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Aerial treatment of the Australian plague locust, *Chortoicetes terminifera* (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes)

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Abstract

Between October 1999 and April 2000, nearly 4000 ha of nymphal bands and adult swarms of *Chortoicetes terminifera* (Walker) were aerially treated using a ULV oil formulation of strain FI-985 of *Metarhizium anisopliae* var. *acridum*. During the mild weather (maxima 22–30°C) of spring (October), there was little change in nymphal bands during the first week but at all doses between 25–100 g (1–4 $\times$ 10^{12} conidia) ha^{-1}, the bands rapidly declined 9–12 days after treatment reaching > 90% mortality by 14 days. *Metarhizium* persisted for some time as there was 50% mortality of locusts fed vegetation collected from the treated blocks seven days after treatment. Persistence was confirmed by the high mortality of bands that invaded from untreated areas and of nymphs that hatched on the plot five to seven days after treatment, though mortality was then delayed until early in the third week. During summer (January), temperatures were high (maxima 36–42°C), and at all doses between 25 and 125 g (1–5 $\times$ 10^{12} conidia) ha^{-1}, there was a rapid decline seven to ten days after treatment. By 12–14 days, there was a > 90% decline in numbers in most blocks which was confirmed by helicopter surveys two weeks after treatment that found very few adults within or near treated areas. Mortality was delayed in the high dose where there were blockages of spray equipment during treatment. The clear demonstration that *Metarhizium* can suppress small local populations of *C. terminifera* led to the limited operational use of *Metarhizium* on an organic farm and in a National Park where nearly 2500 ha of bands and swarms were treated. Continued research is needed to develop a commercially viable product so that *Metarhizium* can form a significant part of a programme of integrated pest management of locusts in Australia.

Introduction

In eastern Australia, most outbreaks of the Australian plague locust, *Chortoicetes terminifera* (Walker) (Orthoptera: Acrididae), develop when there is a sequence of regular rains in the interior that allow population increase (Wright, 1987; Hunter, 1996). Locusts from the interior often invade the agricultural zone where further breeding allows the outbreak to reach its height. During most years, some bands and swarms form but several times a decade there are extended periods of regular rain that in the past have resulted in locusts reaching plague proportions. During the 1990s, outbreaks of the Australian plague locust, *C. terminifera*, the spur-throated locust, *Austracris guttulosa*

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(Walker) (Orthoptera: Acrididae) and the migratory locust, *Locusta migratoria* (Linnaeus) (Orthoptera: Acrididae) were managed using a programme of preventive control. Forecasts were made of when outbreaks were likely and chemical control began as soon as possible after the outbreak commenced (Hunter *et al*., 1998). The small area requiring treatment during early control has successfully reduced both locust damage and insecticide input, but there is a need for a biological alternative because of increasing environmental and market constraints on insecticide use. Market constraints are particularly important because of quality assurance programmes and the production of organic beef in locust source areas in inland Australia.

One of the most promising biological agents is the fungus *Metarhizium anisopliae* var. *acridum* (formerly *Metarhizium flavoviridae*) (Deuteromycotina: Hyphomycetes) (Driver *et al*., 2000). High mortality of locusts and grasshoppers in the field has been demonstrated as part of the ten year research and development programme by LUBILOSA (Lutte Biologique contre les Locustes et les Sauteriaux) in Africa and by CSIRO (Commonwealth Scientific and Industrial Research Organization) in Australia (Lomer *et al*., 1993; Hooper *et al*., 1995; Douro-Kpindou *et al*., 1997; Langewald *et al*., 1997). Both the LUBILOSA and CSIRO programmes have demonstrated that field efficacy is a result of the interaction between *M. anisopliae* and the acridid insect target and that both application and field evaluation requires a detailed understanding of infection of, and subsequent development within, the locust. While droplet spectrum is important with chemical insecticides, it is more even more so when applying *M. anisopliae* spore particles. Most droplets should be between 40–120 μm because very small droplets may contain no spores and very large droplets are extremely wasteful (Bateman, 1999; Bateman & Alves, 2000). Infection is not by ingestion but through penetration of the insect cuticle by spores picked up directly during spraying or subsequently from the vegetation (Bidochka *et al*., 1997) and moderately small droplets will give a more widespread dose on the vegetation facilitating both direct and secondary pick-up. *Metarhizium anisopliae* takes 7–14 days to kill the locusts with the time for mortality depending temperature. Locust body temperatures are of critical importance with mortality being reduced or at least delayed when locusts consistently attain very high body temperatures inhibitory to *M. anisopliae* development (Blanford *et al*., 1998, 2000).

Mortality takes a week or more which makes field evaluation of mortality difficult because the substantial movement common with locusts can result in untreated locusts invading treated plots and mixing with treated individuals. Consequently, while there has often been demonstration of mortality in locusts kept in field cages, determination of the level of mortality within field plots has proven difficult (Kooyman & Godonou, 1997; Price *et al*., 1997). However, by following bands in the field until they die, declines have been demonstrated for *Rhamatocerus schistocercoides* (Bruner) (Orthoptera: Acrididae) in Brazil (Lecoq & Balança, 1998), *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) in Africa (Langewald *et al*., 1997) and *L. migratoria* in Australia (Hunter *et al*., 1999). In Australia, the FI-985 strain isolated from *A. guttulosa* in Queensland, has been shown to cause substantial mortality of *C. terminifera* in laboratory and preliminary field tests (Hooper *et al*., 1995; Milner, 1997). Following promising results against *L. migratoria* (Hunter *et al*., 1999), more extensive field trials were carried out to demonstrate clearly the field efficacy of the FI-985 strain of *M. anisopliae* against the major locust pest in Australia, the Australian plague locust, *C. terminifera*.

Materials and methods

Metarhizium anisopliae *production*

The conidia of *M. anisopliae* var. *acridum* (strain FI-985) were produced by CSIRO and a commercial partner SGB (Seed, Grain and Biotechnology) Pty Ltd Australia. In a two-stage process, mycelia were first produced in shake flasks and then inoculated into sterilized rice with added nutrients in specially manufactured (van Leer, France) 10 l plastic bags having a perforated strip to allow air circulation. After two weeks, the conidia were harvested, dried and stored as dry conidial powder at 4°C. Just prior to use, the conidia were formulated in Summer Oil® mineral oil (Caltex, Sydney, Australia) and then transported to the field.

Metarhizium anisopliae *application*

Moderate scale treatments were carried out during spring and summer 1999–2000, followed by limited operational use during autumn. Prior to application, detailed surveys were undertaken to locate localized infestations that could be completely treated and so reduce the chance of invasion by untreated locusts, and to clearly demonstrate to the local farmers that the applications had worked. During spring (October) 1999, two localized infestations of bands of the Australian plague locust, *C. terminifera* covering a total of 700 ha were treated north east of Griffith (34°17′S 146°02′E) in the agricultural Riverina region of New South Wales. During summer (January) 2000, two infestations covering 670 ha were treated in Mitchell grass (*Astrebla* spp.) (Poaceae) habitats near Windorah (25°25′S 142°39′E) in the arid interior of Queensland. The concentration of the *M. anisopliae*/oil formulation was 1–5 $\times$ 10^{12} conidia l^{-1}, determined by serial dilution of the formulation and counting on a Petroff-Hausser counting slide. The formulation was applied by aircraft to blocks infested with bands of *C. terminifera* at 1.0 or 0.5 l ha^{-1} using an aircraft. The aircraft was fitted with Micronair® AU5000 rotary cage atomizers (50° blade angle) and the formulation was applied from above tree top height (10 m) at a 50 m track spacing. A Micronair monitor logged flow rates and a DGPS was used to set and record track spacing. To ensure the upwind edge of the block was treated, the first spray run was 200 m upwind of the block. Treatment occurred between 0900 and 1600 when winds were consistently 2–5 m s^{-1} which minimized any effects of thermals and ensured a more even coverage (Nguyen & Watt, 1980, 1981).

Tests of efficacy

Population decline in the field was used as the main test of efficacy. For 14–18 days after treatment, 10–15 bands in treated and untreated blocks were followed using techniques of Hunter *et al*. (1999). All blocks were initially traversed at 100 m intervals and intensively searched for 3–4 h to locate every band, and these original bands were followed even if they left the treated area. Every one to two days, band area was determined from band length estimated by pacing the band front and placing flags every 30–40 m

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along its length, and band width estimated by walking at right angles to the main band front at each of the flags. The area of locusts at dense band (1000–2500 nymphs m^{-2}), band (100–1000 nymphs m^{-2}), sub-band (30–100 m^{-2}) or numerous (5–30 m^{-2}) was used to estimate the total number of locusts in each band. Areas of sub-band and numerous between bands were also recorded and added to the total number of locusts in the nearest band. Mortality was estimated from changes in the mean ± standard error of the number of locusts in each band originally present in a block.

Three other tests were made of efficacy (Hunter *et al.*, 1999). At two and five days after treatment, about 50 locusts from each treated and untreated block were placed in separate 1.2 × 0.6 × 0.6 m field cages made of shade cloth (50% shade). Cages were checked twice a day for mortality and any dead locusts counted and removed. A corresponding sample was taken to the laboratory for rearing at temperatures of 25°C (night) to 30°C (day). A third test, designed to assess the persistence of the original spray deposit on the vegetation, involved collecting vegetation from treated and untreated areas on days 1, 4, 7 and 10 and placing it in a cage containing 50 untreated locusts. Locusts were then sent to the laboratory for rearing and mortality assessment.

Limited operational use March–April 2000

The success of the spring and summer trials led to the first operational use of *M. anisopliae* as part of a large locust control programme in locust source areas. *Metarhizium anisopliae* was applied at 1×10^{12} conidia in 500 ml ha^{-1} to the densest infestations of bands and swarms on an organic farm and in a National Park in northern South Australia (29°30′S 139°00′E to 30°35′S 139°20′E).

Results

Treatments during mild spring weather

At the mild spring temperatures (maxima 22–30°C) of October in the agricultural zone, there was a rapid decline in locust numbers in the second week after treatment both at moderate (75–100 g ha^{-1} = 3–4 × 10^{12} conidia) and at low doses (25–50 g ha^{-1} = 1–2 × 10^{12} conidia), with the final mortality being > 90% (fig. 1). Mortality of locusts in field cages began a week after treatment and reached > 80% 7–10 days later. Formulations of the 50 g ha^{-1} and 75 g ha^{-1} which incorporated proprietory ultraviolet protective agent(s) gave no significant increase in efficacy. The formulations also failed to show any benefit in laboratory studies (R.J. Milner, unpublished).

Locusts that invaded the untreated area or hatched after spraying also had high mortality (fig. 1: open circles). A large band from the untreated area invaded treatment 1 on the fifth day and following heavy rain one to two days after treatment, significant numbers of recent hatchlings were seen in two areas. The mortality of both the invading band and hatchlings was slightly later than with locusts present at the time of treatment (fig. 1) but the final mortality was high indicating substantial persistence of *M. anisopliae* on the vegetation. Persistence was confirmed by feeding locusts

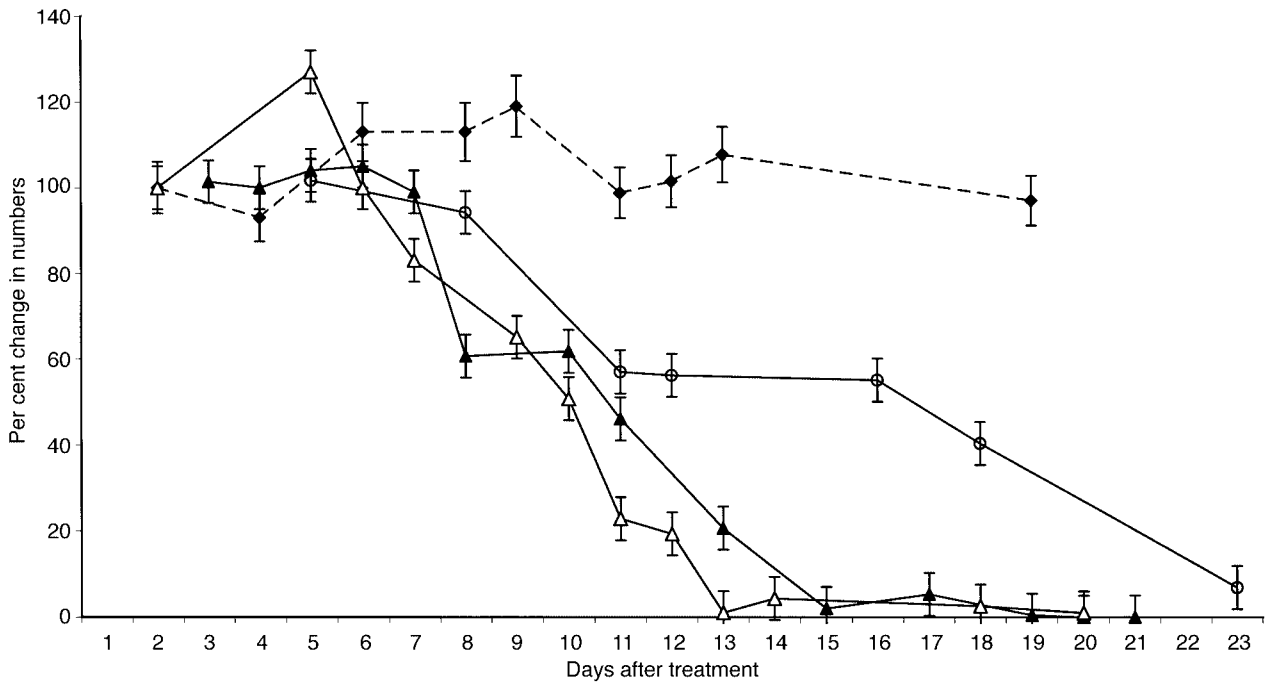

Fig. 1. Decline in numbers of *Chortoicetes terminifera* at intervals after aerial treatment with *Metarhizium anisopliae* at Griffith, New South Wales during spring (maximum temperatures 22–30°C). Data are from five blocks treated at 75–100 g ha^{-1} (▲) and three blocks treated at 25–50 g ha^{-1} (△). ◆, Untreated; ○, hatched or invaded.

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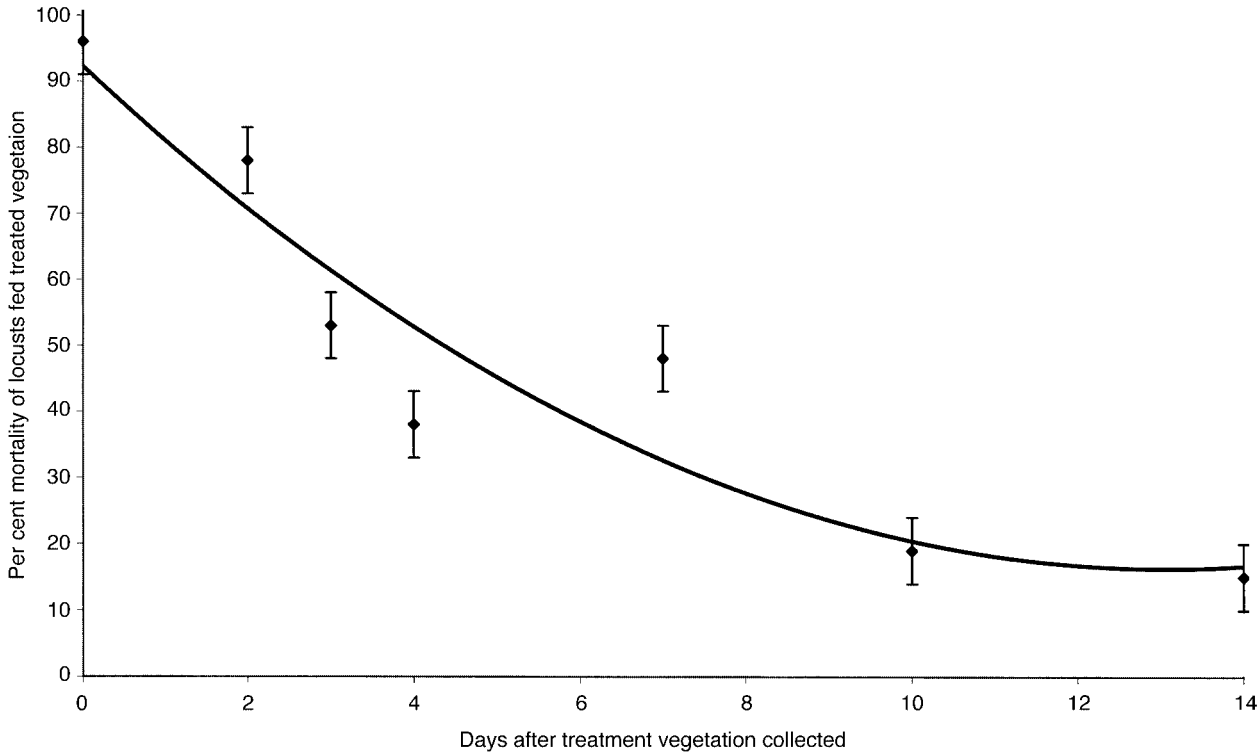

Fig. 2. Per cent mortality of untreated *Chortoicetes terminifera* fed on vegetation from treated blocks at intervals after treatment with *Metarhizium anisopliae* at Griffith, New South Wales during spring.

vegetation collected from the treated blocks: half of the locusts fed vegetation collected seven days after treatment died (fig. 2).

Treatments during hot summer weather

During summer (January), a total of eight blocks in the arid interior were treated at high (125 g = 5×10^{12} conidia ha^{-1}), moderate (75 g = 3×10^{12}) or low doses (25 g = 1×10^{12}) in 1.0 or 0.5 l oil ha^{-1}. Maximum temperatures were 36–42°C following treatment, and mortality occurred in 7–10 days (fig. 3), which was more rapid than the 9–12 days seen during the milder temperatures of spring (fig. 1). Mortality was high both with the laboratory and commercially produced material with a > 90% decline in most blocks which was confirmed two weeks after treatment when helicopter surveys found very few adults within or near treated areas. At the high dose, the formulation was very viscous which reduced the rotation of the Micronair cage from the normal 6100 rpm to 5100 rpm, and led to several blockages during application. The result was a delay in mortality (fig. 3: 125 g ha^{-1}) perhaps because of uneven coverage.

The field assessments were supported by data from locusts kept in field cages. Of the 16 cages from treated blocks (2 and 5 day samples from each of the eight treated blocks), 12 cages had rapid mortality 7–10 days after treatment, with > 90% mortality by day 12. In two cages (locusts from the block treated with 25 g ha^{-1} and sampled day 2, and from the block treated with 75 g ha^{-1} and sampled day 5), the decline was similar but the final mortality was 80%. At the high dose, where there were blockages during application, mortality was delayed and only reached 80%, as in the field. In only one sample was mortality low: in a 25 g ha^{-1} block the day 5 sample (the day 2 sample had > 90% mortality) had only 35% mortality after two weeks, similar to the 20–35% mortality after two weeks with cages from untreated areas. Adult locusts were seen flying in an unmonitored part of a 170 ha treated block on day 9 and these proved to be treated as mortality was 15–20% day^{-1} in locusts collected and placed in field cages, with total mortality reaching > 80% six days later when the experiment was terminated. Locusts fed treated vegetation collected on the day of treatment were reared in a shed at Windorah and had mortality of > 80%, with much sporulation. Subsequent collections, transported 1600 km to headquarters, had low mortality. Whether the decline was a result of problems during transport or reflects decline in efficacy in the field is unclear.

Limited operational use March–April 2000

Following heavy rains in northern South Australia during February 2000, there was widespread laying by locust swarms. Locusts were found in the 7700 km^2 organic beef grazing property Murnpeowie (29°30′S 139°00′E) and in the Gammon Ranges National Park (30°35′S 139°20′E). During late March, a total of 800 ha of nymphal bands were treated: six areas on Murnpeowie and one area in the National Park. Locusts collected from the National Park two days after treatment and placed in field cages began dying ten days after treatment reaching > 90% mortality by day 16.

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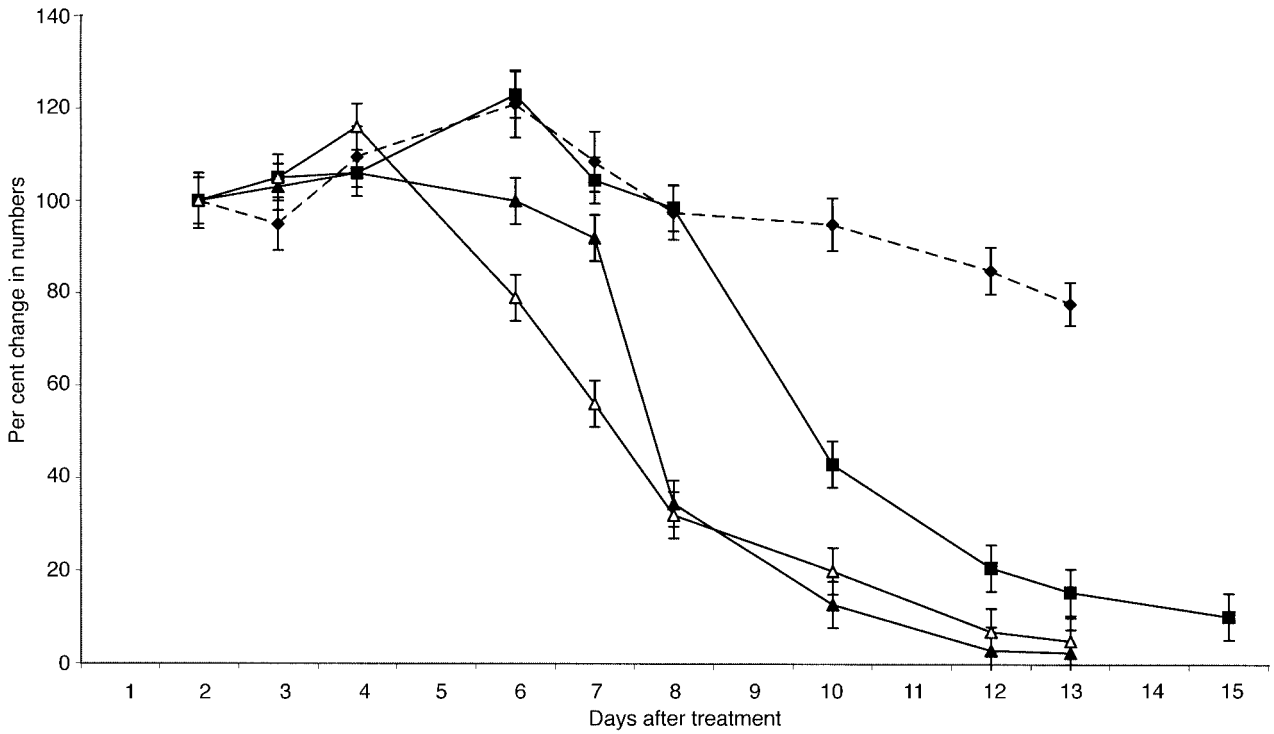

Fig. 3. Decline in numbers of *Chortoicetes terminifera* at intervals after aerial treatment with *Metarhizium anisopliae* at Windorah, Queensland during summer (maximum temperatures 36–42°C). Data are from two blocks treated at 125 g ha^{-1} (■), four blocks treated at 75 g ha^{-1} (▲), and two blocks treated at 25 g ha^{-1} (△). ◆, Untreated.

Field populations had also declined by > 90% by this time, though some of the locusts had reached the adult stage and were flying so it was uncertain if all of the decline was a result of the treatment. Locusts collected from Murnpeowie three days after treatment suffered 80% mortality in field cages while 36% of untreated locusts died, three-quarters of which occurred in the first five days. Dense swarms formed in untreated areas in early April, and three large swarms covering nearly 1700 ha were treated. Access was limited by heavy rains, and by the time the area was checked by helicopter, the swarms had moved.

Discussion

There was high mortality of *C. terminifera* even at a low dose of 25g (1×10^{12} conidia) of *M. anisopliae* per hectare. At this dose, *M. anisopliae* costs about $5 US per hectare which is price competitive with many insecticides. During the October trial, mortality was also high among locusts invading treated areas or hatching afterwards, demonstrating that *Metarhizium* persisted for about a week on the vegetation during the mild October weather. The longer persistence compared to previous trials against *Locusta migratoria* (Hunter *et al.*, 1999) may be a result of milder weather but might reflect the increased robustness of spores being produced using recently improved production methods. The poor results obtained from vegetation samples during the January trials also occurred with previous trials during summer (Hunter *et al.*, 1999) and may reflect difficulties of long distance transport of vegetation and locusts under hot desert conditions.

During the preventive control programmes against *C. terminifera* in the interior, control is often required during summer, when maximum temperatures are 35–40°C or more. These maximum temperatures are above the 25–35°C that the laboratory studies have shown are ideal for development of the FI-985 strain of *M. anisopliae* used here (Milner, 1997) and are in the range where acridids can limit infection by increasing their body temperatures (Blanford *et al.*, 1998, 2000). However, while high temperatures in the middle of the day may be inhibitory, the warm temperatures for the rest of the day and at night are in the range ideal for *M. anisopliae* development, leading to relatively rapid locust mortality. Control is also commonly required during spring outbreaks in the agricultural zone and even though temperatures during the October trials were often below 25°C, *M. anisopliae* develops well during the day when locusts bask to keep their body temperatures above 30°C (Hunter, 1983). It seems then that when it is very hot, *M. anisopliae* develops well at night and when it is mild, it develops well during the day. Further work is required using *M. anisopliae* when temperatures are intermediate as there is sometimes treatment of *C. terminifera* then.

Feeding of vegetation from the field to untreated locusts showed that the *M. anisopliae* formulation used in these trials remained infective for nearly a week in the field. The prolonged infectivity was confirmed by the mortality of locusts invading the plot or hatching after treatment. *Metarhizium anisopliae* has also been shown to persist in the field for at least this long in Africa (Thomas *et al.*, 1997) and models (Scanlan *et al.*, in press) suggest that prolonged infectivity may be of critical importance in increasing the

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effectiveness of low doses. Locusts that do not receive a lethal dose directly can pick up a dose because of their voracious feeding habits and their tendency to climb up and down the vegetation to thermoregulate (Hunter, 1983; Blanford *et al*., 2000).

The delay in mortality until early in the second week after treatment has made demonstration of field efficacy against locusts difficult because the delay gives time for mixing of treated and untreated individuals (Hooper *et al*., 1995; Langewald *et al*., 1997; Price *et al*., 1997). But the technique of following the cohesive bands that form in many locust species has allowed the clear demonstration of the efficacy of *M. anisopliae* against *R. schistocercoides* in Brazil (Lecoq & Balança, 1998) and *L. migratoria* (Hunter *et al*., 1999) and now *C. terminifera* in Australia. Treated locusts reared in field cages or in the laboratory can be used to confirm the field data. However, the delay in mortality is no impediment for *M. anisopliae* being part of a programme of preventive control, the strategy of choice for locust management in Australia. Preventive control begins early in a breeding sequence when populations are moderate and aims to treat at least 40–60% of bands or swarms every generation thereafter. In Australia, there have been successful preventive control programmes against the Australian plague locust, *C. terminifera*, the spur-throated locust, *A. guttulosa* and *L. migratoria* (Hunter *et al*., 1998). Much of the control is outside crops so delay in mortality is of limited economic importance.

The 1 l ha^{-1} application rate may be at the upper limit of application using two Micronairs and may be affecting the droplet spectrum. Mortality was similar when 0.5 l ha^{-1} of oil was used and, during the January trial, was slowest with the viscous high dose material that caused apparent blockages and some areas being underdosed. Work by LUBILOSA suggests that large droplets, as would be produced when a viscous product lowered the rpm of Micronair cages, would contain high numbers of conidia and would overdose some locusts while others are missed altogether (Bateman, 1999). Further work on mechanisms of action and on the best ways to apply *M. anisopliae* to locusts and the vegetation may be fruitful in reducing the dose required. While information needs to be gathered for a registration package to be submitted to the National Registration Authority, the proven field efficacy against *L. migratoria* (Hunter *et al*., 1999) and *C. terminifera* led to *M. anisopliae* being tested semi-operationally. However, the dose required may vary significantly between species. With the very susceptible *C. terminifera,* (LD_{50} of 420 spores per insect in the laboratory (Milner, 1997)), a dose of 25 g ha^{-1} (1×10^{12} conidia) in 0.5 l of summer oil is sufficient. *Locusta migratoria* which is less susceptible (LD_{50} of 4400 spores per insect (Milner, 1997)) and is found in thicker vegetation (Hunter *et al*., 1999) requires 75 g ha^{-1} (Hunter *et al*., 1999). The grasshopper, *Phaulacridium vittatum* (Sjostedt) (Orthoptera: Acrididae) has an intermediate susceptibility, having an LD_{50} of 1200 spores per insect (Milner, 1997), and field trials have indicated that even 75 g ha^{-1} is not completely effective (unpublished data).

Increasing constraints on insecticide use mean that having a biological agent such as *Metarhizium* may be essential to ensure the continued success of preventive control in Australia. Increasing numbers of landholders are resisting the use of chemicals because of the perceived risk of side effects on the environment and because of residues that can limit market access. Many prefer the use of non-chemical alternatives, particularly if treatment occurs when populations are moderate and the potential for direct damage less. The strain (FI-985) used in this study was isolated from an Australian locust, which should facilitate registration and general use in Australia. Government bodies have allowed its use in many environmentally sensitive areas and the National Association for Sustainable Agriculture – Australia, has approved use of current formulations on organic farms. Research currently underway aims to obtain the data required for registration and to ensure a commercially viable product so that in the future *M. anisopliae* can form a significant part of a programme of integrated pest management of several locust species in Australia.

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