Laboratory and field trials of Green Guard® (Metarhizium anisopliae var. acridum) (Deuteromycotina: Hyphomycetes) against the oriental migratory locust (Locusta migratoria manilensis) (Orthoptera: Acrididae) in China

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Laboratory and field trials of Green Guard® (*Metarhizium anisopliae* var. *acridum*) (Deuteromycotina: Hyphomycetes) against the oriental migratory locust (*Locusta migratoria manilensis*) (Orthoptera: Acrididae) in China

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Abstract

In laboratory and field trials in China, the Australian isolate (FI-985) of the fungus *Metarhizium anisopliae* var. *acridum* caused high mortality of nymphs of the oriental migratory locust, *Locusta migratoria manilensis*. In the laboratory, various dilutions of Green Guard®, the commercial *M. anisopliae* var. *acridum* product, caused 70 to 80% mortality of 4th instar nymphs. In field trials conducted at Tianjin and in Henan province, backpack sprayers were used to treat 40 ha (2002) and over 160 ha (2003) with an ULV formulation of Green Guard®. The spray was applied at a rate of 50 to 125g spores in 1125 to 1500 mL of oil/ha and, 8 to 11 d later, field populations had declined by 65 to 97%.

Key words

Metarhizium, migratory locust, biological control, China

Introduction

Locusts and grasshoppers are common pests of agriculture throughout the world. In China, there are a number of locusts and grasshoppers that can cause substantial damage; one of the most important is the oriental migratory locust *Locusta migratoria manilensis* (Meyen), a species that is found in much of eastern China. To limit damage to crops, treatment is required virtually every year, usually with a variety of chemical insecticides. The migratory locust is common near lakes and rivers, thus during the past decade a biological agent, *Nosema locustae* (Microsporida: Nosematidae), has been used near water to limit the use of chemicals there (Zhang *et al.* 2001). Another promising biological agent is the fungus *Metarhizium anisopliae* var. *acridum* (formerly *Metarhizium flavoviride*) (Deuteromycotina: Hyphomycetes) (Driver *et al.* 2000). High mortality of locusts and grasshoppers under laboratory and field conditions has been demonstrated by more than 10-years' research by the LUBILOSA program (Lutte Biologique contre les Locustes et les Sauteriaux) in Africa and by CSIRO (Commonwealth Scientific and Industrial Research Organisation) in Australia (Lomer *et al.* 1993; Hooper *et al.* 1995; Douro-Kpindou *et al.* 1997; Langewald *et al.* 1997; Hunter *et al.* 1999, 2001). An African isolate has been field tested against grasshoppers in China (Li *et al.* 2000), but the high mortality of the Australian migratory locust, *Locusta migratoria* (L.), treated with the Australian isolate (Hunter *et al.* 1999), led to laboratory and field trials to demonstrate the efficacy of the Australian isolate against the oriental migratory locust in China.

Materials and Methods

The conidia of *M. anisopliae var. acridum* (strain FI-985) were produced in Australia at the Becker Underwood factory. The spores were stored as dry conidial powder at 4°C until required. The dry conidial powder was formulated in corn oil at a concentration of 300 g of spores (1.2×10^{13} conidia)/L of oil to form the Green Guard® product, which was shipped to China by air. Within China, the formulated Green Guard® was stored in a cool cellar (10°C in winter, 20 to 25°C in summer) until used.

In a laboratory test to assess whether *L. m. manilensis* was affected by Green Guard®, laboratory-reared 4th instar oriental migratory locusts were treated with Green Guard® concentrate (1.2×10^{13} conidia/L) diluted 50×, 1000×, 3000×, in soybean oil. Each concentration was sprayed onto a sample of 20 nymphs, using a hand-held sprayer. Nymphs treated with various dilutions of Green Guard® or with soybean oil as a control, were then reared at 28°C in 40×10-cm chambers, and the dead were counted at regular intervals thereafter.

The field trials were conducted against infestations of mid-to late-instar nymphs that local authorities had found near waterways. The trial during 2002 at Tianjin was an area of fallow interspersed with areas of short and tall grass near the Dagang reservoir, while the trial during 2003 in Henan province was adjacent to the Yellow River in a 0.5-m soya bean crop, interspersed with strips of grass. Both were localized infestations that allowed treatment of most of the infestation to prevent the invasion of untreated locusts from the outside. The Green Guard® used in the treatments was transported to the field where it was mixed with soybean oil and applied by local authorities at a 10-m track spacing, using backpack ULV sprayers. During 2002, the 2 treatments (50 g of spores in 1500 mL oil, 75 g of spores in 1500 mL oil) were in adjacent 20-ha blocks. On one side of the treated blocks, a 10-ha area was treated with oil alone (oil-treated) while an adjacent 20-ha area was left untreated. During 2003, 2 adjacent 80 ha blocks were treated (50 g of spores in 1125 mL of oil, 125 g of spores in 1125 mL of oil).

Decline in locust density after treatment was the main method of assessing efficacy. Locust density in each block was assessed about the time of treatment and a second assessment made when local authorities reported a noticeable decline in numbers, usually 8 to 11 d later. Density estimates were made in the central part of each block, where at each of 20 to 30 points, the number of locusts/m² was estimated. When a locust band was encountered, the density of the band and its size (length × width) was measured. After each assessment, a quick walk was undertaken diagonally across the whole of the treated area to confirm that locust densities throughout the area were similar to densities in the intensively assessed center. During 2003, the number of locust natural enemies seen was recorded at each point where locust density was estimated.

Ground temperature where the locusts were marching was measured at intervals throughout the day. Thermometers were placed flat on the soil surface in the sun, and in the shade, such that the underside of the thermometer was just touching the soil surface.

Results

In laboratory tests against the oriental migratory locust, there was high mortality at all dilutions tested, but little mortality in the controls (Table 1). These promising results led to field trials at Tianjin during 2002 and in Henan province during 2003. In the initial trial at Tianjin, 3rd to 5th instar nymphal bands were treated and within 8 d, there was substantial mortality at both the 50 and 75g/ha doses (Table 2A). There was little change in locust density in a block treated with soybean oil or in a block left untreated (Table 2A). The Green Guard® was stored for a year in a cellar (10˚C in winter, 20 to 25˚C in summer) and used during the second year of trials. During August 2003, Green Guard® was applied at sites in Henan province at a dose of 50g and 125 g of spores in 1125mL oil/ha to an infestation of 2nd to 4th instar nymphs: there was a 76% and 65% decline, respectively, after 11 d (Table 2B). At the 2 treatment sites, the most common natural enemies were the generalist robber flies (Diptera: Asilidae) (*Ommatius* spp., *Promachus* spp) and Bombyliid flies (*Anastoechus chinensis*), that attack a variety of insects, including locusts. There was no significant change (Student t-test, $p>0.05$) in the number of these natural enemies: at the 50 g/ha site there were 0.85 ± 0.15 natural ememies/m^2 at treatment, and 0.80 ± 0.23/m^2 11 d later. At the 125g/ha site, there were 0.84 ± 0.16 natural ememies/m^2 at treatment, and 1.00 ± 0.19/m^2, 11 d later.

In eastern China, it is very common for the sun to be hidden by haze. During June 2002, in the Beijing-Tianjin area, there were clear sunny skies on only 4 of 16 d. During observations made in July-August 2003, there were clear sunny skies on only 6 of 22 d in Beijing and 3 of 11 d in Shandong/Henan. On clear hot days, when it was hot from mid morning to late afternoon (shade temperature: 27 to 33˚C), locust nymphs were commonly seen marching across the soil surface. The temperature on the soil surface was 10 to 15˚C higher than that in the shade and was above 40˚C from late morning to late afternoon (Fig. 1). But most days were cloudy or hazy and then the temperature on the soil surface was only 4 to 6˚C higher than temperatures in the shade, remaining between 30 to 35˚C for most of the day (Fig. 1).

Table 1. The mortality of fourth instars of the oriental migratory locust, *L. migratoria manilensis*, treated with various concentrations of Green Guard™, using a hand held sprayer in the laboratory. Number of nymphs per treatment: 20.

Dilution	Dose(spores/mL)	% Mortality after 15 d
100% concentrate	1.2×10^{10}	—
1:50	2.5×10^{8}	80%
1:1000	1.2×10^{7}	75%
1:3000	0.4×10^{7}	70%
Oil control	—	0%

Discussion

There was high mortality of *L. migratoria manilensis* at a dose of 50g (2×10^{12} conidia) of *M. anisopliae* per hectare. The dose of 50 g/ha (2×10^{12} spores/ha) is lower than the dose of 5×10^{12} spores/ha of the African isolate applied to grasshoppers in northwest China (Li *et al.* 2000) and is lower than the 3 to 4×10^{12} spores/ha dose that gave high mortality to *L. migratoria* in Australia (Hunter *et al.* 1999). An even lower dose (17 to 25 g/ha) was found to cause high mortality of the Australian plague locust, *Chortoicetes terminifera* (Walker) both during field trials and during control operations where nearly 40,000 ha were treated (Hunter *et al.* 2001, unpubl. data). However, the Australian plague locust is found in habitats with short grass and sparse to moderate vegetation cover, where lower doses are likely to be sufficient according to the modeling of Scanlan *et al.* (2001). Migratory locusts are often found in thick vegetation, which protects most locusts from direct impact at the time of spraying, so infection of many of the locusts is by secondary pickup from the vegetation. In thick vegetation, there are less spores per unit area of vegetation and models (Scanlan *et al*. 2001) indicate that a moderately high dose is required to ensure locusts pick up a lethal infection. Further work at a large number of sites, as is possible under operational conditions, will determine whether or not doses below 50 g/ha are sufficient for control of the oriental migratory locust in China.

Development of *M. anisopliae* is most rapid at 25 to 35°C, with mortality of Australian migratory locusts reaching 50% in 5 to 6 d at constant laboratory temperatures (Milner 1997). But mortality is very temperature dependent: mortality took nearly twice as long (11 d) at 20°C, while at 40°C, there was almost no mortality at all (Milner 1997). In the field on sunny days, grasshoppers and locusts can increase their body temperatures by basking in the sun (Inglis *et al.* 1996, 1997; Blanford 1998, 2000). During spring in mid-temperate Australia, it is generally too cool for *Metarhizium* development at night, but locusts can increase their body temperatures to the 25 to 35°C favored by *M. anisopliae,* for much of the daylight hours, and mortality in the field takes about 2 weeks (Hunter *et al.* 2001). During the hot days of summer, locust body temperatures are high for most of the daylight hours (Blanford *et al.* 1998, 2000), being at or above the 37 to 40°C where development of *M. anisopliae* is nearly completely inhibited (Milner 1997). However, temperatures are more moderate at night, being in the 25 to 35°C range preferred by *Metarhizium*, for much of the night and in the early morning, so that mortality is more rapid (1.5 to 2 w) (Kooyman & Godonou 1997; Langewald *et al*. 1997; Hunter *et al*. 1999, 2001). In eastern China most control of *L. migratoria manilensis* occurs during summer when night temperatures are in the 25 to 35°C range preferred by *Metarhizium*. However, there is often a thick haze that hides the sun, so that locusts are also at 25 to 35°C where *M. anisopliae* development is most rapid (Milner 1997, Scanlan *et al*. 2001). Both the warm nights and hazy days are favorable for *M. anisopliae*, leading to rapid mortality in the field (8 to 11 d) and making this locust a particularly good candidate for control by *M. anisopliae*.

M. anisopliae is ideally suited to the preventive control programs conducted against the oriental migratory locust. *M. anisopliae* provides a way of killing a high proportion of locusts in their favored habitats: in vegetation around lakes and in river floodplains and avoids the need to apply chemicals there. At a dose of 50 g/ha, it is slightly more expensive than the most recent chemical insecticides, but does not have the high level of risk associated with the use of

Table 2. The mortality of mid- to late instar nymphs of the oriental migratory locust, *L. migratoria manilensis*, treated with various doses of Green Guard™ in the field.

Site and date	Vegetation types	Age of Green Guard™	Area and dose of Green Guard™/ha	% change after 8 d	% change after 11 d
A. Tianjin June 2002	0.1 to 0.5-m tall grass	1 mo	20 ha: 75 g in 1500 mL oil	-72%	
			20 ha: 50 g in 1500 mL oil	-97%	
			10 ha: 1500 mL oil	+19%	
			20 ha: Untreated	-12%	
B. Henan Aug. 2003	Dense soybeans 0.5 m tall	14 mo	80 ha: 125 g in 1125mL oil		-65%
			80 ha: 50 g in 1125mL oil		-76%

chemicals near water. Mortality after treatment is slower than that for chemicals, so treatments should begin when locusts are middle instars, a practice that is already common in current preventive programs in China. Locusts treated as 2nd to 4th instars would die in the 2nd week after treatment, before reaching the adult stage. Even treatment of last-instar nymphs is possible if the nymphs are near lakes or rivers and not near crops: locusts will die as young adults, before they can fly well and reach more distant crops.

Increasing constraints on insecticide use mean that biological agents like *M. anisopliae* may contribute to the continued success of preventive control programs against the oriental migratory locust in China. Product efficacy is maintained after storage for over a year and results in moderately high mortality in 10 d or so. The preliminary results here show that Green Guard® does not reduce the number of natural enemies. When mixed with vegetable oils (corn oil, soybean oil), it provides a natural product that will not leave chemical residues, allowing it to be used near water and on products destined for export into markets where chemical residues are not wanted. Large-scale field trials are planned in support of moves towards registration and use of *Metarhizium* as a significant part of a program of integrated pest management of locusts and grasshoppers in China.

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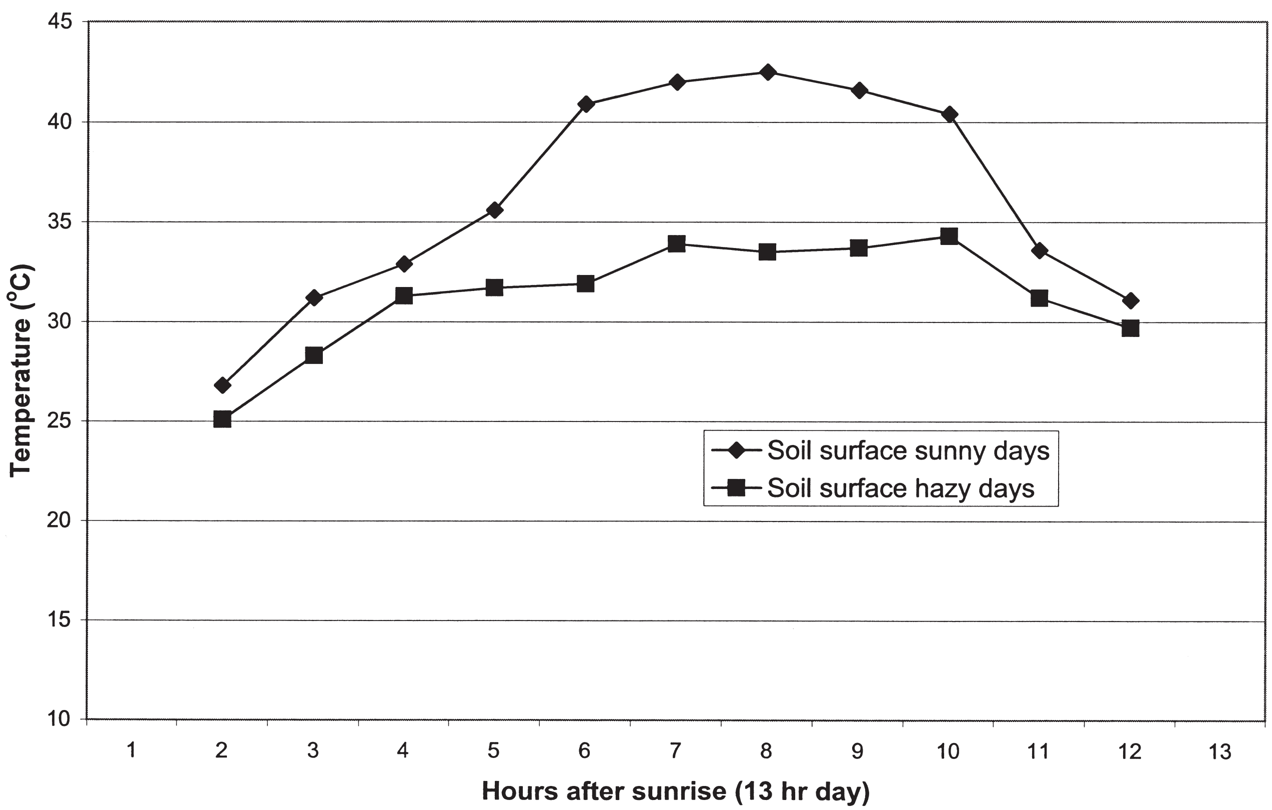

Fig. 1. Temperature (°C) on the soil surface on 4 sunny and 12 hazy days in the Tianjin-Beijing area, May-June 2002.

Literature cited

Blanford S., Thomas M.B., Langewald J. 1998. Behavioural fever in a population of the Senegalese grasshopper, *Oedaleus senegalensis*, and its implications for biological control using pathogens. Ecological Entomology 23: 9-14.

Blanford S., Thomas M.B., Langewald J. 2000. Thermal ecology of *Zonocerus variegatus* and its effect on biocontrol using pathogens. Agricultural Forest Entomology 1: 195-202.

Driver F., Milner R.J., Trueman J.H.W. 2000. A taxonomic revision of Metarhizium based on a phylogenetic analysis of ribosomal DNA sequence data. Mycological Research 104: 131-151.

Duoro-Kpindou O-K., Shah P.A., Langewald J., Lomer C.J., van der Pau H., Sidib A., Daff C.O. 1997. Essais sur l'utilisation d'un biopesticide (*Metarhizium flavoviride*) pour le contrôle des sauteriaux au Mali de 1992 à 1994. Journal of Applied Entomology 121: 285-291.

Hooper G.H.S., Milner R.J., Spurgin P.A., Prior C. 1995. Initial field assessment of *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycotina: Hyphomycetes) for control of *Chortoicetes terminifera* (Walker) (Orthoptera: Acrididae). Journal of the Australian Entomological Society 34: 83-84.

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.

Inglis G.D., Johnson D.L., Goettel M.S. 1996. Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. Biological Control 7: 131-139.

Inglis G.D., Johnson D.L., Goettel M.S. 1997. Effects of temperature and sunlight on mycosis (*Beauveria bassiana*) (Hyphomycetes: Sympodulosporae) of grasshoppers under field conditions. Environmental Entomology 26: 400-409.

Kooyman C., Godonou I. 1997. Infection of *Schistocerca gregaria* (Orthoptera: Acrididae), hoppers by *Metarhizium flavoviride* (Deuteromycotina: Hyphomycetes) conidia in an oil formulation applied under desert conditions. Bulletin of Entomological Research 87: 105-107.

Li B.P., Bateman R., Li G.Y., Meng L., Zheng Y.R.J. 2000. Field trial on the control of grasshoppers in mountain grassland by oil formulation of *Metarhizium flavoviride*. Chinese Journal of Biological Control 16: 145-147.

Langewald J., Kooyman C., Duoro-Kpindou O-K., Lomer C.J., Dahmoud A.O., Mohammed H.O. 1997. Field treatment of Desert Locust (*Schistocerca gregaria* Forskäl) hoppers in the field in Mauritania with an oil formulation of the entomopathogenic fungus *Metarhizium flavoviride*. Biocontrol Science and Technology 7: 603-611.

Lomer C.J., Bateman R.J., Godonou I., Kpindou D., Shah A., Paraiso A., Prior C. 1993. Field infection of *Zonocerus variegatus* following application of an oil based formulation of *Metarhizium flavoviride* conidia. Biocontrol Science and Technology 3: 337-346.

Milner R.J. 1997. *Metarhizium flavoviride* (FI985) as a promising mycoinsecticide for Australian acridids, pp. 287-300. In: Microbial Control of Grasshoppers and Locusts. Goettel M.S., Johnson D.L. (Eds) Memoir 171 of the Entomological Society of Canada.

Milner R., Staples J. 1998. The effect of formulation on field efficacy of *Metarhizium flavoviride* for control of wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt). Journal of Orthoptera Research 7: 83-91.

Price R.E., Bateman R.P., Brown H.D., Butler E.T., Muller E.J. 1997. Aerial spray trials against brown locust (*Locustana pardalina*, Walker) nymphs in South Africa using oil-based formulations of *Metarhizium flavoviride*. Crop Protection 4: 345-351.

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 an entomopathogenic fungus (*Metarhizium anisopliae*). Ecological Modelling 136: 223-236.

Zhang L., Shi W.P., Yan Y.H., Lin B.N., Wu M.F., Ye S.M., Li D.Q. 2001. Relationships between application rate of *Nosema locustae* and infection of *Locusta migratoria manilensis* in Henan province. Journal of China Agricultural University 6: 90-95.