

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.

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 parasitoids 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

1.1. INTRODUCTION

Insects represent three-quarters of all animal species in the world, with the vast majority of them being terrestrial and/or

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,

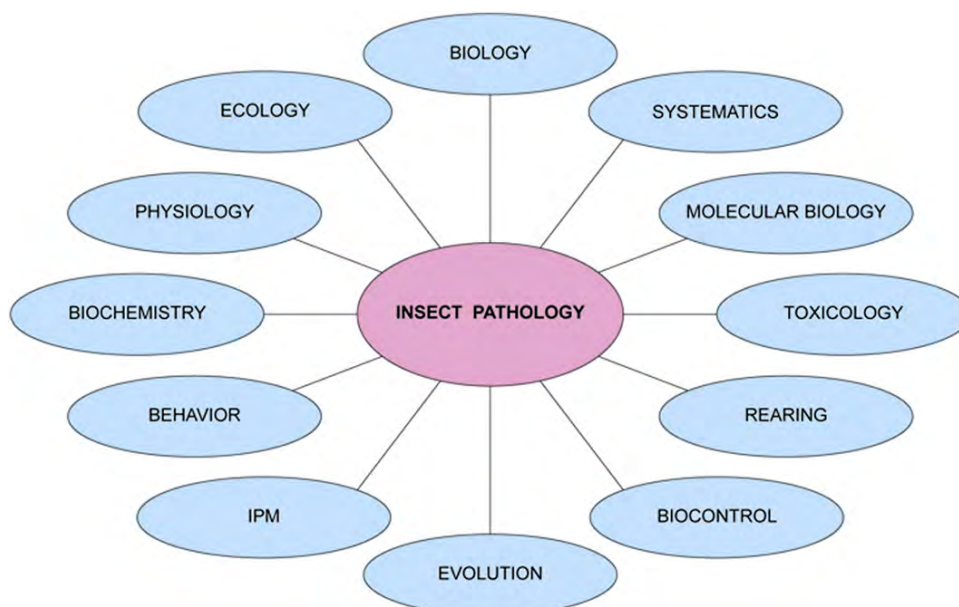


FIGURE 1.1 Relationship between insect pathology and other subdisciplines in entomology.

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 parasitoids 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 nutriment; (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 neoplasm (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 nutriment 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., steinernematids 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 non-cellular 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 steinernematid 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 cultured *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 protists, 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

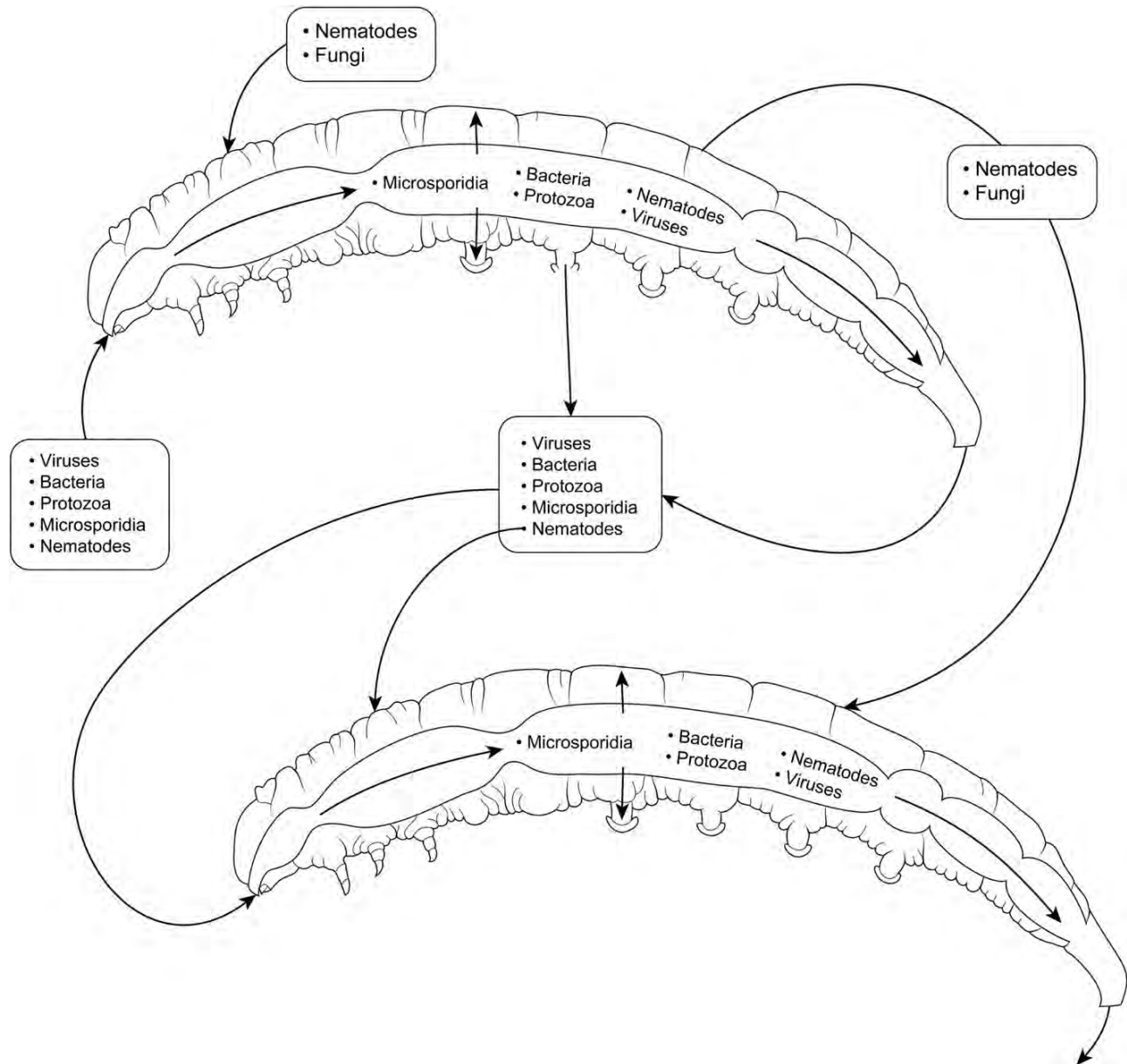


FIGURE 1.2 The primary portal of entry for most entomopathogens (viruses, bacteria, protists, microsporidia, and some nematodes) is primarily through the mouth (*per os*), whereas for fungi and some nematodes, the primary portal of entry is through the cuticle. The entomopathogens that enter through 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.)

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 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 infective 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 protists. 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

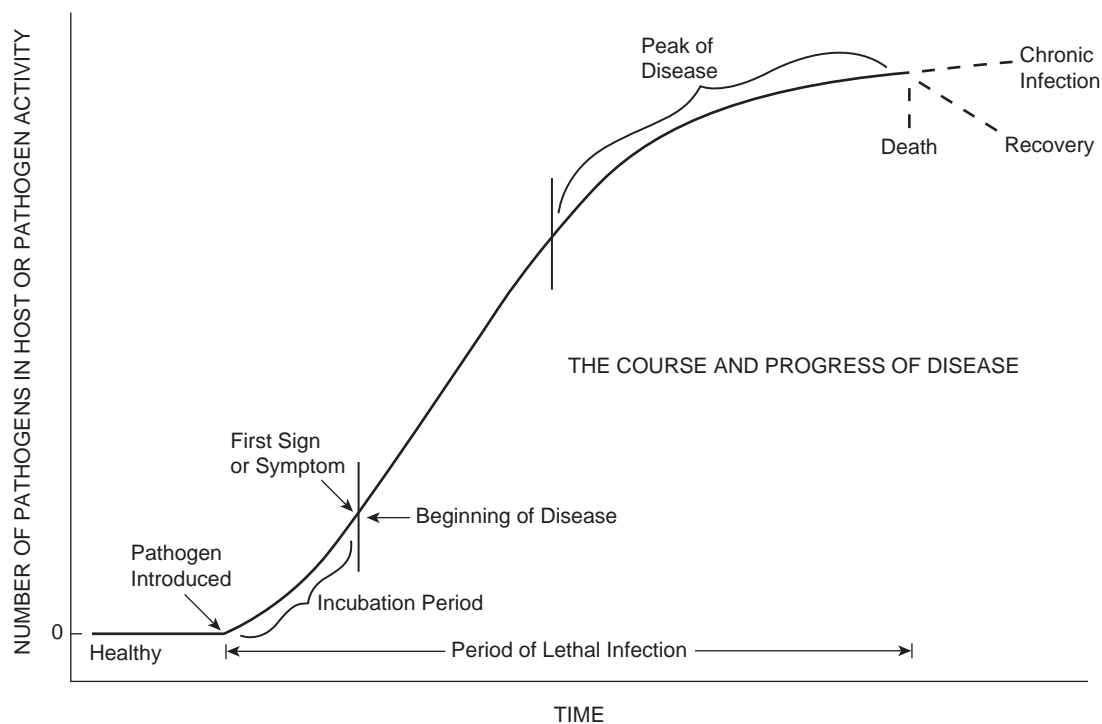


FIGURE 1.3 When a healthy, susceptible insect acquires an infectious concentration of an entomopathogen, it sets into motion a sequence of events referred to as the course and progress of disease. As the pathogen reproduces and/or produces toxins, the intensity of disease increases. The time from when the insect acquires the pathogen until its death is referred to as the “period of lethal infection”. (Modified after Tanada and Kaya, 1993.)

(i.e., the period of lethal infection is short). Chronic infections are often overlooked because their manifestations tend to be less striking and apparent to the observer until very late in the infection process. Chronic infections are of long duration and the hosts may or may not die. If the host dies, the period of lethal infection is long, whereas if the host does not die, it may reach adulthood and reproduce. Latent infections in insects have been detected primarily with viruses. In such cases, the term latent or occult viral infection is used, and the virus is referred to as an occult virus and not as a latent virus (Onstad *et al.*, 2006). Recently, an occult nodavirus belonging to the genus *Alphanodavirus* in the family *Nodaviridae* was described from an insect cell line (Li *et al.*, 2007).

1.3.11. Koch's Postulates

One of the basic tenets in pathology for establishing the etiological or causal agent of a disease involving microorganisms is the application of Koch's postulates. Robert Koch (1843–1910), a German physician who is considered one of the founders of microbiology, made brilliant discoveries on the causal agents of anthrax, tuberculosis, and cholera through the application of postulates that bear his name.

Koch's postulates as stated in Tanada and Kaya (1993) are:

- 1 A specific pathogenic organism must be seen in all cases of a disease.
- 2 This organism must be attained in pure culture.
- 3 The organism from the pure culture must reproduce the disease in experimental animals.
- 4 The same organism must be recovered from the experimental animal.

It is not always possible to fulfill Koch's postulates because one or more steps cannot be performed or can be performed only with great difficulty. For step 1, some organisms are

difficult to detect, although the electron microscope has made it possible to view submicroscopic organisms such as viruses, rickettsia, rickettsiella, and mollicutes putatively in infected insects. However, the mere presence of these organisms in a host does not prove that they are the causal agent of a particular disease without further experimentation by following Koch's postulates. For step 2, certain microorganisms cannot be isolated in pure culture with the present available laboratory methods or are very difficult to culture. These microorganisms are mainly obligate pathogens requiring living cells or tissues. For some obligate microorganisms, insect tissue cultures can be used to fulfill step 2. For step 3, different pathogens may produce similar signs, symptoms, and syndromes which may mistakenly result in designating the wrong organism as the etiological agent.

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

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

^aKoch'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 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.

Some helpful references for diagnosing diseased insects and identifying the entomopathogens include “An Atlas of Insect Diseases” by Weiser (1969), “Laboratory Guide to Insect Pathogens and Parasites” by Poinar and Thomas (1984) and “Manual of Techniques in Insect Pathology” edited by Lacey (1997). Inglis and Sikorowski (2009b) provide an excellent discussion on diagnostic techniques, and Stock *et al.* (2009) edited a book dealing with molecular diagnostic techniques entitled “Insect Pathogens: Molecular Approaches and Techniques.” The reader is referred to these excellent sources to identify some of the more important entomopathogens that infect insects.

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