Biological control of the black field cricket, *Teleogryllus commodus* (Walker) (Orthoptera: Gryllidae), using the fungal pathogen *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes)

Richard J. Milner[A], Lisa Miller[B], George G. Lutton[A] and Felice Driver[A]
[A]CSIRO, Division of Entomology, GPO Box 1700, Canberra, ACT 2601, Australia.
[B]Agriculture Victoria, Colac, Victoria 3250, Australia.

Summary

The hyphomycete fungus, *Metarhizium anisopliae,* is a natural pathogen of black field cricket, *Teleogryllus commodus,* in Victoria. Comparison of 12 isolates, using Random Amplified Polymorphic DNA (RAPDs), from this insect collected in Victoria showed that they were all very similar genetically but quite distinct from the group 3 *M. flavoviride* isolates from grasshoppers and locusts. Screening bioassays showed that isolates from crickets were more virulent for this pest than isolates from other sources. One of these cricket isolates FI1099 was selected for use in field trials. An oil formulation of conidia of FI1099 was tested either as a ultra-low volume (ULV) spray or in a high volume oil/water emulsion at two sites in Victoria. At Warrambeen, a dose of 2×10^{13} conidia per hectare gave a 30–40% reduction in the cricket population after 21 days compared with 80% control in the malathion plots. At Turkeith, the higher dose of 4×10^{13} conidia per hectare gave 60–70% control while the malathion again gave about 80% control. These results are promising and show that *M. anisopliae* has the potential to control black field cricket when applied as an oil-based mycoinsecticide.

Introduction

The black field cricket, *Teleogryllus commodus* (Walker) (Orthoptera: Gryllidae), is a serious pest of pastures in parts of Victoria and South Australia causing over $8m damage in some years (Anon. 1989). It is also a serious pest in parts of the north island of New Zealand (Blank 1982). Populations are particularly high in western Victoria where cracking clay soils provide essential shelter during hot dry weather. *T. commodus* is univoltine: over-wintering diapause eggs are laid in the soil in autumn and hatch in early summer (December); the nymphs develop through five instars before becoming adults usually in the autumn (March). Populations decline with the onset of cooler weather in April and few, if any, adults over-winter. Pasture defoliation can be severe in late summer and autumn (February–April). The only method of control available to farmers is the application of chemical insecticides using malathion cereal baits (Stahle *et al.* 1983). Many thousands of hectares are treated each year, often using aerial application methods.

There is no published information on natural enemies of black field cricket other than pathogens. As part of a programme evaluating pathogens for control of crickets, Reinganum *et al.* (1981) surveyed diseases in Victoria. Live crickets were sampled from 232 sites in 1979 and pathogens either apparent at the time of sampling, or which appeared within two weeks of collection, were identified. Cricket paralysis virus was found most commonly (42.7% of sites) and *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes), the only other pathogen found, infrequently (5.2% of sites). Neither pathogen was sufficiently abundant to have any influence on population density. A number of isolates of *M. anisopliae* were obtained during the survey and many of these were evaluated in the present study (Table 1). Reinganum *et al.* (1981) concluded that the fungus, since it was pathogenic and easily grown on inexpensive media such as rice, was promising for development as a mycoinsecticide, however no field trial results have been published.

Recently, it has been shown that certain isolates known as *M. flavoviride* Gams and Rozsypal group 3 (Prior 1995) comprise a genetically uniform and distinct group, and are virulent for a range of grasshoppers and locusts. Interestingly, natural infections of *M. flavoviride* group 3 have only been found on grasshoppers and locusts which may suggest that they have co-evolved. Furthermore, it has been found that by formulating the conidia of *M. flavoviride* in oil, the fungus can control locusts and grasshoppers when applied using ultra-low volume (ULV) techniques or in an oil-emulsion using high volume boom sprayers (Baker *et al.* 1994). The oil not only prevents evaporation of water during ULV spraying but also aids attachment and penetration of the target host by the fungus. This enables the conidia to germinate under field

Table 1. List of isolates and the sources of *Metarhizium* spp. used in this study.

Isolate	Species name	Original host	Source	Place
FI 610	*M. anisopliae*	termite mound	CSIRO	Brown Mt., New South Wales
FI 985	*M. flavoviride*	*Austracris guttulosa*	ARSEF 324	Rockhampton, Queensland
FI 1030	*M. anisopliae*	*T. commodus*	IMI 351 830	Victoria
FI 1037	*M. anisopliae*	*T. commodus*	ARSEF 448	Victoria
FI 1066	*M. anisopliae*	soil	IIBC Ben 4	Benin
FI 1067	*M. flavoviride*	acridid	IIBC I92–701	Benin
FI 1090	*M. anisopliae*	*T. commodus*	ARSEF 435	Warrnambool, Victoria
FI 1091	*M. anisopliae*	*T. commodus*	ARSEF 437	Victoria
FI 1092	*M. anisopliae*	*T. commodus*	ARSEF 438	Victoria
FI 1093	*M. anisopliae*	*T. commodus*	ARSEF 445	Carapook, Victoria
FI 1094	*M. anisopliae*	*T. commodus*	ARSEF 440	Warrnambool, Victoria
FI 1095	*M. anisopliae*	*T. commodus*	ARSEF 441	Warrnambool, Victoria
FI 1096	*M. anisopliae*	*T. commodus*	ARSEF 442	Hawkesdale, Victoria
FI 1097	*M. anisopliae*	*T. commodus*	ARSEF 443	Camperdown, Victoria
FI1098	*M. anisopliae*	*T. commodus*	ARSEF 444	Byaduck, Victoria
FI 1099	*M. anisopliae*	*T. commodus*	ARSEF 445	Carapook, Victoria
FI 1100	*M. anisopliae*	*T. commodus*	ARSEF 446	Mortlake, Victoria

conditions of low humidity which normally prevent or seriously delay the infection process (Bateman *et al.* 1993, Milner *et al.* 1995). Crickets are also orthopteroid insects, and the present study was undertaken to determine if the grasshopper control strategy could be extended to crickets.

For genetic fingerprinting, randomly amplified polymorphic DNA (RAPD) markers have been used to generate molecular markers (Welsh *et al.* 1991, Williams *et al.* 1991) and to analyse genomes and populations in a variety of organisms including fungi (Fegan *et al.* 1993, Bidochka *et al.* 1994), humans and plants (Williams *et al.* 1991), and insects (review by Haymer 1994). These DNA polymorphisms may arise from repetitive and unique regions and indicate the degree of relatedness between isolates.

The aims of the study were to use molecular fingerprinting techniques to assess the genetic status of isolates from *T. commodus*, select the most virulent isolate for this host and to undertake initial field tests of the efficacy of the selected isolate in oil-based sprays.

Materials and methods

Isolates

A list of isolates used in this study are given in Table 1. The isolates from *T. commodus* were all from the ARSEF collection (USDA Insect Pathogenic Fungi Culture Collection at Ithaca, New York) and originally derived from the survey for pathogens of black field cricket (Reinganum *et al.* 1981). Other isolates were either from the CSIRO Insect Pathogen Culture Collection or from the International Institute of Biological Control, Ascot, United Kingdom.

Genetic analysis

To extract the DNA, the methods of Curran *et al.* (1994) were modified slightly. Briefly, 50–100 mg of dried mycelium was ground to a fine powder using a mortar and pestle. The ground mycelium was transferred to a 1.5 mL microcentrifuge tube containing 750 µL proteinase K buffer (0.1M Tris HCl pH 8.5, 0.05 EDTA, 0.2M NaCl, 1% SDS and 2 mg mL^{-2} proteinase K) and incubated at 65°C for 30 minutes. The DNA was extracted by hand using an equal volume of water saturated phenol. The phases were separated by spinning for two minutes in a microfuge, and the aqueous phase was transferred to a clean tube. The extraction procedure was repeated using 1 volume phenol : chloroform : isoamyl alcohol (50:48:2), then chloroform : iso amyl alcohol (24:1) until the interface was clean. DNA was precipitated using 1 volume of isopropanol. The DNA was recovered by centrifuging for two minutes. The pellet was washed twice with 500 µL of 70% ethanol, then dried in a vacuum and resuspended in 400 µL of 10mM Tris HCl at pH 8.0, 1mM EDTA and 10 µg/mL RNase A. The DNA was incubated at 37°C for 30 minutes and then extracted with 1 volume phenol : $CHCl_3$: isoamyl alcohol (50:48:2), followed by $CHCl_3$: isoamyl alcohol (24:1) until the interface was clean. The DNA was precipitated by adding 40 µL 3M sodium acetate pH 5.4 and two volumes ethanol. The DNA was recovered by brief centrifugation in a microfuge, washed twice with 70% ethanol, then dried in a vacuum. Pellets were resuspended in 5mM Tris HCl, 0.5mM EDTA.

RAPD-PCR (polymerase chain reaction) amplifications were performed in a total volume 25 µL. Each reaction contained 2.5 µL of 10X Bresatec reaction buffer (16.6mM $(NH_4)_2SO_4$, 67mM Tris HCl at pH 8.8, 0.45% Triton X100, 200 µg mL^{-1} gelatin) 3.5mM $MgCl_2$, 200µM each dATP, dTTP, dCTP, dGTP, 30pM primer, 10–15 ng DNA and 2.4 U Taq polymerase (Bresatec). The reactions were covered by a drop of light mineral oil. Primers were those used by Fegan *et al.* (1993) from Random Primer kits H and F (Operon Technologies, Alameda, California). Reactions were placed in a thermocycler (Corbett Research, Australia) and DNA was amplified using the following temperature cycles: one cycle at 94°C for five minutes, 40°C for two minutes, 72°C for three minutes, then 39 cycles at 94°C for one minute, 40°C for one minute 30 seconds, and 72°C for two minutes.

Products were separated in 1.3% TBE agarose gels (Sambrook *et al.* 1989). Gels were run at 7.5 V cm^{-1} for two hours, stained with ethidium bromide and photographed on a UV transilluminater (UVP Inc.).

Bioassay

Five isolates (Table 2) were grown on plates of Sabourauds Dextrose Agar supplemented with 1% yeast extract. After about three weeks at 25°C, the conidia were harvested into Propar 12 oil (Ampol) and sonicated to produce a homogenous suspension. The concentration of conidia was determined using a Petroff-Hausser particle counter and adjusted to 3×10^7 conidia mL^{-1}. Two decimal dilutions were made in Propar 12. Adult crickets were inoculated by placing a 5µL drop of Propar 12 using a micropipette on the ventral thorax and were then incubated individually in 20 mL plastic containers at 28°C . Mortality was recorded every day for 12 days and the dead insects placed in moist chambers to promote sporulation. Twenty insects were used for each of the three doses, 20 were treated as blank oil controls and 20 were left as untreated controls. In the first experiment, the crickets were *T. commodus* while in the second the closely related species *T. oceanicus* was used as they were more abundant in the laboratory colonies. In previous laboratory experiments, it had been found that these species are similar in their susceptibility to *Metarhizium* spp. (Milner unpublished data).

Field trials

Two field trials were set up in February 1995. The first was a replicated small plot trial established at the Alcoa Landcare Demonstration Farm, "Warrambeen" near Colac, Victoria. Each plot was 25 × 25 m and separated by an untreated buffer of 20 m. There were four treatments: untreated control, baited with malathion at a rate of 125 mL a.i. to 10 kg oats ha^{-1}, sprayed with a high volume oil/water emulsion of *M. anisopliae* at 2×10^{13} conidia ha^{-1} in 130 L ha^{-1}, and the same number of conidia applied using a MicroUlva Plus ULV sprayer at 2 L ha^{-1}. The plots were arranged in a randomized block with four replicate plots per treatment. The treatments were applied late in the evening on February 15 as the very hot, windy, day-time conditions were unsuitable for spraying.

The other trial was set up at "Turkeith", a property located about 20 km south of Warrambeen and with a history of serious cricket infestations. There were two unreplicated plots each 50 × 50 m separated by a 20 m untreated buffer. One plot was treated with the same high volume spray as at Warrambeen but at double the dose – 4×10^{13} conidia ha^{-1}, while the other plot was treated with malathion as previously. The residual paddock was used as an untreated control. The plots were treated on the day after those at "Warrambeen" during the afternoon when the weather was cool, cloudy and threatening rain.

The viability of the *M. anisopliae* conidia was checked by plating on Sabouraud's dextrose agar with yeast extract (SDAYE) plates and found to be >90 %. Laboratory tests also confirmed the infectivity of the spray as used in the field trials for crickets. The *M. anisopliae* was formulated in Propar 12. Laboratory studies have shown this mineral oil to be non-toxic for *M. anisopliae* conidia. For the high volume spray, Wetter TX (Monsanto) was added as an emulsifying agent and water added to give a 20:80 oil : water emulsion.

The population density of crickets was determined using folded hessian bags (Murphy and Stahle 1985). The bags, five per plot at Warrambeen and 12 per plot at Turkeith, were placed over cracks and removed periodically to count the number of crickets. At Warrambeen the population was assessed at 2, 3, 15, 21, 33, and 49 days post treatment and at Turkeith 2, 7,

14, 20, 36 and 48 days. The population density is expressed as number of crickets per bag. In addition, samples of live crickets were collected at 2 and 7 days post-treatment at Warrambeen and 6 days post-treatment at Turkeith. These crickets were incubated in the laboratory at 28°C for 21 days and the mortality recorded. All dead crickets were placed in moist chambers to promote sporulation thus confirming that the insects were killed by *M. anisopliae.*

The population data were analysed by ANOVA, following transformation ($\sqrt{n+1}$), using the Minitab 10 software package. At Turkeith, each bag was regarded as an independent sampling unit for the analysis.

Results

Genetic analysis

The DNA extracted from isolates from black field crickets and locusts were subjected to RAPD analysis using primers HO1, HO2, FO6, FO7, FO8 and F10. Fifteen isolates from crickets and three isolates from locusts were included in the analysis. Lanes 2–16 in Figure 1 contain PCR products from the cricket isolates. The bands were identical in mobility and intensity, indicating the reproducibility of this technique. Lanes 17–19 correspond to isolates of *Metarhizium* isolated from locusts. They differed from the cricket isolates for all primers tested. Some primers such as HO2 (Figure 1) identified minor variation within the cricket or the locust isolates, however, all the primers clearly distinguished these two groups. Interestingly where multiple isolates of *Metarhizium* from a single host have been analysed using RAPDs, (Fegan *et al.* 1993, Driver and Milner unpublished data), the variability has been much greater than that exhibited by the cricket isolates in this study.

Bioassay

Only two isolates, FI610 and FI1099, caused both high mortality and produced large numbers of sporulating cadavers (Table 2) with FI1099 producing a higher proportion of sporulating cadavers at all doses. The mortality data for *T. oceanicus* are difficult to interpret due to the high control mortality. However, the fact that only three insects sporulated when dosed with FI985 supports previous laboratory data suggesting that neither species of *Teleogryllus* is susceptible to FI985. In the experiment with *T. commodus* only 15% of the untreated controls died by day 12 and FI1099 again proved to be the most virulent isolate. However many of the crickets even though killed by the *M. anisopliae* treatment (based on a comparison with control mortality) failed to sporulate. FI1067 was also pathogenic but conidia did not develop on any of the cadavers while FI1066 was the least pathogenic. With FI1099, both species of cricket died of *M. anisopliae* 5–7 days after inoculation at the highest dose and after 6–11 days at the lowest dose at the incubation temperature of 28°C. Based on these results, and the propensity of FI1099 to sporulate well on artificial media, this isolate was selected for field trials.

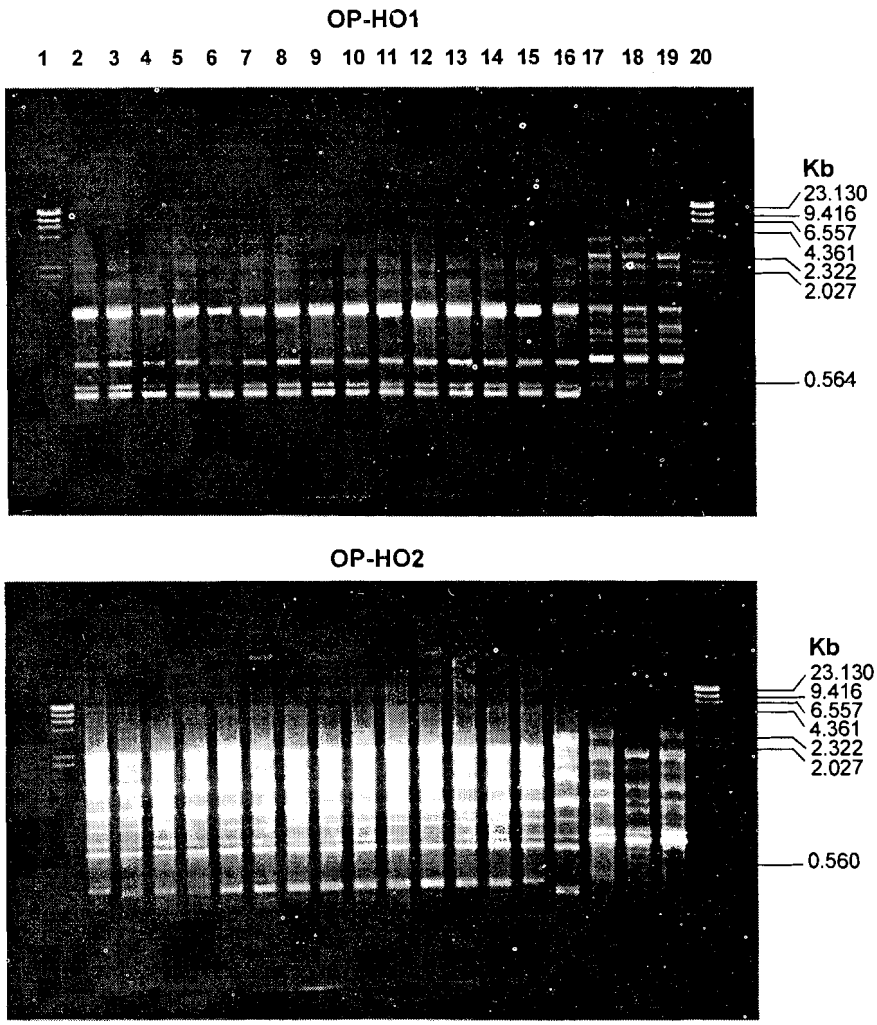

Figure 1. RAPD banding patterns from isolates of *Metarhizium* from black field crickets (lanes 2–16) and acridid grasshoppers (lanes 17–19) as revealed using HO1 and HO2 oligonucleotide probes. Note lanes 1 and 20 are λ Hind III molecular markers.

Field trials

At Warrambeen, the number of crickets remained constant until day 15 when the population in the malathion plots declined to 0.5 per bag compared with 3.8 per bag on average in the control plots (difference significant $P < 0.05$) (Table 3), both *M. anisopliae* treatments were also lower than the controls by day 15, though not significantly different ($P > 0.05$). After day 15, there was a gradual reinvasion of the insecticide plots and by day 49 the number was nearly at the level of the control plots. The numbers in *M. anisopliae* treated plots continued to decline to day 21 indicating continued *M. anisopliae* induced mortality. The ULV application was more effective than the high volume application in reducing numbers though neither were statistical significantly lower than controls or any sampling occasion. However, the live cricket samples gave 20–30 % infection supporting the conclusion that the *Metarhizium* treatment had produced the population reductions observed in the field. As with the insecticide treated plots, there was evidence of reinvasion after day 21 and by day 49 the population densities were similar to those in the controls.

The dose of *Metarhizium* applied at Turkeith was double that used at Warrambeen and the fungus gave a much higher level of control (Table 4). The insecticide treatment produced the most substantial population reduction (to 0.75 crickets per bag by day 14). There was some reinvasion, however, even by day 48 the population in the insecticide treated plot was still significantly lower than in the control (Table 4). This slower rate of reinvasion than at Warrambeen was probably due to the larger plot size. The apparent increase in population density in the control and treatment plots

between day 14 and day 20 in the *Metarhizium* was probably an artefact as the increase in numbers of crickets in the control plots suggest that the weather on March 7 was particularly favourable for sampling crickets under the bags. Nevertheless, the data suggest that the fungus gave a population reduction of around 70% while the insecticide gave about 80% reduction, comparable to that reported by Murphy and Stahle (1985) in a baiting trial with malathion. The live cricket sample from Turkeith gave 43% mortality with 36% sporulation. This was taken seven days after spraying and seems to have underestimated the effect of the fungus, suggesting that some crickets picked up a lethal dose more than seven days after spraying. Observations in the field at the end of April, after the experiment had been completed, suggested that reinvasion had occurred in both the fungus and insecticide plots after day 48.

At neither site was there any evidence of transmission of the disease between crickets. Observations in the laboratory suggest that infected crickets may be less mobile and aggressive than uninfected crickets and may be attacked and eaten before the fungus has developed to the infectious stage. Conidia, the infective stage, are not produced until several days after death. In addition, field conditions may be too dry to promote sporulation. Field transmission, as implied by the findings of Reinganum *et al.* (1981), may require a combination of wet weather and low density cricket populations in which cannibalism is unlikely.

Table 2. Per cent mortality of five isolates of *Metarhizium* spp. against *Teleogryllus* spp. (n=20) assessed 12 days after treatment and subsequent per cent sporulation in parenthesis.

Isolate	Target	150 000 conidia/cricket	15 000 conidia/cricket	1500 conidia/cricket
FI 1099	*T. commodus*[a]	95 (50)	75 (25)	75 (25)
FI 1066	*T. commodus*	50 (5)	10 (0)	20 (0)
FI 1067	*T. commodus*	50 (0)	40 (0)	35 (0)
FI 1099	*T. oceanicus*[b]	100 (70)	95 (60)	95 (55)
FI 985	*T. oceanicus*	100 (5)	75 (5)	95 (5)
FI 610	*T. oceanicus*	100 (50)	95 (35)	100 (40)

[a] control mortality of untreated control 15%.
[b] control mortality of crickets treated with oil was 75% and untreated 100%, however, no control crickets sporulated.

Table 3. Changes in population density of *Teleogryllus commodus* (expressed as average number of crickets per bag) at Warrambeen.

Date	Days post-treatment	High volume *Metarhizium*	Low volume *Metarhizium*	Malathion bait	Control
9 February	-5	5.15 ns	4.50 ns	4.45 ns	5.70
14 February	0	1.95 ns	3.00 ns	2.30 ns	2.40
16 February	2	1.20 ns	0.85 ns	2.25 ns	1.45
17 February	3	1.15 ns	1.20 ns	1.20 ns	1.10
1 March	15	3.00 ns	2.40 ns	0.50*	3.80
7 March	21	2.40 ns	1.75 ns	0.85*	2.75
23 March	33	2.35 ns	2.90 ns	1.15 ns	2.20
4 April	49	3.55 ns	2.70 ns	2.85 ns	3.20

* indicates significantly lower population than controls at 5% level.
ns indicates not significant.

Table 4. Changes in population density of *Teleogryllus commodus* (expressed as average number per bag) at Turkeith.

Date	Days post-treatment	High volume *Metarhizium*	Malathion bait	Control
17 February	2	2.33 ns	1.83 ns	3.16
22 February	7	2.33*	3.41 ns	5.00
1 March	14	1.42*	0.75*	3.00
7 March	20	3.33*	2.33*	8.67
23 March	36	3.67 ns	3.58 ns	6.75
4 April	48	3.42*	1.83*	9.08

* indicates significantly lower than controls at 5% level. (Note: malathion and *Metarhizium* plots never significantly different from each other).
ns indicates not significant.

Discussion

The *M. anisopliae* fungus is being developed as a mycoinsecticide for a range of target insect pests in many countries. Recently two *Metarhizium*-based products, BioPath and BioBlast, have been registered for use against cockroaches and termites respectively in the USA. In Australia BioGreen, a product for control of the red-headed pasture cockchafer, is being considered for registration by the National Registration Authority. Characteristics of *Metarhizium* which make this fungus attractive for development as a mycoinsecticide are that it is safe to produce and use, is readily mass produced on simple substrates such as rice, produces conidia which are robust and thus amenable to formulation and storage, and is genetically diverse with specific strains often having a restricted host range. In order to develop a mycoinsecticide based on *M. anisopliae* for a particular target it is necessary to select a virulent strain which sporulates profusely *in vitro*, and to develop an application strategy that provides acceptable and consistent pest control.

Given that *M. anisopliae* is especially diverse genetically, it was surprising that the genetic analysis of 12 isolates from disparate sites in Victoria showed that they were all practically identical. This suggests that the strain may be quite specific for black field crickets. One of these isolates, FI1099, was selected for use in the field trials as it was virulent for *Teleogryllus* in the screening experiments and sporulated well on the rice used for mass production of the fungus. Laboratory assays have shown that this isolate will not infect *Locusta migratoria* L. (Orthoptera: Acrididae) nor *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) at doses which give a high level of kill on crickets (Milner and Prior, unpublished data), however further work is needed to determine the level of host specificity exhibited by this isolate.

The present study has demonstrated that isolate FI1099 can be mass produced, formulated in oil and applied to control a field population of black field crickets. The doses used in these trials are some tenfold higher than those found effective against wingless grasshopper, *Phaulacridium vittatum* (Sjöst.) (Baker *et al.* 1994) and are likely to be uneconomic. Further research is needed to achieve effective control at a much lower dose, i.e. by using a more virulent isolate and developing more efficient application technology. Given the low level of genetic diversity of isolates naturally occurring on *T. commodus* in Victoria, it seems unlikely

that a more virulent isolate will be found locally. This leaves three options for developing a better isolate: passage of a cricket isolate such as FI1099 through black field cricket and selecting for a more virulent isolate; obtain a more virulent isolate from another host, another part of Australia or another country; or genetically engineer an isolate. At the present time only the first option can be readily achieved and will be pursued with the aim of field testing a passaged isolate in the 1995/6 season. Screening of exotic isolates has not given promising results to date but will continue as the opportunity arises. Improving the virulence of *M. anisopliae* by genetic engineering is not yet possible and might have problems from an environmental point of view if it had a wider host and/or was thought to threaten non-pest species of cricket.

Crickets are nocturnal insects which hide during the day in deep cracks. This makes application difficult both with chemical and biological insecticides since very few crickets are directly exposed to a spray at any one time. The use of baits has proved effective with chemicals because the pesticide persists on the oats (or other cereal) and as the crickets progressively feed on these baits the population is killed. Baits take two weeks or longer to be effective, which may reflect the fact that only a small proportion of crickets come onto the surface to feed each night. Baits were not used in the application of *M. anisopliae*, baits for two reasons: firstly, crickets were not infected in the laboratory when exposed to oats treated with fungal spores; and secondly, the fungus, unlike the chemical, does not act as a stomach poison. Nevertheless, recent laboratory studies have given promising results using rice baited with *Metarhizium* spores and this strategy will be field tested. A combination of an isolate with increased virulence and an improved application strategy it is hoped will provide a higher level of control at a lower dose.

Acknowledgments

Ian and Trish Taylor of Warrambeen and Lachlan Gordon of Turkeith kindly provided the two field sites. Financial support was provided by Alcoa through Kathy Junor and by Graham Woods Agencies through David Looker. Valuable comments on the manuscript were provided by Drs. Gordon Berg, Thom Boyce and Roger Farrow. The constructive advice and help of Dr. Chris Prior is gratefully acknowledged. Research on the genetic aspects of pathogenicity in *Metarhizium* is being funded by Rural Industries Research and Development Corporation.

References

Anon. (1989). The economic impact of pasture weeds, pests, and diseases on the Australian wool industry, 205 pp. (Sloane Cook and King Pty. Ltd.).

Baker, G.L., Milner, R.J., Lutton, G.G. and Watson, D.M. (1994). Preliminary field trial on the control of *Phaulacridium vittatum* (Sjöstedt) (Orthoptera: Acrididae) populations with *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycotina: Hyphomycetes). *Journal of the Australian Entomological Society* 33, 190-2.

Bateman, R.P., Carey, M., Moore, D. and Prior, C. (1993). The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Annals of Applied Biology* 122, 145-52.

Bidochka, M.J., McDonald, M.A., St. Leger, R.J. and Roberts, D.W. (1994). Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA. *Current Genetics* 25, 107-13.

Blank, R.H. (1982). Control of *Teleogryllus commodus* using insecticidal baits and other agents. Proceedings of the Australasian Conference on the Ecology of Grassland Invertebrates, Adelaide 1981, 355-63.

Curran, J., Driver, F., Ballard, J.W.O. and Milner, R.J. (1994). Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequence data. *Mycological Research* 98, 547-52.

Fegan, M., Manners, J.M., Maclean, D.J., Irwin, J.A.G, Samuels, K.D.Z., Holdom, D.G. and Li, D.P. (1993). Random amplified polymorphic DNA markers reveal a high degree of genetic diversity in the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae*. *Journal of General Microbiology* 139, 2075-81.

Haymer, D.S. (1994). Arbitary (RAPD) primer sequences used in insect studies. *Insect Molecular Biology* 3, 191-4.

Milner, R.J., Baker, G.L., Hooper, G.H.S. and Prior, C. (In press). Development of a mycoinsecticide for the Australian plague locust. *Conference on Future Methods in Locust Control*, Bamako, Mali, March 1995.

Murphy G. and Stahle, P. (1985). Examination of baiting techniques against the black field cricket *Teleogryllus commodus*. Proceedings of the 4th Australasian Conference on Grassland Invertebrate Ecology, Canterbury, New Zealand, May 1985, 293-5.

Prior, C. (In press). Safety of *Metarhizium* isolates. *Conference on Future Methods in Locust Control*, Bamako, Mali, March 1995.

Reinganum, C., Gagen, S.J., Sexton, S.B. and Vellacott, H.P. (1981). A survey for pathogens of the black field cricket, *Teleogryllus commodus*, in the Western district of Victoria. *Journal of Invertebrate Pathology* 38, 153-60.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning: a laboratory manual. (Cold Spring Harbour Press, New York, USA).

Stahle, P., Murphy, G. and Conley, D. (1983). Control of field crickets. Agnote 2046/83, Victorian Department of Agriculture, pp. 2.

Welsh, J., Petersen, C. and McClelland, M. (1991). DNA polymorphisms amplified by arbitary primers are useful as genetic markers. *Nucleic Acids Research* 19, 7213-8.

Williams, J.G.K., Kubelik, A.R., Llivak, K.J. , Rafalski, J.A. and Tingey, S.V. (1991). DNA polymorphisms amplified by arbitary primers are useful as genetic markers. *Nucleic Acid Research* 18, 6531-5.