THE EFFECT OF *METARHIZIUM ANISOPLIAE* FORMULATIONS, AND THEIR COMBINATION WITH *SERRATIA ENTOMOPHILA*, ON GRASS GRUB LARVAE

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SUMMARY

Granular mycelial and conidial formulations of *Metarhizium anisopliae* were mixed with soil in two insectary experiments investigating the control of grass grub (*Costelytra zealandica*). In the first experiment three rates of each formulation were used. For each formulation the highest rate of treatment caused significant mortality (>30%) among second instar larvae, with mortality linearly related to dose. In the second experiment the level of mortality was increased to >60% when the bacterium *Serratia entomophila* was added with the fungal preparation. The results suggest that the granular formulation of *M. anisopliae* could have a role in grass grub control especially when combined with *S. entomophila*.

Keywords: *Metarhizium anisopliae*, grass grub, *Costelytra zealandica*, *Serratia entomophila*, mortality

INTRODUCTION

The fungus *Metarhizium anisopliae* is a well known pathogen of scarab and many other insect pests (Glare 1992a). It has been investigated previously as a pathogen of grass grub (*Costelytra zealandica*) by Latch (1965) but considered to have little potential as a practical control agent. The fungus has usually been tested as suspensions of conidia, but the development of a new granular mycelial formulation of *M. anisopliae*, ('Bio 1020') (Andersch *et al.* 1990), together with the recognition of important synergistic effects between the fungus and the bacterium *Serratia entomophila* (Glare 1992b), makes it timely to re-examine the potential of this pathogen for grass grub control.

In this paper we describe two insectary experiments which assessed the efficacy of *M. anisopliae* conidial and mycelial formulations against grass grub larvae and examine the potential of combined applications of *M. anisopliae* and *S. entomophila*.

METHODS

Experiment 1

Two forms of *M. anisopliae* (conidial dust and mycelial granules 'Bio 1020') were combined with heat-treated soil (Templeton deep silt loam), each at three concentrations. Rates of the formulations were; 10^4 , 10^6 , 10^7 conidia/g dried soil and 100, 500 and 2000 mg granules/litre dried soil. Conidial dust was dispersed in sterile water with the addition of 0.01% Tween 80. From this stock, aliquots were combined with water and the soil to establish an appropriate spore/soil rate at approximately 15% soil moisture. Granules were weighed out and thoroughly mixed with soil prior to potting. Soil was added to 500 ml pots and six 2nd instar grass grub larvae were added to each pot. Germinated clover seed was added to the surface of the pot as a food source. The experiment was a randomised block design with eight replicates. Two untreated controls were included with the six treatments in each block. The experiment was carried out in October 1992 in an open sided insectary, where ambient and soil temperatures were monitored. Larval survival was assessed by destructive sampling of the pots after 1 month. Dead larvae were recovered where possible. Analysis of variance was used to compare treatment means.

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Experiment 2

This experiment was carried out in parallel with Experiment 1. High rates of conidia ($10^7/\text{g}$ soil) and granules (2000 mg/litre) were used either alone or together with *S. entomophila* ($4 \times 10^5/\text{g}$ soil). There was an untreated control. The experiment was a randomised block design with five replicates of 2nd and five of 3rd instar larvae. Five larvae were added to each pot. In all other respects the experiment and analysis were identical to Experiment 1.

RESULTS

In Experiment 1, survival of larvae was significantly ($P < 0.05$) reduced at the high rate of both *M. anisopliae* treatments (Fig. 1) and mortality showed a significant ($P < 0.05$) linear correlation with dose in each treatment. Mortality due to *M. anisopliae* in the highest rate treatments, corrected for control mortality by Abbott's formula, was estimated as 33% and 47% for conidia and granules respectively. Few dead larvae showing sporulation of *M. anisopliae* were found, with all but one (4/5) in the high rate granule treatment.

Figure 1: Effect of *Metarhizium anisopliae* formulations on mortality of 2nd instar grass grub larvae. L,M,H - Low medium and high rates of formulation (see text). LSD(a) control vs any one treatment; LSD(b) between treatments.

In Experiment 2, survival among the 3rd instar larvae was uniformly high and ranged between 50 and 60% with no significant treatment mortality effects. With the 2nd instar larvae, however, a significant ($P < 0.05$) level of mortality was obtained with the *M. anisopliae* granule treatment, while the response to the conidial preparation was non-significant (Fig. 2). Addition of *S. entomophila* significantly ($P < 0.05$) increased mortality over fungus alone and all the surviving larvae showed symptoms of amber disease. Mortality due to treatment was calculated as 62% for conidia and *S. entomophila*, and 68% for granules and *S. entomophila* (Abbott's corrected).

DISCUSSION

The experiments showed that 2nd instar grass grub larvae could be killed by *M. anisopliae* applied at high rates. While the formulations are not directly comparable, combining the mortalities from the high rate treatments in both experiments suggests a greater effect from granules than conidia. The rate of granules which gave significant

Figure 2: Effect of *Metarhizium anisopliae* formulations alone and in combination with *Serratia entomophila* on mortality of 2nd instar grass grub larvae. G, granule; C, conidia.

control of grass grub larvae is similar to that reported by Andersch *et al.* (1990) for black vine weevil (*Otiorhynchus sulcatus*) control. They found that conidia produced by the mycelial granule remained viable and active in the soil for several months. Therefore, the short time period of these experiments and the cool conditions in October may have resulted in underestimation of the potential of the fungus.

Few infected sporulating cadavers were recovered from any of the fungal treated soils despite high levels of mortality. The experiments were carried out with a mean ambient temperature of 15.6°C and soil temperatures ranging between 3 and 22°C with a mean of 12°C. It is possible that at these low temperatures larvae were killed by the fungus but colonised by secondary bacteria causing septicaemia and rapid disappearance of the cadavers. Latch (1965) considered *M. anisopliae* to have little potential for grass grub control due to high temperature requirements for germination of the conidia. However, Rath (1992) has obtained high levels of control of the redheaded cockchafer (*Adoryphorus couloni*) in Tasmania with a strain of *M. anisopliae* active at low temperatures.

In the second experiment there was little effect of the fungus on the 3rd instar larvae. This is an unexpected result as mature larvae are generally considered more susceptible than young ones (Glare and Milner 1990). For the 2nd instar larvae, the combination of *M. anisopliae* and *S. entomophila* produced very high levels of mortality within the 1 month assessment period. These were significantly greater than those with *M. anisopliae* alone and faster than would have occurred with *S. entomophila* alone, which causes amber disease but usually no mortality until more than one month after initial infection. As expected, the surviving larvae in the bacterial treatments were all showing symptoms of amber disease.

The results of these preliminary experiments suggest that *M. anisopliae* granular mycelial formulations should be investigated further for control of grass grub and other scarabaeids. It is interesting to note the level of mortality which occurred without sporulation which suggests that the effects of the fungus as a natural mortality factor may be underestimated. This may be particularly true in areas where other pathogens are also present. In particular, the combination of *M. anisopliae* with *S. entomophila* should be pursued further.

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REFERENCES

- Andersch, W. Hartwig, J. Reinecke, P. and Stenzel, K., 1990. Production of mycelial granules of the entomopathogenic fungus *Metarhizium anisopliae* for biological control of soil pests. *Proc. Vth International Colloquium on Invert. Path. and Microbial Control*: 2-5.
- Glare, T.R., 1992a. Fungal pathogens of scarabs. Pp 63-77 *In: The Use of Pathogens in Scarab Pest Management*, T.A. Jackson and T.R. Glare, (Eds.). Intercept Books, Andover.
- Glare, T.R., 1992b. Synergistic and antagonistic interactions among microbes associated with *Costelytra zealandica* (Coleoptera: Scarabaeidae). *Proc. 25th Ann. Meet. Soc. Invert. Path.*: 177.
- Glare, T.R. and Milner, R.J., 1990. Ecology of entomopathogenic fungi. Pp 547-612 *In: Handbook on Applied Mycology*, Vol 2, D.K. Arora, K.G. Mukeriji and J.G.F. Pugh, Marcel Dekker Inc., New York.
- Latch, G. C. M., 1965. *Metarhizium anisopliae* (Metschnikoff) Sorokin strains in New Zealand and their possible use for controlling pasture inhabiting insects. *N.Z. J. Agric. Res.* 8: 384-396.
- Rath, A., 1992. *Metarhizium anisopliae* for control of the Tasmanian pasture scarab *Adoryphorus couloni*. Pp 217-227 *In: The Use of Pathogens in Scarab Pest Management* T.A. Jackson and T.R. Glare, (Eds.). Intercept Books, Andover.