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The efficacy of *Metarhizium acridum* against nymphs of the Italian locust, *Calliptamus italicus* (L.) (Orthoptera: Acrididae) in Uzbekistan and Georgia

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Abstract

Trials with the naturally occurring fungus, *Metarhizium acridum*, were conducted against nymphs of the Italian locust, *Calliptamus italicus*, in Uzbekistan and in Georgia during 2010 and 2011. The trials were conducted in two quite different habitats: in western Uzbekistan, which is desert with a median annual rainfall of about 100 mm, and on the plains of Georgia, which receive about 400-800 mm of rain per year. In three trials at a dose of 500 mL/ha of Green Guard® (containing 50 g *M. acridum* spores/ha in oil) during 2010 in Uzbekistan, there were clear declines in locust numbers by 7 days reaching 69-76% by 14 days. During 2011, a dose of 500 mL/ha resulted in a decline of 78% by 10 days and 90% by day 16, while at a dose of 250 mL/ha (containing 25 g *M. acridum* spores/ha in oil) declines were 69% and 71% by day 10 and 16, respectively. In Georgia, 8 trials were conducted at 500 mL/ha. At four sites in pastures during 2010, declines were evident by 7 days after treatment, reaching about 80% by day 14. With two applications against locusts in young sunflower crops, declines reached 66-83% by day 14. During 2011, at two sites with moderately dense pasture, locusts declined by 74% and 83% after 14 days. Overall, locust mortality was substantial in all of the trials even though the weather was quite hot, with maximum temperatures often near 40°C.

Key words

Biopesticide, locusts, field mortality

Introduction

The Italian locust, *Calliptamus italicus* (L.), has a widespread distribution from western Europe through to central Asia and western Siberia. This locust has one generation per year: eggs hatch in mid to late spring and the nymphs take 5-7 weeks to reach the adult stage. The adults mature a few weeks later and their eggs enter diapause, not hatching until the following year. The species is most common in semi-deserts where there is sagebrush (*Artemisia* spp.), while in areas of irrigated agriculture it can be found on fallow, on the edges of fields, on roadsides and along irrigation canals, especially in overgrown weedy vegetation. The nymphs can form marching bands and attack adjacent crops causing severe damage. In addition, adults can form swarms that can fly to adjacent regions. While in the northern parts of its range (N. Kazakhstan and W. Siberia) the Italian locust inhabits dry steppes, in the south parts (Uzbekistan) it prefers irrigated crops and earned a common name of the "Oasis locust" (Tsyplenkov 1970, Gapparov 2002, Latchininsky *et al*. 2002). The fact that bands of nymphs and swarms of adults can cause substantial damage to pastures and crops means that treatment programmes are common in many of the countries where this locust is found. These control programmes have relied exclusively on broad-spectrum chemical pesticides but the presence of this locust in environmentally sensitive areas such as near irrigation canals and wetlands, has led to a search for a biological alternative to chemical pesticides. A promising alternative has been entomopathogenic fungi: in Uzbekistan, the effectiveness of the fungus *Beauveria tenella* (Ascomycota: Hypocreales) has been demonstrated against a number of locust species – *Locusta migratoria migratoria* L., *Dociostaurus maroccanus* (Thunberg) and *Calliptamus italicus italicus* (L.) (Nurzhanov & Latchininsky 1987). During the 1990s, high mortality of locusts was shown in the field with isolates of the fungus *Metarhizium acridum* (Ascomycota: Hypocreales) in Africa (Douro Kpindou *et al.*1997, Langewald *et al.* 1997) and in Australia. The latter isolate has since been shown to have high efficacy against a number of locust species in Australia and elsewhere (Hunter *et al.* 1999, 2001, Hernández-Velázquez *et al.* 2003, Zhang & Hunter 2005). The Australian isolate has been made into the commercial product Green Guard®, which was first used operationally in Australia during the year 2000 when 23 000 ha were treated against the Australian plague locust *Chortoicetes terminifera* (Walker), with locust mortalities reaching 80-90% or more after 8-14 days, depending on temperature (Hunter *et al.* 2001). Moreover, in the years since, thousands of hectares have been treated with this product as part of locust control operations in Australia. In the current study, we conducted trials against nymphs of the Italian locust in two countries with quite different habitats: in the Karakalpakstan region of western Uzbekistan, where median annual rainfall is about 100 mm and in the two regions of Georgia: one where median rainfall is 400-450 mm/year and a second where about 800 mm of rain falls per year. The aim was to demonstrate the efficacy of Green Guard® *Metarhizium* in three Italian locust habitats with differing rainfall regimes.

Materials and methods

During 2010 and 2011, nymphs of the Italian locust (*Calliptamus italicus*) were treated with Green Guard® SC Premium, which consists of spores of *Metarhizium acridum* formulated in specialized oils. The product was manufactured and formulated at the commercial factory in Australia and was then transported by air to Tashkent in Uzbekistan or Tbilisi in Georgia where it was stored at room temperature until use. When suitable sites infested with locusts were found, the Green Guard® was transported by vehicle and then stored at room temperature until required at the treatment site.

In Uzbekistan, *Metarhizium* was applied against Italian locusts in the lower basin of the Amu Darya River in the Karakalpakstan region during June 2010 and June 2011. The climate is very dry with

an annual rainfall of about 100 mm but the area does benefit from periodic flooding from rains and snow melt in mountains more than 1000 km distant. Vegetation at the sites consisted of grasses and Russian knapweed (*Acroptilon repens*).

In Georgia, experiments were carried out during 2010 and 2011 in the Kartli region that has a median annual rainfall of 400-450 mm. During 2010 trials were conducted in pastures and in sunflower crops, while in 2011 the applications were in pastures. Experiments were also carried out during 2010 in a second region that has a quite different rainfall regime: in the wine growing area of Kakheti where rainfall averages ~800 mm/year. Pastures consisted of moderately dense 0.3-0.6 m tall grasses typical of the region.

In these experiments, 250-500 mL of the commercial product Green Guard® SC Premium was mixed with 100 L of water per hectare and applied with the spray application equipment commonly used locally: either handheld power sprayers (Uzbekistan), backpack sprayers (early July 2010, Georgia), or boom sprayers (late June 2010 and during 2011, Georgia). The active ingredient of the Green Guard® SC Premium formulation is spores of *Metarhizium acridum* at 10% concentration so that the 250 mL/ha dose contained 25g spores/ha while the 500 mL/ha dose contained 50 g spores/ha, doses that have proven efficacious elsewhere (Hunter *et al.* 1999, 2001, Hernández-Velázquez *et al.* 2003). We tested the *Metarhizium* formulated as Green Guard® SC Premium because it can be mixed either with water or with oil. Oil mixes can be applied as Ultra Low Volume spray and ULV spraying is becoming increasingly common in both countries.

Population decline in the field was used as the main indication of efficacy. Prior to spraying, local surveys were conducted to locate sites with substantial densities (~20-50/m^2) of nymphs of the Italian locust. In the central part of each site, counts were made of the number of locusts/m^2 at 10 m intervals with a total of 10-20 estimates made at each site. Counts were made on the day of spraying, and at intervals thereafter over the next 2 weeks or so. In Uzbekistan, counts were made within a wire frame to standardize the size of the area counted while in Georgia counts were made in an estimated meter squared. In Georgia, locusts were also collected from treated and untreated sites 24 hours after application and taken to the laboratory where they were kept at 28°C. The mortality due to *Metarhizium* was assessed from day 4 onwards (by which time any mortality due to handling would have ended) and corrected for control mortality using Abbott's formula (Abbott 1925). Caging of locusts for further observations was not possible in Uzbekistan because of the remote location of the experiments in the far west of the country.

Results

Uzbekistan.—During mid June 2010, 500 mL/ha of Green Guard® SC Premium, which contained 50 g/ha of spores of *M. acridum*, was applied to three 3-ha sites having mostly 4th instar nymphs of the Italian locust. By 7 days after treatment, there were clear declines in locust numbers and there were then further decreases to reach 69-76% (24-31% survival) after 14 days (Fig. 1). The untreated controls declined by only 10% and 19% (81-90% survival) after 7 and 14 days respectively. At the time of the 14-day assessments, most locusts were still nymphs and only a few of the locusts had reached the winged adult stage. Overall, locust mortality was substantial, even though the weather was quite hot and dry with maximum temperatures reaching 40°C during the day.

During 2011, applications were carried out in early June, when nymphs were predominantly in the 1st and 2nd instars. Two sites were treated with Green Guard® SC Premium: one site was treated at a low rate of 250 mL/ha and the other was treated at a higher rate of 500 mL/ha. While there was little change in locust densities at the untreated sites, at the site treated with the 250 mL/ha dose, locust numbers had declined by 38% on day 7, by 69% on day 10 and by 71% (29% survival) on day 16 (Fig. 2). At the higher dose of 500 mL/ha, declines were higher, reaching 48% by day 7, 78% by day 10 and 90% (10% survival) by day 16 (Fig. 2).

Georgia.—During late June and early July 2010, four 3-7 ha sites in pasture were treated with 500 mL/ha Green Guard® SC Premium in 100 L water/ha. Maximum temperatures were ~38-42°C following spraying. While there was little change in locust densities at the untreated sites, the four treated sites revealed clear density decrease by 7 days after treatment, with declines of 77-86% (14-23% survival) by day 14 (Fig. 3). At the two applications against locusts in young sunflower crops, there were only slight declines by day 7; however by the same day 7, densities in the nearby untreated crop had *increased* by 18% (Fig. 4). Declines were clear by day 9 and reached 66-83% by day 14 (Fig. 4).

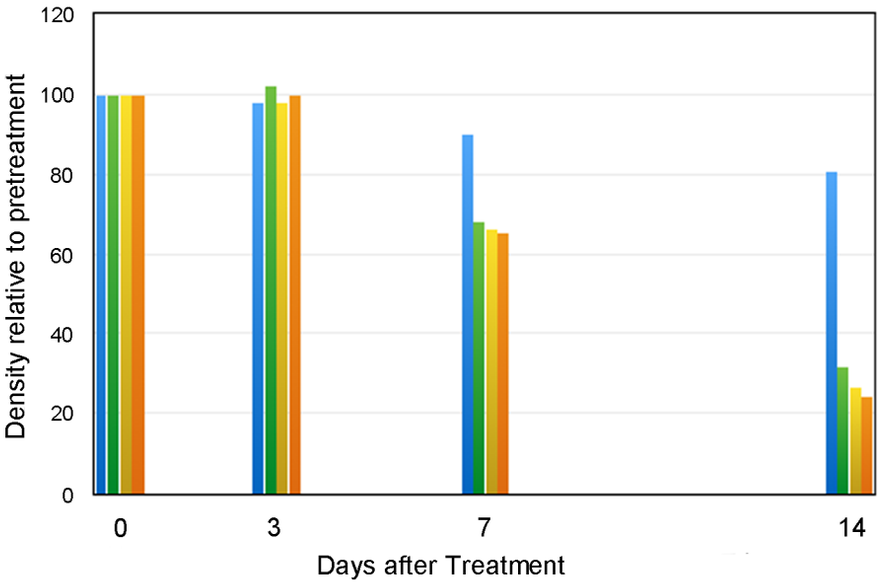

Fig.1. Percentage change in density of *C. italicus* nymphs that were untreated (first column) or three sites treated with *Metarhizium acridum* at 50 g/ha (columns 2-4) in Karakalpakstan, Uzbekistan during 2010. Mean locust densities pretreatment were from 25-27/m^2 at the different sites and these are shown as 100% on day 0.

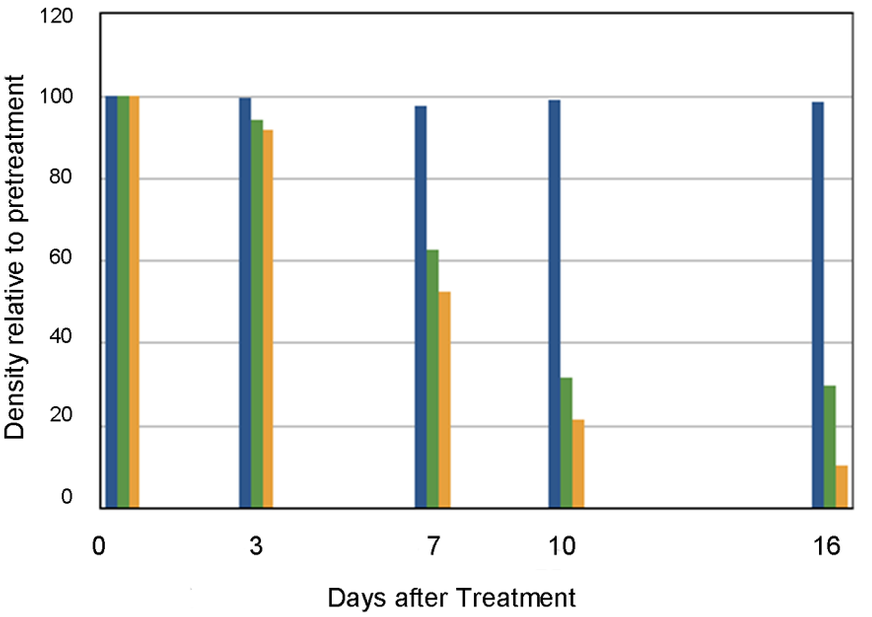

Fig. 2. Percentage change in density of *C. italicus* nymphs that were untreated (first column) or treated with *M. acridum* at 25 g/ha (second column) or 50 g/ha (third column) in Karakalpakstan, Uzbekistan during 2011. Mean locust densities pre-treatment were from 20-23/m^2 at the different sites and these are shown as 100% on day 0.

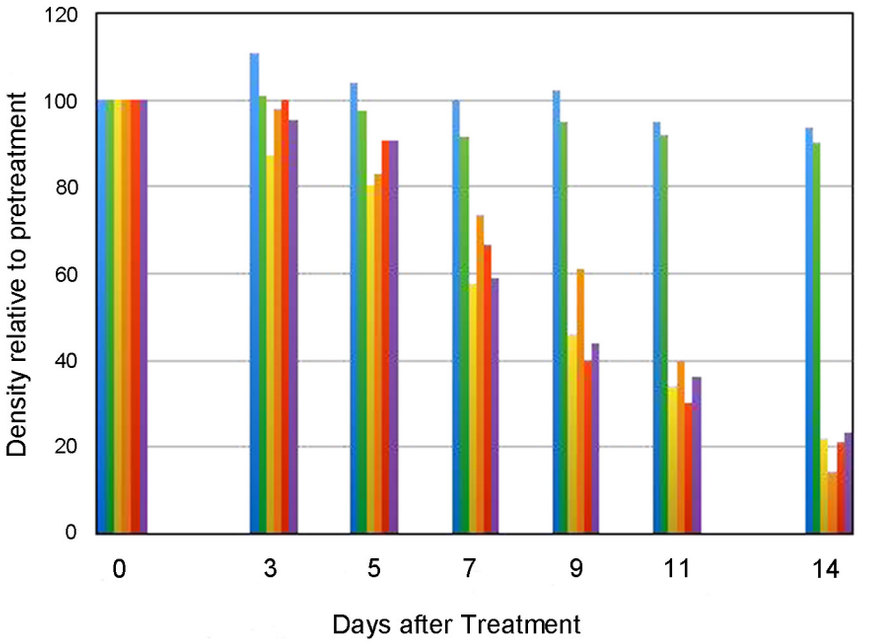

Fig. 3. Percentage change in density of *C. italicus* nymphs that were untreated (first two columns) or treated with *M. acridum* at 50 g/ha (columns 3-6) in in pastures in two regions in Georgia during 2010. Mean locust densities pre-treatment were from 34-54/m^2 at the different sites and these are shown as 100% on day 0.

At 24 hours after each of the applications, locusts were collected from the untreated and one of the treated sites and placed in cages in the laboratory. At the Kartli pasture site, 87% of locusts from the treated pasture died 4-10 days after collection compared to 15% from the untreated plots. The mortality corrected for these controls using Abbott's formula was 85%, similar to the 83% mortality obtained in the field. At the Kartli sunflower crop site, 82% of locusts from the treated pasture died 4-10 days after collection compared to 12% from the untreated plots. The corrected mortality of 79% was similar to the 75% mortality obtained in the field. At the Kakheti pasture site 83% of locusts from the treated pasture died compared to 21% from the untreated plots, giving a corrected mortality of 78%, the same as the 78% decline observed in the field.

During 2011, locusts in moderately dense pasture were treated at 500 mL/ha and, at the summer maximum temperatures of 35-39°C, declines of 83% and 74% were observed after 14 days. Locusts collected the day after treatment and placed in cages in the laboratory had a corrected mortality of 75% by day 10.

Discussion

In trials both in Uzbekistan and in Georgia, there was a high mortality of Italian locusts treated with *Metarhizium acridum*. The mortalities obtained at the 500 mL/ha dose were quite consistent in the different experiments under a variety of conditions: there was a similar pattern in that declines were usually clearly evident by 7 days, reaching about 60-70% after 10-11 days, with further declines to 75-90% two weeks post-treatment. The mortality being evident from day 7 onwards is similar to the mortality seen during hot weather against the Australian migratory locust (Hunter *et al.* 1999), Australian plague locust (Hunter *et al.* 2001), the Central American locust *Schistocerca piceifrons* (Walker) (Hernández-Velázquez *et al.* 2003),

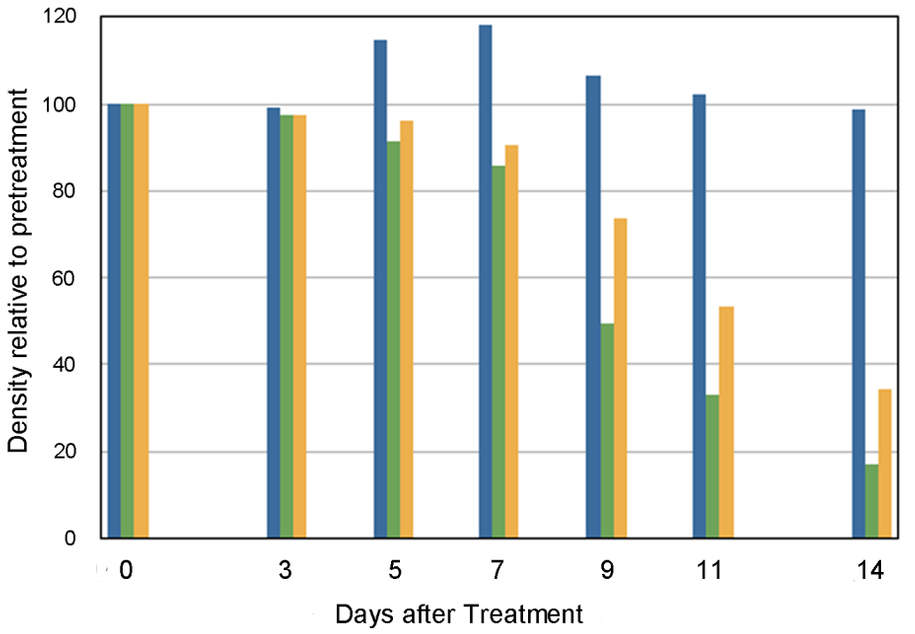

Fig. 4. Percentage change in density of *C. italicus* nymphs that were untreated (first column) or treated with *M. acridum* at 50 g/ha (columns 2,3) in sunflower crops in Georgia during 2010. Mean locust densities pre-treatment were from 25-33/m^2 at the different sites and these are shown as 100% on day 0.

and the desert locust *Schistocerca gregaria* (Forskål) (Langewald *et al.* 1997). And in the current studies, mortality was similar in both the deserts of Uzbekistan and in the moderate and moderately high rainfall areas of Georgia, which is consistent with high mortality in past studies both in deserts (with some Australian plague locust studies and with the desert locust) and in higher rainfall areas (other Australian plague locust studies, migratory locust, Central American locust). Furthermore, in spite of the fact that there is little development of *M. acridum* at temperatures above 36°C in the laboratory (Milner *et al.* 2003), we found that mortality in the field was high even when maximum temperatures were near 40°C. This high mortality when maximum temperatures are high has also been observed in Australia and Africa, and is a result of very hot days having evening, night and early morning temperatures in the 23-33°C range favorable for rapid *M. acridum* development (Hunter 2005). This consistent pattern of mortality against a wide variety of locust species in a variety of situations means that *M. acridum* has the potential to form part of a control program against a wide variety of locust pests under different climate conditions.

The dose required to obtain high mortality does, however, vary depending on the weather and on vegetation type. With the Australian plague locust, which is found in short vegetation, a dose of 25 g of spores/ha is sufficient (Hunter *et al.* 2001) though in recent years a dose of 35 g of spores/ha has often been used during spring when mortality is slower because of lower temperatures (Australian Plague Locust Commission, *pers. comm.*). With the migratory locust and red locust, *Nomadacris septemfasciata* (Audinet-Serville), which are found in taller vegetation, it has been found that a higher dose of 50-60 g of spores/ha is required to ensure infection in the greater vegetation cover. The Italian locust is found in intermediate vegetation and so a higher dose (*e.g.*, 50 g of spores/ha) than that used for Australian plague locust seems to be required to obtain adequate mortality.

Increasing constraints on chemical insecticide use mean it is becoming important to have a biological alternative as part of locust control programmes. The specificity of *M. acridum* to acridids (locusts and grasshoppers) means that it can be used where chemical pesticides cannot or should not be used, such as in national parks, along waterways or near irrigation canals. Using chemical pesticides in such ecologically sensitive areas not only affects non-target organisms but also increases the risk of high levels of chemical residues being detected in foodstuffs either for local consumption or for export. Without a biological alternative, the increasing restraints on chemical use mean increasing numbers of locusts will have to be left untreated. Having a highly efficacious biological alternative like *M. acridum* will ensure the continuing success of locust management programmes.

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