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Review

Mycopesticides: status, challenges and potential*

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SUMMARY

Fungi figure prominently among potential biocontrol agents of major agricultural pests, including weeds and insects. Fungi are among the most important pathogens of plants, and insect pathogenic fungi have long been of interest because of their unique mode of infection and their ability to create epizootics. Despite the fact that mycopesticides have a long experimental history, they have enjoyed only limited commercial success to date. Naturally occurring fungi are considered to be relatively slow acting and unreliable as biocontrol agents. Current research into mass production and formulation problems may provide additional mycopesticides during the current decade. The long-range potential of these biocontrol agents will depend upon the success of new screening programmes, as well as basic research into the molecular mechanisms of host–pathogen interactions.

INTRODUCTION

'Mycopesticides' are fungi intended for use in the biological control of (primarily agricultural) pests. Fungal hosts include numerous major agricultural pests such as insects, weeds, nematodes, and even other fungi [15]. The term 'pesticide' further implies that these agents will be used in an inundative rather than classical biocontrol scheme [22]. In the classical strategy, a new, exotic organism is introduced into an environment and becomes established as part of a modified ecosystem. There are a few successful examples of classical biocontrol involving fungi, such as the control of skeleton weed using *Puccinia chondrollina*, and control of the spotted alfalfa aphid using *Zoophthora radicans* (both in Australia) [17,25,81]. However, relatively few fungi appear to be good candidates for classical biocontrol [15]. Furthermore, this approach raises obvious environmental concerns, and offers no profit motive for commercial development. Inundative application involves heavy inoculation of an endemic fungus, usually by conventional farm equipment [54]. This creates a transient, localized elevation in the natural occurrence of the fungus, presumably with an equally temporary and localized effect on the environment.

Potential microbial biocontrol agents include bacteria, such as *Bacillus thuringiensis*, and numerous viruses. Fungi are of special interest for a number of reasons. They are often the best or only known natural pathogens of certain pests. Impressive epizootics of insects are commonly observed, in which entire insect populations are killed by fungi [17,75,77]. Most diseases of plants (including weeds) are fungal [21,54]. Fungi also characteristically attack their hosts in an unique, aggressive manner. Bacteria and viruses are by comparison passive invaders; insects must directly ingest these agents, and they customarily enter plants through wounds or stomata. Fungal pathogens attack insects directly by breaching the host integument, assisted or enabled by cuticle-degrading enzymes [19,20]. Target insects need not be actively feeding, or even capable of 'eating' (e.g. aphids). In direct analogy, most plant pathogenic fungi enzymatically attack the plant cuticle [24,69]. Furthermore, fungi are known to produce mycotoxins that may enhance the virulence of insect and plant pathogens [54,77].

STATUS OF MYCOPESTICIDES

The paradigm for all mycopesticides may well be the mycoherbicide Collego (Ecogen, Inc.), developed in collaboration by scientists at the University of Arkansas, the US Department of Agriculture, and Upjohn Laboratories [11, 28,99]. Collego is a preparation of *Colletotrichum gloeospo-*

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rioides f. sp. *aeschynomene*, used to control northern jointvetch (*Aeschynomene virginica*), primarily in rice crops. Since its registration in 1982, Collego has been used with safety and effectiveness, and is more or less commercially successful [22,45]. The first registered mycoherbicide, DeVine (Abbott Laboratories, in 1981), is a preparation of *Phytophthora palmivora*, used for control of *Morrenia odorata* (stranglervine or milkweed vine) in Florida's citrus groves, a small and specialized market [16,66,110]. Since the 1960s, the fungus *Cephalosporium diospyri* has been distributed gratis to Oklahoma ranchers for control of *Diospyros virginiana* (weedy persimmon) [45,46]. *Colletotrichum gloeosporioides* f. sp. *cuscutae* has been produced in large scale for control of dodders (*Cuscuta* spp.) in the People's Republic of China [103]. Other potential mycoherbicides are numerous. There are estimated to be over 8000 species of plant pathogenic fungi [15], of which perhaps 100 are at various stages of research and development as potential mycoherbicides [21,22,101]. Two of the most advanced candidates are *Alternaria cassiae*, being developed by Mycogen Corp. (San Diego, CA) for use against sicklepod (*Cassia obtusifolia*) and other soybean pests [7,23], and *Colletotrichum gloeosporioides* f. sp. *malvae*, registered in Canada in 1992 as Biomal by Philom Bios (Saskatoon, Canada), for control of round-leaved mallow (*Malva pusilla* Sm.) [45,84]. These and some of the other most frequently cited potential mycoherbicides are listed in Table 1. *Colletotrichum coccodes* is notable, as its host, *Abutilon theophrasti* (velvetleaf), is an important pest in corn and soybeans [44, 45,90,111].

Table 2 lists some of the entomogenous fungi believed to be the most promising as potential biocontrol agents. *Beauveria bassiana* has perhaps the longest history as an experimental mycoinsecticide; in 1874, Pasteur suggested that this organism might be used to control insect pests [85]. By the turn of the century, large-scale field testing of *Beauveria* was being done in Europe and North America [e.g. ref. 9]. In more recent times, *Beauveria* has been used in the former Soviet Union for control of the Colorado potato beetle, and in the People's Republic of China for control of corn borer, mites and caterpillars [38,77]. Private companies in the USA, such as Abbott Laboratories (Chicago, IL) and Nutrilite Products Inc. (Lakeview, CA), have produced experimental preparations of this organism [75]. Still, *Beauveria* cannot be considered commercially successful. *Metarhizium anisopliae* also has a long and illustrious history, including early experiments by Metchnikoff [80]. *Metarhizium* has found modern use in Brazil for control of pasture and sugarcane spittlebug [38,77]. Also like *Beauveria*, *Metarhizium* has been experimentally formulated in the USA by Nutrilite Products without ultimate success [75]. *Hirsutella thompsonii* was registered in the United States and marketed by Abbott Laboratories for mite control from 1981 to 1986 [75,76]. During this same

TABLE 1

Some potential mycoherbicides

Fungus	Weed host	References
Alternaria cassiae	Sicklepod (*Cassia obtusifolia*)	7,23
Cercospora eupatorii	Crofton weed (*Eupatorium adenophorum*)	31
Cephalosporium diospyri	Wild persimmon (*Diospyros virginiana*)	46
Chondrostereum purpureum	Black cherry (*Prunus serotina*)	92
Colletotrichum coccodes	Velvetleaf (*Abutilon theophrasti*)	44,90,111
Colletotrichum gloeosporioides f. sp. *aeschynomene*	Northern jointvetch (*Aeschynomene virginica*)	11,28
Colletotrichum gloeosporioides f. sp. *clidemiae*	Koster's curse (*Clidemia hirta*)	104
Colletotrichum gloeosporioides f. sp. *cuscutae*	Dodders (*Cuscuta* spp.)	103
Colletotrichum gloeosporioides f. sp. *malvae*	Round-leaved mallow (*Malva pusilla* Sm.)	84
Colletotrichum orbiculare	Spiny cocklebur (*Xanthium spinosum*)	4,5
Colletotrichum truncatum	Hemp sesbania (*Sesbania exaltata*)	12
Phytophthora palmivora	Strangler vine (milkweed vine) (*Morrenia odorata*)	16,66,110

TABLE 2

Some potential mycoinsecticides

Fungus	Insect host	References
Aschersonia aleyrodis	Whitefly	53
Beauveria bassiana	Beetles, caterpillars, etc.	38
Culicinomyces clavospirus	Mosquito	37
Erynia neoaphidis	Aphids	109
Hirsutella thompsonii	Mites	76
Lagenidium giganteum	Mosquito	37,70
Metarhizium anisopliae	Beetles, froghoppers, etc.	38,2
Nomuraea rileyi	Caterpillars	62
Paecilomyces lilacinus	Brown planthopper, etc.	58,108
Verticillium lecanii	Aphids, whitefly, etc.	52

period, *Verticillium lecanii* was marketed in the UK (by Tate and Lyle) for control of whitefly in greenhouses [52, 75]. *Aschersonia aleyrodis* has reportedly been produced in the former Soviet Union for control of whitefly [53]. There are approximately 750 known species of entomogenous fungi, many of which have been promoted as potential mycoinsecticides [17,77]. Others listed in Table 2 include *Lagenidium giganteum* for control of mosquitoes [37,70], *Nomuraea rileyi* for control of caterpillars [62], and *Paecilomyces lilacinus*, a pathogen of insects, nematodes, and recently recognized as a parasite of *Aspergillus flavus*, the source of aflatoxin [58,108].

It is clear that biocontrol fungi have been tested and promoted for a long time, at least 100 years. Despite this, their commercial status in 1993 is not all that different than that in 1893. A number of factors have contributed to this situation, not the least of which is the history of pesticide use. Synthetic chemical pesticides were fairly suddenly developed in the 1940s, quashing the then active interest in biological control [60,75]. Chemical agents are undeniably effective and convenient, and have in large part been responsible for making US agriculture the world's most productive. Interest in alternative control measures has regrown only very slowly during the past 15 years or so, the result of concerns about the personal and environment safety of chemical pesticides. New technology may also be slow to be accepted by producers. For example, although tests indicate that Collego performs as well as chemical agents, it has achieved only 20% market penetration [45]. Moreover, biocontrol agents in general, and mycopesticides in particular, have a not completely undeserved reputation for unreliability.

MYCOPESTICIDE CHALLENGES

Many potential mycopesticides exhibit relatively low virulence or efficacy, and all are slow acting compared to chemical pesticides [54,77,102]. Poor field persistence is a common problem; fungi often require high humidity to survive, and can be sensitive to UV light, temperature, and agricultural chemicals (most obviously fungicides) [15,41,77, 78,82]. Host range can also be a concern. For example, Collego should not be applied within 30 m of cucumber, squash, or other specific crops within its host range [99]. On the other hand, compared with the majority of synthetic chemical pesticides, most fungal pathogens appear to be fairly host specific [12,17,83]. For example, no naturally occurring fungi attack broadleaf weeds as a group. Although narrow host range may be desirable from regulatory and environmental perspectives, this characteristic may thus be limiting with respect to potential market size.

Even when attractive fungi are identified as potential mycopesticides, production and formulation difficulties can be formidable. Submerged culture production methods have been problematical for many fungi, including many species of *Alternaria, Colletotrichum* and *Fusarium* and most entomogenous fungi [14,40,95]. Most potential mycoinsecticides of the Entomophthorales cannot as yet be grown in vitro [77]. Often products lose virulence during propagation and have limited shelf lives [63,77,91].

POTENTIAL FOR MYCOPESTICIDES

New mycopesticides for this decade would likely be well-known organisms, for which production and formulation problems are currently being worked out. Advances in fermentation technology have in general improved the attractiveness of biological pesticides [14]. Work is being carried out to determine culture conditions that promote the retention of virulence [56,64]. Recent work by Roberts and coworkers has demonstrated that mycelial particles may be able to take the place of infective spores or conidia, whose production by submerged culture methods may be difficult [71,87]. A good deal of success in the formulation of fungal spores has involved the use of emulsions [27, 89]. Encapsulation strategies also have good potential for enhancing field persistence and other desirable properties [86,88,107]. Propagation and formulation methods can even influence pathogen virulence and host range [3,13,54,72,93].

The long-term prospects for mycopesticides seem excellent. There is a tremendous natural germplasm of pathogenic fungi remaining to be examined. Templeton has strongly recommended exploration to identify new pathogenic fungi [102], and it seems likely that new screening programmes could be highly profitable.

It should also be possible to genetically enhance mycopesticide fungi directly, to increase virulence and efficacy, and to tailor host range as desired. Genetic engineering strategies have been suggested for mycopesticide systems [6,51,102], and DNA transformation systems have been recently developed for such organisms as *Beauveria, Colletotrichum, Fusarium* and *Metarhizium* [26,39,43,67,100]. Specific genes that specify limiting virulence factors or host range determinants could, in theory, be isolated and modified to produce enhanced organisms.

Although our basic understanding of host–pathogen interactions is far from complete, basic research to identify determinants of fungal virulence and host range is active. Spore adhesion characteristics appear to be an important initial factor [36,77]. Insect cuticle-degrading enzymes appear to be important in virulence [19,20,94], particularly including proteases [8,97], and possibly also chitinases [33,55] and lipases [57,96]. Enzymes are important to most plant pathogens [24,68,105], especially cutinases [10,30] and also cell wall-degrading enzymes such as cellulases, xylanases and pectinolytic enzymes [18,32,35,79,98]. In our own work, we have carried out comparative studies of hydrolytic enzyme production by diverse natural isolates of potential biocontrol fungi, including the insect pathogens *Beauveria bassiana, Metarhizium anisopliae* and *Nomuraea rileyi*, the weed pathogen *Colletotrichum coccodes*, and the mycoparasite *Paecilomyces lilacinus* [1,34,47–50,73,74]. Among isolates of a given species, we found significant strain variability in the production and regulation of host-degrading enzymes, not related to overall virulence in a simple and direct way.

Numerous toxins are also produced by potential biocontrol fungi [15], including the entomotoxins beauvericin and destruxins [42,77] and phytotoxins such as curvulins and eremophilanes [54,60]. Host resistance factors include insect immune systems, plant phytoalexins and lytic and phenolic enzymes [59,65,77].

However, the relative importance of these various factors in any given host–pathogen is difficult to assess. That is, it is unclear which, if any, parameters could be manipulated to result in desired alterations of virulence or host range. Genetic identification of virulence factors is also problematical at this time. Often mutants with altered virulence characteristics have complex phenotypes, and many potential biocontrol fungi are not amenable to classical genetics [61, 77]. Molecular genetic evidence is thus far available for only a few potential virulence determinants [29,106].

Whatever new or improved mycopesticides are ultimately marketed will face the final hurdle of consumer acceptance. Extensive field testing and compatibility with conventional equipment are likely prerequisites for commercial success [45].

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