USE OF ENTOMOPATHOGENIC FUNGI IN BIOLOGICAL CONTROL: A WORLD VIEW

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ABSTRACT - The entomopathogenic fungi inflict widespread destruction to arthropods inhabiting plants, soil, and aquatic environments. Today, about 25 species are recognized worldwide as important biotic factors in the regulation of agriculturally and medically important insect pests of economic significance. Scientists have scrutinized these fungi in biological control as biopesticides, as exotic microbes introduced from one place to another, or as manipulative agents in IPM systems. This paper addresses current basic and applied research on entomopathogenic fungi as biological control agents.

Index terms: entomopathogenic fungi; microbiotic control of insects.

USO DE FUNGOS ENTOMOPATOGÊNICOS NO CONTROLE BIOLÓGICO: UMA VISÃO GERAL

RESUMO - Os fungos entomopatogênicos causam danos enormes aos artrópodes que vivem em plantas, solos e meios aquáticos. Atualmente, cerca de 25 espécies são reconhecidas como importantes fatores bióticos no controle de insetos economicamente importantes para as áreas da agricultura e medicina. Os cientistas têm examinado cuidadosamente a utilização destes fungos no controle biológico como biopesticidas, como micróbios exóticos introduzidos de um lugar para outro ou como agentes de controle em sistemas de manejo integrado de pragas. Este trabalho refere-se a recentes pesquisas básicas e aplicadas sobre fungos entomopatogênicos como agentes de controle biológico.

Termos para indexação: fungos entomopatogênicos; controle microbiano de insetos.

INTRODUCTION

About 750 species of entomopathogenic fungi found in all major taxa of the Eumycota are known to infect arthropods inhabiting plants, soil, and aquatic environments (Fuxa 1987, McCoy 1990). On plants, fungi infect a wide range of organisms including insects, mites, and spiders. Generally, mass destruction of the host occurs after considerable injury to the host plant. In natural and artificial aquatic habitats, mosquitoes, midges, and blackflies are regularly infected by fungal organisms. In soil, where entomopathogenic fungi cause mycosis to all developmental stages of many soil insects, this host/fungal relationship is lesser understood.

The fungi have some advantages that make them unique among entomopathogens. Rather than killing their host by toxigenic action following oral ingestion, they usually invade their host directly through the integument by a germ tube from a germinating spore. Therefore, mycosis is not limited to chewing insects but also occurs in homopterans and other arthropods with piercing-sucking mouthparts.

Ecologically speaking, the efficacy of most entomopathogenic fungi is limited by

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moisture. Spore germination and overall fungal survival are highly dependent upon moisture and probably require free water for many species. In essence, the utility of fungi as biological control agents is affected by an acceptable microclimate, one that favors optimum pathogen survival and spore germination.

About 25 species of entomopathogenic fungi have been scrutinized in biological control projects as biopesticides, as exotic microbes introduced from one place to another, or as manipulative agents in IPM systems. The purpose of this paper is to present a current overview on the use of entomopathogenic fungi in biological control. In addition, current fundamental research pertaining to better application of biological control will be discussed.

ENTOMOPATHOGENIC FUNGI IN CLASSICAL BIOLOGICAL CONTROL

Classical biological control (introduction of exotic fungi, either new species or more pathogenic strains of a species that already exist) has received very little attention in biological control (Carruthers & Hural 1990). Most fungal species have worldwide distribution; therefore, scientists have ignored introduction of new germplasm into a new area since the species was already there.

In the last decade, a number of biochemical methods (isozymes, RFLP, etc.) using electrophoresis (Kendrick 1978, Poprawski et al. 1988) have been developed to separate fungal strains. Comparative biological studies have conclusively demonstrated differences in pathogenicity, host specificity, temperature tolerance, etc. among strains (McCoy & Boucias 1989). In addition, pesticide resistant germplasm has been detected among fungi that infect insects (Osborne et al. 1990). Obviously, virulent strains genetically adapted to a new region of the world or tolerant to a common pesticide regime have potential for introduction from one place to another. The introduction of *Zoophthora radicans* into Australia from Israel for the control of spotted alfalfa aphid, *Therioaphis trifololli maculata*, is an excellent example of establishment of a more efficacious temperature tolerant strain of fungus in another country (Carruthers & Hural 1990). Another interesting classical study currently underway involves the introduction of *Beauveria bassiana* from Brazil into the U.S.A. for the control of the imported fire ant (Carruthers & Hural 1990). Since the fire ant is foreign to the U.S.A. but native to Brazil, an isolate of *B. bassiana* was introduced into Florida. The introduced strain (447) has been established in Florida soil and has reduced ant mound density, reduced colony vigor, and reduced ant foraging (Stimac et al. 1990). More recently, the application of molecular methods (nucleic acid restriction fragment length polymorphism (RFLP) and allozyme) for fungal identification has revealed the presence of *Entomophaga maimaiga* in gypsy moth population in the northeastern U.S.A. nearly 80 years after the fungus was introduced into the U.S.A. from Japan (Hajek et al. 1990).

The opportunity for applying both conventional biological and biotechnological methods is available; classical biological control research using selective entomopathogenic fungi can be expanded in the future.

AUGMENTATION OF FUNGI AS BIOLOGICAL CONTROL AGENTS

An augmentative approach for entomopathogenic fungi is used when endemic natural mycoses require enhancement or supplementation to reach epidemic proportions. This applied ecological approach is usually achieved by modifying the physical environment or managing crop production practices to favor fungal survival and infection or by the timed release of infective fungal propagules.

An excellent example of fungal pathogen augmentation has been recently demonstrated in the research of Brown & Nordin (1982) working with *Zoophthora phytonomi* mycosis of the alfalfa weevil, *Hypera postica,* in the U.S.A. Initially, detailed epizootiological studies showed that a threshold host density of about 2.0 weevils/alfalfa stem was required to induce an epizootic. Using laboratory, field, and simulation modeling experiments, early harvesting strategies were developed to maximize development and spread of the fungus. Early harvesting apparently increased disease prevalence because it (1) concentrated larvae in windrows, (2) injured or stressed weevil larvae to increase susceptibility, and (3) altered microenvironmental conditions (moisture) to enhance sporulation, germination, and thus infection by the fungus.

In China, the yellow-legged fungus gnat, *Phoradonta flavipes,* is an important pest of mushrooms in hothouses. By controlling the artificial application of water to enhance moisture conditions in the presence of the fungus, *Erynia ithacensis,* induced epizootics have been used to control the gnat and eliminate the need for chemical control (Li et al. 1990).

In view of the low cost for implementation, it is likely that augmentative programs using different fungal species will increase in the future as our knowledge of fungal epizootiology is improved and the use of biological control is more common place.

ENTOMOPATHOGENIC FUNGI AS BIOPESTICIDES

During the past 30 years, five deuteromycetous fungal species including *Aschersonia aleyrodis, Beauveria bassiana, Hirsutella thompsonii, Metarhizium anisopliae,* and *Verticillium lecanii* have been developed commercially by private and governmental agencies as mycopesticides and applied routinely to control arthropod pests worldwide (Table 1). In most cases, their use has been short lived due to a multiplicity of problems unique to each situation. The most common problem encountered has been difficulty in mass producing a stable product with acceptable storage properties, environmental persistence, and reliability of control in the field. In all cases, large quantities of infective propagules were needed to achieve control under ideal conditions.

Recently, a number of highly virulent pathotypes with broader host specificity have been mass produced and formulated by industry. All have improved product stability and storage capabilities. These breakthroughs

TABLE 1. Trades names of commercial or experimental preparations of fungi formulated as microbial insecticides.

Fungal species	Trade name	Producer
Aschersonia aleyrodis	Aseronija	All Union Inst. (USSR)
Beauveria bassiana	Biotrol FBB	Nutrilite Products (USA)
	Boverin	Glavmikrobioprom (USSR)
	Divaria	Abbott Labs. (USA)
Hirsutella thompsonii	Mycar	Abbott Labs. (USA)
Metarhizium anisopliae	Biotrol FMA	Nutrilite Products (USA)
	Metaquino	CODECAP (Brazil)
Verticillium lecanii	Vertalec	Tate an Lyle (England)
	Mycotal	Tate and Lyle (England)

combined with and expanding cautious interest by private industry in biopesticide development have stimulated more investigative research.

The development of the entomophthoralean fungi as microbial pesticides has been relatively slow because of problems with conidial survival following mass production. Resting spore, infected cadaver, and dry mycelial preparations have been developed for some species for testing against aphids and leafhoppers; however, there are no current programs designed to exploit their use (Wilding 1990).

There are six deuteromycetous species being investigated as biopesticides of mainly mosquitoes, soil insects, and glasshouse pests (Table 2). Most applied programs involve cooperation with industry in the area of production, safety and formulation research.

Fungi against soil insects

Most soil-inhabiting Coleoptera that feed on the roots of plants, shrubs, and trees as larvae are infected by pathogenic hyphomycetes, mainly *Beauveria* spp., *Metarhizium anisopliae*, and *Paecilomyces* spp. (McCoy 1990). The soil ecosystem can provide many favorable conditions for fungal survival (i.e., no solar effect) and, more importantly, the host is frequently an indirect pest with a low economic threshold. Therefore, pathogen persistence rather than virulence is a priority factor. In addition, fewer chemical pesticides are available for soil insect control, creating a niche for more environmentally safe biopesticides.

In Australia and Tasmania, pasture cockchafer grub populations are being reduced successfully using specific strains of *Metarhizium anisopliae*. Because of the surface foraging behavior of the black-headed cockchafer, cereal and clay conidial baits have been applied to the surface to kill nocturnal larvae that carry the fungus into the soil after feeding (Coles & Pinnock 1984). In Tasmania, a cold-tolerant strain of *M. anisopliae* has been drilled directly into the topsoil at 4.5×10^4 conidia/g of soil to achieve 60-80% control of the red-headed cockchafer, *Adoryphorus couloni*, a grub that feeds on

TABLE 2. Deuteromycetous fungi currently being tested as biopesticides.

Fungus	Field inoculum	Insect host	Location
Metarhizium anisopliae	conidia mycelia	cockchafers root weevil	pasture soil
Beauveria bassiana	conidia	weevils and grubs	soil
Beauveria brongniartii	blastospores	European cockchafer	–
Verticillium lecanii	conidia	whitefly, thrips	glasshouse
Paecilomyces fumoso-rosea	conidia	whitefly	glasshouse
Lagenidium giganteum	presporangia	mosquitoes	rice fields

pasture grass during a 2-year life cycle (Rath et al. 1990).

The Australian sugarcane white grub, *Antitrogus* sp., with a 2-year life cycle in the soil is being treated with a selective strain of *M. anisopliae*. Perliminary field results suggest an LC_{50} of 5 x 10^4 conidia/g of soil (Milner 1990).

In Switzerland, large-scale aerial application of *B. brongniartii* blastospores resulted in 90% infection of swarming European cockchafer, *Melolontha melolontha*, adults (Keller 1986). Subsequently, dead adults transmitted conidia to the soil-inhabiting larvae.

In the U.S.A., both *Beauveria bassiana* and *Metarhizium anisopliae* are being evaluated for the control of European chafer and Japanese beetle in the northeast (Krueger et al. 1990) and weevil larvae in the southeastern region of the country (McCoy 1990). Soil application of *M. anisopliae* conidia compared to dry mycelia showed more rapid mortality of chafer with a mycelial formulation but total mortality was similar (Krueger et al. 1990).

In Florida, 2 strains of a commercially formulated wettable powder of *B. bassiana* have been field tested for control of the larvae and emerging adults of different citrus root weevils (McCoy 1990). Conidia were applied to the soil surface prior to larval invasion of the soil from the citrus tree. Results based on fungal propagule counts and bioassay are encouraging although persistence appears to vary among soils and can be influenced by irrigation. In preliminary studies, a mycelial granular formulation of *M. anisopliae* (BIO 1020) developed by Bayer AG has been shown to sporulate in sandy soils and infect different species of citrus root weevils and other soil pests (Storey et al. 1990). BIO 1020 is a highly stable formulation and is the first commercial mycelial product developed as a biopesticide (Andersch et al. 1990).

Fungi against greenhouse insects

Greenhouse crops grown under controlled temperature and moisture conditions with reduced solar radiation offer excellent potential for pest control with fungi.

The fungus, *Verticillium lecanii* formulated commercially as Mycotal WP by Koppert has improved potency (50-fold increase in sporulation) and has shown excellent performance against greenhouse whitefly, *Trialeurodes vaporariorum*, on tomatoes and cucumbers (Schaaf et al. 1990). At 3 kg/hectare in 3000 ml water/hectare, the fungus has caused a 90% reduction in whitefly populations after 2 wk. The fungus appears to sporulate more abundantly on cucumber compared to tomato leaves.

Selected highly pathogenic strains of *M. anisopliae* are being evaluated for the control of black vine weevil, *Otiorhyneus sulcatus*, a serious pest of glasshouse plants in containers around the world (Moorhouse et al. 1990). Studies have shown that drench treatment of potted strawberries with 100 ml of a conidlal suspension at 5 x 10^7 conidia/ml will reduce larval populations from 75-97%.

In Florida, a strain of *Paecilomyces fumosoroseus* has been discovered with a broad host range and virulence to the sweet potato whitefly, *Bemisia tabaci* (Osborne et al. 1990). This fungus has attracted commercial interest and experimental formulation will be available for testing in the U.S.A. in 1991. This fungus is resistant to many pesticides commonly used in the glasshouse, an attribute favorable to its use in IPM systems.

Fungi against mosquitoes in aquatic habitats

Although a number of entomopathogenic fungi are known to attack mosquitoes, *Lagenidium giganteum* appears to have the greatest opportunity as a biopesticide when applied to rice fields (Jaronski 1990). Numerous field tests have been conducted using both aerial and ground application of either presporangia or oospores with 90% control in plots up to 10 hectares. However, control has been variable and the economics of mass production continue to be a limiting factor.

CONCLUSIONS

All aspects of research relating to entomopathogenic fungi as biological control agents have increased throughout the world in recent years. New novel ways to produce and introduce fungi into the environment are being developed. Molecular methods for identifying strains have changed our view of fungal use. If technological problems relating to product stability and fungal persistence in the environment can be resolved, their use will be expanded greatly in the future.

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