Chapter 5

BIOLOGY, ECOLOGY AND EPIDEMIOLOGY OF MICROBIAL ORGANISMS INFECTING ARTHROPOD PESTS

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INTRODUCTION

Cotton is host to a multitude of insect and mite pests, most of which are known to be infected by one or more entomopathogens. These include viruses, bacteria, fungi, protozoa and entomophagous nematodes. Knowledge of pathogens on these pests is considerable but is far from complete. New host-pathogen relationships, even new groups of pathogens, are still being discovered. The purpose of this chapter is to summarize knowledge of the entomopathogens encountered in pest populations in the cotton field. Their biology, symptomatology, pathology and epidemiology will be discussed as appropriate for each pathogen and host species relationship. This chapter is organized according to pathogen groups rather than pest species in order to prevent redundancy. Each pathogen group shares certain features which do not differ greatly from host to host. Therefore, the general biology of each group will be presented and followed, where appropriate, by special considerations related to specific hosts.

In reviewing the literature on pathogens of cotton pests, it is immediately apparent that, while most cotton pest species have been identified as hosts for various pathogens, relatively little of the research effort on these host- pathogen relationships has been conducted in the cotton system. A great deal of present knowledge of these pathogen-host relationships comes from research efforts on crops such as corn, soybeans, sorghum, vegetables and others. The demand for high levels of control of key cotton pests such as the boll weevil and the bollworm/tobacco budworm have resulted in heavy reliance on chemical insecticides in the past. Because of this dependency there has been relatively little effort made to survey for the pathogens of arthropod pests of cotton or to learn of their actual or potential roles in this agroecosystem. There

are notable exceptions, such as work on the boll weevil, *Anthonomus grandis grandis* Boheman, by R. E. McLaughlin, much of which is cited later in this chapter.

As a side-effect of the recent boll weevil eradication program in the southeastern United States, several pests such as the beet armyworm, *Spodoptera exigua* (Hübner), and southern green stink bug, *Nezara viridula* (L.), among others, are becoming increasingly important in cotton. Several pathogens of the beet armyworm have been noted by the authors in cotton in Alabama and South Carolina during the 1988 field season (unpublished data). It is likely that reports will be forthcoming on pathogens of stinkbugs and similar "new" pests as they demand more attention by field entomologists working with this crop.

The reader should keep in mind that this chapter contains information on relationships that result principally from naturally occurring pathogens. Although information on the biology of several pathogens commonly used as microbial insecticides will be presented, this chapter will not review applied microbial control knowledge. This subject is covered more thoroughly in Chapter 15.

VIRAL PATHOGENS

Viruses are, in themselves, incomplete forms of life. They contain DNA or RNA that has sufficient genetic coding information to cause specific host cells to produce specific products that the cell would not normally produce, thereby causing infection. These products include viral proteins, specific enzymes, new viral nucleic acid, etc. The virus is able to direct the infected cell to produce these products at the expense of energy and material normally channeled toward growth and maintenance of the cell itself. The end result is often destruction of the cell. If sufficient cells are involved, host growth and development may be reduced or abnormal, with death being a frequent result. There are many different families of viruses that infect insects. Only five—Baculoviridae, Reoviridae, Iridoviridae, Ascoviridae, and Polydnaviridae—will be discussed as those best known from insects affecting cotton.

BACULOVIRUSES

Baculoviruses are very numerous within the Phylum Arthropoda, but the majority are known from insects. Several insect orders contain known hosts, but they are recorded principally from the Lepidoptera (Bilimoria, 1986). There are no known counterparts of this group of viruses within the plant kingdom or within the Phylum Vertebrata. The International Committee on Virus Nomenclature places these viruses in the family Baculoviridae (Matthews, 1982). Only one genus, *Baculovirus*, has been described, but there are three subgroups—A, B, and C. The latter are based on the presence or absence of a proteinaceous occlusion body which surrounds the infectious virions and, if present, on the morphology of the occlusion body. In subgroup A, commonly referred to as the nuclear polyhedrosis viruses, many virions are occluded in a polyhedral shaped occlusion body (Figures 1, 2) which forms in the host cell nucleus. In Subgroup B, a single virion is occluded in a small, capsule-shaped occlusion body (Figure 3).

Subgroup C virions are naked and do not produce any type of protective occlusion body. There are currently no known Subgroup C viruses from cotton pests.

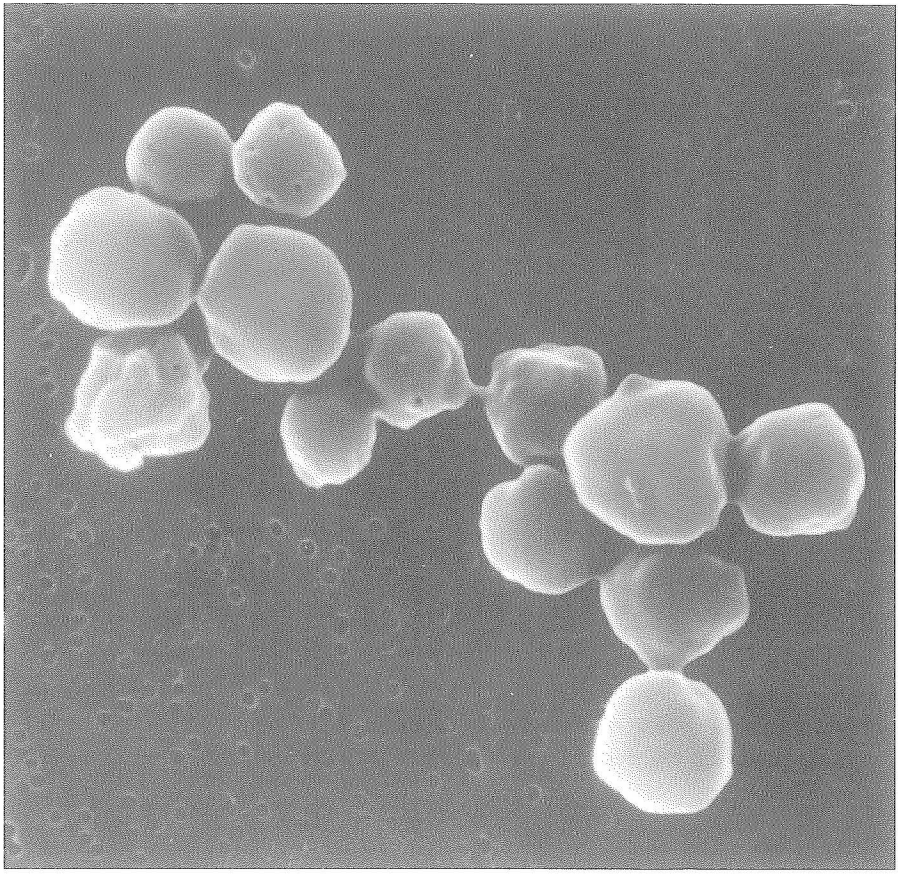

Figure 1. Polyhedral occlusion bodies of *Baculovirus* Subgroup A from infected bollworm larvae. (SEM, 7480X.)

Nuclear Polyhedrosis Virus (*Baculovirus* Subgroup A) — The nuclear polyhedrosis viruses are currently known from over 500 different hosts, mostly Lepidoptera, but also from Coleoptera, Diptera, Hymenoptera and several other orders. Within the Lepidoptera, at least 34 families contain known hosts of the nuclear polyhedrosis viruses (Martignoni and Iwai 1986). Cotton pests from which nuclear polyhedrosis viruses have been isolated include the tobacco budworm, *Heliothis virescens* (F.), the bollworm, *Helicoverpa zea* (Boddie), the beet armyworm, the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), the cabbage looper, *Trichoplusia ni* (Hübner), the cotton leafworm, *Alabama argillacea* (Hübner), the pink bollworm, *Pectinophora gossypiella* (Saunders), most of the cutworms and numerous other Lepidoptera which

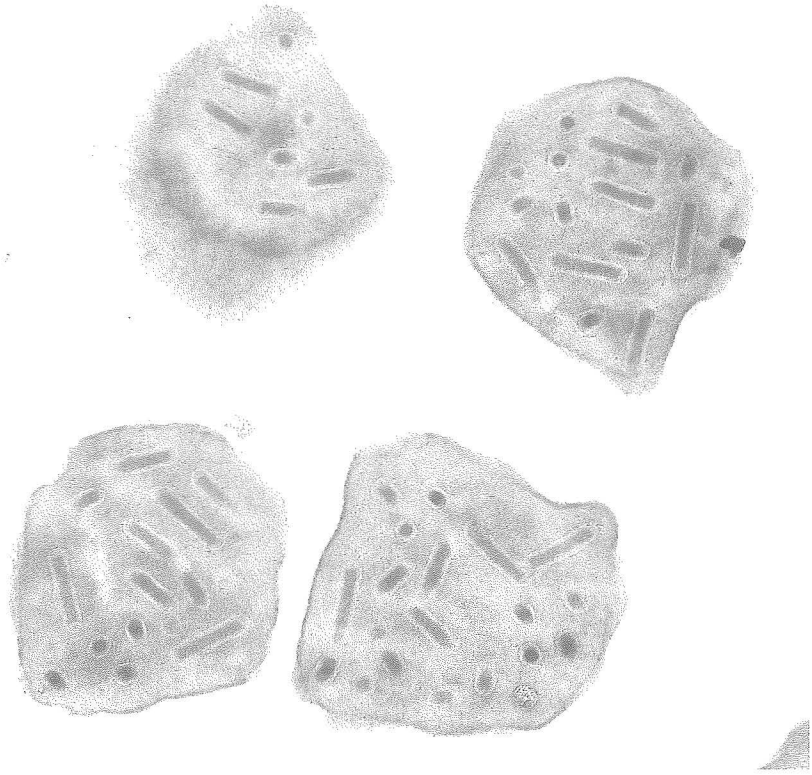

Figure 2. Cross-section of *Baculovirus heliothis* Subgroup A polyhedral occlusion bodies showing embedded virions in transverse and longitudinal sections. (TEM, 25,000X.)

occasionally attack cotton, such as the European corn borer *Pyrausta nubilalis* (Hübner), the saltmarsh caterpillar, *Estigmene acrea* (Drury), the yellowstriped armyworm, *Spodoptera ornithogalli* (Guenée) and others. Outside the United States, many other species could be added to this list.

The virions of baculoviruses, regardless of subgroup, are morphologically similar. The basic virus particle is rod-shaped and consists of a dark, electron-dense core which contains the double stranded DNA-protein complex. This is surrounded by several layers. From inside out the order is: the capsid, an intermediate layer and three outer layers collectively making up the virion envelope (Figure 3) (Federici, 1986). The nucleoprotein core plus capsid are collectively termed the nucleocapsid. Nuclear polyhedrosis virus virions are occluded or embedded in polyhedral shaped occlusion bodies (Figure 1). Occluded virions may be singly-embedded with only one nucleocapsid per envelope (Figure 2), or multiply-embedded, with two or more nucleocapsids per envelope (Figure 4). The occlusion bodies are large, predominantly from 3 to 8 hun-

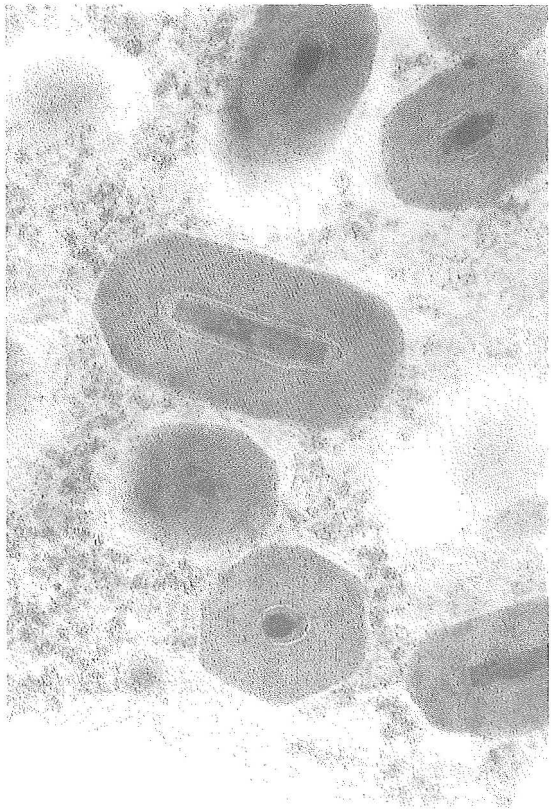

Figure 3. Cross-section of typical capsule-shaped *Baculovirus* Subgroup B occlusion bodies showing the singly-embedded virion consisting of a dense nucleo-protein core and surrounding membrane layers. (TEM, 85,000X.)

dred-thousandths of an inch (0.8 to 2.0 nanometers) in diameter, although a total size range of 2 to 60 hundred-thousandths of an inch (0.5 to 15 nanometers) is generally reported (Federici, 1986). Their size allows them to be readily seen with the light microscope. The occlusion bodies of lepidopteran nuclear polyhedrosis viruses are polyhedral in shape, being either tetrahedral, cuboidal or dodecahedral (Bergold, 1963). Viewed in profile in the light microscope, they may appear triangular, square or roughly circular, respectively. Each isolate produces a characteristic polyhedron type that is presumably genetically controlled. Each of the large occlusion bodies or polyhedra may contain from a few to several hundred occluded virions. Finally, a thin polyhedral membrane surrounds the entire polyhedron (Federici, 1986). The majority of the complete polyhedron consists of proteins. However, the virion envelopes contain

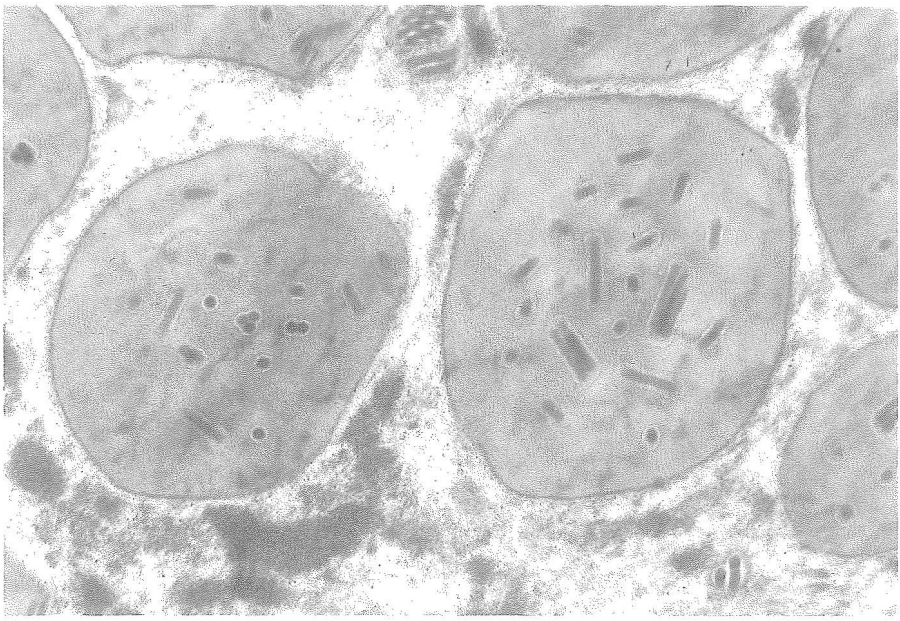

Figure 4. Cross-section of typical polyhedra from *Baculovirus* Subgroup A showing multiple-embedded nucleocapsids. (TEM, 66,500X.)

some lipid and the outer polyhedral membrane is composed principally of carbohydrates (Bilimoria, 1986; Federici, 1986).

All components of the complete polyhedron are functional and play important roles in the life of the virus. The process of infection and replication has been described well by Granados and Williams (1986). Infection is normally initiated when a susceptible insect—for example, a larval bollworm—ingests polyhedra. When the polyhedra reach the midgut, they are rapidly dissolved by the alkaline pH conditions encountered in the gut fluids, and the virions are liberated. Those which come in contact with the gut wall are taken into the cells through a process resembling phagocytosis. The envelope is lost in the process, and only the nucleocapsid enters the cell. This may enter the gut cell nucleus where the DNA is released and is able to replicate itself and produce more nucleocapsids.

Many of these newly produced nucleocapsids, as well as some of the original invaders, will eventually pass to the body cavity side of the gut cell. Via a process that is essentially the reverse of that by which they entered the gut wall cell, they will exit into the lumen of the body, picking up a new coat or envelope of cell wall material in the process. Once in the body cavity, the circulating hemolymph or blood carries the particles until they come in contact with susceptible cells. There they attach and again enter the cells by losing their temporary envelope. They finally reach the cell nucleus where the DNA is liberated and begins replicating (Granados and Williams, 1986).

Virions produced in these tissues are normally occluded into polyhedra which form within the nucleus as the virions are being produced. Occluded virions will not become active again unless ingested and released in a new host. Ultimately, an infected nucleus becomes so filled with polyhedra that it becomes distended and ruptures, releasing polyhedra into the insect's body cavity. Since tens of thousands of cells are infected, death usually occurs just before the time of multiple cell lysis (disintegration). Tissues most commonly infected include the fat body, trachea, integument and certain hemocytes. Other tissues may be involved, depending on the particular host-virus system (Smith, 1976; Granados and Williams, 1986).

The above process is the most commonly encountered. It can have certain variations. For example, the entire replication and polyhedron development process can be confined to the midgut cells with no penetration into the hemocoel and no infection of integument or internal tissues. This condition is seen in sawfly larvae (Order Hymenoptera) (Bird and Whalen, 1953; Smith, 1976) but is not known in any cotton pests. Virions can infect by being injected into the hemocoel through the integument. This occurs through feeding punctures of predators or stings of parasitic wasps that have become contaminated during similar activities on infected individuals (Andreadis, 1987). In these cases, involvement of the polyhedron in the infection process is bypassed.

Following ingestion of a lethal dosage of polyhedra, an infected larva will behave normally for several days or longer. Smaller larvae show symptoms much more rapidly than larger larvae. Time of development of disease and thus time to onset of symptoms is positively correlated with increasing temperature (Hall, 1963) and dosage. Initial symptoms include reduction in movement followed or accompanied by a loss of feeding activity. Shortly before death, the body lightens in color as billions of polyhedra form in the tissues (Figure 5). Many species of virus-infected caterpillars will move upward on the host plants. Just prior to death they attach to the plant by their terminal prolegs. They may then die laying on the leaf surface or may hang head down, attached only by the prolegs (Figure 6). Just prior to death and progressing rapidly following death, the integument (exoskeleton) becomes extremely fragile as the heavily infected cells begin to lyse (disintegrate). A progressive darkening also occurs during this same period until the larva becomes dark chocolate-brown to black (Figure 7). At this point, gently shaking or lightly touching the cadaver results in rupturing the integument and spilling the liquified body contents onto the leaf surface. Frequently, the cadaver dies in place, ruptures, and leaves a large black residue on the leaf surface which remains until washed off by rains.

The nuclear polyhedrosis viruses of cotton pests occur naturally. Infection, especially the occurrence of epizootics or increases in numbers of infected insects above a normal level, are dependent on a number of factors. These factors include presence and susceptibility of host, environmental suitability, and presence of the viable pathogen (Weiser, 1987). In addition, mechanisms of transmission are needed to facilitate infection leading to the development of epizootics (Andreadis, 1987). Nuclear polyhedrosis viruses of Lepidoptera are relatively host specific and principally infect the

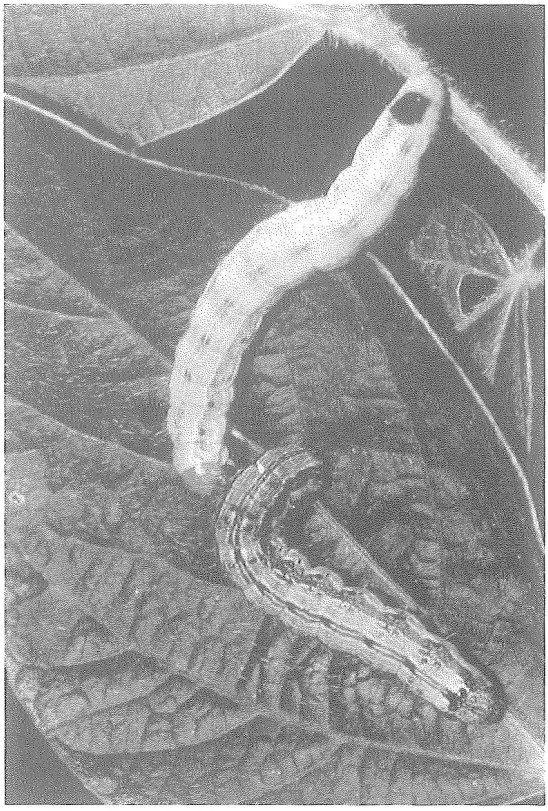

Figure 5. *Baculovirus*-infected (light) and uninfected (dark) larvae of the bollworm on soybean leaves.

larval stages of their hosts. In cotton, mechanisms are needed to facilitate virus survival between host generations within and between seasons. For a given field, this could mean several years if the host insect does not reinfest the crop each year or if the field is rotated into a crop that is not attacked by a particular pest.

Nuclear polyhedrosis viruses can survive for long periods in the soil. New hosts can be infected by consuming the virus particles from previously disintegrated hosts through soil particles splashed on plants during rains or as wind-blown dust deposited on the plant (Thompson and Steinhaus, 1950). Some female moths, sublethally infected as larvae, can carry virus which contaminates the surface of their eggs as they are laid. Larvae hatching from these eggs may become infected when they eat the egg chorion. Once a small number of individuals in a population is infected, transmission is facilitated by the increased inoculum released by the disintegrating infected individuals. Predators and parasites add to the transmission level (Thompson and Steinhaus, 1950; Andreadis, 1987). Weakened, infected individuals are even cannibal-

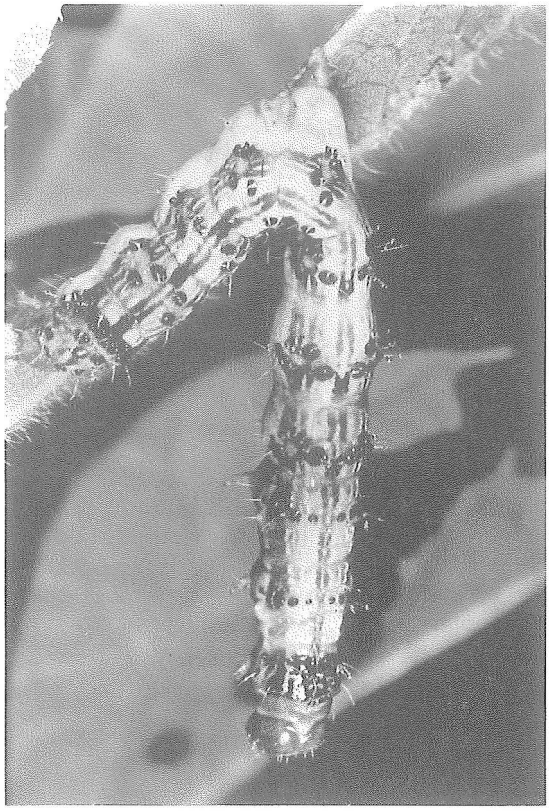

Figure 6. Bollworm larva showing typical hanging posture soon after death from *Baculovirus heliothis* infection.

ized by their healthy cohorts, which in turn are infected. An individual infected larva can produce sufficient virus to kill thousands of insects of its own kind. In an epizootic (epidemic) involving 10,000 larvae per acre or more, the amount of inoculum in a cotton field assures that most larvae will come in contact with the virus.

Environmental factors often reduce inoculum levels. Rain redistributes polyhedra on plant surfaces and spreads them more uniformly, but heavy rain-fall will wash them to the soil. Virus is rapidly inactivated by exposure to certain ultraviolet wavelengths of sunlight. Non-susceptible insects can eat virus on plants and remove it as inoculum for susceptible insects. Thus while virus builds up in the field during an epizootic, it is constantly being lost as well.

Several baculoviruses are frequently encountered in cotton fields. In the Southeast, the cabbage looper suffers routinely from epizootics of a nuclear polyhedrosis virus. Most growers are familiar with this virus-host relationship and have learned to take advantage of the natural mortality provided by this virus. Most other hosts do not show

Figure 7. Bollworm larva several hours after death caused by *Baculovirus heliothis* infection. Note darkened color and swollen body due to liquification of internal tissues and weakening of integument.

the extreme incidence of natural infection noted in the cabbage looper. An exception is the beet armyworm. This pest reached outbreak proportions in many counties of Alabama and South Carolina in 1988. Epizootics of nuclear polyhedrosis virus developed in most infested fields during late July and early August (Carner, unpublished data; Smith *et al.*, 1989).

Fruit feeding insects, while susceptible, rarely suffer from epizootics. The bollworm and tobacco budworm populations are both susceptible to a common nuclear polyhedrosis virus, but epizootics are rarely reported, despite the fact that epizootics of the virus occur in nearby peanut, soybean and corn fields. This situation appears to be related in part to the cryptic (concealed) feeding habits of the insect larvae on cotton and in part to a virus inactivating factor or factors in the cotton plant. Further research is needed to clarify this interrelationship as it has important implications not only for

the natural occurrence of this virus but also for its use as an applied microbial insecticide (see Chapter 15). Most of the nuclear polyhedrosis viruses known from cotton pest insects occur infrequently rather than causing dramatic epizootics. In these cases, they are of more interest for their potential development as microbial insecticides than for their natural impact on pest populations.

Granulosis Viruses (*Baculovirus* Subgroup B) — Discussion of the granulosis viruses parallels that of the nuclear polyhedrosis viruses with only a few exceptions. Morphologically, these viruses consist of a singly-enveloped virion which is identical in structure to that of the nuclear polyhedrosis viruses. This virion is occluded in an individual, capsule-shaped occlusion body (Figure 3) which is relatively small, 0.16 to 0.30 by 0.30 to 0.50 micrometers in dimensions (Federici, 1986). Thus they are the size of very small bacteria and, while visible with the light microscope, are more difficult to diagnose than the larger nuclear polyhedrosis viruses.

Granulosis infections are not as numerous as the nuclear polyhedrosis viruses; approximately 100 have been reported (Granados and Williams, 1986). They are known only from Lepidoptera. Of the major cotton pests in the United States, they have been recorded only from the bollworm, cabbage looper, beet armyworm, fall armyworm and several species of cutworms (Martignoni and Iwai, 1986).

Granulosis virus pathology, symptomatology and epizootiology are very similar to those of the nuclear polyhedrosis viruses. Growth rate of infected larvae is slowed resulting in a prolonged developmental time, which differs from nuclear polyhedrosis virus. Certain granulosis viruses infect the integument while others do not. Host larvae infected by the latter have an integument that remains tough and leathery after death. Those granulosis viruses that do infect the integument produce a very fragile cadaver as was described for nuclear polyhedrosis virus infection (Smith, 1976).

CYTOPLASMIC POLYHEDROSIS VIRUSES

Broadly grouped in the family Reoviridae, the cytoplasmic polyhedrosis viruses have affinities with certain plant and vertebrate viruses. They are known from over 150 different insect hosts, including Diptera and Lepidoptera, but the majority are known from infections of larval Lepidoptera (Matthews 1982). Cotton pests with recorded cytoplasmic polyhedrosis virus infections are the cabbage looper, fall armyworm, beet armyworm, tobacco budworm, bollworm, pink bollworm, many of the cutworms and other armyworms (Martignoni and Iwai, 1986). Thus, they are nearly as numerous among cotton pests as are the nuclear polyhedrosis viruses. On the other hand these recorded infections were usually from insects collected on plants other than cotton.

The cytoplasmic polyhedrosis viruses have been known for many years because they, like the nuclear polyhedrosis viruses, produce large, light microscopically visible occlusion bodies. They differ markedly in virion morphology, however. Cytoplasmic polyhedrosis virus virions are small, subspherical icosahedra. Their diameters range from 50 to 65 nanometers and can only be viewed with the electron microscope. The virions consist of a spherical nucleoprotein core surrounded by an outer shell

(Matthews, 1982). These virions are occluded within proteinaceous polyhedra as are the baculoviruses (Figure 8). The polyhedra can also vary in shape, ranging from tetrahedrons to icosahedrons (Aruga, 1971). A morphological study of seven different cytoplasmic polyhedrosis viruses by Cunningham and Longworth (1968) provided a range of mean polyhedron diameters of 1.13 to 2.49 nanometers.

Following ingestion by susceptible host insects, the polyhedral occlusion bodies are rapidly dissolved in the guts of their hosts. The released virions attach to the midgut wall and enter the cell cytoplasm. Once in the midgut cytoplasm, replication of the nucleic acid and synthesis of all virion and polyhedral components begin. Infection is confined to the cells of the midgut (Watanabe, 1971). Mature polyhedra are released into the gut as infected cells die and may be excreted in large numbers in the frass or fecal material (Aruga, 1971; Boucias and Nordin, 1978). Ultimately, severe infection results in loss of the midgut's ability to absorb food and function properly resulting in the host's death.

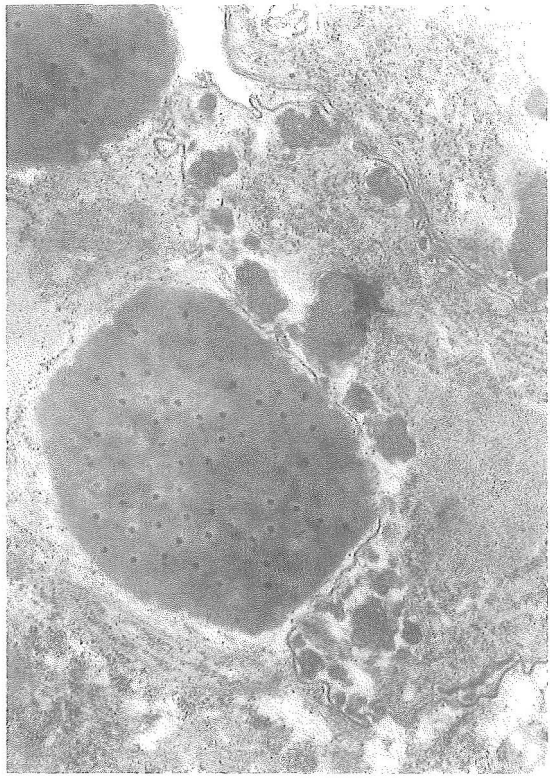

Figure 8. Thin section of midgut tissue from bollworm showing a cytoplasmic polyhedrosis virus polyhedron with many embedded icosahedral shaped virions.

Larvae infected with cytoplasmic polyhedrosis virus show few abnormal symptoms for several days after ingestion of the virus. Close examination reveals a reduction in general activity including a loss of appetite after two to four days. Larvae may regurgitate or pass abnormally wet fecal material as the infection advances. Frass also lightens in color due to presence of large numbers of occlusion bodies (Boucias and Nordin, 1978). In some host species, a white or light patchiness may appear late in infection. Dissection of the larva at an advanced stage of infection reveals a very light-colored yellow to opaque midgut instead of the normal semi-transparent tissue. This is caused by the presence of large numbers of polyhedra packing the cytoplasm of the midgut cells (Smith, 1976). At death, the larva darkens, turning brown to blackish. Unlike the nuclear polyhedrosis infection, it maintains a very tough integument which does not rupture when touched. Larvae infected late in their development often survive, pupate and emerge as adults.

Most virus is transmitted when healthy larvae eat foliage that has been contaminated by fecal material or regurgitate of infected larvae. The virus can also be transmitted from infected females to their offspring as a contaminant on the egg surface which is ingested by newly-hatched larvae. Sublethally infected female larvae that pupate and successfully emerge as moths can carry virus through metamorphosis. The virus then contaminates eggs as they are laid. In the tobacco budworm this mode of transmission occurred even after diapause was complete (Sikorowski *et al.*, 1973).

IRIDOVIRUSES

Iridoviruses are large, icosahedral, DNA viruses belonging to the family Iridoviridae. Those isolated from insects are grouped into two genera based on their sizes and serological relationships. The smaller iridescent viruses (about 130 nanometers) have been placed in the genus *Iridovirus* and include isolates from Diptera (Tipulidae), Coleoptera and Lepidoptera. Larger iridescent viruses (about 180 nanometers) have been isolated from mosquitoes and other Diptera. They are placed in the genus *Chloriridovirus* (Hall, 1985).

The name Iridoviridae is based on the characteristic iridescent green, blue or purple seen in heavily infected hosts, which is caused by the presence of high concentrations of the virus packed in crystalline arrays (Figure 9). The iridoviruses replicate in the cytoplasm of cells in a wide range of tissues, but heaviest concentrations are usually found in the fat body. There are two reports of these viruses infecting *Helicoverpa/Heliothis*. Carey *et al.* (1978) isolated a small iridescent virus (about 130 nanometers) from *Helicoverpa armigera* (Hübner) in Africa, and Stadlebacher *et al.* (1978) recovered a similar virus from bollworm larvae collected from clover and vetch in Mississippi. Infected bollworm larvae turned an iridescent lavender-blue, blue, or blue-green. The virus from the bollworm ranged in size from 131 to 160 nanometers with an average diameter of 145 nanometers. Therefore, both the Africa and United States isolates probably belong to the genus *Iridovirus*.

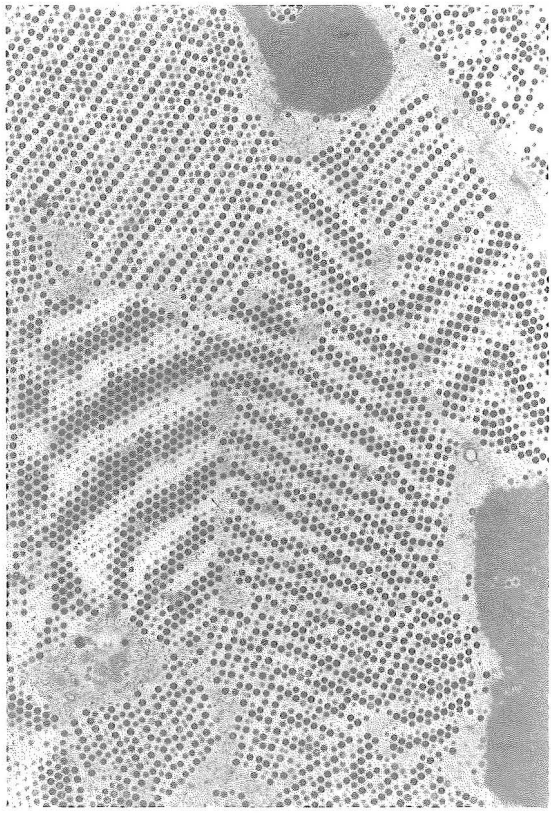

Figure 9. Thin section of fat body cells with virions arranged in the crystalline array pattern typical of *Iridovirus* infection. (TEM, 7,400X.)

ASCOVIRUSES

Ascoviruses are a recently discovered group of viruses which have been isolated from several species of noctuid larvae. They are non-occluded, enveloped, DNA viruses that measure 150 by 400 nanometers. Federici (1983) proposed the name Ascovirus to describe the many virus-containing vesicles found in the hemolymph of infected larvae (Figures 10 and 11). The virus was first reported from bollworm/tobacco budworm larvae in Mississippi (Adams *et al.*, 1979) and South Carolina (Carner and Hudson, 1981). Similar viruses have been reported from the cabbage looper (Federici, 1983) and the fall armyworm (Hamm *et al.*, 1986). Symptoms in infected larvae include sluggishness, reduced feeding and stunted growth. Larvae may remain alive for several weeks after infection. As the disease progresses the

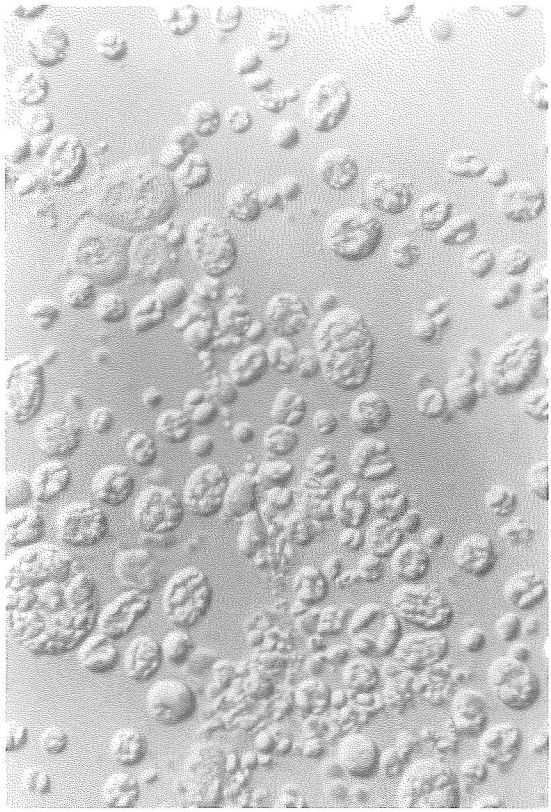

Figure 10. Hemolymph from a bollworm larva with Ascovirus infection. Note the large number of vesicles that are granular in appearance due to the presence of clumps of virus particles. (Nomarski interference contrast, 1000X.)

hemolymph turns milky and becomes filled with virus-containing vesicles. The vesicles are formed by a unique developmental sequence in which the host cell cleaves into a cluster of vesicles as virus formation progresses. The host cell then ruptures, releasing the vesicles into the blood or hemolymph.

The virion is allantoid in shape and consists of a DNA/protein core of similar shape surrounded consecutively by an inner membrane and an outer envelope (Figure 11). The external surface of the inner membrane and the outer envelope have a reticulate appearance in negatively stained preparations. Replication is initiated in the nucleus, but virion assembly does not occur until after disruption of the nuclear envelope. Subsequently, host cells are cleaved into vesicles in which replication and assembly appear to continue. Ascoviruses vary in their tissue specificity. The isolate from boll-

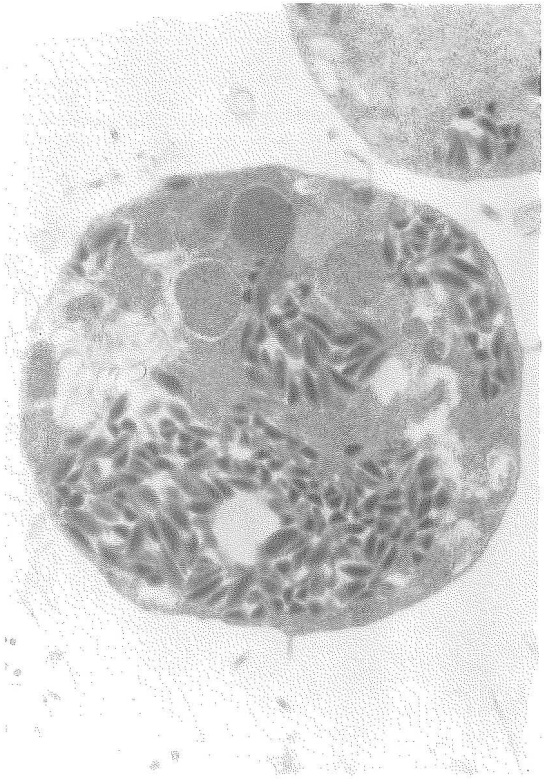

Figure 11. Thin section of a single vesicle from hemolymph of Ascovirus-infected bollworm larva. Note clumps of dark-stained virions. (TEM, 23,000X.)

worm/tobacco budworm and the cabbage looper replicates primarily in epidermal and fat body cells. It has also been observed in tracheal matrix and midgut epithelium. The isolate from *Spodoptera* spp. is restricted primarily to the fat body.

In some cotton fields in South Carolina, infection levels in bollworm/ tobacco budworm populations have reached 20 to 30 percent, but usually levels are much lower. Field collections have shown that infection levels are usually higher in fields where parasitoid populations are high, leading one to believe that virus transmission is mediated by parasites. Hamm *et al.* (1985) demonstrated that the braconid parasite, *Cotesia marginiventris* (Cresson), could transmit the virus between larvae of the fall armyworm. In South Carolina, the parasite, *Microplitis demolitor* (Wilkinson), was used successfully to transmit the virus between bollworm larvae (Carner, unpublished data). In the laboratory it is difficult to transmit the virus to larvae by feeding. However,

piercing the cuticle of larvae with a contaminated pin usually results in 100 percent infection, presenting more evidence that parasites may play an important role in the transmission of this virus in the field. In both cases of parasite transmission the virus killed not only the host larva, but also the developing parasite, putting in question the beneficial nature of this virus.

POLYDNAVIRUSES

Polydnavirus is the name used by researchers to refer to a unique group of nonoccluded viruses found in the ovaries of parasitic wasps. The name was derived from the characteristic multi-segmented DNA of variable molecular weight found in all of these viruses. Morphologically, these viruses can be divided into two main groups. Those found in braconid wasps such as *Cardiochiles nigriceps* (Viereck) consist of a short rod-shaped nucleocapsid surrounded by a double envelope. They are very similar to some of the nonoccluded baculoviruses and were originally classified by some researchers as a subgroup of the Baculoviridae (Stoltz and Vinson, 1979). Viruses found in the oviducts of ichneumonid wasps such as *Campoletis sonorensis* (Cameron) are spindle shaped and do not resemble any known group of viruses. Stoltz *et al.* (1984) proposed that these ichneumonid viruses be placed in a new family, the Polydnaviridae. Most researchers agree that eventually both the braconid and ichneumonid viruses will be grouped together in a separate family because of similar characteristics of the DNA genome.

These parasite viruses replicate in the nuclei of calyx cells and high concentrations of the virus accumulate in the lumen, forming what is referred to as the calyx fluid (Figure 12). This fluid is injected into the hemolymph of the host at the time of oviposition. The major function of these injected viruses is to interfere with the immune system of the host and prevent encapsulation of the parasite egg (Edson *et al.*, 1981). Some viruses also prevent development of the host by affecting hormone levels, and thus make the host more suitable for development of the parasite (Dover *et al.*, 1988). Each parasite species possesses a virus which is unique for that species, and the virus is present in all female individuals of that species.

Many of the viruses described to date have been from parasites which affect cotton insect pests. These include the braconids: *Cardiochiles nigriceps* Viereck, *Microplitis croceipes* (Cresson) and *Cotesia marginiventris*; and the ichneumonid, *Campoletis sonorensis* (Stoltz and Vinson, 1979). It is likely that most, if not all, of the braconid and ichneumonid parasites found in cotton possess a calyx virus characteristic for their family.

FUNGAL PATHOGENS

The fungal pathogens of insects are unique in that they are able to invade their hosts by penetration through the integument. Every major pest of cotton in the United States is infected by at least one known fungal pathogen; most are infected by several. Insects which feed by piercing and sucking, e.g. aphids, whiteflies, thrips, spider mites and

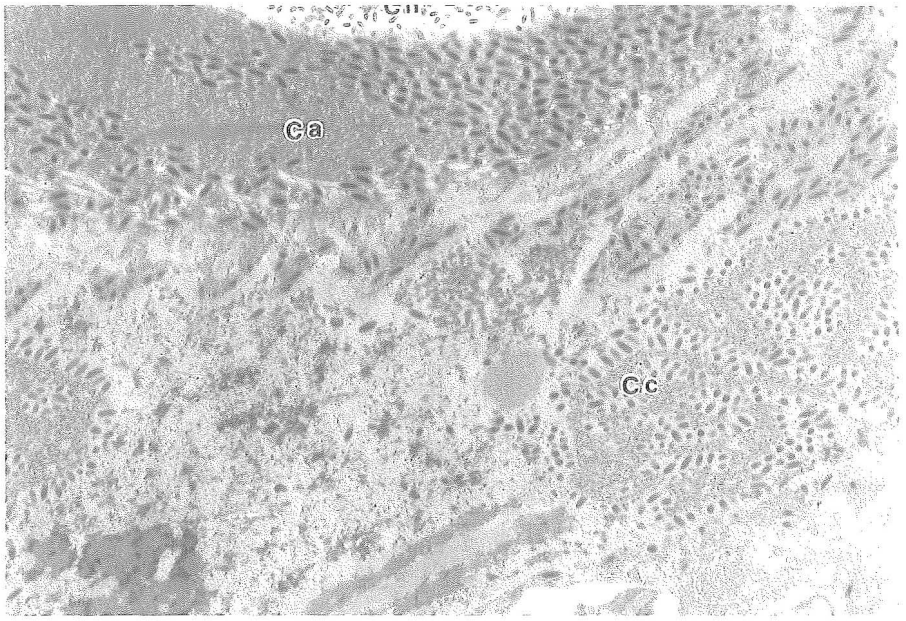

Figure 12. Thin section from the calyx region of an oviduct from the parasite *Campoletis sonorensis* showing Polydnavirus virions in the calyx fluid (Ca) which is adjacent to the chorion (Ch) of an egg. Virus replication is taking place in the calyx cell (Cc). (TEM, 10,500X.)

stinkbugs rarely are reported to have pathogenic infections of bacteria, viruses, or protozoa since ingestion of inoculum would be rare. Fungal infections are recorded in each of these groups, and dense populations frequently support striking epizootics (epidemics). Factors favoring epizootics of entomopathogenic fungi are both biotic and abiotic. Fungi are dependent on host density and on specific climatic factors such as wind, humidity, temperature, light and others to initiate and maintain infection in a host insect. Epizootics are dependent on these same factors plus various host population parameters such as density, age structure, distribution within fields and seasonal occurrence or distribution. Initiation and maintenance of epizootics are thus dependent on many specific conditions.

The canopy of the cotton plant provides an ideal situation for the development of fungi, especially in fields where the canopy is closed between rows. Late season populations of insects and mites are usually infected by one or more species of fungal pathogens. Because of the dependence of fungal pathogens on favorable moisture conditions, incidence of these pathogens in pest populations may vary considerably from one season to the next. Most of the studies dealing with fungal pathogens of cotton insects are concerned with natural occurrence and epizootiology of these pathogens. There has been very little work on development of these pathogens as microbial insecticides.

Fungal pathogens reported from cotton insects and mites fall into two main groups: those belonging to the order Entomophthorales and those which are members of the imperfect fungi.

ENTOMOPHTHORALES

The order Entomophthorales is made up of a large group of highly specialized fungi in the class Zygomycetes which are mainly parasitic on insects and arachnids (mites and spiders). The classification of this group has been the subject of considerable controversy in recent years. Early reports placed most species in a single genus, *Entomophthora*. However, as new species were added to the group and the number of species in the genus exceeded 100, attempts were made to develop a more manageable system of classification. Several revisions of the group have been published including those by Batko (1964), Remaudiere and Keller (1980), and Humber (1989). For this chapter we will use Humber's classification which divides the order into six families and 21 genera. Representatives of this group which are found in cotton include the genera *Erynia*, *Pandora*, and *Entomophaga* in the family Entomophthoraceae and *Neozygites* in the family Neozygitaceae.

The vegetative phase of the Entomophthorales fungi occurs within the body of the live host, usually in the form of hyphal bodies. These increase rapidly by fission or budding, completely filling the hemocoel and killing the host. Shortly after the death of the host, conidiophores grow out from the hyphal bodies and emerge through the less resistant portions of the cuticle. In some cases the conidiophores will form a mat which completely covers the body of the host (Figure 13). Conidia are formed singly on the tips of the conidiophores and are forcibly ejected from the host cadaver. The spores have a sticky coating and will adhere to any substrate with which they come in contact. The aureole or opaque circle of ejected conidia usually seen around a host cadaver is a diagnostic characteristic for this group of fungi.

The conidia are the primary infective units which spread the fungus through a population. In some species the primary conidia serve this purpose. In other species specialized secondary conidia are formed at the tips of slender vertical stalks. Hosts become infected by walking over the leaf surface and brushing against these spores. Conidia can be spherical, pear-shaped, or slender, depending on the species and can vary in length from 10 to 30 micrometers (Figure 14a, b, c).

Most species of Entomophthorales also form thick-walled resting spores (Figure 15) which aid in the survival of the fungus during harsh conditions and when hosts are not present. These spores are formed inside the host, often in individuals other than those on which conidia are formed. Hosts containing resting spores usually will display symptoms completely different from those infected with the conidial stage of the fungus.

Carner *et al.* (1975) reported a species of Entomophthorales with pear-shaped conidia (Figure 14b) infecting larvae of the bollworm in soybeans in South Carolina. Hamm (1980) found a similar species infecting bollworm/tobacco budworm larvae in sorghum and identified it as *Entomophthora aulicae*. Both reports describe what is now known as *Entomophaga aulicae* (Reichardt) Humber (Humber, 1989). This same

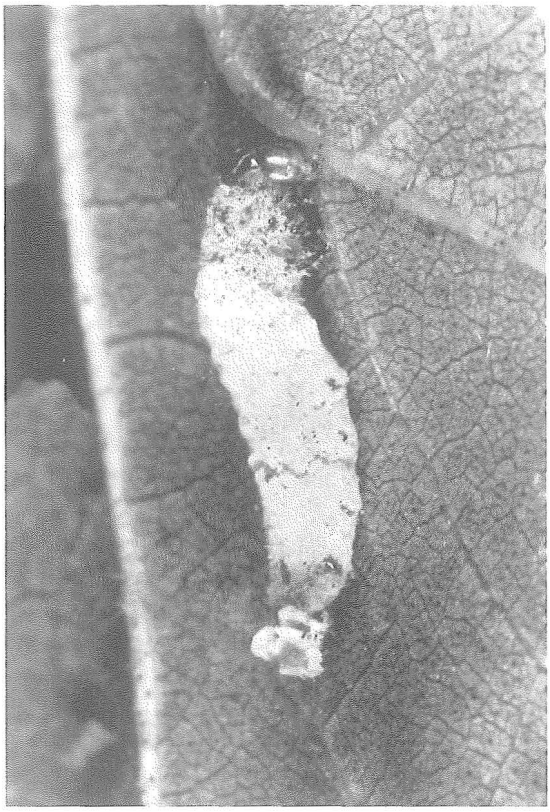

Figure 13. Bollworm larva killed from infection by *Erynia* sp. The cadaver is covered with a dense mat of hyphae and sporulating conidiphores.

fungus has been observed infecting bollworm/ tobacco budworm larvae in late-season cotton in South Carolina. The fungus usually infects late instar larvae and produces large pear-shaped conidia which contain 10 to 12 nuclei (Figure 14b). Bollworm/ tobacco budworm larvae in these same populations were also infected by a different species of Entomophthorales, which infected smaller larvae (mainly 2nd and 3rd instars) and differed from *Entomophaga aulicae* in that it produced an extensive mycelial mat over the exterior of the larval cadaver. Conidia were also smaller, more fusiform (spindle-shaped) than pyriform (pear-shaped), and contained only one nucleus per spore (Figure 14a). This second fungus is probably a species of *Erynia*.

The predominant species of looper on cotton is the cabbage looper. However, populations of the soybean looper, *Pseudoplusia includens* (Walker), sometimes build up in late-season cotton. Both species can be infected by *Pandora gammae* (Weiser) Humber, a fungus which plays a significant role in reducing looper populations in soybeans (Harper and Carner, 1973). In cotton this fungus is usually found in late season

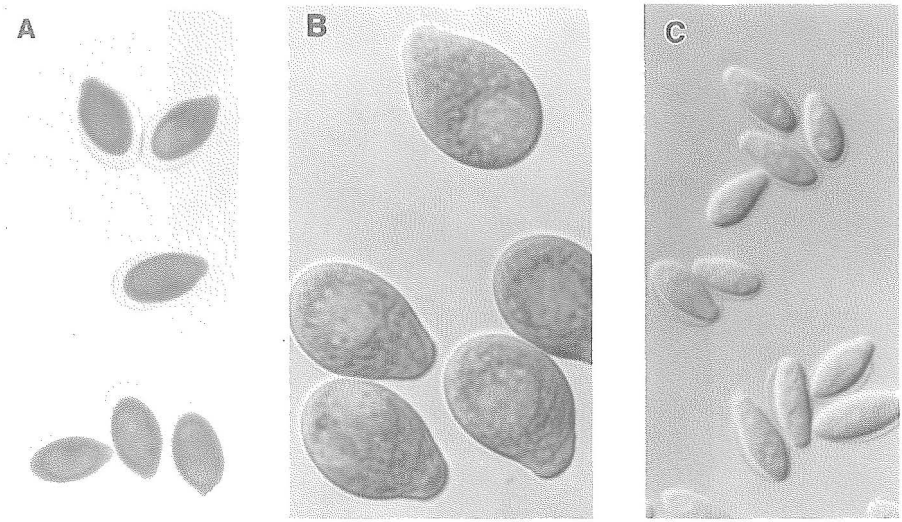

Figure 14. Conidia from the different genera of Entomophthorales. A. *Erynia* sp. from bollworm (800X). B. *Entomophaga aulicae* from bollworm (1,000X). C. *Pandora gammae* from soybean looper (1,100X).

populations which are predominantly soybean looper. Loopers infected with *Pandora gammae* display different symptoms depending on the type of spore produced. Larvae infected with the conidial stage are completely covered with a mat of tan-colored conidiophores (Figure 16). After conidia (Figure 14C) are produced, larvae turn brown and become shriveled. Larvae infected with the resting spore stage of the same fungus are black and swollen and have no external growth (Figure 17). Unidentified species of *Erynia* and *Entomophaga* have also been seen infecting loopers in cotton (Carner, unpublished). These fungi produce symptoms similar to those described in bollworm/tobacco budworm larvae.

The beet armyworm has been a serious pest of cotton in recent years. Although the nuclear polyhedrosis virus has usually been reported as the predominant pathogen of this species, a species of *Erynia* has also caused high mortality in populations of this pest in South Carolina. This *Erynia* spp. appears similar to the one which infects bollworm/tobacco budworm larvae. The yellow-striped armyworm is also a host for this fungus. Both the yellowstriped and beet armyworms have been found infected with *Pandora gammae* (Carner, unpublished).

The cotton aphid, *Aphis gossypii* Glover, has become increasingly important as a pest of cotton in the southeastern United States during the past decade. The fungus *Neozygites fresenii* (Nowakowski) Batko is a common mortality agent of this pest in many states in most years. Dramatic epizootics or outbreaks of the fungus are frequently observed, with high percentages of population reductions. The authors have noted these in Alabama and South Carolina and reports from other states have been common (Steinkraus, 1991).

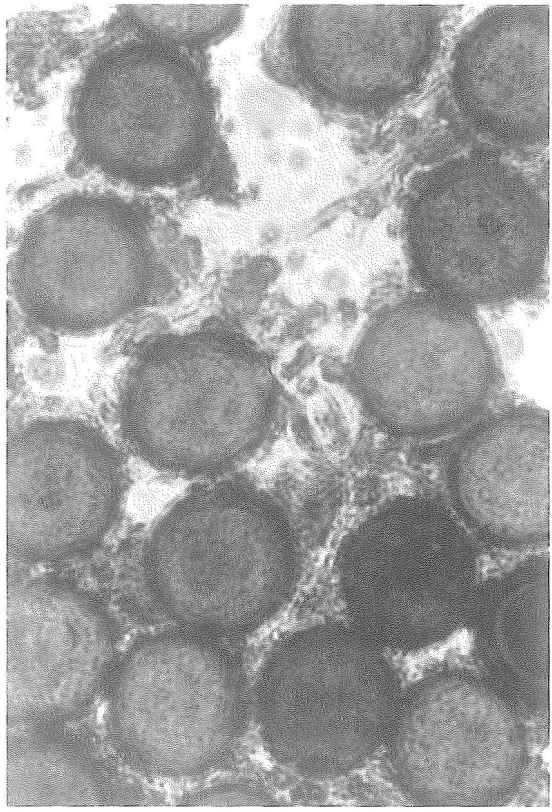

Figure 15. Resting spores of *Neozygites floridana* from the twospotted spider mite. (1370X.)

The predominant mortality factor in populations of the twospotted spider mite, *Tetranychus urticae* (Koch), is the fungal pathogen, *Neozygites floridana* (Weiser) Remaudiere and Keller (Figure 18). Epizootics of this fungus usually occur when mite populations reach high levels and can completely decimate populations within a period of one to two weeks (Carner and Canerday, 1970). Mites infected with the conidial stage of *Neozygites floridana* become mummified and turn a light tan color immediately after death. Under high humidity conditions conidiophores and conidia will develop over the entire external surface. Primary conidia are spherical with a prominent papillar base and contain four nuclei. The infective stage of this fungus appears to be a specialized secondary spore which is produced at the tip of a slender vertical stalk which grows out from the primary conidium (Figure 19). Mites infected with the resting spore stage of this fungus are black with no external growth (Carner, 1976).

Populations of the western flower thrips, *Frankliniella occidentalis* (Pergande), are sometimes infected with the fungal pathogen, *Neozygites parvispora* (MacLeod and Karl) Remaudiere and Keller, a species originally described from the onion thrips,

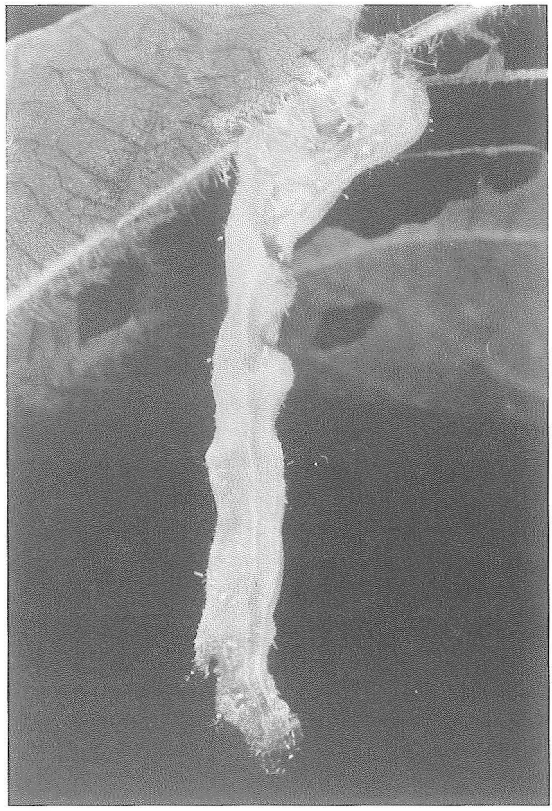

Figure 16. Soybean looper larva hanging from a soybean leaf early in the morning following death the previous evening from *Pandora gammae* infection in which conidia were formed. The body is covered with a layer of tan-colored conidiophores.

Thrips tabaci (Lindeman) (Carner, unpublished). This fungus has a life cycle very similar to that described for *Neozygites floridana* in spider mites.

Members of the Entomophthorales are specialized pathogens with a high degree of adaptation to the species which they infect. They are generally observed causing epizootics when host populations are high. Like most fungi they are dependent on favorable environmental conditions for their development, but not to the extent that imperfect fungi are. These fungi are closely tied to the life cycle and behavior patterns of their hosts and can maintain infection in a population with the normal periods of high humidity that occur at night. For example, the mite fungus, *Neozygites floridana* kills its host in the late afternoon and early evening when conditions are favorable for spore production. Conidiophore production begins immediately and is completed

Figure 17. Soybean looper larva hanging from a cotton leaf following death from *Pandora gammae* infection in which resting spores were formed internally.

within several hours. As soon as primary conidia are ejected and land on the leaf surface, they germinate to form slender upright stalks on which secondary conidia are formed. All of this development takes place during the night while the humidity is high. The secondary spores are more resistant than the primary conidia and are able to survive the warm dry conditions that normally occur during the daylight hours. Spider mites are inactive at night and begin to move around on the leaves as temperatures rise during the morning. As they move around on the leaf surface they brush against the secondary spores and the spores become attached to the cuticle. Germination of these infective secondary conidia does not occur until conditions become favorable again the following evening. Spore germination and penetration of the cuticle requires humidities close to 100 percent, but once the fungus is inside the mite it does not require high humidity for its development until it kills the mite several days later.

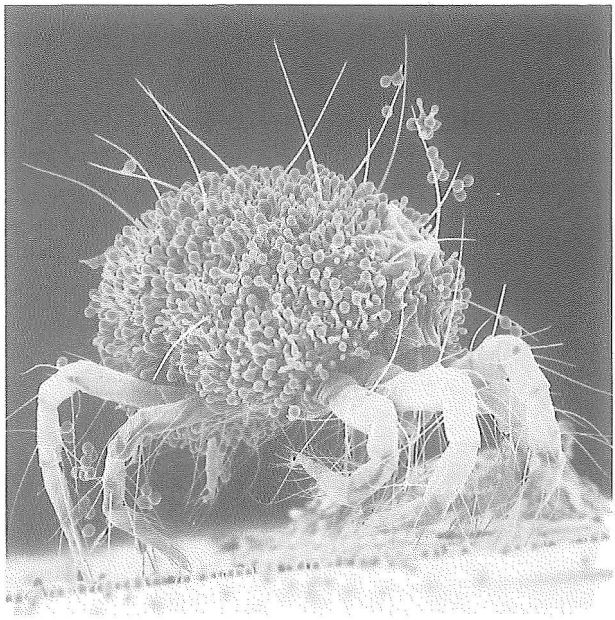

Figure 18. Twospotted spider mite infected with *Neozygites floridana* showing the formation of primary conidia. (SEM, 140X.)

NOMURAEA RILEYI

Nomuraea (Spicaria) rileyi (Farlow) Samson is a member of the class Hyphomycetes. As such it produces conidia but has no known sexual method of reproduction. There is only one other species in the genus, *Nomuraea atypicola* (Yasuda) Samson, which is distinctively different based on conidial color. It is not known to infect any cotton pests.

Nomuraea rileyi is commonly encountered as a pathogen in many species of lepidopterous larvae in cotton fields (Ignoffo, 1981). It frequently infects tobacco budworms, bollworms, cabbage loopers and the armyworms associated with cotton. On other crops—corn, sorghum, soybean, and crucifers—it is found on additional species of Lepidoptera. *Nomuraea rileyi* has been reported from most agricultural areas around the world, ranging from tropical to temperate climates (Ignoffo, 1981). Most records appear to be associated with larval noctuids, but the species is recorded from a spider (Samson, 1974) and from several Coleoptera (Ignoffo, 1981), so the potential host range may be large.

Nomuraea rileyi is very similar in morphology to *Penicillium* (Samson, 1981). It produces oval conidia which are green in color. These are produced in chains on short-necked phialids which are in turn produced in dense whorls along the filament-like conidiophores (Figure 20).

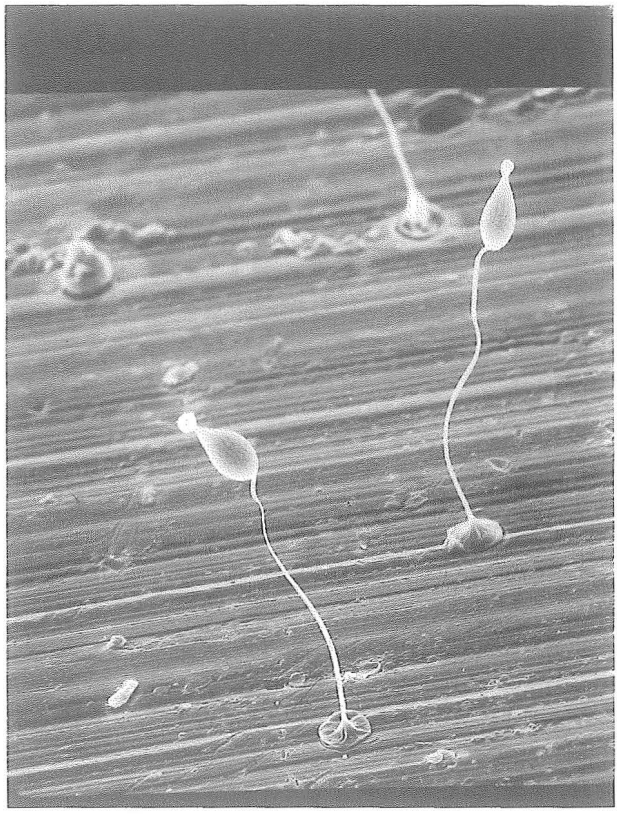

Figure 19. Capilliconidia of *Neozygites floridana*. Note the development of the capillary stalk from the primary conidium on the substrate with the subsequently formed capilliconidium and adhesive tip. (SEM, 750X.)

Insect larvae infected with *Nomuraea rileyi* exhibit symptoms typical for many Deuteromycete infections. Little external differences are noted between infected and healthy individuals for several days following infection. Larvae then become less active. Neonate cabbage looper larvae are killed in six to seven days, depending on temperature (Getzin, 1961). Following death, larvae may hang from the plant structures on which they are sitting with their prolegs attached to the plant surface. On cotton, infected bollworm or tobacco budworm larvae are sometimes seen hanging head down from their feeding holes in the bolls. On leaves and stems, the larvae often assume a curved posture, arching upward and forward from their attached prolegs with the forward portion of the body held rigidly above the substrate. If the correct environmental conditions are present, the fungal mycelium or hyphal bodies which have proliferated and filled the hemocoel of the cadaver will begin to produce conidiophores. These grow through the body wall in large numbers and ultimately cover the

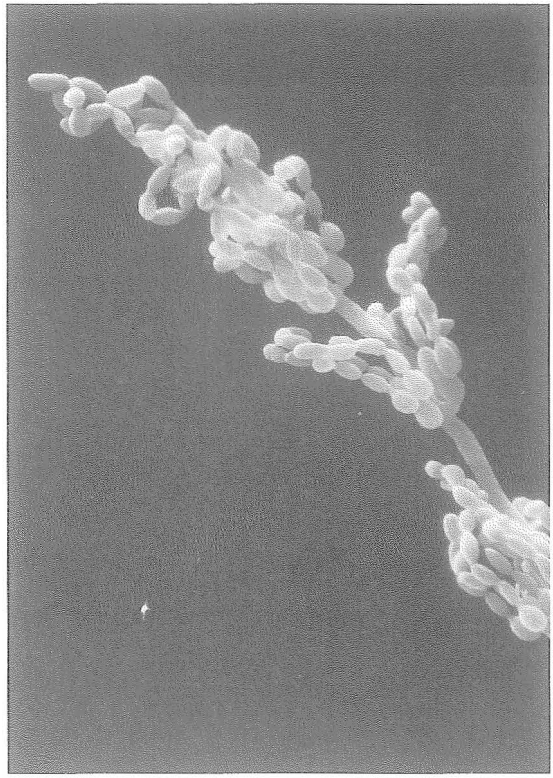

Figure 20. SEM of *Nomuraea rileyi* reproductive structures showing chains of spores produced from dense whorls of conidiogenous cells. (1,300X.)

entire cadaver as a dense, bright white bloom. It is this stage that is most frequently noticed by growers. If environmental conditions continue to be favorable, this stage is followed by the production of hundreds of thousands of green conidia which cause the cadaver to turn from bright white to a light green color (Figure 21). Touching or shaking such cadavers results in dislodging conidia as a green dust.

Infection of a larva by *Nomuraea rileyi* begins when conidia which have either adhered to the integument or have been ingested, germinate and produce germ tubes which penetrate through the integument or gut wall by both mechanical and chemical mechanisms. Once penetration occurs, the germ tube begins to produce cells beneath the integument by growth and division at the penetration site. Growth continues as cells break away and grow in the hemolymph as short, stocky hyphal bodies, often called blastospores. These proliferate by budding, eventually causing death of the host. They continue to grow until they fill the host abdomen. At this stage, they produce elongate conidiophores and conidia as discussed under symptomatology.

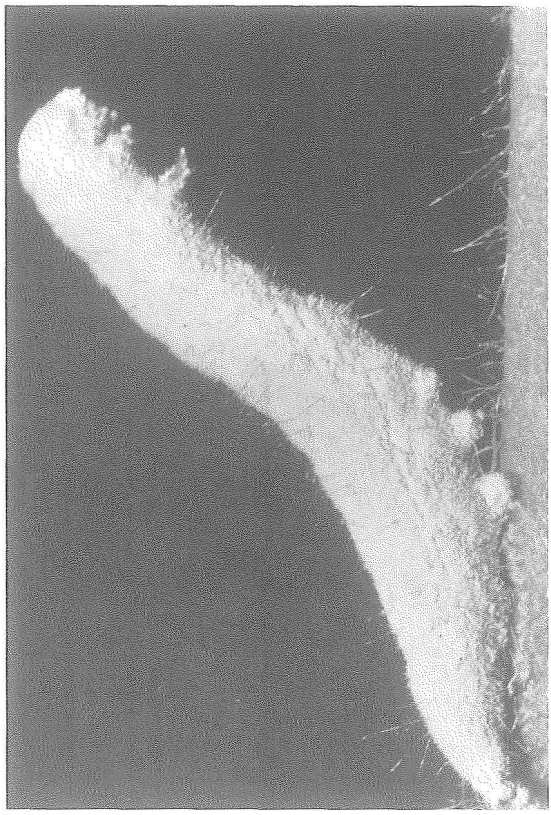

Figure 21. Typical *Nomuraea rileyi*-infected lepidopterous larva in complete sporulation stage. Larvae are light green in color at this stage.

Nomuraea rileyi is typically abundant under conditions of high humidity and high host density. In most cotton growing areas of the United States such conditions are not continuously present. Infection is frequently noted in individual larvae in low incidence during much of the growing season, but the fungus is normally prevalent in highest incidence in host populations during late summer. This is probably due to development of microclimates within the closed crop canopy conducive to fungal infection and spread, as well as to higher host populations increasing the probability of infection. Production of conidiophores and conidia from dead cadavers is dependent on moisture conditions, especially on high humidity. Once produced, conidia are easily dislodged from the cadavers by wind or other physical disturbance and contact new hosts by air movement or by gravity. If conditions for conidiophore and conidia production are not appropriate, the fungus can remain dormant inside the intact dead larva for extended periods of time. The fungus overwinters inside the host or as free conidia (Sprenkel and Brooks, 1977; Ignoffo, 1981).

BEAUVERIA BASSIANA

Beauveria bassiana (Balsamo) Vuillemin is a deuteromycete whose life cycle, epizootiology and ecology is very similar to that of *Nomuraea rileyi*. However, this fungus has a much wider host range than *Nomuraea rileyi*. It has been isolated from many different orders of insects. It, too, has been isolated from all major temperate and tropical regions of the world.

Morphologically, *Beauveria bassiana* differs from *Nomuraea rileyi* in the structure of its conidiophores (Samson, 1981). Conidia are produced along zig-zag shaped conidiophores rather than in chains (Figure 22). This configuration is very distinctive and characteristic of the genus, but requires high magnification and careful specimen preparation to be able to discern. The conidiophores resemble those of *Nomuraea rileyi* in that the conidia-bearing cells are produced in whorls along the conidiophores. *Beauveria bassiana* is also distinctive in its production of snowy white conidia which are generally produced in a layer that closely covers the cadaver. In some insects, the

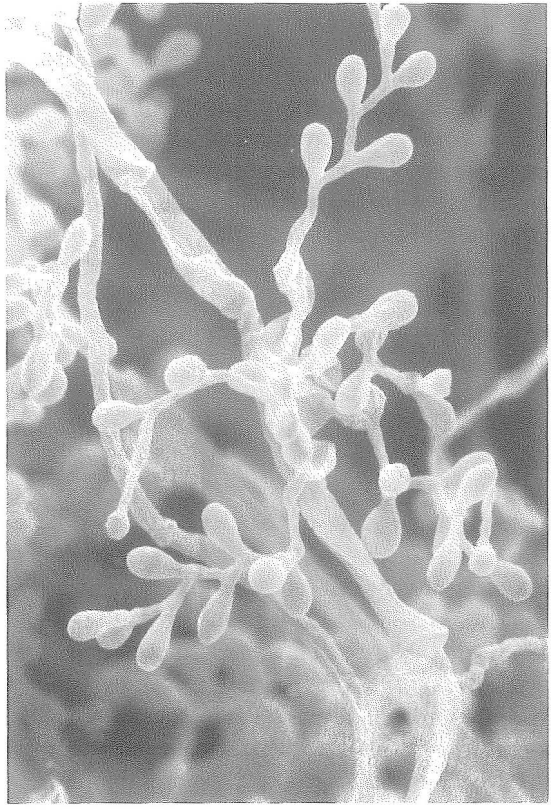

Figure 22. SEM of *Beauveria bassiana* reproductive structures showing spores produced in a zig-zag pattern from single conidiogenous cells. (6,500X.)

conidia are produced in clumps over the body surface, creating a more granular appearing surface.

Beauveria bassiana has been found in nearly every major order of insects including Coleoptera, Lepidoptera, Orthoptera, Hemiptera, Homoptera, Diptera, Hymenoptera and many others. Cotton pests known to have been infected include adult boll weevils (McLaughlin, 1962; Smith, 1991), the tarnished plant bug, *Lygus lineolaris* (Paliot de Beauvois) (Unpublished data, M. J. Gaylor, Entomology Department, Auburn University, Auburn, Alabama), the western plant bug, *Lygus hesperus* (Knight) (Dunn and Mechalis, 1963), and several armyworms, *Spodoptera* spp. (Gardner and Fuxa, 1980; Kenneth and Olmert, 1973). All of these records were encountered under laboratory conditions. Naturally infected insects are rarely found in the field. Wright and Chandler (1991) recently isolated a strain of *Beauveria bassiana* from boll weevil in the Rio Grande Valley of Texas. In addition to the weevil, Wright (1992) has successfully infected the sweetpotato whitefly, *Bemesia tabaci* (Gennadius) and the cotton fleahopper, *Pseudatomoscelis seriatus* under both laboratory and field conditions. The strain is currently being developed as a potential microbial insecticide for use against these and other pests in cotton (see Chapter 15).

Infection, growth, development and sporulation of *Beauveria bassiana* is essentially as described for *Nomuraea rileyi*. Symptoms are also similar with the exception of the color of conidia produced by each. The pure white color of *Nomuraea rileyi* in the conidiophore bloom stage is similar to the color of both the conidiophore and conidial stages of infection by *Beauveria bassiana*. The two can be separated by holding them under humid conditions for conidia production or by microscopic examination of the conidiophores.

The principles relating to transmission, dispersion, and survival for *Nomuraea rileyi* are also applicable to *Beauveria bassiana*.

BACTERIAL PATHOGENS

Bacterial infections in natural populations of cotton pests are not well documented. Isolations of numerous non-pathogenic bacteria from boll weevils and armyworms (McLaughlin, 1962; McLaughlin *et al.*, 1966) and of *Bacillus cereus* Frankland and Frankland from cotton leafworms (Agudelo and Falcon, 1977) in Colombia have been reported. Bacteria such as *Serratia marcescens* Bizio and others are frequently problems in laboratory rearing of insects but are not considered to be important pathogens in field populations (Bucher, 1963). A possible exception is the report by McLaughlin and Keller (1964). They reported weevil larvae shipped from Mexico being heavily infected by this organism. While pathogenic bacteria probably contribute to low levels of disease in many cotton insect pest populations, they are generally unnoticed in routine scouting of fields.

Bacillus thuringiensis, the entomopathogenic bacterium used in commerce under various trade names, is present in most soils, but again, is not known to cause any appreciable natural mortality in populations of insect pests on cotton. This bacterium

is of potential value as a microbial insecticide and is discussed from that standpoint in Chapter 15.

Bacterial infections have very typical symptoms in lepidopterous larvae. Infection requires that the bacterial cells reach the hemocoel of the insect, either by penetrating the gut wall or through wounds. Once in the hemolymph, bacteria grow rapidly by cell division, utilizing the hemolymph as a particularly rich growth medium. The time sequence of progress of infection may vary with bacterium, host, inoculum level, temperature and other factors, but the infected insect will generally show reduced activity within two to three days. Death of the insect occurs soon after reduced activity is evident. Just prior to death, general body color may begin to darken. After death, cadavers become dark brown to black in color. The integument of caterpillars often remains relatively tough and leathery, resisting rupture when handled. Sucking insects usually darken and retain their body shape, but body contents are initially watery. Microscopic examination of tissue smears of these insects will usually reveal heavy concentrations of bacterial cells.

Diagnosis of bacterial infections is often difficult. Insects dying from physical wounding by predators, physiological causes and pesticide poisoning often show typical bacterial symptoms. Further, they may contain large numbers of saprophytic bacteria which have grown opportunistically in the dead cadavers. Diagnosis of bacteria as cause of death requires demonstration of pathogenicity using Koch's postulates, a time consuming and expensive process. Determination of the species of bacteria associated with a cadaver may reveal those that are known pathogens, but specific isolates of such species may or may not exhibit the characteristic of pathogenicity. Thus, one must be extremely careful in diagnosing bacteria as the cause of death in dead, field-collected larvae.

PROTOZOAN PATHOGENS

Protozoa are single celled animals which have a wide variety of ecological roles, ranging from primary producers to consumers. A large number of species are also parasitic or pathogenic in insects and are, in fact, quite widespread throughout the Class Insecta. Major protozoan groups which contain insect pathogens include the amoeba, ciliates, flagellates, sporozoa and microspora. All members of the latter two groups are obligate pathogens and are highly adapted to this mode of existence. While a considerable volume of literature is available on protozoan infections in species of insects that attack cotton, most of the literature again has dealt with these pest species as they affect other crops, particularly soybean and corn. With the exception of the boll weevil, almost no information is available on the interrelationships between protozoa, pest insects and cotton. One reason for this paucity of information undoubtedly is related to protozoan mode of action and the direct nature of damage that many pests cause in cotton. Many protozoan infections are not lethal, but cause debilitating effects (Brooks, 1988) which may include reductions in feeding, movement and fecundity. Such characteristics would reduce, but not prevent, damage to cotton by bollworms,

tobacco budworms, armyworms, weevils and other insect hosts that feed directly on the flowers and fruits. Thus, heavily infected populations might show reduced damage within generations, but that level of damage could be economically important. The same insect species attacking corn or soybean at similar population levels may be satisfactorily regulated because their economic thresholds are higher.

Only three groups of protozoa will be discussed: the Subphylum Mastigophora; the Class Sporozoea; and the Class Microsporea. The Mastigophora are the flagellated protozoa, and only a small portion of the members of the phylum are insect parasites. All members of the latter two subphyla are parasitic (Brooks, 1988), and both contain members that are obligate insect pathogens. Both the Sporozoa (Class Sporozoea) and Cnidospora (Class Microsporea) characteristically produce spores which provide a mechanism for survival outside of the host insect and which provide mechanisms for infection when ingested by their hosts. One major difference between the two subphyla is the presence of one or more polar filaments in the cnidosporan spore and the absence of this structure in sporozoan spores (Brooks, 1974). These are important features in the infection process, as will be discussed.

FLAGELLATE INFECTIONS

Members of the subphylum Mastigophora characteristically move by means of a flagellum or flagella. Normally, flagellates do not cause high mortality in their hosts, but can cause symptoms that include diarrhea and vomiting. They are typically found within the lumen of the alimentary tract or in organs emptying into it. Flagelletosis is frequently seen in hemipterans, including the green stink bug and certain of the staining bugs of the family Pyrrochoridae. The species *Leptomonas serpens* Gibbs was described from the southern green stinkbug, and *Leptomonas pyrrhocoris* Zotta was originally isolated from a stainer bug, *Pyrrhocoris apterus* (L.) in France (Lipa, 1963). In neither case was the work done on these insects as pests of cotton, but the southern green stinkbug can be a serious cotton pest and related stainer bugs, such as *Dysdercus suturellus* (Herrich-Schaffer) (the cotton stainer), are very likely candidate hosts for *Leptomonas pyrrhocoris* or related flagellates.

Transmission of organisms occurs through eating infective stages of the protozoa which have been excreted or regurgitated onto or into host substrates. *Leptomonas serpens* is known to be passed in this way to plant sap where it can grow and be picked up later by subsequently feeding insects (Gibbs, 1957). The organisms normally attach to the gut or other organ walls and grow and reproduce to large numbers at these sites. Diarrhea is the principal symptom in these cases. In some hosts, the flagellate is able to enter the hemocoel and cause more serious damage, evidenced in *Pyrrhocoris apterus* as lowered activity, lighter color and thicker, whitish hemolymph (Lipa, 1963).

SPOROZOAN INFECTIONS

McLaughlin (1965 a,b; 1967,1971) conducted extensive work on the relationship between the sporozoan *Mattesia grandis* McLaughlin and the boll weevil. This pathogen infects larvae following ingestion of spores. These release numerous smaller

infectious sporozoites which are able to pass through the intestinal wall and reach the fat body inside the body cavity. There, they multiply rapidly through several developmental stages until they ultimately form more spores (Figure 23). Mortality may begin as early as seven days but McLaughlin found peak mortality to occur at 14 days post-inoculation. Adults were also susceptible, and infection reduced both egg production and adult longevity in laboratory studies (McLaughlin, 1965b).

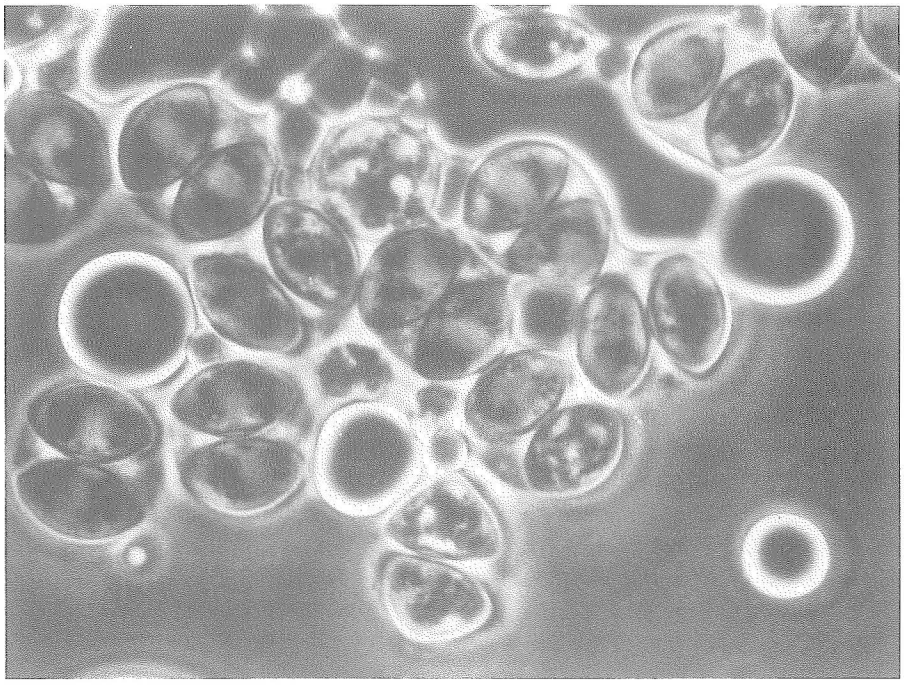

Figure 23. Gametocytes of the sporozoan *Mattesia grandis,* each containing two oocysts, from boll weevil fat tissue. (Phase contrast microscopy) (Courtesy of R. McGaughlin.)

This pathogen was found originally in laboratory cultures of weevils in Mississippi, but McLaughlin (1965a) speculated that it may have entered the colonies from material that was field collected in Tamaulipas, Mexico. He and his colleagues conducted extensive field tests with this pathogen (see Chapter 15).

CNIDOSPORAN INFECTIONS

A second protozoan group, the microsporidia, are found in a large number of insect species that attack cotton, but little work has been done on them as they influence this crop. The pathogens are members of the genus *Nosema* and are found in most lepidopterous pests of cotton, the green stinkbug (Personal communication, J. Maddox,

Illinois National History Survey, Champaign, Illinois), the boll weevil (McLaughlin, 1969) and probably many others. These pathogens, depending on dosage and on the specific host-pathogen involved, may be lethal or debilitating in their action on their hosts.

The microsporidia, like the sporozoans, have a complex life cycle composed of many successive and morphologically distinct stages (Brooks, 1974). The extrahost stage is a somewhat resistant spore (Figure 24) which can exist outside of the host for some

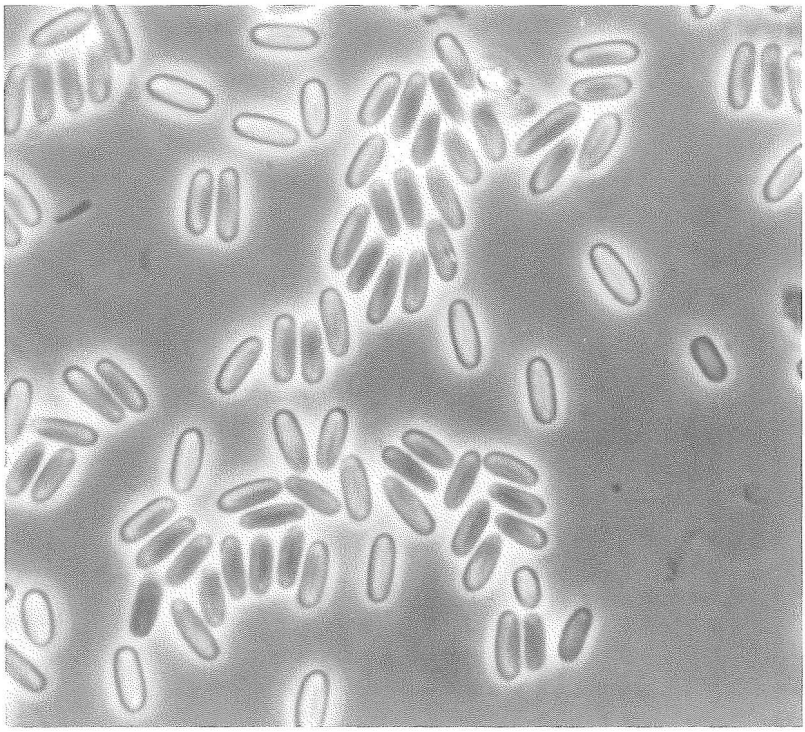

Figure 24. Mature spores of a microsporidian typical of those seen in the hemolymph of infected larvae. (Phase contrast microscopy, 2000X). (Courtesy of W. M. Brooks.)

period of time. It must be ingested to initiate an infection. The spore contains a long hollow tube, the polar filament, which lies tightly coiled within the spore (Figure 25). This filament is forcefully released from the spore once inside the insect's gut. It serves as a sort of living hypodermic needle to aid in infecting the host. A small piece of tissue, the nucleated sporoplasm, is ejected through this tube. If the polar filament is oriented in the gut in such a way that its eversion, or forceable release, results in penetration of the gut wall, the sporoplasm will be placed inside the hemocoel or fat body and will begin developing. Inside the cytoplasm of susceptible tissues, the pathogen multiplies through a series of stages involving nuclear divisions, nuclear and cytoplasmic division, formation of several morphologically distinct stages and ultimately spore formation.

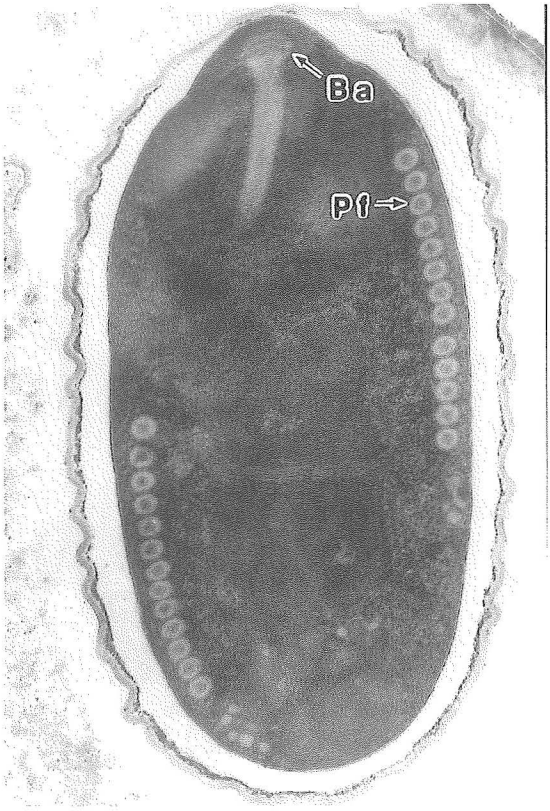

Figure 25. Thin section of a microsporidian clearly showing the coiled polar filament (Pf) and its basal attachment point (Ba). (TEM, 20,000X.) (Courtesy of C. B. Moore.)

Tissues infected vary with host and microsporidian species involved, but most frequently infection involves the fat body (Figure 26) with subsequent infection of silk glands, epidermis, gut epithelium, Malpighian tubules, nerve tissue and hemocytes (Brooks, 1974). The infectious process, from spore ingestion to spore production may require from few to many days, depending on temperature, dosage, and other factors.

Infection by microsporidia may be either chronic or acute, depending on the host, pathogen, dosage, environment and other factors. Frequently, mortality does not result from infection, but feeding and reproductive capacity are reduced. Thus, infected populations tend to cause much less damage than would be caused by equal numbers of healthy individuals.

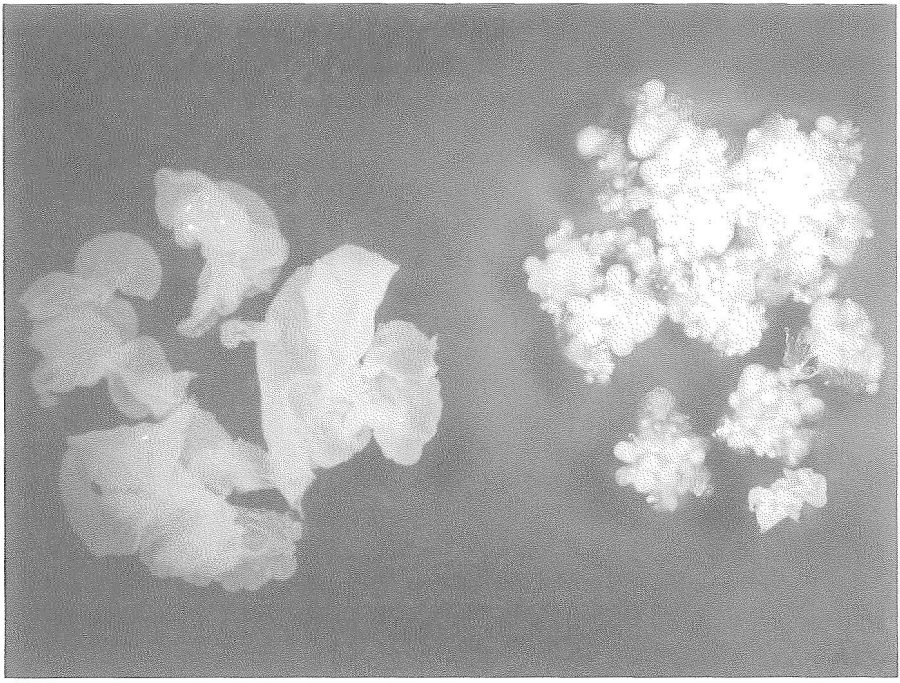

Figure 26. Comparison of fat body tissue from healthy (left) and *Vairimorpha*-infected (right) bollworm larvae. Note milky cloud around infected tissue caused by released spores. (About 5X.) (Courtesy of W. M. Brooks.)

Cotton pests known to suffer from microsporidiosis and the pathogens involved include: *Heliothis* and *Helicoverpa* spp. by *Nosema heliothidis* Kramer (Figure 27) and *Vairimorpha necatrix* (Kramer) (Figure 24); the southern green stinkbug by an undescribed microsporidian (Unpublished data, J. Maddox, Illinois National History Survey, Champaign, Illinois), the boll weevil by *Nosema gasti* (McLaughlin) (McLaughlin, 1969); and the cabbage looper by *Nosema trichoplusia* Tanabe and Tamashiro (Tanabe and Tamashiro, 1967). This list is not exhaustive or complete. A complete list would be complicated by the question of cross-infectivity (Brooks, 1988) since all of the above pathogens have been shown to be highly cross-infectious to other species. For example, *Nosema gasti* was infectious to many Lepidoptera including the important cotton pests—the tobacco budworm, pink bollworm, bollworm and cabbage looper (Ignoffo and Garcia, 1965).

While little work has been done on these pathogens in relation to cotton, they are very likely present and possibly at times prevalent in field populations. If cotton production should become less dependent on chemical pesticide inputs in the future, these pathogens will likely receive much more attention.

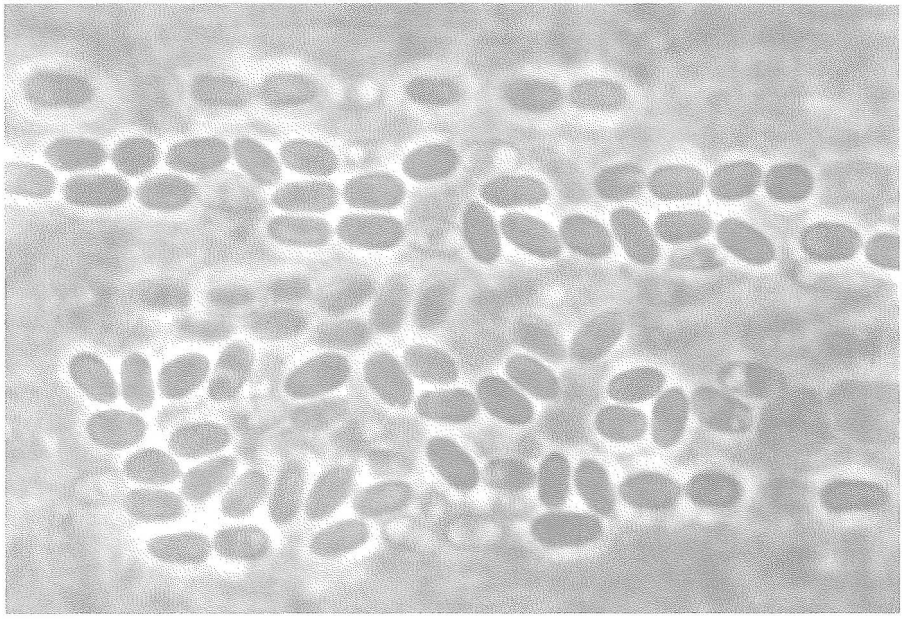

Figure 27. Spores of *Nosema heliothidis* in midgut tissue of bollworm (wet mount, phase contrast microscopy, about 1800X). (Courtesy of W. M. Brooks.)

NEMATODES

A wide variety of nematodes have been reported as obligate or facultative endoparasites of many insect species. Parasitism by nematodes may result in sterility, reduced fecundity, delayed development, aberrant behavior or host death. These effects may play a significant role in regulating insect populations (Kaya, 1987). Although numerous reports of nematode parasitism in insect populations have been published, only a few of these are from insects found in cotton. Those reported from cotton insects fall into three families: Mermithidae; Steinernematidae; and Heterorhabditidae. Members of the Mermithidae can easily be distinguished from the other two families by the size and numbers of nematodes found in each insect host. If a host contains only one or two worms that measure one inch or longer, the nematode is probably a mermithid. Hosts parasitized by steinernematids and heterorhabditids will usually contain several thousand or more juvenile nematodes (0.2 to 0.5 millimeter) and some larger adult nematodes (1 to 5 millimeter) (Nickle, 1974).

MERMITHIDS

The mermithids constitute a large group of obligate parasites of invertebrates. They are common in both terrestrial and aquatic environments and are generally host spe-

cific for a small group of insects. Mermithids are nearly always lethal to their host and most are considered to have potential as biological control agents (Peterson, 1982).

Mermithids are relatively long nematodes, with some adults reaching a length of 11 inches (30 centimeters) in insect hosts. They are usually light colored, appearing as whitish worms when observed emerging from their hosts. Most mermithids have a direct type of life cycle. The short-lived, non- feeding infective-stage juvenile emerges from an egg and searches for a host. Using its stylet, it bores through the insect's body wall and enters the hemocoel where it develops. After a development period of five days to several months, depending on the species, the full-grown parasite emerges, molts to the adult stage in the environment, mates and deposits eggs (Poinar, 1983).

Although there are hundreds of reports of mermithid parasitism, very few are from cotton insects and most of these are from host plants other than cotton. Stadlebacher *et al.* (1978) reported parasitism levels of 39 to 47 percent by *Hexamermis* spp. in bollworm larvae collected from clover and vetch in Mississippi. Nickle (1978) reported parasitism of the fall armyworm by *Hexamermis* spp. in Nicaragua. This same nematode infected the beet armyworm, under laboratory conditions. Puttler *et al.* (1973) reported a 64 percent level of parasitism by *Hexamermis arvalis* in the black cutworm, *Agrotis ipsilon* (Hufnagel), from corn.

STEINERNEMATIDAE AND HETERORHABDITIDAE

Members of these two families are entomogenous nematodes which have been recovered from many areas throughout the world. They are selective for insects and a few other arthropods, but do not adversely affect mammals or plants. Because they kill their hosts rapidly (24 to 48 hours) and have a wide host range there has been a great deal of interest in their use as biological control agents (Woodring and Kaya, 1988). Because of similarities in their life cycles, the two families will be discussed together.

Like most members of the order Rhabditida, the steinernematids and the heterorhabditids are bacterial feeders, but they differ from other rhabditids by having a mutualistic association with specific bacteria in the genus *Xenorhabdus*. Two species of these bacteria are recognized. *Xenorhabdus nematophilus* (Poinar and Thomas) is a non-pigmented associate of steinernematids, and *Xenorhabdus luminescens* Poinar and Thomas is a red-pigmented, bioluminescent symbiont of heterorhabditids. These bacteria do not have an environmentally resistant stage and have never been isolated except from their nematode vectors or their vectors' insect hosts (Woodridge and Kaya, 1988).

Like most nematodes, members of these two families have a simple life cycle that includes the egg, four juvenile stages and the adult. The infective stage is a special third stage juvenile or dauer larva which is ensheathed within a separate, but still intact, cuticle from the previous juvenile stage (Figure 28) and is particularly resistant to environmental conditions. These infective juveniles contain live cells of the mutualistic bacterium in their intestines and function as vectors of the bacterium. The infective nematodes locate a host and enter through natural body openings—mouth, anus or spiracles. They then penetrate through the midgut wall or tracheae into the hemo-

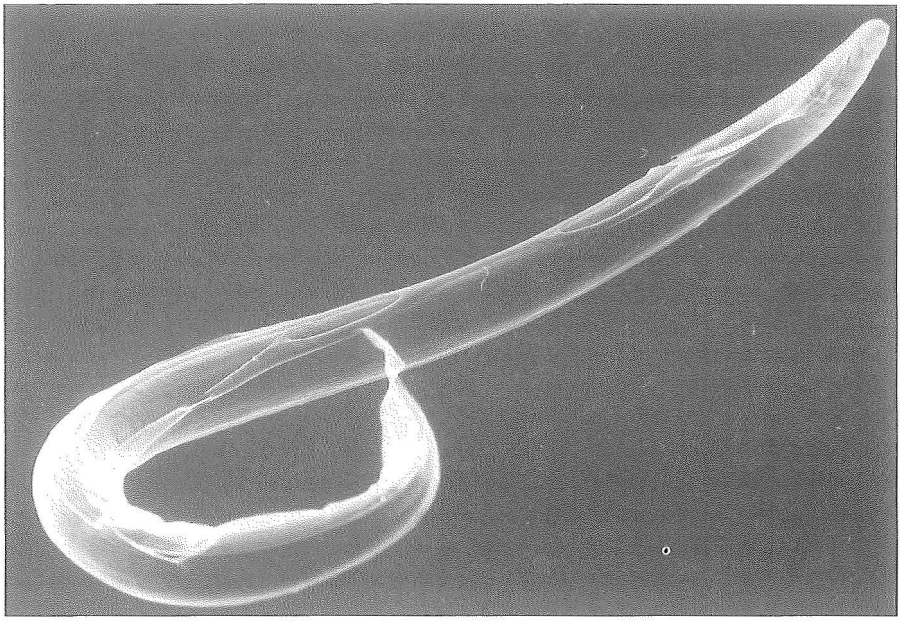

Figure 28. Infectious third stage juvenile or dauer larva of *Steinernema carpocapsae* (Family Steinernematidae). Note the wrinkled outer sheath evident near the lower posterior end of the body. (SEM, 560X.)

coel. The infective juveniles of the heterorhabditids differ from the steinernematids by having a tooth. They are able not only to enter through natural openings, but also may directly penetrate through soft areas of the cuticle of a host. Once in the hemocoel, the nematodes release the bacterium which rapidly multiplies and kills the host within 48 hours. The immature nematodes feed on the bacteria and develop to adults which mate and produce progeny. The nematodes will pass through two or three generations within the host. When conditions are suitable, the infective juveniles will exit the cadaver to seek new hosts. The entire cycle requires 10 to 14 days (Woodridge and Kaya, 1988).

Although members of these two families are known to be widespread and are commonly isolated from soils, there are very few reports of their natural occurrence in cotton insects. Poinar (1975), in his original description of the family Heterorhabditidae, reported that the original collection of the type species, *Heterorhabditis bacteriophora* Poinar, was from a pupa of *Helicoverpa punctigera* from Australia. Kahn *et al.* (1976) described another species of nematode in the same genus, *Heterorhabditis heliothidis* Kahn, Brooks, and Hirschmann from prepupal and pupal specimens of the bollworm from North Carolina. Poinar (1990) now considers both of these to be the same species, *Heterorhabditis bacteriophora*. Recently, Cabanillas *et al.* (1994) described a new species of steinernematid nematode, *Steinernema riobravis,* which was originally found infecting bollworm and fall armyworm in corn fields in the Lower Rio Grande

Valley near Weslaco, TX (Raulston et al., 1992). This species is of interest because it appears to be adapted to a semi-arid environment and can survive at higher soil temperatures than can other species in this genus (Cabanillas *et al.* 1994). No reports have been made on its natural occurrence in cotton, but it may have promise for development as a control agent for these and other cotton pests because of its adaptation to hot, semi-arid environments. Akhurst and Brooks (1984) conducted a survey for steinernematid and heterorhabditid nematodes in North Carolina. They collected over 500 soil samples from cropland, pasture, and forest habitats at 53 sites. Nematodes were isolated from 25 of the sites and were more common in cropland and pastures than in forest soils. Heterorhabditids were more abundant (84 percent of isolates) than steinernematids. It is probable that nematodes of both families are present in cotton fields and cause mortality in those insects which spend part of their life cycle in the soil.

SUMMARY

It is evident from the information presented in this chapter that the large number of pest insects and mites associated with cotton has an even larger number of microbial and nematode pathogens associated with them. Most of these do not occur predictably at sufficiently high incidence levels to produce noticeable natural reductions of their host populations. Notable exceptions do occur, such as the epizootics of nuclear polyhedrosis virus in cabbage looper populations and of the fungus *Neozygites fresenii* in cotton aphid populations. Some, such as the NPV of the beet armyworm or the fungus *Neozygites floridana* on twospotted spider mites may occur in high incidence in some fields in some years, but not consistently. Most, however, occur either in such low levels that they are rarely noticed, or they are overlooked because their symptoms do not allow for ready diagnosis or recognition.

Individually and collectively these pathogens are very important in cotton pest management, and have far greater potential than has been realized to this date. As natural mortality factors, some contribute significantly to suppression of their host popultions. Others, as has been pointed out throughout this chapter, are currently of value or have potential future value for development as microbial control agents. As the boll weevil eradication program expands across the cotton belt of the United States, less pesticide use on cotton will result in opportunities for managing other pests in different ways than at present. Reliance on naturally occurring or artificially manipulated pathogens has considerable promise for current and future insect and mite management programs.

There is a definite information gap on pathogen-host relationships in cotton. As stated previously, most information on pathogens from cotton pests has been collected from other crop systems. Until this knowledge gap is filled, we will be unable to fully appreciate and take advantage of the roles that these organisms are playing or can play in cotton insect and mite pest management.