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Review Article

Current status of *Metarhizium* as a mycoinsecticide in Australia

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Abstract

BioGreen is currently the only mycoinsecticide registered in Australia. Research has shown that mycoinsecticides based on conidia of naturally occurring strains of *Metarhizium* are potentially valuable for control of a range of pests including canegrubs, termites and locusts. Recent developments with commercialization of products for these three target pests are reviewed. It is expected that BioCane, a granular product based on isolate FI-1045 of *M. anisopliae* var. *anisopliae*, for control of greyback canegrub, *Dermolepida albohirtum*, will be registered shortly following successful large-scale trials involving almost 9 tonnes of product in 1999. Another isolate from the same clade, FI-610, has been found to be effective against a range of termite species and is currently in commercial trials. Finally, large-scale testing of an isolate of *M. anisopliae* var. *acridum* against locusts has shown that doses as low as 1×10^{12} conidia/ha can effectively control Australian plague locust, *Chortoicetes terminifera*, while slightly higher doses have given good results against migratory locust, *Locusta migratoria,* spur-throated locust, *Austracris guttulosa,* and wingless grasshopper, *Phaulacridium vittatum*. It is expected that this product, recently given the name Green Guard™, will be used as a component of the preventive control programmes by the Australian Plague Locust Control Commission on properties where an organic control method is preferred. Australia, with two commercial producers of *Metarhizium*-based products, is poised to take advantage of the many benefits of using mycoinsecticides based on the *Metarhizium* fungus.

Introduction

Under natural conditions, fungi are a frequent and often important natural mortality factor in insect populations. All groups of insects may be affected and over 700 species of fungi have been recorded as pathogens. Some species are facultative generalist pathogens, such as *Aspergillus* and *Fusarium*. However, most species are obligate pathogens, often quite specific and rarely found, e.g. many species of *Cordyceps*. Entomophthoran fungi are often important in natural control of flies and aphids under warm humid conditions but attempts to use these fungi as biological insecticides have usually failed because they are too difficult to mass produce and are often ineffective under conditions of moderate humidity common in the field. Research has focussed on the relatively easily produced asexual spores (conidia) of the hyphomycete genera *Metarhizium, Beauveria, Verticillium* and *Paecilomyces*. These fungi often have a wide host range although there is considerable genetic diversity within species and some clades show a high degree of specificity (Driver *et al.*, 2000). For example, *Metarhizium anisopliae* var. *acridum* Driver & Milner is only effective against acridid insects (grasshoppers and locusts).

Unlike other potential biocontrol agents, fungi do not have to be ingested to infect their hosts but invade directly through the cuticle, and so can, potentially, be used for control of all insects including sucking insects. The main drivers behind the push for mycoinsecticides are the need for more specific agents as components of integrated pest management programmes, concerns over residues in grazing animals and crops, and the difficulty of controlling some insects using chemical insecticides. Mycoinsecticides can be produced locally, sometimes on waste products from other manufacturing processes, and therefore their potential is being investigated in less developed countries such as China and Brazil. Of importance is whether mycoinsecticides are classified as 'organic', which opens up increasingly significant markets. In Australia, Green Guard™ has been so-classified by the National Association for Sustainable Agriculture Australia (NASAA), by which organic beef producers are accredited.

There is currently only one mycoinsecticide registered in Australia. BioGreen is a granular product consisting of broken rice particles on which spores of *Metarhizium flavoviride* Gams & Roszypal have been grown. It is produced by BioCare Technology Pty Ltd in Somersby, New South Wales (NSW), and used to control redheaded pasture cockchafer, *Adoryphorus couloni* (Burmeister) (Col., Scarabaeidae), in turf and pasture in Victoria and Tasmania. Other products are registered in Europe, USA, and South Africa (Table 1).

Table 1. Examples of currently registered mycoinsecticides.

Country	Name	Fungus	Target	Crop
USA	Mycotrol/Botanigard	*Beauveria bassiana*	whiteflies/aphids/thrips	glasshouse tomatoes/ornamentals
USA	Naturalis	*B. bassiana*	sucking insects	cotton/glasshouse crops
USA	BioBlast	*Metarhizium anisopliae*	termites	houses
USA/Europe	PFR-97™	*Paecilomyces fumosoroseus*	whiteflies/thrips	glasshouse crops
UK	Vertalec/Mycotal	*Verticillium lecanii*	aphids/thrips	glasshouse crops
South Africa	Green Muscle	*M. anisopliae*	locusts	natural bushland
Réunion	Betel	*B. bassiana*	scarabs beetle larvae	sugarcane
Switzerland	Engerlingspilz	*Beauveria brongniartii*	scarab beetle larvae	pasture
Switzerland	Beauveria Schweizer	*B. brongniartii*	scarab beetle larvae	pasture
France	Ostrinol	*B. bassiana*	corn borer	maize
Australia	BioGreen	*M. flavoviride*	red-headed cockchafer	pasture/turf

Metarhizium as a Mycoinsecticide

In Australia, research is mainly directed at developing *Metarhizium* as a biological insecticide for use against a wide range of insects (Milner & Jenkins, 1996) including soil insects, locusts, and cattle ticks. The main groups are CSIRO Entomology in Canberra working in collaboration with the Bureau of Sugar Experimental Stations (BSES) and the Australian Plague Locust Commission (APLC), the Queensland Department of Primary Industries (Qld DPI) working on ticks in Brisbane, and Dr David Holdom, working on peanut scarabs and heliothis control for the Qld DPI in Brisbane. Two commercial companies undertaking research on production of *Metarhizium* are Biocare Technology Pty Ltd and SGB Pty Ltd (part of the IAMA group) in Albury. This paper will concentrate on the commercialization of products derived from CSIRO research.

Mycoinsecticides are usually formulated products with live conidia as the active ingredient. The conidia germinate on contact with the cuticle of the insect, produce a penetrating germ tube and establish a systemic infection which kills the host in 7 to 21 days depending on conditions, especially temperature and dose. At death, the host insect is a hard cadaver full of mycelium which grows out through the cuticle to form a new generation of conidia on the outside of the cadaver. This process and the initial germination and invasion are dependent on very high humidity and warm conditions. In Australia, suitable conditions occur in the soil in summer. However, for epigeal insects such as locusts, it is necessary to formulate the conidia in oil to overcome the humidity barrier at infection. The infection rarely develops to the stage of forming new conidia with locusts because conditions after death are usually too dry and because the cadavers are rapidly scavenged by predators such as ants. The live conidia are produced by solid-substrate fermentation on sterile moist rice, and then used directly as natural granules, or removed by sieving and formulated as a powder, granule or oil concentrate. Ideally 1-3 kg of substrate will produce enough conidia after 2-3 weeks incubation to treat a hectare.

Metarhizium is a genus composed of three species divided into ten clades (or varieties) (Driver *et al.*, 2000). The most common form is the genetically highly diverse *Metarhizium anisopliae* var. *anisopliae* (Metsch.) Sorokin. The soil forms its normal habitat, although it does not grow saprophytically in soil but exists as dormant conidia which infect susceptible hosts on contact. The soil-inhabiting larvae of scarab beetles are typical hosts and co-evolution has led to some isolates being specific to one or two genera of scarab. Thus the most virulent strains are usually those which cause natural epizootics in that particular host. This host specificity is dose-dependent: a high dose will infect a very wide range of hosts. At present, while the DNA profile is a guide to finding a virulent isolate, bioassay is the only way to find an effective isolate. Besides virulence and host specificity, temperature is an important factor in selecting an isolate for development as a mycoinsecticide. Most isolates grow well between 15° and 30°C, although some develop at temperatures as low as 5-10°C and others grow even at 35-40°C. Some isolates produce one or more members of a family of toxins called destruxins and while production of destruxin does sometimes correlate with virulence (Kershaw *et al.*, 1999), their role in pathogenicity is controversial. Certainly these compounds are toxic to some non-hosts when injected directly into the body cavity and one, destruxin E, is toxic *per os* for other insects such as Diptera, leading to speculation that destruxins could be used as insecticides. They can also be antifeedants (Amiri *et al.*, 1999).

Development of BioCane for Sugarcane Scarab Control

Insects attacking the roots of growing sugarcane cause reduced sugar yield, make the crop more vulnerable to damage at harvest and reduce the amount of subsequent ratooning. Some 18 species of scarab beetles (canegrubs) as well as the larvae of soldier fly, *Inopus rubriceps* (Macquart) (Dipt., Stratiomyidae), are the major pests which together cost the industry an estimated Au$10-15 million per year in Queensland (Agnew, 1997). Chemical pesticides (mainly suSCon® Blue with chlorpyrifos as the active ingredient) are widely used against canegrubs but are expensive and not effective against all species in all locations. Consequently there is a demand for a more specific, environmentally benign, alternative. The first mycoinsecticide developed for this purpose is BioCane which is a product of collaborative research between CSIRO, the BSES and BioCare Technology Pty Ltd funded by the Sugar Research and Development Corporation. This is a natural rice granule formulation of *M. anisopliae* var. *anisopliae* isolate FI-1045. The main target pest is the greyback canegrub, *Dermolepida albohirtum* (Waterhouse) (Col., Scarabaeidae), which is the worst single pest causing damage of over $5M a year. It is particularly serious in the Burdekin region, a large sugar-growing irrigation area near Townsville in Queensland where chlorpyrifos is ineffective because of breakdown associated with alkaline soils (Robertson *et al.*, 1998).

BioCane is produced in self-aerating bags, mixed with a clay to minimize dust problems and aid application, and applied at a rate of 33 kg/ha or 6.6×10^{13} conidia/ha (Logan *et al.*, in press). The application is made at fill-in. At this stage the plant has grown out of the set but the furrow is still open, enabling the material to be placed 10-15 cm below the soil surface. Recent studies have shown that it is important to cover the BioCane within an hour to avoid killing the conidia by over-heating, desiccation and exposure to UV. Greyback canegrubs have an annual lifecycle with eggs being laid in the cane in November/December. The grubs cause most damage as third-instars in March-June. Extensive field trials over several seasons have shown that BioCane applied correctly at 33 kg/ha will give 50-60% control of that season's grubs (Samson *et al.*, 1999). The conidia are known to persist in the soil profile for at least three years and so control should be as good or better in subsequent ratoons. The present cost is around Au$275/ha which is much less than the cost of the main alternative in the Burdekin – acidified suSCon®.

Almost 9 tonnes of product was used in 1999 in commercial trials under an experimental use permit and registration is expected early in 2000 with full commercial release in about June 2000.

Metarhizium for Termite Control

Hänel & Watson (1983) in their classic paper concluded that "unknown factors" were responsible for limiting the spread of *Metarhizium* in termite colonies thereby limiting the control potential. The present CSIRO project took the work of Hänel and Watson as the starting point and explored the nature of these "unknown factors". The main factors considered were the suitability of the isolate, the temperature and humidity conditions within the termite nest, possible antifungal compounds produced by termites and behavioural factors.

To obtain possible 'termite' isolates and also to assess the role of *Metarhizium* in natural control of termites, a survey was conducted of the occurrence of this fungus in termite nests and associated material in Australia (Milner *et al.*, 1998a). While *Metarhizium* was found to be widespread and over 100 isolates were obtained, the fungus was rarely found infecting termites and most isolates were thought to have originated from the surrounding soil used by the termites to construct their mounds. A diverse range of isolates was obtained which confirmed that termite-adapted isolates probably did not exist. Screening of these isolates resulted in the selection of FI-610 as being the most virulent for *Coptotermes* spp. (Isopt., Rhinotermitidae) and *Nasutitermes* sp. (Termitidae). FI-610 was shown to infect over a wide range of humidity and also to infect and kill termites at temperatures up to 35ºC (Milner *et al.*, 1998b). Thus the physical conditions in the nest were not inhibitory, nor was there any evidence that termite secretions played any role in defence of the colony.

Application of FI-610 to cut sections of the mounds of *Nasutitermes exitiosus* (Hill) resulted in those treated areas being walled off by the termites clearly suggesting a behavioural component in the defence. Subsequent laboratory and field experiments have confirmed that termites can detect and avoid conidia of FI-610. Other isolates may be less readily detected and while this is generally correlated with virulence, some isolates such as FI-1186 are reasonably virulent but less repellent than FI-610 (Staples & Milner, 2000). Termites effectively clean each other by allogrooming and this is often thought to aid the spread of pathogens such as *Metarhizium*; however, this is probably an effective defence because the groomed conidia end up in the gut of the termites where they are unable to infect, and the faeces may be used to line the galleries. Thus the conidia, even though still viable, are not available to contact the cuticle and invade the insect.

Despite these limitations, a number of strategies have been developed to use *Metarhizium* for termite control (Milner & Staples, 1996). Conidia of FI-610 can be blown into the termite galleries in buildings and these conidia will infect and kill those termites that are hit by the dust. The conidia will remain viable in the galleries and serve to prevent further invasion of the house, forcing the termites to feed elsewhere or to develop a new access point. This use could form part of an IPM strategy which includes making the house less prone to termite attack. If the colony can be found, then pure conidia blown into the central nursery can kill the colony. In Fiji, treatment of *Neotermes* sp. (Kalotermitidae) colonies feeding in plantation mahogany trees has been very successful using conidia blown into the hollowed trunks of the trees. Current research is directed at using the less repellent strains, as well as FI-610 formulated to make it non-repellent, in baits to destroy colonies from remote feeding sites.

Commercial trials are currently being undertaken by SGB Pty Ltd who are exploring the possibility of developing a commercial product.

Metarhizium for Locust Control

Australia's four major acridid pests have all been found to be susceptible to the same isolate of *M. anisopliae* var. *acridum* (FI-985) (Milner, 1997). Control of these pests is currently based on the use of chemical pesticides, particularly fenitrothion; however, there is an increasing demand for a non-chemical method of control with the increasing production of 'organic' beef in southwestern Queensland and a reaction against the use of chemical insecticides by many farmers. CSIRO research has extended the findings of the international LUBILOSA programme (LUtte BIologique contre les LOcustes et les SAuteriaux; http://www.cgiar.org/iita/research/index.htm; Lomer *et al.*, 1997) developing *Metarhizium* for control of locusts in Africa and has found that oil-formulated conidia kill more quickly than water formulations and are effective at low humidities. However it still requires about 2 weeks to work effectively in the field. The slow kill is no impediment to its use in programmes of preventive locust control used by APLC and the Queensland Department of Natural Resources (Qld DNR). Locust control begins early in outbreaks when locusts are mainly in pastures and aims to limit invasions of high-value crops. Following promising small-scale field trials, a national committee was established in 1997 to oversee and coordinate development of this product. The project is now a collaboration between CSIRO, APLC, NSW Agriculture and Qld DNR.

In the past year, about 2000 ha of pasture have been sprayed with dried conidia of FI-985 suspended in a mineral oil at doses in the range 1-5 $\times 10^{12}$ conidia/ha using fixed wing aircraft fitted with AU-5000 micronairs applying one litre of formulation per hectare. A dose of 3×10^{12} conidia/ha has consistently given over 90% control of migratory locusts, *Locusta migratoria* (L.), in the Central Highlands of Queensland over a range of weather conditions (Hunter *et al.*, 1999). Promising results have been obtained from field trials against Australian plague locust, *Chortoicetes terminifera* (Walker), spur-throated locust, *Austracris guttulosa* (Walker), and wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt). Factors affecting the efficacy of the product include the moisture content of the spores, the amount of vegetation cover, the dose of conidia and temperature. The fungus develops best when temperatures are 20-35ºC. Control may be delayed when night temperatures are low and when hot sunny conditions enable the locusts to raise their body temperatures to over 40ºC by basking (Blandford & Thomas, 1999). Under suitable weather conditions, control of very susceptible targets such as the Australian plague locust may be achieved at doses of 1×10^{12} conidia/ha or lower (Milner, 2000). The spray has not been shown to affect other insects except for bee workers, a small proportion of which may pick up a lethal dose while foraging.

Current field trials are aimed at determining if lower doses are efficacious in a variety of conditions against different species of pest grasshoppers and locusts. In addition, new formulations which improve the handling properties and ease of application are being developed. In collaboration with a commercial partner, SGB Pty Ltd, more efficient methods for production, harvesting and drying are being developed. The overall aim is to reduce the cost of using *Metarhizium* so that it becomes competitive with chemical pesticides. The name Green Guard™ has recently been registered for this product.

Conclusions

Metarhizium has a number of advantages as a mycoinsecticide: it is relatively easy to mass produce, strains can be found with appropriate levels of virulence and specificity, and the conidia can be dried to ensure good persistence in the field and storage for over one year in cool conditions. Research is concentrating on increasing the range of products registered and available for sale in Australia. One product, BioGreen, is already available and a second, BioCane, is expected to be registered this year. Products for use against termites and locusts are not far behind. Promising results have been obtained with other pests including cattle ticks, black field crickets, house flies, autumn gum moth, diamondback moth, thrips and cockroaches. In the USA, products with a similar fungus, *Beauveria bassiana* (Bals.) Vuill., as the active ingredient are registered and used against sucking insects such as whiteflies and mirids (see Table 1). While fungi account for only a small part of the pest control market at present, they offer many advantages as components of integrated pest management programmes, and Australia is poised to take advantage of this potential.

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