Bacterial, Viral, and Fungal Insecticides

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Summary. Microorganisms that are pathogenic to insects provide a wealth of biological material that can be exploited by humans to control insect pests. Innovative applications of a few such entomopathogens are found throughout the world, but widespread commercial production of microbial insecticides awaits further studies of the biology, ecology, and pathogenicity of the agents. Genetic engineering techniques may be used to increase the virulence of these microorganisms, as well as to make them more tolerant of physical and chemical conditions and perhaps to broaden their host ranges. The use of microbial insecticides could decrease our dependence on chemical pesticides.

Approximately 1500 naturally occurring microorganisms or microbial by-products have been identified as potentially useful insecticidal agents. The utilization of these microorganisms to regulate insect populations within a defined geographical area constitutes the field of microbial insect control and represents an exciting challenge in applied molecular biology and ecology.

This interest in microbial insecticides is largely a result of the many problems associated with the extensive use of chemical pesticides. Not only do chemical pesticides generally affect beneficial insects as well as pest species, but insects tend to acquire resistance to the chemicals so that new pest problems rapidly develop. Furthermore, chemical residues pose environmental hazards and health concerns. Thus microbial insecticides are seen as an alternative means of pest control that can play an important role in integrated pest management systems and reduce our dependence on chemical pesticides. Microorganisms that are pathogenic to insects (entomopathogens) have relatively narrow host ranges, which makes it possible to reduce specific pest populations while natural predators and beneficial insects are preserved or given the opportunity to become reestablished. Insect resistance to microbial pesticides tends to be less common or to develop more slowly than resistance to chemical pesticides. As far as is known, entomopathogens now produced commercially do not affect humans or animals other than their target species, and their by-products are biodegradable.

A microbial insecticide is expected to be (i) convenient to apply (as a dust, spray, or bait, alone or in conjunction with chemicals, predators, parasites, or other pathogens), (ii) storable, (iii) economical, (iv) easy to produce, (v) virulent, and (vi) safe and aesthetically acceptable (*1*). Because they must be ingested to cause infection, viruses and bacteria need to be applied at the time the target insect begins feeding, that is, before it has caused any major economic damage. Pathogenic fungi do not necessarily have to be ingested in order to cause infection, but may disable or kill the insect by colonizing its surface. The agents should reach the target insect in sufficient quantity to kill or disable it in a vehicle that does not harm the pathogen. No harmful residues should remain on the commodity treated, especially when diluents or carrier materials are used.

Knowledge of the relation between the particular host insect and the pathogen is important in determining the strategy to be used in controlling the pest. Knowledge of the effect of environmental and physical factors on the host and pathogen is also important. For example, sunlight, temperature, rain, and relative humidity can influence both the behavior of the insect and the ability of the microorganism to infect it; the mode of application of the agent, the carrier material, dose standardization, and scheduling of the application can all determine the effectiveness of short- and long-term insect control. The spores of some bacteria (for example, *Bacillus thuringiensis*) can survive most environmental changes that might be encountered by their insect hosts, whether the spores are applied to field crops or to commodities held in storage. Many other microbial agents, however, particularly viruses, require the use of various techniques to protect them.

Over the past decade, technologies involving fermentation, insect cell culture, and insectaries have been developed for the production of microbial insecticides. Fermentation methods are used for producing bacterial and fungal insecticides, whereas insectaries are used for virus production. Some techniques, such as cell culture for the propagation of obligate parasites or pathogens, have not yet been adapted for large-scale commercial production of microbial agents.

Entomopathogens that are proposed for use as control agents must be scrutinized for their possible effects on the health of humans and other animals, as well as for their effects on the environment. Microbial insecticides that are already being sold commercially have met certain safety, efficacy, and environmental criteria that have been established by the Environmental Protection Agency (EPA). In this article we discuss some of the properties of certain bacterial, viral, and fungal pesticides that are already in commercial production or that show promise of being of some use economically (*2, 3*).

Bacterial Insecticides

Most bacteria pathogenic to insects are members of the families Pseudomonadaceae, Enterobacteriaceae, Lactobacillaceae, Micrococcaceae, and Bacillaceae. Except for the Bacillaceae, these families contain nonsporulating microorganisms. Most spore-forming bacteria pathogenic to insects belong to the family Bacillaceae. About 100 bacteria have been reported as entomopathogens, but only four (*Bacillus thuringiensis*, *B. popilliae*, *B. lentimorbus*, and *B. sphaericus*) have been closely examined as insect-control agents. These species are sporeformers; the first two produce, in

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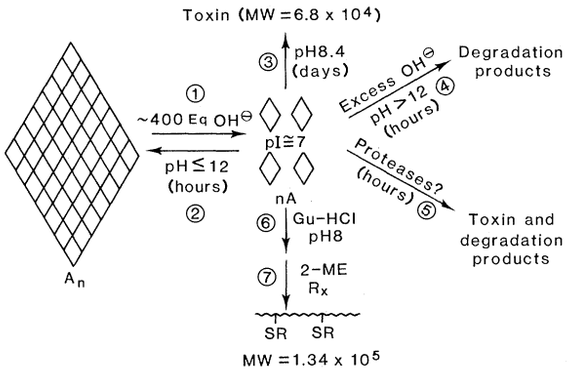

Fig. 1. Schematic diagram of the behavior of the protoxin from *B. thuringiensis* in solution. The reactions are described in the text. Abbreviations: *Eq OH*$^-$, equivalent base; *Gu-HCl*, guanidine hydrochloride; *2-ME*, 2-mercaptoethanol; R_x, alkylation reaction; *SR*, alkylated disulfide linkages.

addition to the spore, discrete crystalline inclusions within the sporulating cell; the last two normally do not. Of these four organisms, *B. thuringiensis* is the only bacterium that has been developed successfully as a commercial insecticide on a very large scale and now is sold in several countries. The EPA has registered it for use on field crops, trees, ornamentals, home vegetable gardens and, more recently, stored products (primarily grain and grain products) to control lepidopteran larvae.

The primary insecticidal activity of *B. thuringiensis* resides in a parasporal glycoprotein crystal that is synthesized and crystallized within the parent cell (*4, 5*). Most studies of *B. thuringiensis* have focused on the field application and efficacy of the organism. However, there has been a recent upsurge of interest in the molecular biology of *B. thuringiensis*, particularly as it relates to toxin synthesis. This organism provides an ideal model for studying the formation and regulation of a protoxin and its subsequent conversion to a toxin.

Bacillus popilliae and *B. lentimorbus* are pathogens of scarabaeid larvae and cause "milky disease" of larvae of the Japanese beetle (*6*). Characteristics of these pathogens are those of an effective biological control agent. They grow in the hemolymph of the insect larvae and ultimately accumulate billions of spores before the host dies. The spores permit the pathogen to survive in an inert state for long periods in the soil and are the means of disease transmission. To produce spores of *B. popilliae* and *B. lentimorbus* commercially, the bacteria must be grown in living larvae of the Japanese beetle; artificial culture methods are not yet available. *Bacillus sphaericus* apparently synthesizes a toxin (*7*) that is active against mosquito larvae, but the toxin has not yet been characterized.

The Toxin of *B. thuringiensis*

Subspecies *kurstaki*

The insecticidal activity of *B. thuringiensis* subspecies *kurstaki*, as well as that of other subspecies, is associated with a parasporal glycoprotein crystal that is synthesized within the organism during its sporulation cycle. In its native state, the glycoprotein is a protoxin that is solubilized and activated after ingestion by an insect that is susceptible to the toxic product (*8*). The protoxin can be activated in vitro by exposure to alkaline buffer. Maximum solubility of the crystal protoxin occurs within several hours after the crystal is titrated with alkali. The protoxin is stable for several hours thereafter, but then begins to degrade into small fragments with a concomitant loss in insecticidal activity. Reaggregation also occurs, especially after the *p*H is lowered to near neutrality or near the experimentally determined *p*I of the protoxin, *p*H 7 (*9*). A range of molecular weights has been reported (*10*) for the protoxin (native subunit), from 0.9×10^5 to 1.3×10^5, depending on the method used for solubilization and for size determination. When material solubilized by a mild titration procedure (*9*) is examined, all molecular weight determinations give essentially the same result of 1.34×10^5. Also, a single NH_2-terminal residue is detectable in quantitative yield. The crystal protein may be an intact product of translation since methionine is the NH_2-terminal residue.

Upon prolonged incubation of the protoxin at slightly alkaline *p*H, a glycoprotein toxin (apparent molecular weight, 6.8×10^4) is generated (*11*). This glycoprotein remains toxic at room temperature for several months at neutral *p*H. It is the smallest toxic component that has been found by alkali treatment and any further breakdown appears to be detrimental to toxic activity. When compared to the protoxin, the 68,000-dalton polypeptide has very similar toxicity and, like the protoxin, is two to three times more insecticidal than the native crystal.

Figure 1 shows a schematic diagram of the behavior of the protoxin and toxin in solution. The crystal (A_n) is made up of many subunits that are dissociated in native conformation (*n*A) by mild alkali titration (reaction 1). Reaggregation occurs slowly (reaction 2), especially at *p*H 12; also, the subunit may break down to smaller fragments (reaction 4). Degradation may be further stimulated by contaminating proteases that cofractionate with the crystals during isolation. Consequently, the subunit must be stabilized

Table 1. Characteristics of the subspecies of *B. thuringiensis*.

israelensis	*kurstaki, berliner, alesti, tolworthi*
1. Crystal size and shape varies	1. Crystals are about the same size and have a bipyramidal shape
2. Protoxin subunit has apparent molecular weight of 1.34×10^5	2. Protoxin subunit has apparent molecular weight of 1.34×10^5
3. Major crystal breakdown product has apparent molecular weight of 2.6×10^4	3. Major crystal breakdown product has apparent molecular weight of 6.8×10^4
4. More lysine, threonine, proline, valine, methionine, and isoleucine in crystals	4. More arginine, serine, glutamine, and glycine in crystals; four strains very similar
5. Ineffective against Lepidoptera; toxic to mosquitoes	5. Ineffective against mosquitoes; toxic to Lepidoptera
6. Crystals are immunologically different from other strains	6. Crystals are immunologically related
7. Crystals contain glucose, mannose, fucose, rhamnose, xylose, and galactosamine	7. Crystals contain glucose and mannose
8. Crystal peptide map has major spots not found in maps of other strains	8. Crystal peptide maps are similar in the four strains
9. Crystal protein contained in spore coat along with low molecular weight proteins; the size of the protein differs from that in the other strains	9. Crystal protein contained in spore coats along with low molecular weight proteins; similar sizes in the four strains

by placing it in a denaturing solvent and alkylating the disulfide linkages (SR) (reactions 6 and 7). The toxin can be generated by prolonged incubation at slightly alkaline *p*H (reaction 3). Whether this reaction is similar to the mechanism of activation in vivo (reaction 5) is not known.

Insecticidal Proteins from Other Subspecies of *B. thuringiensis*

Bacillus thuringiensis subspecies *kurstaki* is important in agriculture and forestry because it kills leaf-eating Lepidoptera (particularly larval forms of moths). Recently, another potentially useful strain, *B. thuringiensis* subspecies *israelensis*, was isolated. This microorganism produces a toxin effective against Diptera such as mosquitoes and blackflies (*12*). Tyrell *et al.* (*13*) have shown that subspecies *israelensis* produces crystals that are structurally, biochemically, and immunologically different from the protoxin produced by the *kurstaki* subspecies. It appears that the closely related subspecies that are toxic to Lepidoptera, including *kurstaki*, *berliner*, *alesti*, and *tolworthi*, all produce crystals that are similar to each other structurally, biochemically, immunologically, and functionally (Table 1). These four subspecies synthesize bipyramidal crystals, and usually each bacterium contains only one such crystal. Subspecies *israelensis* forms crystals of various shapes and each bacterial cell contains two or three crystals. Electrophoretic profiles of solubilized crystal proteins from the five subspecies reveal a common protein of 1.34×10^5 daltons, similar to the size of the *kurstaki* protoxin (*9*). Presumably, this component is converted to a 68,000-dalton toxin in all of the subspecies. Proteins of approximately this size are the smallest found in electrophoretic profiles of the crystals toxic to Lepidoptera. Crystals of the subspecies *israelensis* have a smaller major component (26,000 daltons) which may also be a product of proteolysis during solubilization and activation of the 134,000-dalton protoxic molecule. However, the 26,000-dalton protein does not appear to be toxic.

All five subspecies have six major tryptic peptides in common, and peptide maps of the subspecies toxic to Lepidoptera are almost identical (Table 1). However, peptide fingerprints of subspecies *israelensis* crystals show four major peptides not present in the other four subspecies. Differences also occur in the number and amount of minor peptides, in the amino acid composition, and in the carbohydrate content (Table 1). Crystal protein is a major spore coat component in all of the subspecies, and extracts of the spore coat protein from subspecies *kurstaki* and *israelensis* are insecticidal (*14*).

The Insect Toxin Gene of *B. thuringiensis*

To better understand the structure of the insecticidal crystalline protein of *B. thuringiensis* and the regulation of its synthesis, investigators in several laboratories have cloned the gene that codes for the protein in subspecies *kurstaki* (*15–17*). In particular, a recombinant Charon 4A phage, C4K6c, was isolated by Held *et al.* (*16*) and found to produce, when grown in *Escherichia coli*, a protein that reacts with antibodies specific for the crystalline protoxin protein of *B. thuringiensis* subspecies *kurstaki*. Alkali extracts of C4K6c lysates are toxic to insect larvae, and the degree of toxicity is consistent with the quantity of antigen found in lysates of phage-infected cells. Cells of *E. coli* infected with C4K6c produce a polypeptide antigen of the same size as the protoxin molecule obtained by alkali solubilization of *kurstaki* protoxin. Held *et al.* (*16*) identified a 4.6-kilobase-pair (kbp) Eco RI fragment from C4K6c that contains all or part of the toxin gene and subcloned it into two plasmids, pBR328 and pHV33. Both *E. coli* and *Bacillus subtilis* containing these recombinant plasmids produced antigen that cross-reacted with antibody directed against the protoxin. The 4.6-kbp Eco RI fragment is of chromosomal origin, although a homologous protoxin gene is also present on a 45-kbp plasmid carried by the *kurstaki* subspecies. Several acrystalliferous nontoxic mutants that have been isolated lack the 45-kbp plasmid and, in some mutants, all plasmids are absent. All of the mutants contain the chromosomal gene but do not produce protoxin antigen. The function of the plasmid gene in the expression of insecticidal activity is not known.

Viral Insecticides

Many viruses that belong to the families Baculoviridae, Poxviridae, Reoviridae, Iridoviridae, Parvoviridae, Picornoviridae, and Rhabdoviridae, as well as a number of unclassified viruses, are pathogenic in insects (*17–20*). Some of these viruses cause natural epizootic diseases within insect populations and a few have been commercially developed as pesticides. In the United States, the EPA has registered, or is considering for registration, several baculoviruses including the nuclear polyhedrosis viruses (NPV's) that infect *Heliothis zea* (cotton bollworm), *Orgyia pseudotsugata* (Douglas fir tussock moth), *Lymantria dispar* (gypsy moth), *Autographa californica* (alfalfa looper), and *Neodiprion sertifer* (European pine sawfly) and a granulosis virus (GV) that infects *Cydia pomonella* (codling moth).

The NPV and GV baculovirus subgroups have received the most attention as viral pesticides for several reasons: (i) viruses within the family Baculoviridae are known to cause lethal infections only in invertebrates and, therefore, are thought to be inherently safer than other insect viruses that are more apparently related to vertebrate or plant viruses (*21*); furthermore, members of the Baculoviridae usually have a relatively narrow host range. (ii) Some baculoviruses are highly pathogenic in insects and can produce sufficient progeny virus per insect [for example, 10^{10} occluded (embedded) virus per larva] to allow commercial production. (iii) The virus particles of NPV's and GV's are occluded in proteinaceous crystals. The viruses are thus more stable in the environment, have a long shelf-life as commercially prepared microbial pesticides, and are compatible with pesticide formulations, other agrochemicals, and pesticide application methods that are currently used in the field.

When used as pesticides, occluded viruses are usually sprayed on foliage; insects that consume virus-contaminated foliage acquire disease. Semiautomated technology has been developed for insect rearing, diet production, and virus preparation (*22*). When the insect hosts are not amenable to mass rearing by the established technology (for example, sawflies) it is sometimes possible to produce NPV's commercially by infecting insects collected in the field (*23*). Some viruses replicate well in insect cell cultures and the feasibility of producing them in such cultures is being considered (*24*). Before field application, viruses are formulated with substances that will protect them from ultraviolet light and with other agents that will optimize the storage, wetting, suspension, flow, and dispersal of the virus during spraying (*25*).

Nonoccluded baculoviruses, such as those infecting the rhinoceros beetle and the citrus red mite, are also used in pest management programs (*26*). Instead of being applied by spraying, nonoccluded viruses are usually introduced within in-

fected insects which then initiate an epizootic disease. For those viruses that spread rapidly in an insect population, horizontally and vertically, such methods can be effective in reducing populations below the economic damage threshold for a number of years. Viruses that are not highly virulent but produce chronic, persistent infection have been used with the idea of stressing the insect population (*27, 28*).

Baculovirus Infections

The important role of baculoviruses in insect control has stimulated interest in their biochemistry and mechanisms of infection [for reviews see (*18, 19, 29–32*)]. Baculoviruses possess large (about 100 to 200 kilobases), double-stranded, circular, covalently closed DNA genomes that are packaged in enveloped, rod-shaped capsids approximately 40 to 140 by 250 to 400 nanometers.

In the occluded forms of baculoviruses, the virions (enveloped nucleocapsids) are embedded in a crystalline protein matrix. Occluded baculoviruses belong to two subgroups based on the morphology of the occlusion; NPV's contain many virions embedded in a single, large (up to 15 micrometers) polyhedral crystal, whereas GV's contain a single virion embedded in a small crystal. The crystalline protein matrix is primarily composed of a single 25,000- to 33,000-dalton polypeptide. The virions are thus protected in the environment during transmission of the virus from insect to insect and in their passage through the foregut of the insect to the midgut where the alkaline *p*H in the gut lumen solubilizes the crystal, possibly by ionizing tyrosine residues clustered at the NH_2-terminus of the matrix proteins (*33*).

The virions are released from the matrix and begin infection of the midgut columnar cells by fusion with microvillar membranes. Infections with some baculoviruses are confined to these midgut cells. Infections with others may be more extensive, with the virus replicating in the midgut cells and producing nucleocapsids that bud through the basement membrane into the hemolymph of the insect (*34*). The nonoccluded virions that are released into the hemolymph are responsible for the systemic infection in the insect. Virus replication in the secondary tissues results in both the budding of nonoccluded virus into the insect hemolymph and the formation of occluded virus in the nucleus. Nonoccluded viruses, but not occluded viruses, continue the spread of infection within the insect; occluded viruses require an alkaline *p*H (> 10) to release the virions. Upon death of the insect and disintegration of the integument, the occluded viruses are released and, if consumed by susceptible hosts, spread the infection.

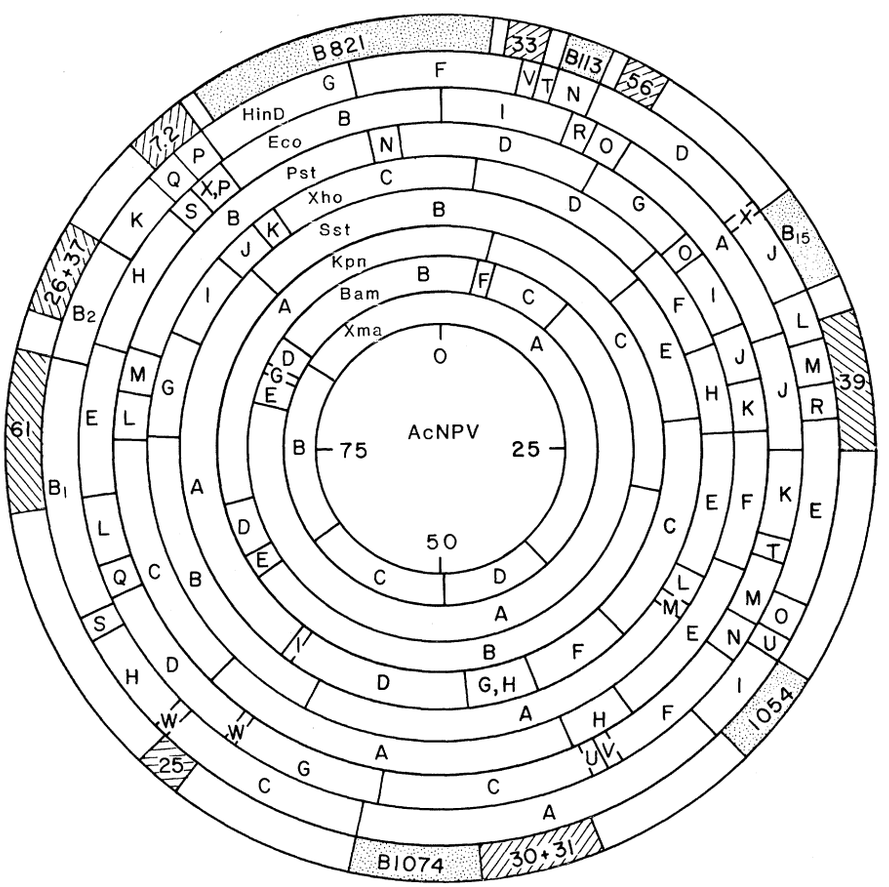

Fig. 2. The physical map and gene organization of the baculovirus AcNPV. The physical map of the restriction endonuclease sites of the 128-kilobase circular, double-stranded DNA genome is presented in the inner eight concentric rings. The outer concentric circle presents information on the gene organization of AcNPV. The stippled areas refer to the position of some of the temperature-sensitive mutations (for example, ts-B113) which have been mapped by marker rescue. The hatched areas refer to the regions encoding various structural and nonstructural proteins of AcNPV as determined by hybrid selection and translation of messenger RNA in vitro. The size of the proteins are given in number of kilodaltons.

Molecular Biology of a Baculovirus

The most extensively studied baculovirus is the *Autographa californica* nuclear polyhedrosis virus (AcNPV) which has a relatively broad host range: it productively infects over 28 different lepidopteran species. This virus replicates in several lepidopteran cell cultures producing both occluded and nonoccluded forms. With the use of plaque purification in cell culture, a genetic characterization of AcNPV has been initiated with the isolation and characterization of temperature-sensitive (ts) mutants (*35*). Other mutants, which either have morphologically altered occluded forms (*36*) or produce only a few occluded viruses per cell (*37, 38*) have also been isolated and characterized.

Plaque purification and restriction endonuclease analysis of a wild population of AcNPV revealed the presence of many closely related variants differing in the number and size of the DNA fragments produced by restriction endonuclease digestion (*39*). The use of a virulent, plaque-purified virus for pesticide purposes has been recommended to minimize the presence of defective interfering viruses (*40*). Restriction endonuclease analysis has been recommended as a means of quality control and detecting genetic variations in commercial virus preparation.

The AcNPV DNA genome (128 kilobases) has been extensively mapped by restriction endonucleases (*41–44*) (Fig. 2) and is primarily composed of unique nucleotide sequences (*41, 43*). Correlation of the physical restriction map with specific viral proteins or functions is being approached in several ways (*45,*

46). A marker rescue technique has been developed which involves gene replacement. The technique can be used to correlate AcNPV mutations with respect to the physical map and also to genetically engineer AcNPV (47, 48). The "hybrid-selection" technique is being used to correlate specific regions of the AcNPV physical map with specific AcNPV proteins (46) (Fig. 2).

The synthesis of virus-induced proteins in AcNPV-infected cells is a temporally controlled process (49–51). Working with a close relative of AcNPV, Kelly and Lescott (51) defined four stages of viral-induced protein synthesis on the basis of function-blocking experiments; an early α phase approximately 2 to 3 hours after infection, an intermediate β phase (6 to 7 hours after infection) requiring functional protein and DNA synthesis, a γ phase (at 10 hours) including virion structural proteins, and a late δ phase (at 15 hours) associated with occlusion. Infectious nonoccluded progeny of AcNPV are found in the culture medium approximately 10 hours after infection. Immunoperoxidase techniques detected the synthesis of polyhedrin, the major protein of the occlusion matrix, by 12 hours after infection, and this preceded the appearance of occluded virus at 16 or 18 hours after infection (52). Temporal control of transcription is at least in part responsible for the temporal control of AcNPV protein synthesis. The transcription of the polyhedrin gene is first observed at 12 hours after infection and the 1.2-kilobase transcript progressively increases in quantity through 24 hours after infection (48).

Fungal Insecticides

Natural infections by fungi play a major role in the control of many economic insect pests. Occasionally, the resultant disease reaches epizootic levels causing a complete collapse of the pest population. Bassi (53) originally isolated *Beauveria* from a diseased silkworm in 1834, and although many early attempts were made to exploit fungal disease for the control of economic insect pests, they were largely unsuccessful (54). However, in the early 1900's, the introduction of *Aschersonia* species resulted in complete control of citrus white fly in Florida, and control by these species is still effective today.

More than 500 species of fungi are capable of infecting insects (55) and there are susceptible host species in all the major orders of the Insecta. Some fungi can maintain themselves in susceptible populations through many insect generations without requiring repeated introduction. Since most species do not have to be ingested to cause infection, they can be used to control insect populations in nonfeeding stages, often before any economic damage has been done.

In spite of their potential as agents of insect control, however, worldwide use of fungi on a commercial basis is limited. Comparatively little is known about the mode of pathogenesis of many entomopathogenic fungi and many are inhibited by micro- and macroclimate. Technology for the large-scale production of these fungi is difficult and in many cases not yet developed, and the safety of the agents in regard to nontarget insects and other animals has not been adequately established, in part because of a lack of reliable bioassay procedures. The few fungal insecticides that are used on a large scale are as follows. Boverin, prepared from the conidia of *Beauveria bassiana*, is used for control of the Colorado potato beetle (*Leptinotarsa decemlineata*) in the Soviet Union. It is usually applied in combination with reduced dosages of trichlorphon (56) or malathion (57). A preparation of *Metarhizium anisopliae* called Metaquino is produced in Brazil and is used primarily for controlling a spittle bug (*Mahanarva posticata*) in sugarcane (58). Considerable use of *B. bassiana* for control of European corn borer (*Ostrinia nubilalis*) is reported in China (59). In the United States, *Hirsutella thompsonii* is commercially produced and used for control of the citrus rust mite. *Nomurea rileyi* is being used in large-scale field trials as a control for the cabbage looper and the velvet bean caterpillar (60). *Verticillium lecanii* is produced commercially in Great Britain for use against aphids and other pests of glasshouse crops.

Most fungal entomopathogens progress through consistent steps in the infection process (61). Initially the conidium or zoospore attaches to the insect cuticle. If conditions in the microclimate are appropriate the attached spore germinates and penetrates through the cuticle by means of a germ tube or by infection pegs arising from a knoblike structure on the end of a hypha called an appressorium. Once entrance to the haemocoel is gained, yeastlike cells are produced and the host is killed as a result of mechanical disruption or the production of toxic metabolites. After death of the host, a mycelial growth phase usually follows with eventual repenetration of the cuticle of the insect to the exterior where new reproductive units can form.

Beauveria bassiana and *Beauveria brongniartii* cause the disease commonly known as white muscardine. Although their host range is broad, these fungi occur most commonly in populations of hypogean pests or in pests occurring in locations with temperature, moisture, and other physical conditions similar to those in soil. Their pathogenicity and ability to overcome host defense mechanisms (62) is in part due to production of toxins. *Beauveria bassiana* produces the cyclic depsipeptide beauvericin, which is reported to be toxic to mosquito larvae (9) and adult houseflys (57). However, beauvericin was not found to be toxic to lepidopteran larvae (63). Not all isolates of *B. bassiana* produce beauvericin (64). Other cyclodepsipeptides, including the beauverolides (65), bassianolides (66), and isarolides (67), are produced by *B. bassiana* and *B. brongniartii* but, as with beauvericin, broad toxicity screening has not been conducted and conclusive evidence for the role of these metabolites in the pathogenicity of *Beauveria* has not been obtained.

The efficacy of *Beauveria* is affected in part by temperature and humidity in the macroclimate (68); microclimate conditions may also be important (69). Sunlight (70) and possibly biological activity of other organisms (71) affect the ability of *B. bassiana* to survive and to initiate infection. *Beauveria bassiana* (72) and *B. brongniartii* (69) applied with low doses of chemical insecticide can act synergistically to cause mortality in host insect populations. These species are important candidates in integrated pest systems.

Two varieties of *Metarhizium anisopliae*, var. *anisopliae* and var. *major*, cause the syndrome known as green muscardine. *Metarhizium anisopliae* is able to infect the Old World desert locust (*Schistocerca gregaria*) (73) and a weevil, *Hylobius pales* (74), by ingestion. Consequently, bait traps and attractants could be used in conjunction with *M. anisopliae* for some insects.

Common hosts of *Verticillium lecanii* are the Homoptera, but *V. lecanii* also infects Collembola, Diptera, and various mites and spiders. Although seemingly widespread in nature, it only produces epizootics in tropical climates or in greenhouse environments that approximate tropical conditions (75). Virulence of the organism in the absence of a host seems to be remarkably stable (76). Use of *V. lecanii* may be restricted by the inability of its conidia to survive dry conditions (77), making it difficult to produce and store the organism. There is evidence that *V. lecanii* must be applied directly to aphids in wet conditions to be

effective (*78*). It can be grown and distributed on a local basis for immediate use (*77*) and is being commercially produced in Britain.

In Florida, natural epizootics among populations of the citrus rust mite, *Phyllocoptruta oleivora*, are common during the summer. The epizootics are caused by *Hirsutella thompsonii*, which is one of the few fungal pathogens to be produced commercially in the United States. Mygar, produced by Abbott Laboratories, is registered for use on citrus crops. Generally, experimental applications of *H. thompsonii* have been successful, but this success has depended on the type of preparation, weather conditions, and citrus grove cultural practices. The fungus intially causes large reductions in the mite populations, then maintains the populations at low levels for relatively long periods (6 months) (*79, 80*). *Hirsutella thompsonii* can be applied as a conidial formulation or as mycelial fragments. Growth and sporulation can occur after application on leaf surfaces if the agent is applied with a carrier that can act as a substrate for fungal growth (*80*). This fungus may not be particularly suited for incorporation into integrated pest management programs because it is sensitive to many fungicides and moderately sensitive to many insecticides (*76, 81*).

Nomuraea rileyi produces natural infections in many Lepidoptera. Infection of the host insect is typically through the integument, and toxins may be involved in death of the host (*82*). There are several reports that the application of *N. rileyi* can advance the occurrence of natural epizootics by 2 weeks, thus providing earlier protection (*83*). Conidia, produced on semisolid media, are the usual form of the applied agent (*84*), but it may also be possible to use blastospores or mycelium. Nutrients are required for germination of the conidia and invasion of the host may be through the integument or via the alimentary tract (*85*).

Entomophorales species produce disease in a wide variety of insects, although individual strains or species are often host specific. In spite of their widespread occurrence in nature and ability to produce epizootics, use of these fungi in pest control is virtually nonexistent. Epizootics are influenced a great deal by climate. Conidia of many species are extremely short-lived (*86*) in air at a relative humidity of less than 80 percent. Even culture maintenance was difficult until liquid nitrogen storage systems for the *Entomophorales* were developed (*87*). Resting spores produced by some species are more resistant to temperature, heat, and chemicals than conidia and could be used in control programs, but the spores do not readily germinate, and although they can survive for several years (*88*), they may be less infective after storage. The growth of *Entomophorales* species in artificial media is difficult, but some species can be produced in the form of hyphal bodies and media suitable for production of resting spores have been developed (*89*).

Culicinomyces clavosporus, *Lagenidium giganteum*, and *Coelomomyces* are mosquito pathogens. *Culicinomyces* produces conidia on the surface of artificial media (*90, 91*). Conidia are infective by ingestion and produce mortality in larvae of several major mosquito genera (*90, 92*). *Lagenidium giganteum* grows saprophytically in aquatic environments or parasitically in mosquito larvae (*93*). Infection is by a zoospore which encysts on the larval cuticle and then penetrates by formation of a germ tube. Reproductive structures are produced (*94*) externally and zoospores are liberated in water. *Lagenidium giganteum* can be grown on artificial media and, if supplemented with various sterols, the fungus produces significant quantities of the infective zoospores (*94, 95*). Production of *L. giganteum* zoospores in artificial media and successful laboratory and field trials make it a particularly likely candidate for mosquito control. Species of the genus *Coelomomyces* are often reported as obligate parasites of mosquitoes. Although widespread and capable of producing epizootics, the discovery of a complex life cycle involving intermediate hosts (*96*), as well as the general inability of these fungi to grow well on other than highly complex media (*94*), indicate that more basic information is needed before they may be seriously considered for use in mosquito control programs.

Future Considerations

Currently, microbial pesticides have the greatest potential in intelligently designed and carefully applied pest management programs. Expanded use of these pesticides will depend heavily on the balance between production costs and ecological considerations. Broad-range chemical pesticides disrupt ecosystems (*97*) and affect natural balances in insect populations. In those cases where disruption of the ecosystem cannot be tolerated (for example, national forests), the increased cost of microbial pesticides may be preferred to the irreparable changes caused by less costly broad-range pesticides. Ironically, the more narrow host range of microorganisms makes them less attractive to industry from a profit perspective.

The genetic engineering of microbial insect pathogens to develop more potent or virulent strains, to improve their physiological tolerance of physical and chemical stresses encountered in nature, and to expand their host spectrum holds great promise. The abundance and variety of extrachromosomal elements in *B. thuringiensis*, for example, should allow their isolation and purification in sufficient quantities for detailed investigation of the structure and biological properties of these molecules. Genetic manipulation of promising plasmids, by simple transformation or recombinant DNA techniques, may ultimately improve the efficacy, pathogenicity, and commercial production of *B. thuringiensis* as well as other entomopathogenic bacteria. As already described, the toxin gene of *B. thuringiensis* subspecies *kurstaki* has been isolated by means of recombinant DNA technology (*15–17*). New and different toxins may be developed by the genetic manipulation of this toxin gene or by its combination with toxin genes of other subspecies.

Likewise, the genetic manipulation of viruses by both classical selection techniques and recombinant DNA technology may achieve increased efficacy and broadened host range. Thus far, classical genetic selections have been used to increase resistance to ultraviolet light and to increase occluded virus production. Genetic engineering of AcNPV by recombinant DNA techniques is currently under way. Future possibilities include the introduction of an insect-specific toxin (for example, a paralytic neurotoxin) gene into the genome of AcNPV by recombinant DNA technology. Such engineering could hasten the rate at which the virus kills the host and possibly broaden the host range of the virus, thus making these microorganisms more attractive to industrial producers and pest control managers.

Continued expansion of the use of fungal insecticides will require a better understanding of the physiology, genetics, and pathogenicity of these agents. Basic research concerning the production and action of toxic metabolites is needed to establish their role in pathogenesis. Genetic studies should lead to a better understanding of virulence or to the development of strains that are more easily produced in virulent form on artificial culture. Such studies are in their infancy but they are crucial if the role of fungal insecticides is to be enhanced.

In addition to the practical value of investigations of the molecular genetics of entomopathogens, there is consider-

able potential for answering fundamental biological questions about microorganisms in general. For example, better understanding of the origins and functions of plasmid molecules and the control of their expression in bacilli would enhance substantially our understanding of the molecular basis of bacterial sporulation. An important future use of the *A. californica* nuclear polyhedrosis virus will be as a vector for recombinant DNA research and genetic engineering in invertebrates (*49, 50*). The advantages of the AcNPV vector system include the ability to package large segments of passenger DNA in the rod-shaped viral capsid and the availability of at least one strong promoter (the polyhedrin promoter) which is turned on following the production of infectious nonoccluded viruses. It may be possible to use genetically engineered AcNPV in the production of insect-derived compounds such as pheromones, since all the genes encoding enzymes involved in a biosynthetic pathway can be incorporated into the expandable AcNPV genome.

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