TEMPERATURE LIMITATIONS ON FIELD EFFECTIVENESS
OF *METARHIZIUM ANISOPLIAE* AGAINST *COSTELYTRA*
ZEALANDICA (WHITE) (COLEOPTERA: SCARABAEIDAE)
IN CANTERBURY

T.R. GLARE, R.J. TOWNSEND and S.D. YOUNG

AgResearch, PO Box 60, Lincoln New Zealand

SUMMARY

A New Zealand isolate of the entomopathogenic fungus, *Metarhizium anisopliae*, was applied against grass grub (*Costelytra zealandica*) larvae by direct drilling into pasture in March, 1993, to give a concentration of approximately 4×10^5 conidia/g dry soil. Spore survival over the next year was good, but few infected larvae were recovered and larval numbers did not decrease in treated plots. To investigate if temperature was limiting infection by the applied *M. anisopliae*, soil from treated sites was collected, seeded with healthy larvae, and kept at 15°C and 20°C for over 80 days. Larvae held at 15°C remained healthy while those at 20°C succumbed to *M. anisopliae* infections. This demonstrated the requirement for cold-temperature active *M. anisopliae* for Canterbury conditions.

Keywords: *Metarhizium anisopliae*, *Costelytra zealandica*, biological control, temperature

INTRODUCTION

The fungus, *Metarhizium anisopliae* (Deuteromycetes), is one of the most common insect pathogenic fungi in the world. In New Zealand, it has been described from 10 insect species (Glare *et al.* 1993) and it is a common pathogen of both grass grub, *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae) larvae and porina caterpillars, *Wiseana* spp. (Lepidoptera: Hepialidae). The prevalence of the fungus in grass grub and porina populations led to early investigation of its biological control potential. The most detailed study in New Zealand was conducted by Latch (1965), who examined strains isolated from grass grub, porina and another scarab, *Pericoptes truncatus* F. He concluded that the temperature limitations of *Metarhizium* spp. would severely reduce the effectiveness of the fungus as a biological control agent in New Zealand pastures. This conclusion has rarely been tested in the field in New Zealand. Despite the temperature limitations found by Latch (1965), *M. anisopliae* is still commonly isolated from pastures in both the North and South Islands of New Zealand, although it rarely (if ever) causes recognisable epizootics.

Several recent advances with *Metarhizium* spp. overseas has again raised the possibility of using *M. anisopliae* in New Zealand. The successful development of new formulations, such as oil-based *M. flavoviride* for locusts (Bateman *et al.* 1993) and dry mycelial preparations of *M. anisopliae* (reviewed by Zimmermann 1993), has increased the use of this genus throughout the world. In addition, Rath (1992) and colleagues have developed *M. anisopliae* as a commercially-prepared control agent for cockchafer larvae in Tasmania, an area traditionally considered too cold for effective fungal-based biological control. Because of these results, *M. anisopliae* was reevaluated as a biological control agent for New Zealand pasture pests. A field trial was established to test whether a) inoculum would survive during the year and b) *M. anisopliae* could be an effective control agent if applied in Canterbury.

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MATERIALS AND METHODS

Fungal isolates and culture

The *M. anisopliae* isolate F16 was originally isolated from a *C. zealandica* larva collected from Nelson, in 1990. A cold tolerant isolate, F36, also isolated from a grass grub larva, was collected near Riversdale, Otago, in 1991. These isolates have been maintained on Potato Dextrose Agar (PDA) (GIBCO, Paisley, Scotland) at 15°C–25°C, by subculturing every 2 to 4 weeks, and holding them at -70°C. For field trials, F16 was grown on rice in autoclavable bags. Each bag containing 100 g of polished short grain rice and 50 ml distilled water, was sterilised at 121°C for 20 minutes, then allowed to cool prior to addition of the inoculum. Inoculum for field trials was produced by scraping conidia directly from PDA cultures into 0.01% Triton x-100. After inoculation, the bags were stored at 25°C for 5 weeks, by which time all contents were covered by a mass of dark green spores. Conidia were removed from the rice by addition of 200 ml 0.01% Triton x-100 to each bag, followed by vigorous shaking. Conidial density was estimated by plating dilutions onto PDA and incubating at 25°C for approximately 3 days.

Field application

The field trial was conducted on a farm near Methven, on the Canterbury plains. *M. anisopliae* was applied in March, 1993, as a solution using the same method as developed for the bacterium, *Serratia entomophila* (Jackson *et al.* 1988). A Duncan triple-disc drill modified for liquid application was used to apply the fungal suspensions ($8.2\text{--}9.6 \times 10^7$ colony-forming units (CFUs)/ml) in 400 litres of water/ha at a depth of 10–30 mm and at a rate resulting in approximately 4×10^5 CFUs/g dry soil. Fungal inoculum was applied to five replicate (15 x 5 m) plots arranged in a randomized block design. Untreated control plots were not drilled.

Twenty, 2.5 cm diameter soil samples (depth, 8 cm) were taken per plot at regular intervals throughout the year for serial dilution of soil suspensions, which were plated onto a selective medium modified from Doberski and Tribe (1980) (1% neopeptone, 4% glucose, 1.5% agar, 0.025% cycloheximide and 0.05% chloramphenicol). Plates were incubated at 25°C for 3–7 days before microscopic examination for *Metarhizium* spp. colonies. To estimate *C. zealandica* larval numbers and disease incidence, 20 10.2 cm diameter (depth, 20 cm) or 25 6.2 cm (depth, 12.5 cm) cores were taken from each plot approximately every 10 weeks, sorted in the field and larvae assessed in the laboratory for fungal infection by incubating at 20°C.

Due to equipment failure, no soil temperature readings were available from the trial site. Subsurface (10 cm) temperature readings discussed are from pastures at Winchmore Research Station, 30 km from the trial site. Due to the soil type at Winchmore, it is likely these temperatures were slightly higher than those at the trial site. Soil moisture was calculated from plot samples and ranged between 26 to 48% (w/v).

Incubation of larvae in treated and untreated soil

To test whether temperature was limiting the development of disease in grass grub larvae in treated pasture, soil from the field experiment treated with *M. anisopliae* F16 was brought into the laboratory in December, 1993, and stored for 2 days at 4°C prior to use. Twelve grams of soil was added to each of 20 universal bottles. For an untreated control, 20 universals of 10 g of heat-sterilized soil were used, wetted with 2 ml 0.01% Triton x-100. Third instar grass grub larvae collected from a Canterbury farm, thought to be free of fungus, were used. Tubes were arranged in a randomized block design. Twenty larvae were incubated individually in soil at 15° and 20°C for over 80 days. Larvae were examined and fed at regular intervals. Results for day 60 were statistically examined by χ^2 analysis using 2 x 2 factorial contrasts.

RESULTS

Field application of *M. anisopliae* isolate F16

Application of *M. anisopliae* F16 by modified disc drill resulted in high inoculum survival. Sampling on the drill rows on the day of application, and after 1 week, gave an average of 37.94 and 19.47×10^4 CFUs/g dry soil, respectively. Only occasionally

Figure 1: a) Number of colony forming units (CFUs) of *Metarhizium anisopliae* recovered on selective media after application to trial plots (mean of five replicates $\pm$ SEM). b) Larval numbers/m² in field plots after application with *M. anisopliae* (F16) ($\pm$ SEM). In both graphs, week 0 = March, 1993.

Figure 2: Effect of incubating third instar *Costelytra zealandica* larvae in soil from *Metarhizium anisopliae* (F16) treated plots and sterilized soil at 15°C and 20°C.

was any *M. anisopliae* detected on the selective medium in untreated control plots. Sampling continued through to February 1994, and it was found that fungal inoculum remained viable in soil throughout the year (Fig. 1a). In Figure 1a, samples taken in week 0 and 1 were from the application furrow, whereas other samples were taken

randomly within each plot. To compensate, on-row samples (0 and 1 week) have been divided by 10 in Figure 1a. Despite the high levels of *M. anisopliae* present, larval numbers remained unaffected, and there were as many larvae in the treated plots as in the untreated controls (Fig. 1b). Larval numbers in the treated plots actually increased substantially in the second season compared with the control plots. Few *M. anisopliae*-infected cadavers were recovered and few larvae incubated at 20°C in the laboratory showed mycoses. Temperature records at Winchmore Research Station showed that during the larval and pupal stages (March–November) soil temperature averaged below 15°C. Only for January and February, 1994, did soil temperature exceed 15°C.

Incubation of larvae in treated soil

As larval numbers had not declined significantly, despite good inoculum survival, it was suspected that the low soil temperature was affecting fungal infection. Plate counts of *M. anisopliae* on selective medium from the treated soil was equivalent to 4.2×10^5 CFUs/tube of soil. The effect of incubating healthy larvae in treated and sterile soil differed significantly between the two temperatures (day 60, $P < 0.01$) (Fig. 2). At 15°C few larvae died in either sterile or treated soil, whereas at 20°C all larvae died in treated soil but few larvae died in sterile soil. There were no viable fungi in the control soil. Although the larvae used were thought to be from a fungus-free farm, there was some fungal infection in the untreated soil, indicating the prevalence of *Metarhizium* in Canterbury farms.

DISCUSSION

Application of isolate F16 to Canterbury *C. zealandica* populations resulted in good inoculum survival, but poor larval control, even in the warmer summer months. Direct drilling fungal conidia into pastures was highly successful in establishing inoculum in soil. Incubation of larvae in treated soil at higher temperatures in the laboratory demonstrated that temperature was a major factor limiting infection, as predicted by Latch (1965). It is interesting that larvae from treated plots rarely succumbed to disease when removed from soil and incubated at higher temperatures in the laboratory, whereas supposedly healthy larvae incubated in treated soil at 20°C did become diseased. This suggests that it is germination and host penetration that is inhibited by low temperatures, not the progress of disease within the host. The rise in the larval populations in treated plots in the second season was unexpected. Previously, it has been shown that scarab adults can be attracted to *M. anisopliae* mycelia in the soil (Villani *et al.* 1994), but whether this would account for the increased number of larvae is questionable. It is possible egg laying in this pasture was not even in the second year and further sampling of the whole paddock containing the trial site will give a better understanding of larval distribution.

The recent discovery of a strain (F36) which kills 100% of larvae at 15°C in bioassays (which F16 does not; Glare, unpubl. data), provides a means to possibly circumvent the temperature limitation on *M. anisopliae* effectiveness. The strain of fungus used in the field experiments (F16) is, genetically, closely related to *M. anisopliae* var. *anisopliae*, the most common strain found throughout the world (Curran *et al.* 1994). The F36 strain is more closely related to another species, *M. flavoviride*, than to F16 (Curran *et al.* 1994; Glare and van der Wielen, unpubl. data) and also genetically related to the cold-temperature active strain used in the successful Tasmania project (F. Driver, pers. comm.). The work of Rath (1992) and colleagues developing *M. anisopliae* for cockchafer control in Tasmania has demonstrated that by careful strain selection, it is possible to utilize this fungus, even in colder regions. Inoculum application and survival in the present trial was good and therefore if an appropriate strain is used, control may be possible. The strain F36 is difficult to grow and was easily contaminated at the time of the experiments. This isolate will be used in future field experiments once production problems have been overcome.

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