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WORLD PICTURE OF BIOLOGICAL CONTROL OF INSECTS BY FUNGI

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The entomopathogenic fungi fill an extremely important niche in microbial control of insect pests. Virtually all insect orders are susceptible to fungal diseases. Fungi are particularly important in the control of pests which feed by sucking plant juices, because these insects have no means of ingesting pathogens; and they are important for Coleoptera control since viral and bacterial diseases are uknown for most of the coleopteran pests. There are approximately 700 species of entomopathogenic fungi in almost 100 genera (Table I). Accordingly, there is the potential for developing microbial control programs with fungi for virtually all pest insect species. Only a very small percentage of possible fungus/insect combinations have been tested for their potential as microbial control systems. Some of these are listed in Table II.

Fungal genetic diversity

In addition to having a wide spectrum of hosts, entomopathogenic fungi also have, in most cases, wide geographic ranges. Considerable genetic diversity can be found among various isolates of single species from different hosts and localities. For this reason, it is important to collect, culture, and conserve germplasm from a wide variety of collection sites and hosts. As with other organisms such as plants and animals, conservation of germplasm of entomopathogenic fungi merits high priority, since genotypes will be lost from nature as environmental conditions in a given locality change. In addition to the general repositories of fungi which exist in several countries, the US Department of Agriculture maintains a specialized, permanent entomopathogenic fungus culture collection (ARSEF) on the Cornell University, Ithaca, New York campus (R. Humber, Curator). This collection currently numbers approximately 3,000 cultures. ARSEF also provides an identification service. Since fungal cultures are often lost to laboratory accidents, contamination, or abandoned at retirement or with the change of interest of senior scientists; it would be prudent for scientists, including those residing in Brazil, to submit their living isolates to ARSEF or another established culture collection for permanent storage in liquid nitrogen.

In addition to naturally occurring fungal isolates, there currently is the potential to modify genetic characteristics of entomopathogenic fungi through molecular biology techniques. One of the most common of these fungi, *Metarhizium anisopliae*, was recently, for the first time, transformed using such techniques (Goettel et al., 1989). A gene for benomyl resistance from another fungus, *Aspergillus nidulans*, was inserted by protoplast fusion into *Metarhizium*. The gene was expressed and caused *Metarhizium* to become resistant to the fungicide. It is assumed that the basic transformation system will be useful for other entomopathogenic fungi and other genes, thereby affording the opportunity to produce improved strains of these fungi.

Invasion of insect cuticle

In virtually all cases, the infective unit for an entomopathogenic fungal species is a spore, usually a conidium. The course of disease development is initiated by the spore adhering to the cuticle of an insect. The spore germinates and the resulting germ tube either penetrates the cuticle directly or produces an appressorium from which an infection peg grows into the insect cuticle. The fungus penetrates to the hemocoel where it normally grows in a yeast-like phase called hyphal bodies or blastospores. A fungus may produce toxins in the hemocoel which aid in overcoming the immune response of the host, or in causing other disruptions of

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TABLE I

Classification of entomopathogenic fungal genera[a]. This classification generally follows that in Ainsworth et al. (1973a, b). (Modified slightly from Roberts & Humber, 1981)

Entomogenous taxa	Known or suspected associated entomogenous fungal genera
Subdivision Mastigomycotina	
Class Chytridiomycetes	
Order Blastocladiales	
Coelomomyces	
Coelomycidium (possibly in Chytridiales)	
Myiophagus (possibly in Chytridiales)	
Other genera on insect eggs	
Class Oomycetes	
Order Lagenidiales	
Lagenidium	
Crypticola	
Order Peronosporales	
Pythium	
Order Saprolegniales	
Leptolegnia	
Pathogenicity uncertain for other genera	
Subdivision Zygomycotina	
Class Zygomycetes	
Order Entomophthorales	
Batkoa	
Conidiobolus	
*(*subgenera: *Conidiobolus, Delacroixia, Capillidium)*	
Entomophaga	
Entomophthora	
Erynia	
Eryniopsis	
Furia	
Massospora	
Meristacrum	
Neozygites (= Triplosporium)	
Pandora	
Strongwellsea	
Tarichium	
Thaxterosporium	
Zoophthora	
Order Mucorales	
Sporondiniella	
Subdivision Ascomycotina	
Class Plectomycetes	
Order Eurotiales	
Cephalotheca[b]	*(Beauveria)*
Pseudeurotium[b]	*(Beauveria)*
Sartorya[b]	*(Aspergillus)*
Class Ascosphaeromycetes	
Order Ascosphaerales	
Ascosphaera	
Class Pyrenomycetes	
Order Clavicipitales	
Cordyceps	*(Acremonium, Akanthomyces, Desmidiospora, Hirsutella, Hymenostilbe, Paecilomyces, Pseudogibellula, "Sporotrichum", Stilbella)*
Helminthascus	
Hypocrella	*(Aschersonia)*
Torrubiella	*(Acremonium, Akanthomyces, Aphanocladium, Gibelulla, Hirsutella, Paecilomyces, Stilbella, Verticillium)*
Order Hypocreales	
Cordycepioideus	*(Paecilomyces)*

Entomogenous taxa	Known or suspected associated entomogenous fungal genera
Lisea	
Nectria	*(Fusarium, Verticillium)*
Pyxiodiophora	*(Thaxteriola)*
Stereocrea	
Order Sphaeriales	
Cantharosphaeria (entomopathogenicity doubtful)	
Broomella	
Class Loculoascomycetes	
Order Myriangiales	
Myriangium	
Angatia	
Order Pleosporales	
Podonectria	
Class Laboulbeniomycetes	
Laboulbeniopsis (probably an ascomycete; not in Laboulbeniales)	
Order Laboulbeniales	
ca. 115 genera of ectoparasitic fungi	
Subdivision Basidiomycotina	
Class Teliomycetes	
Order Septobasidiales[c]	
Septobasidium	*(Harpographium, Johncouchia,* and other unnamed genera*)*
Uredinella (? = Septobasidium)	
Subdivision Deuteromycotina[d]	
Class Coelomycetes	
Aschersonia	*(Hypocrella)*
Tetranacrium	*(Podonectria)*
Class Hyphomycetes	
Acariniola	
Acremonium	*(Cordyceps, Nectria, Torrubiella)*
Aegeritella	
Akanthomyces	*(Cordyceps, Torrubiella)*
Amphoromorpha (? = Basidiobolus; Entomophthorales)	
Antennopsis	
Aphanocladium	*(Torrubiella)*
Arthrobotrys	
Aspergillus	*(Sartorya* associated with entomogenous *Aspergillus* species; no *Sartorya* species are entomopathogenic*)*
Beauveria	*(Pseudeurotium, Cephalotheca* – both are associated with insects but neither is entomopathogenic*)*
Chantransiopsis	
Clathroconium	
*Coreomycetopsis (*always found with *Laboulbeniopsis)*	
Culicinomyces	
Desmidiospora	*(*with *Hirsutella, Cordyceps)*
Endosporella	
Engyodontium	
Funicularius	
Fusarium	*(Nectria)*
Gibellula	*(Torrubiella;* also with *Granulomanus)*
Granulomanus	*(Torrubiella;* also with *Gibellula)*
Harpographium	*(Septobasidium)*
Hirsutella	*(Cordyceps, Torrubiella, "Calonectria", "Podonectrioides")*
Hymenostilbe	*(Cordyceps)*
Johncouchia	*(Septobasidium)*
Mattirolella	
Metarhizium	
Myriodontium	
Muiaria	

Entomogenous taxa	Known or suspected associated entomogenous fungal genera
Muiogone	
Nomuraea	*(Cordyceps)*
Paecilomyces	*(Cordyceps, Torrubiella, Cordycepioideus)*
Paraisaria	*(Cordyceps)*
Peziotrichum	
Pleurodesmospora	
Pseudogibellula	*(Cordyceps)*
Sorosporella	(with *Syngliocladium)*
Sporothrix	
Stilbella	
Termitaria	
Termitariopsis	
Tetracrium	*(Podonectria)*
Thaxteriola	*(Pyxidiophora)*
Tilachlidiopsis	*(Cordyceps:* also with *Hymenostilbe?)*
Tolypocladium	
Trichothecium	
Verticillium	*(Nectria, Torrubiella)*
(Mycelia Sterilia)	
Aposporella	
Tompetchia (= "Aegerita")	(possible associate with Septobasidium)
Endosclerotium (not validly published)	
Hormiscioideus	
Hormiscium (sensu Thaxter)	

a Most taxa above the familial level are not provided with Latin diagnoses and are not validly proposed even though these taxa are exempt from any nomenclatural rules. There is no universally accepted scheme for higher fungal classification.

b Not known to be entomopathogenic.

c *Septobasidium* and *Uredinella* have long been classified as Hymenomycetes; recent studies confirm their closer affinities with rusts and/or smuts.

d No orders of families are accepted in the current taxonomy for conidial fungi based on conidiogenesis. The Saccardoan system of orders and families cannot be superimposed meaningfully upon this current classification.

host physiology. The fungus eventually invades virtually all internal organs of the insect after which it penetrates to the outside of the cuticle and produces new conidia. In dry conditions, the fungus may lie dormant within the dead host for long periods of time rather than emerging to the exteror and producing conidia.

Recent studies with *M. anisopliae* have clearly demonstrated that a protease is a key factor in the penetration of insect cuticle by this fungus (St. Leger et al., 1988). Insect cuticle normally is approximately 70% protein, so it is understandable that proteases could be more important than chitinases. During the penetration phase, 80% of protein synthesized by the fungus is the chymoelastase (protease) enzyme. Similar, but not identical, proteases have been detected in cultures of a number of other entomopathogenic fungi (St. Leger et al., 1987). The *Metarhizium* protease gene has been cloned and we are currently determining its nucleic acid sequence at Boyce Thompson Institute. This gene is a prime candidate for introduction into other entomopathogenic fungi with narrower host ranges and lower virulence. Expression of the gene in these transformed strains may significantly increase their efficacy.

Toxin production

A possible indirect use of entomopathogenic fungi for insect control would be the detection, isolation, characterization, and, finally, commercial development of toxins produced in culture by these fungi. The secondary metabolites of entomopathogenic fungi include a number of compounds toxic to insects (Roberts, 1981). These compounds should have a major advantage over many of the currently used synthetic chemical insecticides in that they almost certainly would be biodegradable. The known toxic compounds are quite diverse chemically, but a number of them, e. g. destruxins and bassianolide, are depsipeptides. This is an underexplored, but very promising, research area.

TABLE II

Some fungi with potential for development as microbial control agents[a]
(slightly modified from Roberts et al., 1990)

Fungus	Target insects	Status[b]	References
Aschersonia aleyrodis	Whitefly	A-USSR, C-elsewhere	Rombach & Gillespie, 1988. Fransen et al., 1987.
Beauveria bassiana	Pine Caterpillar	A-PRC	Hussey & Tinsley, 1981.
	Potato Beetle	A-USSR, C-elsewhere	Ferron, 1981. Campbell et al., 1985.
	Corn Borer	A-PRC, C-elsewhere	Hussey & Tinsely, 1981.
	Codling Moth	A-USSR	Ferron, 1981.
	Grasshoppers	C	Marcandier & Khachatourians, 1987.
	Cinch Bug	D	Billings & Glenn, 1911.
	Boll Weevil	D	Camargo et al., 1985.
	Cowpea Curculio	E	Bell & Hamalle, 1970.
	Pecan Weevil	E	Gottwald & Tedders, 1984.
	Mosquitoes	E	Chapman, 1985.
	Lygus Bug	E	Dunn & Mechalas, 1963.
	Granary Weevil	E	Dunn & Mechalas, 1963.
	Brown Planthopper	E	Rombach et al., 1986.
	Termites	E	Lai et al., 1982.
	Spider Mite	E	Rombach & Gillespie, 1988.
	Sugarcane Borer	E	Lecuona & Alves, 1988.
	Fire Ants	D	Alves et al., 1988.
Beauveria brongniartii	European Cockchafer	D	Keller et al., 1986.
	Sugarcane Borer	E	Lecuona & Alves, 1988.
Coelomomyces spp.	Mosquitoes	D	Chapman, 1985.
Conidiobolus obscurus	Aphids	C	Latge & Papierok, 1988.
Conidiobolus thromboides	Aphids	C	Latge & Papierok, 1988.
Culicinomyces clavisporus	Mosquitoes	C	Chapman, 1985.
Entomophaga grylli	Grasshoppers	C	MacLeod et al., 1980.
Entomophthora muscae	Muscoid Flies	D	Steinkraus & Kramer, 1987.
Entomophthora planchoniana	Aphids	D	Latge & Papierok, 1988.
Hirsutella citriformis	Brown Planthopper	E	Rombach et al., 1986.
Hirsutella thompsonii	Citrus Rust Mite	B-USA	Rombach & Gillespie, 1988.
	Spider Mite	E	Rombach & Gillespie, 1988.
Lagenidium giganteum	Mosquitoes	C	Chapman, 1985.
Leptolegnia chapmanii	Mosquitoes	E	Chapman, 1985.
Metarhizium anisopliae	Spittle Bugs	A-Brazil	Roberts & Wraight, 1986. Alves, 1986.
	Mosquitoes	D	Chapman, 1985.
	Rhinoceros Beetle	D	Latch & Falloon, 1976.
	Boll Weevil	D	Camargo et al., 1985.
	Black Vine Weevil	D	Rombach & Gillespie, 1988.
	Cowpea Curculio	E	Bell & Hamalle, 1970.
	Pecan Weevil	E	Gottwald & Tedders, 1984.
	Brown Planthopper	E	Rombach et al., 1986.
	Termites	E	Lai et al., 1982.
Metarhizium flavoviridae	Brown Planthopper	E	Rombach et al., 1986.
	Black Vine Weevil	D	Rombach & Gillespie, 1988.
Nomuraea rileyi	Velvetbean Caterpillar	C	Ignoffo, 1981.
	Armyworms	D	Ignoffo, 1981.
	Corn Earworm	D	Ignoffo, 1981.
Paecilomyces farinosus	Potato Beetles	D	Samsinakova & Kalalova, 1978.
	Cabbage Looper	D	Agudelo & Falcon, 1983.
	Beet Armyworm	D	Agudelo & Falcon, 1983.
	Black Vine Weevil	D	Rombach & Gillespie, 1988.
Paecilomyces fumosoroseus	Whitefly	D	L. Osborne, pers. comm.
Paecilomyces lilacinus	Nematodes	A-Philippines	P. Jatala, pers. comm., 1987.
	Brown Planthopper	E	Rombach et al., 1986.
Tolypocladium cylindrosporum	Mosquitoes	D	Chapman, 1985.
Verticillium lecanii	Aphids	B-UK	Rombach & Gillespie, 1988. Latge & Papierok, 1988.
	Whitefly	B-UK	Rombach & Gillespie, 1988.

Fungus	Target insects	Status[b]	References
	Thrips	B-UK	Rombach & Gillespie, 1988.
	Grasshoppers	E	Johnson et al., 1988.
	Mites	E	Rombach & Gillespie, 1988.
Zoopthora (=Erynia) sp.	Alfalfa Weevil	D	Brown & Nordin, 1986. Carruthers & Hural, 1989.
Zoophthora (=Erynia) neoaphidis	Aphids	D	Latge & Papierok, 1988.
Zoophthora (=Erynia) phytonomi	Clover Leaf Weevil	D	USDA, 1956.
Zoophthora (=Erynia) pieris	Cabbageworm	E	Li & Humber, 1984.
Zoophthora (=Erynia) radicans	Aphids	D	Latge & Papierok, 1981. Papierok et al., 1984.
	Leafhoppers	E	Papierok et al., 1984.
	Psyllids	E	Papierok et al., 1984.
	Leaf-rollers	E	Papierok et al., 1984.

a Only a sampling of entomopathogenic fungi, target hosts and references are presented.
b A = Registered and in production; B = Registered but not commercially available; C = Intensively studied; D = More studies needed to evaluate full potential; E = Little studied.

Production of fungi

The planned approach to fungal release seriously affects the amount, type, and formulation of the fungal preparation. For example, for colonization attempts wherein the fungus is introduced into an area for the first time, it may be sufficient to simply introduce a few sporulating cultures of the new pathogen into the pest insect population, if the pathogen has high epizootic potential. On the other hand, for use as a microbial insecticide, massive amounts of the fungus must be produced and it must remain in an infective state for a considerable period of time.

Entomopathogenic fungi have been produced for use as insect control agents from time to time during the past 100 years. The methods employed have been extremely diverse. Cadavers of infected insects have been collected and introduced into fields either in the same season, or – after storage – into pest populations the next season. Insects have been artificially infected in the laboratory and released into pest populations while still active or as cadavers covered with sporulating fungus. The most common methods, however, involve the production of the fungus on artificial media. For colonization, the placing of dishes of conidiating cultures grown on artificial agar media in fields may be sufficient. Most mass-production schemes have utilized vegetable materials as the medium, e. g., rice or wheat bran, cracked barley, rice, etc. Even peat soil has been used with success in the People's Republic of China (PRC). The substrate is held during incubation in covered trays, bottles, or autoclavable plastic bags. The amino acid composition of the medium is known to influence the storage and virulence of conidia (Goral, 1978). Nevertheless, fungi are usually produced on very simple, inexpensive media of undefined composition. For example, when produced in liquid cultures, media may include corn steep liquor, yeast extract, molasses, etc.

Like most fungi, the majority of entomopathogenic fungi will not produce conidia in submerged culture, and there have been a number of approaches to cure this problem. One has been to encourage the yeast-like stage of the fungus in submerged culture. The resulting thin-walled spores are referred to as blastospores. There have been successful procedures devised for also producing conidia in submerged culture, but there is some question as to their direct comparability to conidia produced on aerial mycelium. It is suspected that such conidia have thinner walls than those produced in air. In some cases, the liquid medium has been absorbed into a substrate such as vermiculite which provides a large surface area on which the fungus can grow. The cultures may be tumbled in a slowly revolving drum during growth to maximize the exposure of particle surfaces to air. Large-scale production of conidia has been accomplished in the USSR by growing the fungi in fermentors to produce large amounts of mycelia, which were then placed in shallow pans to a depth of approximately 1.0 cm where, after several days, conidia were produced. Conidia have also been produced on thin layers of liquid without previous

growth in a fermentor. A system specially devised for this approach calls for the liquid to be held during incubation in large plastic bags kept inflated with positive sterile-air pressure.

A recent innovation in mass production has been developed around the idea that in nature infected insects normally remain in the insect habitat for considerable periods of time after death and produce conidia over this time. At death, a fungus-infected insect normally is completely filled with hyphae. Under humid conditions, conidiophores are produced on the outside of the insect where conidia are produced in massive amounts. The first step of the new procedure is to mass-produce mycelium in submerged culture. This is easily accomplished for almost all entomopathogenic fungi. The fungus is then filtered from the medium to produce thin mats approximately 3-6 mm thick which are treated with a sugar solution (usually 10% maltose or sucrose) as a desication protectant. The mat is air-dried at room temperature until crisp and then milled to particles approximately 2 mm in size. When introduced into the field, these particles, on contact with soil moisture, dew, or rain, commence production of conidia. As with fungus-filled cadavers, the particles of mycelium can produce new conidia for several consecutive days in the field; thereby providing fresh inoculum for a considerable period after introduction of the fungus. Entomophthorales tend to produce conidia within a few hours of wetting of the dried mycelium. Deutermocytina may require one or more days to commence sporulation.

Formulation of fungal preparations

Formulation is a crucial issue with fungal preparations. The half-life of conidia on leaves in sunlight can be as little as two hours. Some approaches normally used in formulating chemical insecticides, such as use of organic solvents, are not applicable to fungi because they cause death of the organism. Some commonly used sticker-spreaders are not detrimental to fungi, and extenders such as clay usually are beneficial to longevity. Microencapsulation of dried mycelium has been successfully accomplished using alginate and pre-gelatinized starch (Pereira & Roberts, 1989). Despite its importance, there are few scientists available at present with expertise in formulation of microbial products, particularly fungi.

Approaches to microbial control with fungi

Intentional manipulation of micro-organisms for insect pest control is called microbial control. The basic approaches to this type of biological control using entomopathogenic fungi have included the following: (1) Conservation (environmental augmentation as used by Fuxa, 1987) which involves enhancement of naturally-occurring microbial pest suppression by means, usually cultural manipulations, other than the addition of disease agents into the environment; (2) Colonization (or introduction-establishment as used by Fuxa) wherein the microorganism is introduced into the pest population where it maintains and spreads itself. A subset of colonization is seasonal colonization (inoculative augmentation as used by Fuxa), where the pathogen is re-introduced into the pest population periodically, usually each year. This is sometimes done to initiate epizootics earlier in the season than would occur if natural build-up of disease were allowed to occur within the population. The earlier epizootic will protect crops, whereas naturally occurring epizootics tend to be too late to do so; (3) Microbial insecticide (innundative augmentation as used by Fuxa) where microorganisms are disseminated in the pest population in large quantities in a manner similar to chemical insecticide application. Some microbial products, e. g., *Bacillus thuringiensis (Bt)*, are not expected to spread in the pest population and are introduced in large amounts each time the pest population exceeds the economic threshold.

Conservation

As mentioned previously, conservation of entomopathogenic fungi involves manipulation of conditions to encourage efficient action of these pathogens in reducing pest populations. Due to a lack of knowledge of the pathogen and/or its host, or the inability to manipulate the environment, there are few examples of this type of microbial control with fungi. Conservation of the entophthoralean pathogen *Zoophthora* nr. *phytonomi* infecting the alfalfa weevil, *Hypera postica*, has been accomphised in Kentucky, USA (Brown & Nordin, 1986). Cutting alfalfa early and leaving it in windrows in the field for a few days concentrates weevil and the fungus and thereby encourages epizootics. The system was devised after considerable computer modelling of the disease/insect interaction in

nature, and it is now a recommended procedure for alfalfa weevil control in Kentucky.

Colonization

Colonization of entomopathogenic fungi into new localities has been attempted on a number of occasions. Unfortunately there is, as with many biological control introductions of parasitic insects, very little information available on the impact of these organisms.

In 1978 an isolate of *Zoopthora radicans* was introduced into spotted alfalfa and blue-green lucerne aphid populations in Australia (Milner, Soper & Lutton, 1982). The spotted alfalfa aphid was first discovered in Australia in 1977. The insect apparently had arrived without concurrent arrival of *Zoopthora* spp. pathogens. After bioassay tests of several strains of *Z. radicans* from around the world, one from Israel was selected for release because it was virulent and its original site of collection resembled the environmental conditions in much of Australia. Initial infection was low, but by the end of the first growing season the disease had spread several meters from the release points and induced up to 88% disease. By the second growing season, infected aphids were found up to 150 km from the release site (Milner & Lutton, 1986), and as of 1988 had spread 300 km from the release site (Carruthers & Hural, 1989).

Seasonal colonization has been used with *Hirsutella thompsonii* to control citrus rust mite. This fungus occurs at epizootic levels in rust mite populations in Florida citrus almost every year, but the high incidence of disease normally is not attained until after mite damage to the fruit. It is assumed that the inoculum in the orchard is too low early in the season to initiate epizootics. Accordingly, the fungus is introduced early in the season in an effort to initiate earlier epizootics and thereby maintain the pest below economic thresholds. Although the fungus is a registered product in the United States (Mycar^{R}), it is currently not marketed. An improved formulation is being sought prior to marketing efforts. Seasonal colonization is also being considered with *Nomuraea rileyi* which occurs in epizootic levels in soybean noctuid caterpillar populations each year in the United States and frequently in Brazil. Conidia of this fungus are difficult to produce on solid media, but it is likely that dried mycelium produced in submerged culture as described under "Production of fungus" may prove effective for seasonal colonization attempts.

Microbial insecticides

The use of fungi as microbial insecticides is conceptually a very straightforward approach, but it is technologically the most difficult. Fungi must be produced in immense quantities and the resulting product must be maintained in a viable and virulent state, both during long periods of storage and after introduction into the insect habitat. There is virtually no specialized equipment designed specifically for application of fungus-based products; so these products must be designed to be applied with existing chemical-pesticide, fertilizer, or seeding equipment. Since the half-life of the fungus in the field may be short, and the susceptible stage(s) of the pest may be restricted, the need for precise timing – with its associated frequent population monitoring – may be more important than with many chemical insecticides. Effective dose levels are often quite high, and some experiments have been conducted with too-low dosages (effective doses usually are 10^{12}-10^{14} conidia per hectare, depending on target insect), and without sufficient attention to appropriate conditions such as low insolation, high humidity, and most susceptible host stage. To date, tests have almost invariably failed to effectively control the pest. It should be pointed out that direct comparisons between chemical insecticides and mycoinsecticides are difficult to interpret. The fungi tend to kill more slowly, but if they have epizootic potential they may suppress the population longer than a non-systemic chemical. Also, the minor impact of the mycoinsecticide on other natural enemies (parasites and predators) tends to extend the effectiveness of the fungus.

Foliage-feeding Hemiptera/Homoptera – A very interesting use of the microbial insecticide approach to fungus use, and one that has caught the attention of insect pathologists worldwide, is that of using *M. anisopliae* against pasture and sugar cane spittle bugs in Brazil (Alves, 1986; Moscardi, 1989). Over 100,000 hectares of sugar cane are treated annually. The fungus is produced by grower associations, plantation owners, and small industrial concerns. The fungus is more effective against *Mahanarva posticata* nymphs than chemical insecticides, and it has the added benefit that it is not deleterious to *Apanteles* parasites produced and released by grower as-

sociations for control of sugar cane borer *(Diatrea saccharalis)*. Spittle bug mortality can range up to 70% and higher, but usually is about 40%. This is adequate to increase sugar content in the cane sufficiently to justify fungus application. In all cases the fungus is grown on rice in bottles or autoclavable plastic bags and introduced into the field as conidia.

Another homopterous insect, the leafhopper *Empoasca kraemeri*, is a major pest of common beans and cowpeas in Brazil. The fungus *Zoophthora radicans* has occurred regularly in epizootic proportions in *E. kraemeri* populations at the National Research Center for Rice and Beans (CNPAF) in Goiania, Goias, each year since it was first noted there in 1984. The levels of infection apparently have been augmented in each of the past two years by the introduction of dry mycelium (S. G. and S. P. Wraight, pers. comm.). The same fungus when released at the edge of an alfalfa field in Ithaca, New York, spread approximately 100 meters across the field in a month and virtually eliminated the *E. fabae* leafhopper population (S. G. and S. P. Wraight, pers. comm.). An attempt to colonize an isolate of *Z. radicans* from Wisconsin into central Illinois apparently succeeded (McGuire et al., 1987). *Hirsutella guyana* has been found in the states of Rio Grande do Norte and Ceara of Brazil causing epizootics in *E. kraemeri* populations on cowpeas (R. A. Daoust, D. W. Roberts, B. P. Magalhaes & E. D. Quintela, unpub.). This fungus has proved very amenable to mass production by the dry mycelium method (S. P. Wraight, pers. comm.), and may prove very useful in northeast Brazil for leafhopper control.

In recent years a number of field tests have been conducted in rice in Southeast Asia against the brown planthopper (BPH), *Nilaparvata lugens*, and the black bug *Scotinophara coarctata* (Rombach et al., 1986a, b). Several fungal species were used, but most of the tests were conducted with *B. bassiana* and *M. anisopliae* dry mycelium or conidia. In most cases, population reduction took at least one week to occur and population suppression normally extended to at least three weeks. Both formulations were efficacious. Results sometimes were not satisfactory during the rice dry season. The brown planthopper is thought by many rice entomologists to be a pest only after the use of chemical insecticides has caused virtual elimination of parasite and predator populations. These natural enemies normally hold BPH in check. Although not formally studied, the parasites and predators were not noted to be infected in fungus-treated fields; and accordingly, the use of fungi may be considerably more efficacious in the long term than chemical insecticides.

Foliage- and stem-fedding Lepidoptera – A number of foliage-feeding lepidopterous insects are susceptible to entomopathogenic fungi. A very large microbial control project has been conducted for at least fifteen years in the People's Republic of China to control the pine moth larval stage (*Dendrolimus* spp.) with conidia of *Beauveria bassiana*. Over one million hectares in 12 provinces are treated annually (Li Zhenzhi, pers. comm.; and Xu Qingfeng, 1988). The fungus is applied by ground or by air as sprays or dusts. Low volume (2-4 liters per hectare) formulations have been developed which cause 70-80% mortality and cost between $US2-3 per hectare (Pan et al., 1988). This fungus is also used in Northeastern PRC and in France for control of corn borers (*Ostrinia* spp.). Commercialization of the fungus is expected in France since tests in recent years have afforded results similar to those attained with chemical insecticides (Riba, 1984; and C. Riba, pers. comm.). In the United States, the insect was found to feed for several days in the moist leaf axil region before penetrating to the stalk. The placing of the *B. bassiana* conidia into the whorls caused high levels of disease (Feng et al., 1988).

Soil insects – The soil environment is considered particularly promising for use fungi against pest insects because the temperature of soil is usually moderated compared to air temperatures, and humidity is normally quite high. A new *M. anisopliae*-based product is expected to enter the marketplace soon in south-central Australia (D. E. Pinnock, pers. comm.). The fungus is produced on a substrate which is attractive to *Aphodius tasmaniae* larvae. This scarabeiid pest differs from most in that it comes to the surface of the earth to feed upon pasture grasses each night. It carries the fungus-infested bate into its tunnel where the insect becomes infected. High levels of infections (ranging above 80%) have been attained in field trials. A cost analysis has shown that the fungus is more attractive than chemical insecticides for several reasons. First, animals are allowed to feed on the pasture immediately after treat-

ment; second, the product is more efficacious than known chemicals; and third, at projected production costs, the cost-per-hectare treatment will be less than with currently available chemical products. Another attractive feature is that some of the commonly used chemicals have had their registration for pasture use withdrawn in recent years in Australia and the fungus is a much needed alternative. This project is a consortium effort between industry and a university, and it may well mark an example that other academic and industrial units may wish to emulate. *Metarhizium anisopliae* is also being tested in Australia for control of another soil insect, a scarabeiid of sugar cane roots *(Antiprogus parvulus).*

In 1985 in Switzerland 14 areas of high concentration of adult beetles (a total of 89 hectares) were treated with blastospores of *Beauveria brongniartii* at the rate of 2.6×10^{14} blastospores per hectare. Infected females subsequently carried the disease agent into breeding sites. The infection rates of adult beetles averaged 85% and a second treatment on five of the sites increased the infection level to 95% (Keller et al., 1986). This is one of the few studies where the effect of fungus application on non-target insects has been examined. Infection was virtually non-existent in these non-target insects (Baltensweiler & Cerutti, 1986).

One of the most important pests of cowpeas in northeast Brazil is the cowpea curculio, *Chalcodermus bimaculatus.* Larvae feed in the pods and drop to the soil at maturity to pupae. Treatment of the soil with conidia of *B. bassiania* and *M. anisopliae* has caused approximately 95% mortality in cage experiments and over 50% in field experiments (E. D. Quintela and S. P. Wraight, pers. comm.). Fire ants in Brazil were reported to be susceptible to introductions of certain strains of *B. bassiania* into their nests (Alves et al., 1988), and the potential of these strains for control of fire ants in the USA is currently under investigation.

Conclusions

It is clear that if all possible permutations of fungal species, isolates, hosts, and approaches to fungus use are examined, there is an almost unlimited number of as yet unexplored, but promising, approaches to insect control with fungi. Many of the attempts to date must be counted as failures, but these failures should have been expected in light of the almost complete lack of background information prior to field application. With better understanding of the basic characteristics of the fungal pathogens through genetic and biochemical studies, improvement of fungi with molecular genetics techniques, along with sound epizootiology inputs, the potential for success in the future will be greatly increased. Nevertheless, some rather large programs already have been mounted successfully and these serve as examples of the high potential of fungi for microbial control.

It is interesting that some of the features that are attractive about a fungal species for one type of insect control may be considered a disadvantage in another setting. For example, some fungi have broad host spectra. This is attractive to commercial developers in that a single preparation can be used against several pest insect species. On the other hand, for integrated pest management systems, host specificity is usually preferred. Fortunately, despite the fact that a fungal species may be known to infect many insect species, the individual strains of that fungus usually show some degree of host preference. The strains of *B. bassiana* used in the PRC against *Dendrolimus* and *Ostrinia* are almost innocuous for commercial silkworms. The ability to colonize is a definite plus in programs where simple introductions and little follow-up is intended. However fungi which fail to spread and have short residual in the field are attractive for commercial producers since this insures, as with *Bacillus thuringiensis,* repeated sales.

The need for non-chemical approaches for insect control is great. The entomopathogenic fungi are currently playing an important role in natural control of insect pests; and, with the help of modern biotechnology, their level of use as microbial control agents should be sufficiently high to make them significant factors in insect control in the future.

REFERENCES

AGUDELO, F. & FALCON, L. A., 1983. Mass production, infectivity, and field application studies with the entomogenous fungus *Paecilomyces farinosus. J. Invertebr. Pathol., 42:* 124-132.

AINSWORTH, G. C.; SUSSMAN, A. S. & SPARROW, F. K., 1973a. *The Fungi: An Advanced Treatise,* Vol. 4A. Academic Press, New York.

AINSWORTH, G. C.; SUSSMAN, A. S. & SPARROW, F. K., 1973b. *The Fungi: An Advanced Treatise,*

Vol. 4B. Academic Press, New York.

ALVES, S. B. (Coordinator), 1986. *Controle Microbiano de Insectos.* Editora Manole, Ltda., São Paulo, Brasil.

ALVES, S. B.; STIMAC, J. L. & CARMARGO, M. T. V., 1988. Susceptibility of *Solenopsis invicta* Buren and *S. saevissma* Fr. Smith. to isolates of *Beauveria bassiana* (Bals.) Vuill. *Anais da Sociedade Entomologica do Brasil, 17:* 379-388.

BALTENSWEILER, W. & CERUTTI, F., 1986. Bericht über die Nebenwirkungen einer Bekämpfung des Maikäfers (*Melolontha melolontha* L.) mit dem Pilz *Beauveria brongniartii* (Sacc.) Petch auf die Arthropodenfauna des Waldrandes. *Bulletin de la Société Entomologique Suisse, 59:* 267-274.

BELL, J. V. & HAMALLE, R. J., 1970. Three fungi tested for control of the cowpea Curculio, *Chalcodermus aeneus. J. Invertebr. Pathol., 15:* 447-450.

BILLINGS, F. H. & GLENN, P. A., 1911. Results of the artificial use of the white fungus disease in Kansas. *U. S. Dept. Agric. Bull. No. 107:* 58 p.

BROWN, G. C. & NORDIN, G. L., 1986. Evaluation of an early harvest approach for induction of *Erynia* epizootics in alfalfa weevil populations. *J. Kans. Entomol. Soc., 59:* 446-453.

CAMARGO, L. M. P. C. A.; FILHO, A. B. & CRUZ, B. P. B., 1985. Susceptibility of boll weevil (*Anthonomus grandis* Boheman) to fungi *Beauveria bassiana* (Bals.) Vuillemin and *Metarhizium anisoplia* (Metsch.) *Sorokin Biologico, 51:* 205-208.

CAMPBELL, R. K.; ANDERSON, T. E.; SEMEL, M. & ROBERTS, D. W., 1985. Management of the Colorado potato beetle using the entomogenous fungus *Beauveria bassiana. Amer. Potato J., 62:* 29-37.

CARRUTHERS, R. I. & HURAL, K., 1989. Fungi as naturally occurring entomopathogens. In T. Baker & P. Dunn, *New Directions in Biological Control.* UCLA Symposia on Molecular and Cellular Biology, 112, in press.

CHAPMAN, H. C., 1985. Biological Control os Mosquitoes. Fresno: *Amer. Mosq. Control Assoc. Bull.,* 6.

DUNN, P. H. & MECHALAS, B. J., 1963. The potential of *Beauveria bassiana* as a microbial insecticide. *J. Insect. Pathol., 5:* 451-459.

FENG, Z.; CARRUTHERS, R. I.; LARKIN, T. S. & ROBERTS, D. W., 1988. A phenology model and field evaluation of *Beauveria bassiana* (Bals.) Vuillemin (Deuteromycotina: Hyphomycetes) mycosis of the European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera: Pyralidae). *Can. Ent., 120:* 133-144.

FERRON, P., 1981. Pest control by the fungi *Beauveria* and *Metarhizium,* p. 465-482. In H. D. Burges, *Microbial Control of Pests and Plant Diseases 1970-1980.* London: Academic Press.

FRANSEN, J. J.; WINKELMAN, K. & VAN LENTEREN, J. C., 1987. The differential mortality at various life stages of the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), by infection with the fungus *Aschersonia aleyrodis. J. Invertebr. Pathol., 50:* 158-165.

FUXA, J. R., 1987. Ecological considerations for the use of entomopathogens in IPM. *Annu. Rev. Entomol., 32:* 225-252.

GOETTEL, M. S.; ST. LEGER, R. J.; BHAIRI, S.; ROBERTS, D. W. & STAPLES, R. C., 1989. Transformation of the entomopathogenic fungus *Metarhizium anisopliae* using the benA3 gene from *Aspergillus nidulans. J. Cell. Biochem., S13A:* 170 (Abstract).

GORAL, V. M., 1978. Effect of cultivation conditions on the entomopathogenic properties of Muscadine fungi. p. 217-228. In C. M. Ignoffo, *Proc. First Joint US/USSR Conf. on the Production, Selection and Standardization of Entomopathogenic Fungi of the US/USSR Joint Working Group on the Production of Substances by Microbiological Means.* National Science Foundation, USA.

GOTTWALD, T. R. & TEDDERS, W. L., 1984. Colonization, transmission, and longevity of *Beauveria bassiana* and *Metarhizium anisopliae* on pecan weevil larvae in the soil. *Environ. Entomol., 13:* 557-560.

HUSSEY, N. W. & TINSLEY, T. W., 1981. Impression of insect pathology in the People's Republic of China. p. 785-795. In H. D. Burges, *Microbial Control of Pests and Plant Diseases 1970-1980.* London: Academic Press.

IGNOFFO, C. M., 1981. The fungus *Nomuraea rileyi* as a microbial insecticide. p. 513-538. In H. D. Burges, *Microbial Control of Pests and Plant Diseases 1970-1980.* London: Academic Press.

JOHNSON, D. L.; HUANG, H. C. & HARPER, A. M., 1988. Mortality of grasshoppers (Orthoptera: Acrididae) inoculated with a Canadian isolate of the fungus *Verticillium lecanii. J. Invertebr. Pathol., 52:* 335-342.

KELLER, S.; KELLER, E. & AUDEN, J. A. L., 1986. Ein Grossversuch zur Bekämpfung des Maikäfers (*Melolontha melolontha* L.) mit dem Pilz *Beauveria brongniartii* (Sacc.) Petch. *Bulletin de la Société Entomologique Suisse, 59:* 47-56.

LAI, P. Y.; TAMASHIRO, M. & FUJII, J. K., 1982. Pathogenicity of six strains of entomogenous fungi to *Coptotermes formosanus. J. Invertebr. Pathol., 39:* 1-5.

LATCH, G. C. M. & FALLOON, R. E., 1976. Studies on the use of *Metarhizium anisopliae* to control *Oryctes rhinoceros. Entomophaga, 21:* 39-48.

LATGE, J. P. & PAPIEROK, B., 1988. Aphid pathogens. p. 323-335. In A. K. Minks & P. Harrewijn, *Aphids: Their Biology, Natural Enemies and Control.* Elsevier.

LECUONA, R. E. & ALVES, S. B., 1988. Efficiency of *Beauveria bassiana* (Bals.) Vuill., *B. brongiartii* (Sacc.) Petch. and granulose virus on *Diatraea saccharalis* (F., 1794) at different temperatures. *J. Appl. Ent., 105:* 223-228.

LI, Z. & HUMBER, R. A., 1984. *Erynia pieris* (Zygomycetes: Entomophthoraceae), a new pathogen of *Pieris rapae* (Lepidoptera: Pieridae): description, host range, and notes on *Erynia virescens. Canad. J. Bot., 62:* 653-663.

MACLEOD, D. M.; TYRRELL, D. & WELTON, M. A., 1980. Isolation and growth of the grasshopper pathogen, *Entomophthora grylli. J. Invertebr. Pathol., 36:* 85-89.

MARCANDIER, S. & KHACHATOURIANS, G. G., 1987. Susceptibility of the migratory grasshopper, *Melanopus sanguinipes* to *Beauveria bassiana:* Influence of relative humidity. *Can. Entomol., 119:* 901-907.

MCQUIRE, M. R.; MADDOX, J. V. & ARMBRUST,

E. J., 1987. Effect of temperature on distribution and success of introduction of an *Empoasca fabae* (Homoptera: Cicadellidae) isolate of *Erynia radicans* (Zygomycetes: Entomophthoraceae). *J. Invertebr. Pathol., 50:* 291-301.

MILNER, R. J. & LUTTON, G. G., 1986. Microbial control of insects: Biological control of spotted alfalfa aphid. *CSIRO Division of Entomology Biennial Report 1983-85.* Canberra.

MILNER, R. J.; SOPER, R. S. & LUTTON, G. G., 1982. Field release of an Israeli strain of the fungus *Zoophthora radicans* (Brefeld) Batko for biological control of *Therioaphis trifolii* (Monell) f. *maculata. J. Aust. Entomol. Soc., 21:* 113-118.

MOSCARDI, F., 1989. Production and use of entomopathogens in Brazil, p. 53-60. In D. W. Roberts & R. R. Granados, *Proc. Conf. on Biotechnology, Biological Pesticides and Novel Plant-Pest Resistance for Insect Pest Management,* Boyce Thompson Institute, Ithaca, New York.

PAN, W.; TAN, D. & YIN, F., 1988. Oil formulation of *Beauveria bassiana* in ultra-low capacity against the Masson's pine caterpillar, p. 72-76. In Chinese Society of Mycology. *Study and Applications of Entomogenous Fungi in China,* Vol. I, Academic Periodical Press.

PAPIEROK, B.; VALADAO, L.; TORRES, B. & ARNAULT, M., 1984. Contribution a l'etude de la specificite parasitaire du champignon entomopathogene *Zoophthora radicans* (Zygomycetes, Entomophthorales). *Entomophaga, 29:* 109-119.

PEREIRA, R. M. & ROBERTS, D. W., 1989. Dry mycelium preparations of entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana. J. Invertebr. Pathol.,* in press.

RIBA, C., 1984. Application en essais parcellaires de plein champ d'un mutant artificiel du champignon entomopathogene *Beauveria bassiana* (hyphomycete) contre la pyrale du mais, *Ostrinia nubilalis* (Lep. Pyralidae). *Entomophaga, 29:* 41-48.

ROBERTS, D. W., 1981. Toxins of entomopathogenic fungi, p. 441-464. In H. D. Burges, *Microbial Control of Pests and Plant Diseases 1970-1980.* London: Academic Press.

ROBERTS, D. W.; FUXA, J. R.; GAUGLER, R.; GOETTEL, M.; JAQUES, R. & MADDOX, J., 1990. Use of pathogens in insect control. In D. Pimentel, *Handbook of Pest Management in Agriculture.* 2nd Ed. Boca Raton, Florida. CRC Press, in press.

ROBERTS, D. W. & HUMBER, R. A., 1981. Entomogenous fungi, p. 201-236. In G. T. Cole & B. Kendrick, *Biology of Conidial Fungi, Vol. 2.* New York: Academic Press, Inc.

ROBERTS, D. W. & WRAIGHT, S. P., 1986. Current status on the use of insect pathogens as biocontrol agents in agriculture: fungi. p. 510-513. In R. A. Samson; J. M. Vlak & D. Peters, *Fundamental and Applied Aspects of Invertebrate Pathology.* Wageningen: Fndn 4th Colloq. Invertebr. Pathol.

ROMBACH, M. C. & GILLESPIE, A. T., 1988. Entomogenous Hyphomycetes for insect and mite control on greenhouse crops. *Biocontrol News & Information, 9:* 7-18.

ROMBACH, M. C.; AGUDA, R. M.; SHEPARD, B. M. & ROBERTS, D. W., 1986a. Infection of the rice brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) by field application of entomopathogenic Hyphomycetes (Deuteromycotina). *Environ. Entomol., 15:* 1070-1073.

ROMBACH, M. C.; AGUDA, R. M.; SHEPARD, B. M. & ROBERTS, D. W., 1986b. Entomopathogenic fungi (Deuteromycotina) in the control of the black bug of rice, *Scotinophara coarctata* (Hemiptera: Pentatomidae). *J. Invertebr. Pathol., 48:* 174-179.

SAMSINAKOVA, A. & KALALOVA, S., 1978. The fungus *Paecilomyces farinosus.* A study of conditions of its use for the control of the Colorado potato beetle. *Z. Ang. Ent., 87:* 68-75.

STEINKRAUS, D. C. & KRAMER, J. P., 1987. Susceptibility of sixteen species of Diptera to the fungal pathogen *Entomophthora muscae. Mycopathologia, 100:* 55-63.

ST. LEGER, R. J.; COOPER, R. M. & CHARNLEY, A. K., 1987. Distribution of chymoelastases and trypsin-like enzymes in five species of entomopathogenic Deuteromycetes. *Arch. Biochem. Biophys., 258:* 123-131.

ST. LEGER, R. J.; DURRANDS, P. K.; CHARNLEY, A. K. & COOPER, R. M., 1988. Role of extracellular chymoelastase in the virulence of *Metarhizium anisopliae* for *Manduca sexta. J. Invertebr. Pathol., 52:* 285-293.

U. S. DEPT. AGRICULT., 1956. The clover leaf weevil and its control. *Farmer's Bulletin 1484.*

XU, Q., 1988. Some problems about study and application of *Beauveria bassiana* against agricultural and forest pests in China, p. 1-9. In Chinese Society of Mycology. *Study and Application of Entomogenous Fungi in China,* Vol. I, Academic Periodical Press.