

GREEN GUARD®

Richard Milner from CSIRO Entomology in Canberra, Australia, describes a new biopesticide for control of locusts and grasshoppers

Introduction

Last year, Australia was threatened with the largest plague ever recorded as conditions conspired to produce vast numbers of Australian plague locusts (Figure 1) across New South Wales, Victoria, South Australia and Western Australia. So for the first time in living memory locusts threatened agricultural production on both sides of the continent in the same year. But according to the head of Australia's Plague Locust Commission (APLC), Dr Graeme Hamilton, it was 'timely that, after large scale field trials last season, CSIRO's new *Metarhizium*-based biopesticide Green Guard® has been granted a permit for widespread operational trials and that large quantities are being produced by SGB Australia Pty Ltd (known hereafter as SGB), which has CSIRO's licence. In the event, some 23,000 ha (about 10% of the area sprayed by the APLC in 2000/2001) was aerially treated with Green Guard® with great success, and Green Guard® is now one of just three products used by the APLC for locust control. Because of its environmental benefits, such as the lack of adverse effects on other insects, on the aquatic environment and the avoidance of residues, Green Guard® can be used on organic properties and in key environmental areas where the other two chemical pesticides, fenitrothion and fipronil cannot be used. This article summarises the story of Green Guard®.

What is Green Guard®?

Green Guard® is presently being supplied as a concentrate of viable spores of the fungus *Metarhizium anisopliae* var.

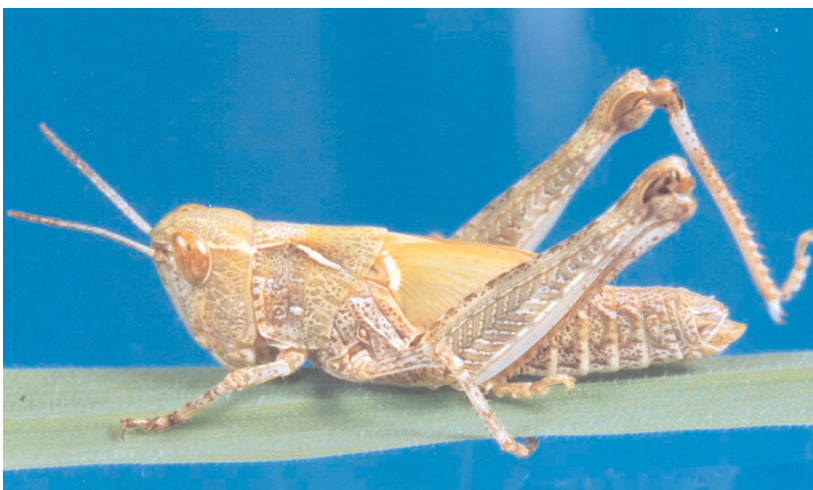


Figure 1. Nymph of an Australian plague locust.

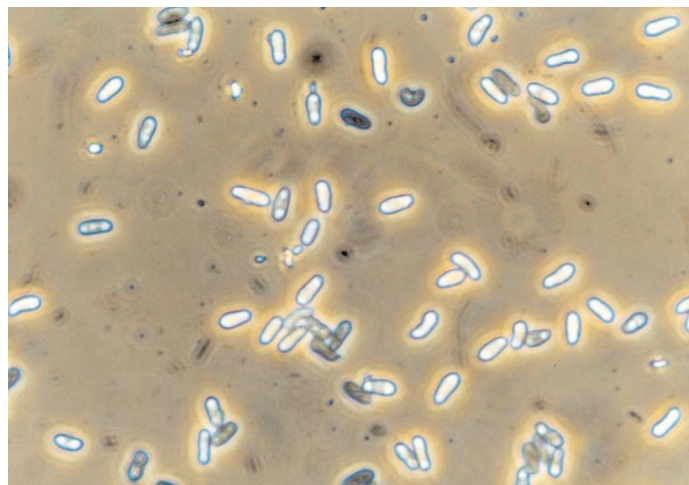


Figure 2. Spores of FI-985 as seen with a phase contrast microscope.

acridum (isolate FI-985) (Driver *et al.*, 2000) (Figure 2) in corn oil in sealed 14 litre plastic pails (Milner and Hunter, in press). The material is diluted with a light mineral oil immediately prior to use with each litre of concentrate making about 6 litres of spray. This is then aerially applied through Micronair AU 5000 nozzles at the rate of 500 ml ha⁻¹ (Figure 3). For Australian plague locusts, the recommended dose is 25 g of spore powder ha⁻¹, however recent trials at half that rate have given very good control while treatments at 6 g/ha were ineffective. The cost of the concentrate when used at 25 g ha⁻¹ is about \$US5.00 ha⁻¹. A rate of 75 g ha⁻¹ is currently recommended for treatment of migratory locusts and wingless grasshopper, however these rates may be reduced in future. There are several advantages in providing the material as an oil concentrate. It avoids the hazard and potential problems with clumping associated with mixing spore powder with the oil in the field. In earlier trials, we mixed the spores directly into the mineral oil and supplied the material as 'ready to use' formulation in 200 L drums. The problem with this was that the spores settled out rapidly in transit and were very difficult to resuspend. By using a concentrate in corn oil, this settling problem has been much reduced and there is a much smaller amount of fragile material to transport and store. Good quality control in the factory has ensured that the product flows freely



Figure 3. Aerial spraying of Green Guard®.

and there have been no problems with clumping or blocking of nozzles.

How does Green Guard® work?

The fungus used for Green Guard® is designated FI-985 and was originally obtained from an infected spur-throated

locust collected in north Queensland in 1979 (Milner, 1997). It has a simple life-cycle: the infectious stage is the spore which is a small ovoid green asexual conidium. When the surface of a locust is contaminated with spores either directly from being sprayed and/or indirectly from the vegetation, the spores send out a germ tube which penetrates through the cuticle into the haemocoel of the locust. Inside the locust, the fungus grows as hyphal bodies or hyphae and eventually kills the insect by preventing normal metabolism. Under ideal conditions, death can occur just 4 or 5 days after inoculation, but in the field, 9–14 days or more may be required as temperature conditions are rarely ideal. At death, the locust takes on a characteristic pink colour (Figure 4, LEFT) and is full of hyphae, which may then grow to form spores either internally (if conditions are dry) or externally as well (if conditions are wet) (Figure 4, RIGHT). In practice, while many pink cadavers are found after spraying, sporulating cadavers have never been found. This is thought to be because the cadavers are scavenged by ants *etc.* Even in apparently favourable habitats, Green Guard® does not persist and locusts are only found infected for about 30 days after spraying.

The two main factors which affect the efficacy of Green Guard® are the dose of spores received by the locust and the locust's body temperature. Laboratory studies have shown that only about 1000 spores are needed to kill most Australian plague locusts—not many when there are 40,000,000,000 spores in a gram of powder! The fungus grows best between 20°C and 32°C with little or no growth



Figure 4. The effects of Green Guard®. ABOVE LEFT – Recently killed Australian plague locusts showing characteristic pink colour. RIGHT – Wingless grasshopper showing typical post mortem sporulation with Green Guard®.

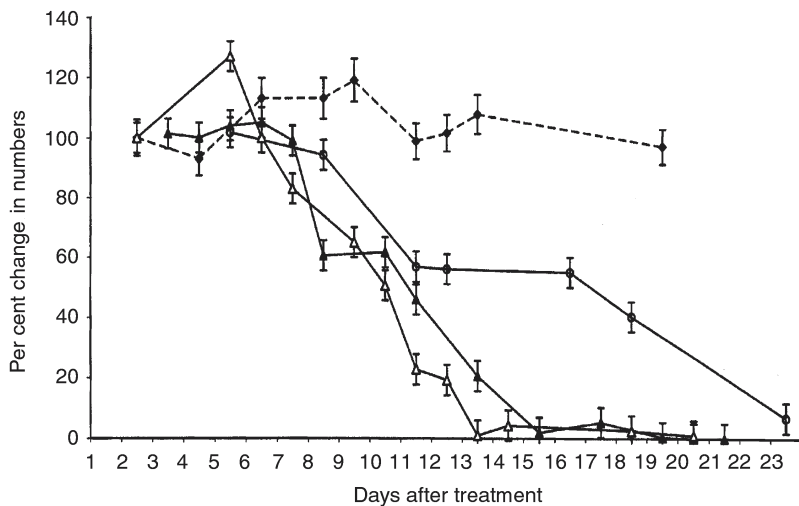


Figure 5. Effect of Green Guard® on population decline in hopper bands of the Australian plague locust, *Chortoicetes terminifera*, at intervals after aerial spraying at Griffith, New South Wales, during spring. Data (▲) are from 5 blocks treated at high dose (75–100 g spores/ha), 3 blocks treated at a low dose (25–50 g/ha) (△), controls (◆) and some plots where insects had hatched or been invaded during the week after spraying (○).

below 15°C or above 37°C. At the extremes of temperature, many more spores are required to infect and kill, so Green Guard® may not give adequate control, for example, if temperatures do not exceed 20°C for the first few days after spraying. At high temperatures, the fungus is also inhibited but the ensuing cooler night-time conditions are usually suitable. Locusts thermoregulate thereby increasing the body temperature during the day. This means that on a sunny day, when the air temperature is about 30°C, the locust body temperature will be over 37°C thus inhibiting fungal growth.

How effective is Green Guard®?

Laboratory studies have shown that all the major acridid pests in Australia are susceptible to FI-985. In the field, most

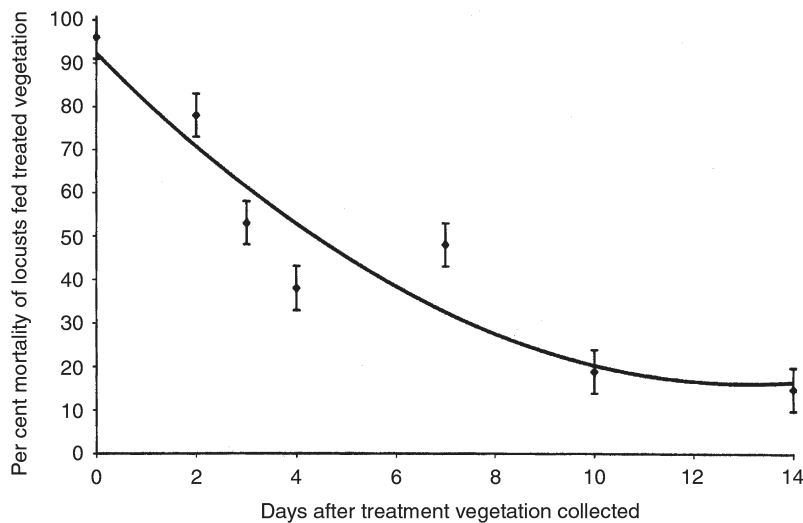


Figure 6. Percentage kill of Australian plague locusts, *Chortoicetes terminifera*, exposed to vegetation collected from sprayed plots at intervals after treatment with Green Guard® at Griffith, New South Wales, during spring.

studies on efficacy have been against the hoppers of the Australian plague locust while less extensive trials have been undertaken against migratory locust, spur-throated locust and wingless grasshoppers. Hunter *et al.* (2001) treated 4000 ha of nymphal bands and adult swarms of the Australian plague locust between October 1999 and April 2000. Treatments under mild spring conditions at doses down to 25 g ha⁻¹ showed a steady population decline from 9 to 14 days by which time control was >90% (Figure 5). Treatments under hot summer conditions also showed good efficacy at doses down to 25 g ha⁻¹ with a more rapid decline and a >90% control by days 12–14. The fungus persisted on the vegetation to give over 50% infection in exposed locusts for about 1 week (Figure 6). This persistence was effective in controlling some bands of hoppers which marched into treated areas during the week after treatment.

Earlier field trials against migratory locust (Hunter *et al.*, 1999) had also shown good declines starting about 6 or 7 days after spraying and resulting in about 90% control by day 12–14 at a dose of 75 g ha⁻¹. Modelling showed that the level of vegetation cover was an important factor in control with doses as low as 25 g ha⁻¹ predicted to give good control under low cover conditions, while a dose of 75 g/ha gave good control under all conditions of cover (Scanlan *et al.*, 2001). Improvements in formulation and drying have been made since these trials suggesting that lower doses may now be effective.

Promising, but less definitive, results have been obtained with field trials against wingless grasshopper and spur-throated locust. Field trials with both these species are confounded by movement of the target, though recent trials on wingless grasshopper at 75 g ha⁻¹ have given good results in vineyards near Mt Gambier, South Australia (Hunter, personal communication).

The mortality caused by Green Guard®, like most biopesticides, is less sensitive than chemical pesticides to dose. Thus very low doses will kill some of the locusts while extremely high doses do not provide 100% kill. The fact that a small proportion of locusts will survive even the best treatment and the 10–14 day delay before control is achieved can be disconcerting to people previously working only with chemical insecticides for locust control.

Safety and non-target effects

As with all isolates of *M. anisopliae* var. *acridum*, FI-985 has only been recorded in nature from acridids. After 20 years of studying biodiversity in the genus, I (Milner) can confidently say that this variety is quite rare—I have only found two naturally infected locusts in Australia! In field trials, it has been

repeatedly shown that other orthopterans such as tettigoniids and crickets are not affected. Once we sprayed a flowering lucerne crop where bees were very active and a small proportion of these bees died and sporulated showing they were infected with the fungus. Pitfall traps have been used to compare the numbers of ground dwelling insects in plots treated with fenitrothion in comparison with untreated plots and those treated with *Metarhizium*. While numbers were very variable, there was a clear adverse effect of fenitrothion on collembola and some other insects while the *Metarhizium* plots gave similar numbers to those of the controls. In the laboratory it has been shown that FI-985 has a much narrower host range than the more common *M. anisopliae* var. *anisopliae* isolates. Studies on aquatic life have shown that cladocerans can be killed but only at doses far higher than might ever be expected in the field. Thus there is very little hazard to other than acridids or foraging bees from the use of Green Guard®.

It is also generally accepted that *Metarhizium* does not pose a risk to vertebrates or to people provided some protection is used to prevent inhalation of the dry spores.

How is Green Guard® produced?

The spores of FI-985 are produced by solid substrate fermentation on moist sterile rice using special self ventilating pouches. Stocks of the FI-985 culture are maintained at -70°C to minimise genetic change. From the starter culture on an agar plate, liquid medium is inoculated and incubated to produce a slurry of mycelium. This is used to inoculate the moist rice in the sterile pouches. The rice is then incubated at about 25°C for 2 weeks, before the pouches are opened and placed in an environmentally controlled room to dry. Once the spore/rice mixture is dry, then the free spore powder is separated using a special reciprocating multi-layered electric sieve. Each pouch contains about 2.5 kg of rice and CSIRO tests have shown that over 250 g of pure dry spore powder can be harvested from each one (Milner and Hunter, in press). The spore powder can then be stored or formulated in corn oil and stored. In either form, the material can be stored for over 24 months in a cool room.

CSIRO have licensed SGB as the sole producer of Green Guard® and they have made significant improvements to the mass production, drying and formulating processes which are proprietary. A major problem with locusts is the variable demand for control products. SGB have optimised the production system and are maximising the storage capacity with the aim of managing this variable demand. Quality checks are conducted by SGB's in house laboratory facility at various points throughout the production process to ensure the product complies to strict production standards and contaminants are kept to a minimum. Product samples from production are also sent to CSIRO for verification of standards.

How does Green Guard® differ from Green Muscle™?

Much of the basic technology used to develop Green

Guard® was derived from a collaboration with the LUBILOSA group working in Africa and England to develop Green Muscle™, a similar product registered in parts of Africa and produced under licence by Biological Control Products in South Africa (Thomas and Blandford, 1998). Green Muscle™ contains viable spores of another strain of *M. anisopliae* var. *acridum* (IMI 330 189) which originated in Niger, Africa. Isolates of var. *acridum* differ in their mass production capacity, virulence, temperature response, and susceptibility to UV. Each of these factors can make a difference to the cost of the product by making it cheaper to produce or effective at a lower dose, while other factors make it better suited to particular conditions. Small differences in the way the two fungi are produced, dried and formulated may also make a difference in field efficacy. While no direct field comparisons have been undertaken, studies suggest that FI-985 may have an advantage in terms of ease of mass production and efficacy at low doses, potentially making this a cheaper product. There are however concerns about the use of 'exotic' strains in different countries which have inhibited comparative studies, as well as causing registration problems. Recently, Green Guard® has been successfully field trialed in Indonesia and China with trials in Mexico and Madagascar currently being planned, showing that this product may be useful outside Australia.

The future

Locust control today requires a range of strategies to satisfy a diversity of consumer demands. So Dr Hamilton regarded the availability of Green Guard® as 'timely' because it provided the APLC with a biological control strategy for the first time. Thus they were able to control locusts in areas where chemical pesticides were undesirable. For example on organic beef properties, in environmentally sensitive areas and where concerns over residues and the other side effects of chemicals were sufficient to make control with chemical pesticides impractical. The APLC now considers Green Guard® an integral part of locust control operations. Green Guard® is still being developed and improvements to the production, formulation and use are constantly being made. In addition, the use permit in Australia is currently confined to use by the APLC and moves are underway to obtain full registration so that there is unlimited use in Australia.

References

- Driver, F.; Milner, R. J.; Trueman, J. W. H. (2000). A taxonomic revision of *Metarhizium* based on sequence analysis of ribosomal DNA. *Mycological Research*, 104, 135–151.
- Hunter, D. M.; Milner, R. J.; Scanlan, J. C.; Spurgin, P. A. (1999). Aerial treatment of the migratory locust, *Locusta migratoria* (L.) (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) in Australia. *Crop Protection*, 18, 699–704.
- Hunter, D. M.; Milner, R. J.; Spurgin, P. A. (2001). Aerial treatment of the Australian plague locust, *Chortoicetes terminifera* (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). *Bulletin of Entomological Research*, 91, 93–99.
- Milner, R. J. (1997) *Metarhizium flavoviride* (FI985) as a mycoin-

BIOPESTICIDES

- secticide for Australian acridids. *Memoirs of the Canadian Entomological Society*, 171, 287–300.
- Milner, R. J.; Hunter, D. M. (2002). Recent developments with the use of fungi as biopesticides against locust and grasshoppers. *Journal of Orthopteran Research* (submitted).
- Scanlan, J. C.; Grant, W. E.; Hunter, D. M.; Milner, R. J. (2001). Habitat and environmental factors influencing the control of migratory locusts (*Locusta migratoria*) with a biopesticide (*Metarhizium anisopliae*). *Ecological Modelling*, 136, 223–236.
- Thomas, M.; Blanford, S. (1998). Current and future strategies for locust and grasshopper control. *Pesticide Outlook*, 9(4), 13–16.

Richard Milner gained his PhD from Newcastle-upon-Tyne, England, for his research on the microsporidan pathogen, *Nosema*, in the flour beetle, *Tribolium*. In 1970 he became a research scientist in CSIRO based at Armidale, New South Wales, Australia and undertook research on the diseases of pasture insects for the next 10 years. Since moving to Canberra his work has shifted to focus on fungi as biopesticides and two products based on *Metarhizium anisopliae*, BioCane™, for control of sugarcane whitegrubs, and Green Guard®, for locusts and grasshoppers are now widely used in Australia. He has published over 120 papers in a wide range of scientific journals and contributed to many books.

Useful Web pages

- <http://www.ento.csiro.au/research/biotech/biot07.html>
<http://www.lubilosa.org/>
<http://www.affa.gov.au/aplc>

The BioPesticide Manual, which is edited by one of our Board members, Leonard G Copping, is the authoritative world compendium of commercial biopesticide products. This new, revised and updated second edition, is complementary in content and style to the long-established *The Pesticide Manual*.

The BioPesticide Manual will be invaluable for all those concerned with biotechnology, natural product research, crop and environmental protection, conservation, integrated crop management, horticulture, food production, processing and marketing.

The following sample entries can be viewed on the web at <http://www.bcp.org/bookshop/ref>

spinosad	<i>Bacillus subtilis</i> QST 713	<i>Amblyseius californicus</i>
gossypure	phosphinothricin acetyl transferase gene	