact with the pathogen (Vega et al., 1995, 2007). In one example of leveraging movement of a non-host, the bumble bee, Bombus impatiens, was used to carry B. bassiana for control of the greenhouse whitefly, Trialeurodes vaporariorum (Kapongo et al., 2008). Autodissemination has been used against a variety of pests, including G. morstians (Kaaya and Okech, 1990; Maniania, 1998, 2002), the Japanese beetle, Popillia japonica (Klein and Lacey, 1999), B. germanica (Kaakeh et al., 1996), house flies, Musca domestica (Renn et al., 1999), the spruce bark beetle, Ips typographus (Kreutz et al., 2004), and P. xylostella (Furlong and Pell, 2001). In other novel approaches to using fungal entomopathogens, endophytic relationships with the host plant or colonization of the rhizosphere might be utilized to develop low-cost microbial control strategies (Vega et al., 2008; Bruck, 2010).

Successful microbial control applications can be facilitated through improved formulation. Research on improved entomopathogen formulations is directed toward ease of handling and enhanced persistence in the environment. Significant work has been undertaken to mitigate the effect of UV radiation on entomopathogens via improved formulation of microbial control products (Burges, 1998; Jackson et al., 2010). Recent advances in formulation of entomopathogenic nematodes that have facilitated above-ground use, a major barrier to expanding use of this pathogen group, using mixtures with a surfactant and polymer (Schroer and Ehlers, 2005), postapplication protective covers using foam (Lacey et al., 2010), and a sprayable gel thought to provide resistance to UV radiation and desiccation (Shapiro-Ilan et al., 2010b). UV radiation-protecting formulations have also been developed for fungal entomopathogens (Behle et al., 2011), and optical brighteners have been demonstrated to protect entomopathogenic viruses from UV radiation (Shapiro, 1992). Protective formulations have also substantially improved the persistence of Bt (Garczynski and Siegel, 2007). Bait formulations can enhance entomopathogen persistence and reduce the quantity of microbial agents required per unit area; e.g., baits have been developed for entomopathogenic nematodes (Grewal, 2002), fungi (Geden and Steinkraus, 2003), and Bt (Navon et al., 1997), yet thus far, the market impact of baits has not been substantial relative to other formulations.

Another approach to improving application methodology and achieving higher levels of microbial control efficacy is to combine entomopathogens with each other or with other biotic agents. Although combinations of entomopathogens can result in synergistic levels of mortality, interactions may also be antagonistic or additive (Kreig, 1971; Koppenhöfer and Grewal, 2005). The nature of the interaction depends on several factors, including the specific combination, the host species, and the timing and rate of application (Kreig, 1971; Koppenhöfer and Grewal, 2005). Some examples of entomopathogen combinations that have been reported to be synergistic include Bt combined with entomopathogenic nematodes for scarab grub control (Koppenhöfer and Kayya, 1997), and entomopathogenic nematodes combined with M. anisopliae against the white grub Hoplia philanthus (Ansari et al., 2006). Examples of antagonism among entomopathogens include the microsporidium V. necatrix combined with Heliothis NPV (except at very high concentrations of the microsporidium) (Fuxa, 1979), combinations of Bt and Anagropa haf falcifera multiple NPV against three lepidopteran corn pests (H. zea, O. nubilalis, Spodoptera frugiperda) (Pingel and Lewis, 1999), and the bacterium S. marcescens combined with entomopathogenic nematodes when targeting the pecan weevil, Curculio caryae (Shapiro-Ilan et al., 2004c).

![Image](image_url)
Entomopathogens may also be combined with chemical agents to enhance microbial control efficacy (Benz, 1971). Similar to combination with biotic agents, combination with abiotic agents can vary and result in synergy, additivity, or antagonism, and the nature of the interaction can depend on various factors including application parameters such as timing and rate (Benz, 1971; Koppenhöfer and Grewal, 2005). Positive interactions include the addition of optical brighteners to NPVs. The brighteners not only provide UV protection (as mentioned above), but also have been shown to enhance the virulence of NPV (Shapiro and Argauer, 1995; Shapiro, 2000; Boughton et al., 2001). The combination of NPV and the neem compound azadirachtin reduced time to death of Spodoptera litura larvae (Nathan and Kalaivani, 2005) most likely owing to synergistic effect on gut enzymic activity (Nathan et al., 2005). Foliar applications of the chemical insecticide carbofuran to corn plants treated with B. bassiana reduced tunneling of O. nubilalis (Lewis et al., 1996), and the LC50 value for N. pyrausta-infected O. nubilalis larvae fed Bt was significantly lower than for uninfected larvae (Pierce et al., 2001).

The impact on efficacy varies depending on the specific chemical agent and target pest (Benz, 1971; Koppenhöfer and Grewal, 2005). Granulovirus infection of S. litura was synergistic with chlorpyrifos, additive with fenvalerate and endosulfan, and antagonistic with carbaryl hydrochloride (Subramanian et al., 2005). Imidacloprid was reported to be synergistic with nematodes used against white grubs (Koppenhöfer and Kaya, 1998; Koppenhöfer et al., 2000) or with B. bassiana against D. abbreviatus (Quintela and McCoy, 1998), but antagonistic when combined with B. bassiana against B. argentifolii (James and Elzen, 2001).

Entomopathogen efficacy may also be enhanced through combination with physical agents. For example, a synergist effect of diatomaceous earth combined with B. bassiana has been observed with a number of coleopteran stored grain pests (Lord, 2001; Akbar et al., 2004; Athanassiou and Steenberg, 2007) as well as with the microsporidium T. solenopsae infecting the red imported fire ant (Brinkman and Gardner, 2001). While the exact details of the interaction between diatomaceous earth and pathogens are currently unclear, it appears to involve a combination of increased availability of water and other nutrients, removal or mitigation of inhibitory materials, alteration of adhesive properties, and physical disruption of the cuticular barrier (Akbar et al., 2004).

**Improving the Environment**

Manipulation of the environment at the target site can increase microbial control efficacy through a variety of mechanisms such as decreasing exposure to harmful biotic or abiotic factors or enhancing entomopathogen reproduction, virulence, and exposure to the host. Thus, the persistence of efficacy of entomopathogenic nematodes can be enhanced through the addition of soil amendments such as mulch or crop residues (Shapiro et al., 1999b; Lacey et al., 2006b). Addition of compost also enhanced the persistence of B. bassiana (Rosin et al., 1996, 1997).

Various cultural practices can also affect entomopathogen efficacy within a cropping system. Tillage or the movement of cattle can enhance NPV efficacy by increasing the amount of virus on the host plant (Fuxa, 1987). Narrowing soybean rows to increase relative humidity enhanced the activity of N. rileyi (Spenkel et al., 1979). Gaugler et al. (1989b) observed enhanced persistence of B. bassiana in soil that was tilled versus untilled. In contrast, detrimental effects of tillage on the efficacy or persistence of hypocrealean fungi, such as B. bassiana, have been observed in other studies (Sosa-Gomez and Moscardi, 1994; Hummel et al., 2002; Shapiro-Ilan et al., 2008d). These discrepancies emphasize the need to test environmental manipulation approaches in a variety of cropping systems as their impact can vary based on biotic and abiotic factors.

### 3.3.4. Approaches to Microbial Control

Similar to biological control approaches that are defined for the use of insect predators and parasitoids (and as categorized in Chapter 1), microbial control can be classified into four approaches: classical, inoculation, inundation, and conservation. In the classical approach (also termed “introduction and establishment” approach), entomopathogens are released into areas where they do not occur naturally in an attempt to control a targeted pest (or conceivably, a complex of pests). The target pest can be endemic or exotic to the area; if it is the latter, the introduced entomopathogen might ideally be from the putative center of origin of the invasive pest. The ultimate goal is the establishment of the pathogen or pathogens for the total or partial suppression of the pest on a long-term basis. Optimally, introduction and establishment of exotic entomopathogens result in the reduction of the pest below the EIL. The pathogen should have a narrow host range, preferably specific to the pest, and have little or no impact on beneficial organisms. Several entomopathogens have been used as classical biological control agents, including viruses, bacteria, fungi, and nematodes. Classical biological control can also include exotic pathogens that have been accidentally introduced or are pathogens of unknown origin.

Unlike classical microbial control, pathogens that are released using the inoculation or inundation approaches are not expected to become permanently established. In inoculative microbial control, some recycling of the entomopathogen is expected; thus, seasonal or in some cases multiyear pest suppression may occur before reapplication is required. The inundative approach can be likened to a “pesticidal” approach; it is intended for short-term pest
suppression and little or no recycling is expected. This approach may require repeated applications depending on the number of generations of the targeted insect and the duration of the stages that are injurious.

Insect pathogens are ubiquitous in nature, but endemic pathogens are frequently insufficient to keep pests below the EIL. Many may cause epizootics in pest populations, but often when the host population density is high and after the EIL has been surpassed. However, there are also occurrences of natural epizootics that hold pests in check, allowing the delay or avoidance of pesticide applications (Steinkraus, 2007a). Conservation microbial control relies on conserving or enhancing the activity of entomopathogens that occur naturally in the pest’s habitat. In this approach, pathogen species are not added directly to the system. Rather, they are conserved or enhanced through agricultural and environmental practices that favor their survival or efficacy. Such practices could include selective timing or application of chemical pesticides (e.g., proper timing or reduction of pesticides that are inimical to the pathogen), or environmental modification, such as increased irrigation to provide moisture for some pathogens (e.g., fungi, entomopathogenic nematodes), or reduced use of conventional plowing. Under optimal environmental conditions, many entomopathogens have the natural ability to cause disease at epizootic levels. Several examples of conservation of naturally occurring entomopathogens are presented by Steinkraus (2007a), Cory and Evans (2007), and Elkinton and Burand (2007).

Case Studies: Classical Biological Control

One of the most successful case studies on the introduction and establishment of a microbial control agent is the virus of the coconut rhinoceros beetle, Oryctes rhinoceros (Huger, 2005). In 1966, Huger described a non-ocluded virus of O. rhinoceros from Malaysia that demonstrated potential for long-term control of the beetle, a serious pest of oil and coconut palm (Huger, 1966, 2005). The beetles are infected through oral contact with the virus and subsequently serve as reservoirs and disseminators. Although there are no external symptoms of the disease in adults, the virus shortens the insect’s lifespan and reduces fecundity (Zelazny, 1973). Transmission to larvae occurs when virus-infected females defecate in breeding sites during oviposition (Zelazny, 1972, 1973). Infection of larvae is always lethal (Zelazny, 1972). Pheromone lures and other methods have been used to capture, infect, and release beetles to disseminate the virus further (Lomer, 1986; Young, 1986). Introduction of the virus in conjunction with cultural practices such as the removal of larval habitats (e.g., rotting palm logs and the like) has significantly reduced O. rhinoceros populations (Huger, 2005). One of the key factors responsible for success of the virus is the persistence of the virus in adult and larval habitats. Adults also serve as a reservoir of the virus. However, a ban on burning of palm logs and the recent invasion of other islands have resulted in the resurgence of the beetle in some locations (Jackson et al., 2005).

The European spruce sawfly, Diprinon hercyniae, is an invasive pest of spruce in North America. An NPV found infecting D. hercyniae was accidentally introduced from Europe into Canada (Bird, 1955). When the NPV appeared in New Brunswick in 1938, disease spread throughout most of the area infested by D. hercyniae and by 1942 it was considered to be the major factor in the collapse of the outbreak (Bird and Elgee, 1957). Natural epizootics of the NPV have since kept populations of the sawfly under control in most locations. The virus was also introduced into a moderately infested area, near Sault Ste. Marie, where it became established and spread rapidly (Bird and Burk, 1961). Virus epizootics recurred each year and have prevented excessive increases in sawfly populations.

Another example of long-term control produced by an introduced viral pathogen is that of the NPV of the European pine sawfly, Neodiprinon sertifer, which is an exotic pest of various pine varieties. The virus was isolated from N. sertifer in Canada by Bird and Whalen (1953). Isolates of the virus were also introduced from Sweden (Bird, 1953). Several field trials using aerial and mist blower applications of the imported virus produced mortalities exceeding 90%. Bird (1953) concluded that without treatment, defoliation would have been almost complete. These and other small-scale applications produced epizootics in N. sertifer populations that resulted in suppression of the pest below the EIL (Bird, 1950, 1953, 1955).

Introductions of fungal entomopathogens and microsporidia for classical biological control outnumber those of other entomopathogens. Among 136 programs using different groups of arthropod pathogens, 49% have introduced fungal pathogens (Hajek and Delalibera, 2010). The introduction of the fungus Entomophaga maimaiga for control of L. dispar is one of the most successful. Lymantria dispar was accidentally introduced into the north-east USA from Europe in the late 1860s and has steadily spread into other areas (Liebold et al., 1993). Although the fungal pathogen (concluded to be E. maimaiga) was obtained from gypsy moth in Japan and released at several locations in the Boston area in 1910–1911, no transmission was detected (Hajek, 1999). The first reports of epizootics caused by the fungus were published by Andreedis and Weseloh (1990) and Hajek et al. (1990). Since then, E. maimaiga has been observed in the north-eastern USA, producing dramatic epizootics in L. dispar (Elkinton and Burand, 2007). Entomophaga maimaiga is expanding its geographical range naturally but also with human assistance (Elkinton et al., 1991; Hajek et al., 1995). Smitley et al. (1995) reported on the distributions of resting spores of the fungus in Michigan; fungus-infected larvae were observed two
years after inoculation. Epizootics in *L. dispar* induced by *E. mainaiga* resulted in up to 99% mortality and were documented at the same sites three years after inoculation. Resting spores survive under the bark of trees and surrounding soil where epizootics have occurred and are capable of producing disease in *L. dispar* for several years (Hajek et al., 1998).

The *Sirex* woodwasp, *Sirex noctilio*, was accidentally introduced from Europe into New Zealand pine plantations (*Pinus* spp.). It is now one of the most important pests of pine in the southern hemisphere and threatens pine production on eight million ha in Australia, New Zealand, South America, and South Africa (Bedding and Iede, 2005; Carnegie et al., 2005). In addition to damage due to feeding by larval *S. noctilio*, the ovipositing female introduces a tree pathogenic fungus, *Amylostereum areolatum*, which can result in death of the tree. It is the most important insect pest of *Pinus radiata* in Australia. Consequently, the first concerted use of biological control agents (parasitoids and nematodes) in an integrated program for control of the wasp was in Australia. The entomogenous nematode, *Deladenus* (= *Beddingia*) *siricidicola*, was imported from Hungary and, after preliminary trials in northern Tasmania, introduced into the state of Victoria in the early 1970s (Bedding and Akhurst, 1974). The nematode has two separate life cycles, one parasitic and the other free living (Bedding, 1967, 1972). Parasitism by the nematode sterilizes female wasps. Rearing of the infective stage of *D. siricidicola* on *A. areolatum* cultures enabled large-scale application to infested plantations (Bedding and Iede, 2005). Successful dispersal of the nematode to new sites was facilitated by infected females wasps ovipositing eggs filled with up to 200 juvenile nematodes (Bedding, 1972). The initial success of the program provided up to 100% infection in *S. noctilio* and a decline in wasp populations and tree damage in treated plantations (Bedding and Iede, 2005). However, subsequent outbreaks of the wasp in Australian pine plantations where the nematode had not been introduced resulted in severe tree damage (Haugen and Underdown, 1990). Subsequent introductions of *D. siricidicola* provided effective control of the wasp (Haugen and Underdown, 1993; Bedding and Iede, 2005; Hurley et al., 2008). Despite the variable successes, overall *D. siricidicola* is still regarded as the most important means of controlling *S. noctilio* (Bedding and Iede, 2005). In the absence of control agents, principally *D. siricidicola*, the wasp has the potential to cause 16—60 million US dollars of damage each year (Bedding and Iede, 2005).

Introduction and establishment of *S. scapterisci* in populations of invasive mole crickets, *Scepteriscus* spp., were reported by Hudson et al. (1988). The exotic *Scepteriscus* spp., a serious pest of lawn and turf, arrived in Florida from South America. The nematode was collected in Uruguay and introduced into Florida in small plot field tests (Hudson et al., 1988). After demonstrating effective control and persistence, it was introduced into several locations around the state. *Scepteriscus scapterisci* has become established in most of these locations and is dispersing from sites where applications were made (Parkman et al., 1993, 1996).

**Case Studies: Inoculation**

Klein (1992) and Klein et al. (2007) reported on the inoculation of *Paenibacillus* spp. into turf and lawn habitats. Inoculation produced localized epizootics and often resulted in persistence of the pathogen with periodic outbreaks of disease. The most widely used species is *P. popilliae* for control of *P. japonica* (Klein, 1992). The bacterium is an obligate pathogen of the beetle and must be ingested and subsequently gain access to the hemocoel. Mortality is due to bacteremia rather than toxemia (Garczynski and Siegel, 2007). Feeding or injecting spores into larvae is necessary for production of the spores that will be used for inoculative application. Production on artificial media has consistently failed to produce spores. *Paenibacillus popilliae* spores plus a carrier, such as talc, are typically applied to the surface of lawns (Klein et al., 2007). Infected larvae turn white, a characteristic sign of the disease referred to as milky disease or milky spore. The spores may persist for years, and at unpredictable intervals cause epizootics. Naturally occurring epizootics of milky disease have been reported (Klein, 1992), as has failure of the bacterium to control *P. japonica* (Redmond and Potter, 1995).

Klein and Georgis (1992) demonstrated that applications of *S. carpocapsae* and *H. bacteriophora* for control of *P. japonica* produced an inoculative effect and resulted in 51% and 60% mortality, respectively, a month following application, and 90% and 96% mortality, respectively, the following spring. *Heterorhabditis bacteriophora*, but not *S. carpocapsae*, continued to control *P. japonica* larvae until the following autumn, resulting in up to 99% mortality. Their findings document reproduction of the nematodes in *P. japonica* larvae, survival in the field, and continuation of control for a longer period than previously demonstrated for inundative control of scarabs. For entomopathogenic
nematodes, this level of recycling is an exception and may have been due to the high population density of *P. japonica* and favorable environmental conditions.

**Case Studies: Inundation**

*Bacillus thuringiensis* is by far the most widely used inundative microbial control agent for control of insect pests of annual and perennial crops, forests, and pests of humans and domestic animals (Beegle and Yamamoto, 1992; Gelernter and Lomer, 2000; Lacey et al., 2001). A multitude of case histories is present in the literature (Beegle and Yamamoto, 1992). As with viruses, the active moiety of the bacterium, the so-called delta endotoxins, must be ingested in order to be larvicidal. Various toxins have been isolated from the delta-endotoxin, each of which has specific activity for a certain groups of insects (Garczynski and Siegel, 2007; Crickmore et al., 2011). *Bacillus thuringiensis* subspecies *kurstaki* (Btk) has been commercially produced for several decades for control of lepidopteran pests (Beegle and Yamamoto, 1992). Typically, it is applied to pest populations using conventional ground and aircraft spray equipment (Hall and Menn, 1999). In general, the bacteria are applied at regular intervals owing to rapid degradation due to UV radiation. The host range and larvicidal activity of several varieties and toxins are presented by Garczynski and Siegel (2007) and Crickmore et al. (2011). In addition to UV radiation, a potential limiting factor is the development of resistance. Resistance to Btk by lepidopterans was reviewed by Shelton et al. (2007), who also present strategies to manage resistance to Bt toxins in various orders of insects.

The host range of *B. thuringiensis* subspecies *tenebrionis* (Btt) is considerably narrower than that of other commercially produced Bt subspecies. It was discovered in Germany by Langenbruch et al. (1985) and has been used principally against the Colorado potato beetle, *Leptinotarsa decemlineata*, in potato-growing regions worldwide. However, with the advent of a number of new pesticide chemistries for *L. decemlineata* control, commercial success of Btt has lagged considerably behind that of other Bt subspecies (Gelernter and Lomer, 2000).

After its discovery in Israel (Goldberg and Margalit, 1977), *B. thuringiensis* subspecies *israelensis* (Bti) was rapidly developed by the biopesticide industry (Margalit and Dean, 1985). Larval control of a multitude of mosquito species using Bti has been demonstrated worldwide (Lacey and Undeen, 1986; Lacey, 2007). Bti is applied using a variety of conventional aerial and ground spray equipment and formulations. Flowable concentrates, granules and slow-release formulations are used to target mosquito habitats. Certain mosquitoes such as *Anopheles* spp. are particularly difficult to target because they are water surface feeders and most Bti formulations rapidly sink from their feeding zone. The most economical and efficacious means of application of Bti to large mosquito habitats uses the Beecomist® ultralow volume (ULV) sprayer by air (Sandoski et al., 1985). The 80 μm droplets generated by the ULV sprayer tend to float on the water surface and within the feeding zone of *Anopheles* spp.

Bti is also used for effective control of black fly larvae (Molloy, 1982; Lacey and Undeen, 1986; Merritt et al., 1989). Its use in the Onchocerciasis Control Program in West Africa helped to control populations of *Simulium damnosum* that had become resistant to organophosphate and carbamate insecticides. Rapid development to insecticide resistance in non-resistant black fly populations was prevented by alternating Bti with chemical larvicides (Guillet et al., 1990).

Another bacterium, *B. sphaericus*, has also been used for control of mosquito larvae, principally in organically enriched habitats (Lacey, 2007). Its larvicidal activity in these habitats is significantly prolonged relative to that of Bti. It is only effective for control of mosquitoes and, relative to Bti, it has a considerably narrower range of activity, primarily in the Culicidae. For example, it is very effective against *Culex quinquefasciatus*, an important vector of the filaroid nematodes that cause elephantiasis, but inactive against *Ae. aegypti*, the vector of dengue and yellow fever viruses. Another potential caveat to its use as an exclusive larvicide over longer periods is the development of extremely high levels of resistance (Rao et al., 1995; Nielsen-LeRoux et al., 2002; Mulla et al., 2003).

Control of codling moth, *Cydia pomonella*, with its host-specific granulovirus (CpGV) is an excellent example of inundative control using an entomopathogenic virus (Fig. 3.4A). CpGV is one of the most widely used and successful baculoviruses. It is commercially produced in Europe and North America and used by both organic and conventional orchardists. Several locations where CpGV has been used worldwide are cited by Lacey et al. (2008a). Although CpGV can be used as a standalone means of control it must be applied at seven- to 14-day intervals to provide effective suppression, especially if there are multiple generations of the moth each year (Arthurs and Lacey, 2004; Arthurs et al., 2005). The principal limiting factor of CpGV is UV radiation (Lacey et al., 2008a). Another severely limiting factor is the development of CpGV resistance in some populations in Western Europe, where it has been used for 20 years or more as the principal means of *C. pomonella* control (Eberle and Jehle, 2006). An integrated approach where CpGV is used in conjunction with other means of control could forestall development of resistance and provide a broader range of control for *C. pomonella* and other orchard pest insects (Lacey and Shapiro-Ilan, 2008).

A uniquely successful inundative program using an NPV for control of *A. gemmatalis* in soybean is described by Moscardi (1999, 2007). Moscardi and colleagues
discovered, developed, and implemented the virus in a large-scale control program. At present, the NPV is used on approximately two million ha of soybeans in Brazil, representing the largest program worldwide for the use of an entomopathogen to control a pest in a single crop. Following the development of an efficient means of in vivo production (Moscardi et al., 1997), farmer cooperatives were instructed on the methods for virus production and began producing the virus for use on their crops (Moscardi, 2007). Several other entomopathogenic GVs and NPVs are used for inundative control of lepidopterous pests (Hunter-Fujita et al. 1998; Moscardi, 1999; van Frankenhuysen et al., 2007). Some NPVs also provide inundative control of a limited number of hymenopteran forest pests (Moreau and Lucarotti, 2007).

Unlike virus and bacteria, fungi gain entry to the host through the integument (Hajek and St. Leger, 1994). This makes them especially valuable as microbial control agents for insects with piercing and sucking mouthparts such as aphids, whiteflies, psyllids, and other hemipterans (Wraight et al., 2007, 2009; Goettel et al., 2010). Many fungal species in the Hypocreales are pathogenic to a broad range of insect pests and are the main contenders among fungi for inundative control and commercial development. Of these, B. bassiana, Isaria fumosorosea, and Metarhizium spp. are the most widely used for insect and mite control (Alves, 1998; Goettel et al., 2010). Although less commonly used, Lecanicillium spp. have been developed as effective means of inundative control for insect pests such as aphids and whiteflies in greenhouse crops.

A multitude of case studies of Hypocreales species used as inundatively applied microbial control agents is found throughout the literature. Field and greenhouse applications of Hypocreales are reported for control of whiteflies, aphids, psyllids, thrips, beetles, and other insect pests (Burges, 2007; Goettel et al., 2010; Lacey et al., 2011). For instance, successful control of B. tabaci and B. argentifolii with B. bassiana has been achieved in various places in the USA (Wraight et al., 2000; Faria and Wraight, 2001; Lacey et al., 2008b). One of the most successful large-scale inundative microbial control efforts using a fungus is the LUBILOSA program in West Africa (Moore, 2008) (see Chapter 6). Aerial application of an oil-based formulation of M. acridum (as M. flavoviride) provided effective control of locusts and grasshoppers despite hot, dry conditions (Lomer et al., 1997, 1999; Bateman, 2004).

The predominant use of entomopathogenic nematodes (Steinernema and Heterorhabditis spp.) in microbial control is via inundation (Grewal et al., 2005a; Georgis et al., 2006). The inundative approach is taken as the nematodes usually do not recycle at levels that provide continual host suppression. Exceptions to this are presented above in the “Case Studies: Inoculation” and “Case Studies: Classical Biological Control” sections of this chapter. The nematodes are most effective in soil and cryptic habitats where the infective stage of the nematode will not desiccate before penetrating a host insect. One well-documented example of successful inundative application is the use of S. riobrave for control of D. abbreviatus (McCoy et al., 2002, 2007; Stuart et al., 2008), with high levels of mortality (e.g., 90% or greater) (Shapiro-Ilan et al., 2002a, 2005b). The use of irrigation systems or herbicide boom sprayers for application of S. riobrave has
been effective in delivering IJs into the zone below trees where larvae enter the soil. Other entomopathogenic nematode species attacking *D. abbreviatus*, with rates and percentage mortality, were summarized by Shapiro-Ilan *et al.* (2002a). Examples of nematode evaluation and efficacy in lawns and turf are presented by Klein and Georgis (1992), Grewal *et al.* (2005b), and Klein *et al.* (2007), and against several pest insects in orchard habitats (Fig. 3.4B) by Shapiro-Ilan *et al.* (2005b), Lacey *et al.* (2006a), and Lacey and Shapiro-Ilan (2008). Numerous other examples of insect control using entomopathogenic nematodes are provided by Grewal *et al.* (2005a) and Georgis *et al.* (2006).

**Case Studies: Conservation**

One of the most notable cases of conservation microbial control is the delay in insecticide application in order to promote epizootics caused by *Neosyzygites fresenii* in populations of the cotton aphid, *Aphis gossypii* (Steinkraus, 2007b; Abney *et al.*, 2008). Abney *et al.* (2008) showed that fungal epizootics caused by *N. fresenii* reduced aphid numbers below the EIL in 1999, 2000, and 2001, and occurred consistently in early to mid-July in their three-year study. The key factor in the success of this program is the prediction of epizootics caused by the fungus, and knowing when to advise cotton farmers to delay application (Hollingsworth *et al.*, 1995; Steinkraus *et al.*, 1996). The program was so successful that it expanded to include Louisiana and Mississippi (Steinkraus *et al.*, 1998).

Another example of the effect that agricultural practices can have on entomopathogen survival is presented by Hummel *et al.* (2002). Two tillage types (conventional plow and disk versus conservation strip tillage), two input approaches (chemical versus biologically based), and two cropping schedules (continuous versus rotation) were compared. A bait-trap bioassay (using *Galleria mellonella*) was used to monitor the abundance of *S. carpocapsae*, *H. bacteriophora*, *B. bassiana*, and *M. anisopliae* populations. Entomopathogens were significantly higher in conservation compared with conventional tillage systems. Pesticide use significantly reduced the detection of fungal entomopathogens. Ground cover (rye mulch and clover intercrop) that resulted in lower temperatures positively affected the abundance of *S. carpocapsae*. Hummel *et al.* (2002) concluded that although the type of tillage was the key factor affecting the abundance of entomopathogens, its benefit could be negated by pesticide use and ground cover that resulted in high temperatures.

### 3.4. FUTURE RESEARCH DIRECTIONS

Although substantial progress has been made in the discipline of epizootiology, additional advances and broader integration of epizootiology into other components of invertebrate pathology are needed. More research is needed in all aspects of epizootiology, including factors that impact epizootics and epizootiological modeling. Owing to the complex nature of epizootiology, multidisciplinary research is likely to be particularly fruitful. Towards this end, collaboration among specialists from various fields, including pathology, microbiology, physiology, and ecology (quantitative, behavioral, etc.), is to be encouraged. Incorporation of molecular tools in epizootiological studies related to microbial control will allow disease prevalence or history and pathogen movement to be tracked, and gene flow among pathogens and host populations to be studied. Furthermore, the discipline of epizootiology can be advanced through integration and emphasis in graduate and undergraduate curricula; epizootiology can be taught as a standalone course or incorporated into existing courses such as insect pathology, entomology, biological control, general biology, zoology, and ecology. Expansion and improved understanding in epizootiology will lead to enhanced microbial control efforts.

As illustrated in this chapter, there have been numerous successes in microbial control, yet there have also been many failures. The definition of success is debatable. For the purposes of this discussion, the criterion for success in microbial control can be considered commercial application of the entomopathogen as a suppressive agent on a significant scale. The key elements for achieving success can be boiled down to two factors: appropriate match of the entomopathogen to the target pest, and cost competitiveness (Shapiro-Ilan *et al.*, 2002a). To constitute an appropriate match, the entomopathogen must possess sufficient virulence, infection, dispersal capabilities, environmental tolerance, niche overlap (with the target), and other traits that facilitate an acceptable level of control. Although some attributes of the host—pathogen match can be estimated in the laboratory, the bottom line for determining an appropriate match is establishment of efficacy in the pest’s habitat. Regardless of the level of efficacy achieved, if the microbial control application is not economically viable relative to other tactics then implementation is destined to fail. The economics of a putative control approach depends on a number of factors, including the value of the crop (a higher crop value being more favorable to microbial control approaches), the proportion of the target area that must be treated, and the ease of delivery or compatibility with existing practices.

If a microbial control tactic is not successful, elements for success might be reached using approaches discussed in the sections above, e.g., by improving production, formulation, or delivery mechanisms. Strain discovery and improvement techniques can also substantially enhance microbial control potential. Additional research is needed to develop new techniques or approaches for the improvement of microbial control agents and their application.
The greatest barrier to expanded implementation of microbial control is a lack of economic feasibility. Thus, research is required to facilitate the use of microbial control agents in a cost-effective manner. A larger emphasis on conservation approaches can augment microbial control usage without necessarily increasing the costs of crop production. In addition, the integration of microbial control tactics into more holistic IPM programs may allow for improved cost competitiveness. A microbial control agent that is incorporated into a multitactic approach may be more cost-effective than a standalone pest control solution, e.g., the microbial could be combined directly with other tactics (in a synergistic approach), or might account for partial suppression of the pest as part of a multiapplication or multistage approach.

Finally, to expand the success of microbial control, translational research must be promoted. Advances in applied microbial control can be made through greater linkage with basic research. Fundamental research in insect pathology, such as on host–pathogen relationships at the molecular and organismal level, ecological relationships, physiology, etc., is the foundation for microbial control and serves as a basis for the future growth and development of the discipline.

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