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Synthesis:

Recent developments in the use of fungi as biopesticides against locusts and grasshoppers in Australia

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Abstract

Since October 2000, the commercial product Green Guard®, based on the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* produced by SGB Pty. Ltd. Wodonga, Victoria, has been used by the Australian Plague Locust Commission for operational control of the Australian plague locust, *Chortoicetes terminifera*. The production, formulation, storage, efficacy and non-target effects are reviewed. Between October 2000 and January 2001 over 23,000 ha were treated at doses of 12 - 25 g/ha in 500 ml of oil. Under most temperate or hot conditions, control of over 90% of Australian plague locusts was obtained by day 14 post spraying, but under less favorable conditions control could take up to 21 d. The factors which make Green Guard® attractive for locust control in Australia include low cost, the absence of environmental effects, especially on aquatic organisms, and its suitability for use in organic beef production areas and where the use of chemical pesticides is undesirable. Large-scale field trials against migratory locusts, *Locusta migratoria,* have shown that doses of 50-75 g/ha are effective. Spur-throated locusts, *Austracris guttulosa*, are very susceptible in the laboratory, but field trials have proved difficult to assess because of the high mobility of the adults and the absence of hopper bands at the nymphal stage. Results with the wingless grasshopper, *Phaulacridium vittatum*, have been variable, with the control being difficult to achieve during cool weather. Laboratory studies on *Beauveria bassiana* have shown that isolates of this fungus, derived from field-infected grasshoppers, are very virulent and will kill better under cool conditions.

Key words

Fungi, Metarhizium, locust control, biopesticide

Introduction

In 1993 a project commenced to extend and develop the findings of the 'Lutte Biologique contre les Locsustes et Sauteriaux' (LUBILOSA) programme (Lomer *et al.* 2001), by investigating the potential for biological control of locusts and grasshoppers in Australia using the insect pathogenic fungus, *M. anisopliae* var. *acridum* Driver and Milner (Milner 1997, Milner *et al.* 1997, Driver *et al.* 2000). Since then investigations have concentrated on four of the most serious acridid pests in Australia: the Australian plague locust, *Chortoicetes terminifera* (Walker); the spur-throated locust, *Austracris guttulosa* (Walker); the migratory locust, *Locusta migratoria* L. and the wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt). The ecology, distribution and pest status of these insects have been reviewed by Baker (1993), while the preventative control strategy for *C. terminifera* is outlined by Hunter *et al.* (1998).

The initial research focused on the selection of an appropriate isolate and on the molecular taxonomy of the *Metarhizium* genus. Isolate FI-985 was selected for commercialization because it was virulent for a wide range of Australian acridids (Milner & Prior 1994, Milner *et al.* 1996), amenable to mass production, and effective in early field trials (Baker *et al.* 1994, Milner *et al.* 1997, Milner 1997). Taxonomic studies resulted in an extensive revision of the genus *Metarhizium* with the acridid-adapted strains being recognized as a new taxon – *M. anisopliae* var. *acridum* (Driver *et al.* 2000). The lifecycle of *Metarhizium* on locusts and grasshoppers is similar to that on other insect hosts. The asexual haploid conidia germinate on contact with the host's cuticle, and penetrate directly into the haemocoel in about 24 h. The fungus then multiplies as hyphal bodies and mycelium in the body of the locust which, at death, is a pink cadaver filled with mycelium. Under ideal conditions in the laboratory, the fungus can kill in 4-7d (Milner & Prior 1994). After death, new conidia may form in a few days on the inside and, if conditions are moist and warm, on the outside of the insect. Internal sporulation is a unique feature of grasshoppers and locusts killed by *M. anisopliae* var. *acridum*.

Green Guard®, a commercial product with FI-985 as the active ingredient, is being produced by SGB Pty. Ltd. in Wodonga, Victoria, (Milner 2000a) and was used operationally for locust control by the Australian Plague Locust Commission for the first time in the 2000/2001 season with some 23,000 ha being treated between October 2000 and January 2001. Pending full registration, the Australian National Registration Authority has approved use of Green Guard® for aerial ULV spraying by the APLC in situations where chemical control is inappropriate. Some of the key elements of the production and use of Green Guard® are reviewed in this paper.

Green Guard® Production

FI-985 is stored as dry conidia at –70°C to minimize genetic strain. The production starts with a pure fresh sporulating colony of FI-985 on a Petri dish plate, grown for about

2 w at 25°C on Sabouraud's dextrose agar with 1% yeast extract added (SDAYE). The conidia are used to inoculate a yeast extract/dextrose broth which is then incubated on an orbital shaker for 5-6 d at 25°C. The solid substrate is long grained parboiled rice (rice partially cooked before milling to retain nutrients). Two and a half kg of rice are placed in a 10-l autoclavable, self-aerating, culture bag (van Leer, France or Unicorn, Texas, USA) and sterilized. When cool, this is inoculated with the broth culture and sterile water added; the inoculum is well mixed with the rice. The bag is then incubated at 25°C for 14 to 18 d.

The rice/conidia mixture is then air-dried and the conidia removed by sieving. This results in a very clean conidial powder which can be stored or formulated for use.

At all stages, the process is checked visually for contamination and any contaminated bags are autoclaved and discarded. The final quality control includes assessment of moisture content, germination and contamination.

Key factors affecting yield are the incubation temperature, the moisture of the rice and the duration of incubation. Radial growth of the mycelium of FI-985 is in the range 14 to 36°C with an optimum around 30 to 32°C (Milner 1997). Moisture also has a major impact, with the standard water level giving about 200 g of dry conidia/bag, and higher moistures giving up to 250 g or more. Finally, time is important, with a rapid increase in conidial production between days 5 and 15, which then asymptotes by about day 30. Using the standard time/moisture combination, the yield is 5-6 X 10^9 conidia/g.

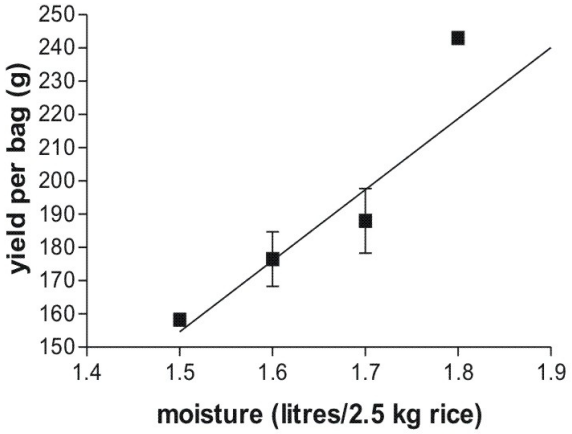


Fig. 1. Effect of initial moisture level on production of FI-985 in van Leer bags. Bars represent standard error of the mean.

Formulation.— Formulation can be critical to the commercial success of a microbial biopesticide. Formulation has the potential to stabilize the living active ingredient, improve its storage and field persistence, make the product safe and easy to handle and improve field efficacy. The formulation of FI-985 has been developed as a interactive process between CSIRO and the APLC (Milner 2000b). Formulation in oil substantially improves the efficacy when compared with a water-based spray (Prior *et al.* 1988, Milner *et al.* 1997). For the 2000/2001 season, the conidial powder was formulated as an oil-concentrate in corn oil, using 322 g of dry conidia per litre. This was then diluted in a low-viscosity mineral oil in the field to give the required concentration for ULV spraying. The oil concentrate was supplied in 20-l sealed wide-mouthed plastic buckets each containing 14 l, or enough to spray about 180 ha at 25 g/500 ml. Corn oil was chosen to minimize the settling of spores. It was found that vegetable oil was much better than mineral oils in minimizing settling out and that the higher the viscosity the lower the rate of settling. However it is not possible to make an oil concentrate using very viscous oils and so the corn oil is a compromise (Milner 2000b). The advantages of this oil concentrate over dry conidial powder or a ready-to-use mineral oil formulation are: 1) it can be quality controlled in the laboratory; 2) there is a relatively small amount of the fragile living material to transport; 3) there is a reduced problem with settling out of the conidia; 4) the conidia are less hazardous than in a dry powder form.

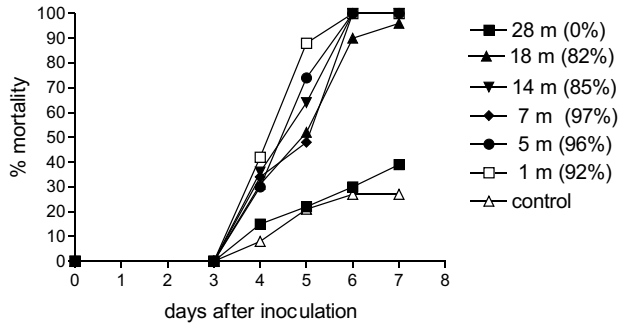
Two important characteristics of an effective formulation are stability and particle size. Stability is evaluated by assessing the survival of conidia in the formulation at 45°C and 55°C. At the lower temperature, 50% of the conidia survive for about 14 d while at the higher temperature the period is reduced to about 3 d (Milner unpub.). The particle size has been determined using a laser light scanner. There was no evidence of clumping of spores and the mean size of the particles was 5.287 µm $\pm$ 3.34 (*s*).

The current formulation could be improved by the addition of compounds such as surfactants to aid dispersal or reduce settling, as well as the use of UV protectants and synergists to enhance speed of kill.

Storage.— Viable conidia of FI-985 are stored either as a dry powder or as formulated product at 4°C. Viability is checked periodically by assessing the germination on SDAYE plates, incubated at 25°C for 24 h. To be considered still satisfactory, over 80% of the conidia should germinate. Results (unpub.) have shown that FI-985 stores well for at least 18 mo under either condition.

Five batches stored for up to 18 mo were similar in their virulence for the locusts, with germination over 80%, while a batch stored for 28 mo was no longer viable.

Field Efficacy.— The field efficacy of *M. anisopliae* as Green Guard® for control of locusts and grasshoppers in Australia has been widely published (Hunter *et al.* 1999, Scanlan *et al.* 2001, Hunter *et al.* 2001), while Lomer (2001) has reviewed the worldwide use of *M. anisopliae* for control of locusts and grasshoppers. Milner (1997) summarized early field trials against the Australian plague locust, *C. terminifera*, spur-throated locust, *A. guttulosa* and the wingless grasshopper, *P. vittatum*. Many of these trials used what is now regarded as a high dose of 100 g conidia/ha in a large volume (2 l). A wide range of measurements were taken to assess field efficacy, checking germination of the conidia before and after spraying, daily population assessments, the use of field cages, glasshouse incubation and bioassay of treated vegetation to assess persistence. More recent trials have concentrated on detailed assessment of population effects, backed up by field cages and less detailed persistence studies. Effects



Note: all insects dying before day 4 omitted as handling mortality.

Fig. 2. Results of bioassays of formulated FI-985, stored over an increasing number of months (m) against Australian plague locusts, *Chortoicetes terminifera*. Percentage germination shown in parentheses.

on non-targets have been undertaken on an opportunistic basis.

Hunter *et al.* (1999) reported on large-scale field trials against *L. migratoria*, undertaken in Queensland in 1998/99. At doses of 75 and 100g/ha, control was about 90% over a range of habitats. Dense woodland delayed mortality. Lower doses generally gave inadequate control; however recent improvements to the product suggest that lower doses may well give effective control. Modelling of this and additional data (Scanlan *et al.* 2001) suggested that a dose of 20 g/ha would be effective when vegetation cover was low, resulting in good target coverage. This paper also supported the notion that secondary pickup of spores by locusts from vegetation was critically important at low doses and where vegetation cover was moderate or dense. The Australian plague locust, *C. terminifera*, is a more susceptible species (Milner *et al.* 1996) and Hunter *et al.* (2001) reported that doses down as low as 25 g/ha were effective against hopper bands of this pest, resulting in a 90% decline in 12-14 d. In addition, other papers have provided data on the efficacy of FI-985 against the wingless grasshopper , *P. vittatum* (Milner *et al.* 1997, Milner & Staples 1998).

In the 2000/2001 season, doses down to 12.5 g/ha were field-tested against *C. terminifera* nymphs and gave high levels of control even at this low dose (Fig. 3). Mortality was delayed in one plot (Fig. 3, plot 3), consisting of a densely vegetated drainage channel. In this habitat initial contact was perhaps low and subsequent access from the vegetation may have contributed substantially to the observed mortality.

In 1997, a large-scale field trial was undertaken against the wingless grasshopper, *P. vittatum*, in comparison with the standard rate of fenitriothion. FI-985 was applied at the rate of 100g/ha in 2 l of oil. A total of 9 plots (40-80 ha) were treated with *Metarhizium*, two plots treated with fenitrothion and three left untreated. The results from three comparable plots at one site are given in Fig. 4. The chemical insecticide reduced the population by over 90% very quickly, but there was reinvasion over the next 2 w. Similar results with IMI 330 189 were reported by Langewald *et al.* (1999). The *Metarhizium* reduced the grasshopper population between week 1 and week 2 by about 70%, but there was only a marginal increase in control in the following week. Control populations remained high.

Since 1997, there have been considerable improvements in the drying and formulation of FI-985, however recent field trials against the wingless grasshopper have confirmed that over 80% control is difficult to achieve against this target pest (Milner, Baker and Hunter, unpub. data). Four reasons have been hypothesized: 1) that application from the air is suboptimal, since there is minimal impaction of spray drift on the prostrate vegetation in the largely bare habitat preferred by *P. vittatum*; 2) in grassed areas, the prostrate broad-leafed plants preferred by *P. vittatum* occur in summer as an understory below dry annual grasses, a situation unfavorable for both direct contact and residual pickup of the mycoinsecticide by grasshoppers; 3) any growth by pasture in response to reduced grasshopper density is discerned by dispersing macropterous morphs, resulting in recolonisation of successfully treated areas; 4) cool conditions post-treatment adversely affect the treatment by delaying germination, slowing residual uptake through reduced

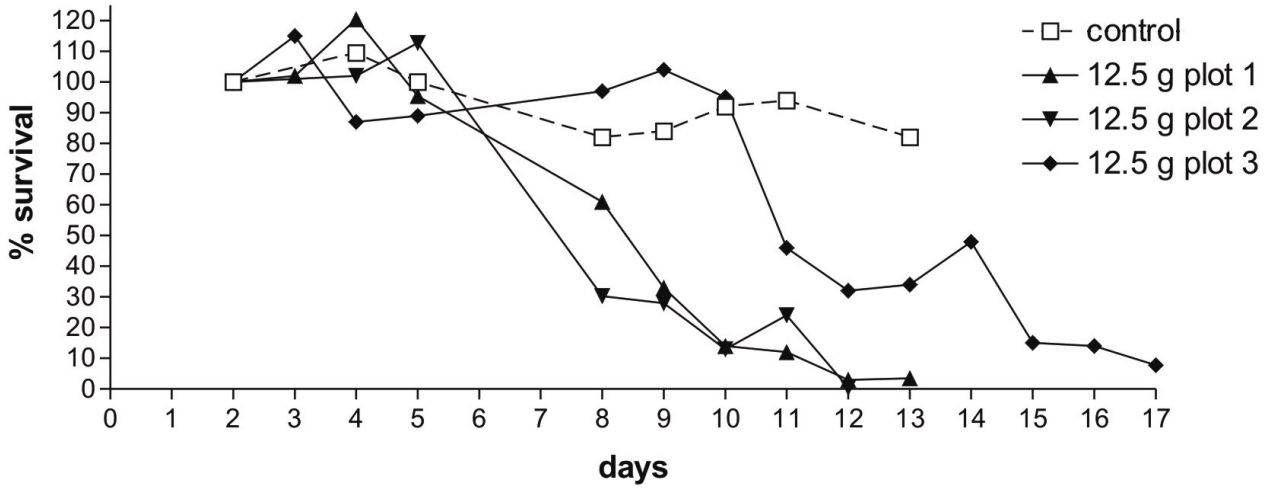


Fig. 3. The effect of low doses of FI-985 on control of Australian plague locust, *Chortoicetes terminifera*, nymphs.

feeding by hosts on steadily degrading residual conidia. (This degradation is due to intense UV light at the altitudes at which *P. vitattum* occurs). Under the latter conditions *B. bassiana* might be more effective than *Metarhizium* (see below).

Bioassays of treated vegetation have generally shown that the fungus persists well for 5-7 d and is largely dissipated by day 14 (Fig. 5).

Overall, these field trials together with the recent operational experience, have shown that Green Guard® is effective against the Australian plague locust at doses down to 12.5 g/ha. Generally 90% control is achieved in 14 d, however this time may increase under certain weather conditions and where vegetation inhibits coverage of the spray. The disease

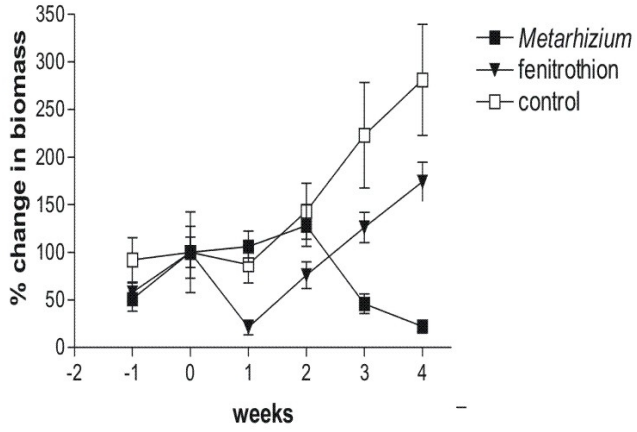


Fig. 4. Effect of FI-985 on control of a grasshopper complex dominated by the wingless grasshopper, *Phaulacridium vittatum* at Dutton Plain, Cooma, New South Wales, in December 1997. Each point is a mean with the bars representing standard error.

does not spread from the treated area and the effects of treatment are largely confined to the month after spraying.

Non-target Effects.— *M. anisopliae* var. *acridum* is only known to occur in nature on acridoid insects (Prior 1997) and the majority of isolates from acridoid hosts are not var. *acridum* but var. *anisopliae*. Thus of the 150 or so isolates obtained by LUBILOSA from acridoids only 50 are var. *acridum* (Lomer *et al.* 2001). In Australia, detailed collecting and surveying in the past has resulted in over 1000 isolates of *Metarhizium* added to the CSIRO Insect Pathogen Culture Collection over the past 20 y and only 2 of these isolates are var. *acridum*. Both were field-collected from infected acridids. This is strong evidence of host-specificity.

In a laboratory study (Milner, unpub.), the susceptibility of a range of 10 insects and a woodlouse was tested to FI-610, a var. *anisopliae* and FI-985, a var. *acridum*, using a single high dose. Neither isolate killed the woodlouse. The FI-610 caused over 50% mortality in 9 of the 10 insect species, while FI-985 caused over 50% mortality in just one non-target species, *Coptotermes lacteus* (Frogatt) (Isoptera Rhinotermitidae). Again this supports the conclusion that the common var. *anisopliae* isolates are quite polyphagous while the var. *acridum* isolates are host-specific.

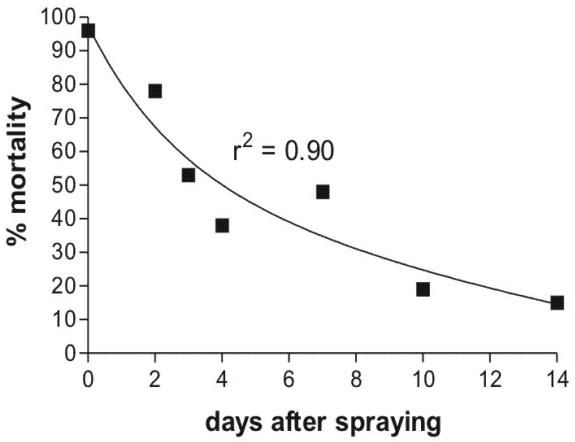


Fig. 5. Effect of time after spraying on mortality of locusts exposed in a glasshouse to field-sprayed vegetation.

The effects on nontargets in field trials have been studied using pitfall traps. A transect of ten 10-cm diameter pitfall traps was placed in the center of each of three 1-ha plots sprayed with fenitrothion, *Metarhizium* or left untreated. The catch was recorded weekly. The results for the 7-14 d and 14-21 d periods, when the *Metarhizium* would be having maximum effects on the grasshoppers, are given in Table 1. Fenitrothion caused a substantial drop in the numbers of Collembola and Hemiptera, with some adverse effects seen on other groups, particularly in the 14 to 21-d collection. These data confirmed the lack of any impact on non-targets by *Metarhizium*.

The risks to aquatic life have also been tested (Milner *et al.* forthcoming). The initial laboratory tests at a single very high dose (2 X 10^6 conidia/ml) revealed that FI-985 was not harmful to fish, *Melanotaenia duboulayi* Castelnau or mayfly larvae, *Ulmerophlebia* sp. (Ephemeroptera: Leptophlebiidae). However a dose of 2 X 10^6 ml of either dead or viable conidia caused relatively rapid mortality of the cladoceran, *Ceriodaphnia dubia* Richard (Crustacea: Cladocera) though doses of <5,000 conidia/ml were not toxic. Laboratory studies showed that the conidia generally dropped to below 15 cm of the surface of a container within 24 h. Field studies during operational control and also in a simulated control situation, showed that contamination of water did not exceed 130 conidia/ml. It is thus concluded that the product does not pose a hazard to aquatic organisms.

Wingless grasshoppers are frequently parasitised by nemastrinid flies and by entomogenous nematodes, therefore it was important to study the effect of this parasitism on susceptibility to *Metarhizium*. Detailed laboratory studies have shown that there is no increase in susceptibility of parasitised hosts to infection by *Metarhizium* and therefore no change in the relative abundance of parasitised and unparasitised hosts following treatment (Milner *et al.* 2002).

Table 1. Summary of results from pitfall traps (mean count per trap ± standard error (*s*) for each transect of 10 traps) in a field trial on pasture, comparing untreated control plots with plots treated with FI-985 and fenitrothion.

	7 - 14 days			14 - 21 days		
Group	Control	*Metarhizium*	fenitrothion	Control	*Metarhizium*	fenitrothion
Arachnida	28.67 ± 10.9	44.1 ± 9.2	79.2 ± 9.2	502.1 ± 154.8	105.8 ± 21.7	41.1 ± 7.8
Diptera	47.2 ± 18.8	15.1 ± 2.6	19.8 ± 3.7	81.8 ± 51.9	11.4 ± 3.6	2.7 ± 0.9
Coleoptera	68.4 ± 7.0	62.0 ± 5.1	45.5 ± 5.4	103.7 ± 9.7	104.6 ± 13.7	59.3 ± 8.6
Lepidoptera	13.0 ± 3.0	7.5 ± 0.8	9.9 ± 1.6	10.4 ± 2.9	10.9 ± 2.0	3.2 ± 0.5
Hymenoptera	35.3 ± 7.2	41.0 ± 16.4	19.6 ± 6.5	77.8 ± 35.2	58.6 ± 16.9	26.5 ± 15.6
Hemiptera	27.2 ± 9.5	14.4 ± 2.3	0.3 ± 0.3	16.1 ± 5.8	14.3 ± 2.9	1.3 ± 0.4
Collembola	172.1 ± 53.9	441.8 ± 88.6	2.5 ± 0.4	796.3 ± 224.6	964.8 ± 444.7	2.6 ± 1.0
Total	56.0 ± 20.4	89.4 ± 59.2	25.2 ± 11.4	226.9 ± 114.4	181.5 ± 131.5	19.5 ± 8.8

Conclusions

The apparent 'success' of Green Guard® is a result of a combination of technical and social factors. Technical factors include the high virulence of FI-985, the suitability of the Australian plague locust for control with this biopesticide, the efficiency of mass producing FI-985, and the good formulation and quality control. Social factors include the receptiveness of the APLC and other government agencies with responsibility for locust control, to participation in its development, the existence of an effective commercial partner and the congruent demands of Australian agriculture, particularly the organic beef producers.

Beauveria bassiana for wingless grasshopper control.— As mentioned above, field trials against the wingless grasshopper have given less than the 90% control regularly obtained with locusts. This is a univoltine species and control is most effective when achieved early in the season, when the insects are small and have not started to disperse. It is a montane species and early season temperatures are often in the range 10-20°C, too cool for *Metarhizium* to be effective. In nature, *B. bassiana* is frequently found killing wingless grasshoppers and it is known that isolates of this fungus will kill locusts and grasshoppers at lower temperatures than those required by *Metarhizium* (Inglis *et al.* 1997). For these reasons, a new study commenced recently to assess the potential of *B. bassiana* for control of the wingless grasshopper.

Initial bioassays revealed that isolates obtained from field-infected grasshoppers, both wingless and other species in the complex, were highly virulent and more virulent than the other isolates tested. One isolate, FI-1437, was selected for further study and found to kill grasshoppers effectively at temperatures as low as 10°C. The *in vitro* growth profile showed that this isolate has an advantage of 5°C or more over FI-985 (Fig. 6) If suitable field sites can be found, it is hoped to test this isolate in the field in comparison with Green Guard® during the 2001/2002 season.

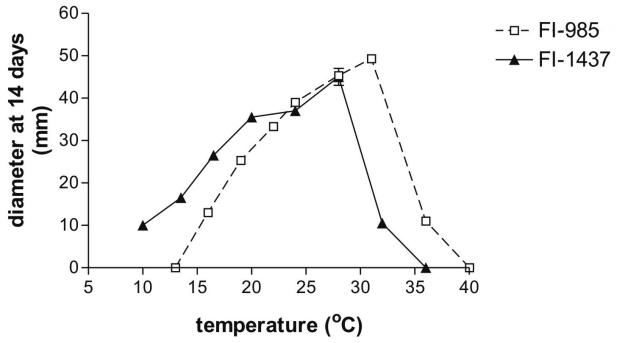


Fig. 6. Comparison of radial growth of *Beauveria bassiana* (FI-1437) and *Metarhizium anisopliae* var. *acridum* (FI-985).

Future

Biopesticides are expected to have a significant and increasing role in control of locusts and grasshoppers in Australia. The use against Australian plague locust, so far confined to New South Wales and South Australia, is expected to be extended to Western Australia and possibly Queensland this coming season. It is hoped that full registration will be obtained soon and that the dose required for the various species will become more firmly established. Future work will be concerned with further understanding the role of thermobiology in determining the rate of control

of locusts and with the potential use of *B. bassiana* for wingless grasshopper control. Collaborative research is currently being undertaken in China, Mexico and Indonesia and this may lead to the use of Green Guard® in these countries or the commercial production of their isolates in Australia.

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