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# Field trials of *Metarhizium anisopliae* var. *acridum* (Ascomycota: Hypocreales) against oriental migratory locusts, *Locusta migratoria manilensis* (Meyen) in Northern China

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ABSTRACT

During 2002–2006, nymph bands of *Locusta migratoria manilensis* (Meyen) were treated by ground and aerial applications in 6000 ha of grasslands and the nearby beach of Yellow river using a soybean oil miscible suspension ULV formulation of *Metarhizium anisopliae* var. *acridum* isolate CQMa102. The formulation was also applied in Tianjin, Henan, Hebei, Shandong and Shanxi provinces of Northern China by ground and aerial applications. During field studies, cage tests were carried out in corresponding field plots in order to estimate the mortality accurately. Doses of $3.3 \times 10^{12}$ and $5.0 \times 10^{12}$ conidia $ha^{-1}$ were equally effective and caused 90% mortality 9–13 days after treatment. In the ground spray trial, $3.3 \times 10^{12}$ conidia $ha^{-1}$ killed >90% of *L. migratoria manilensis* 11–15 days after treatment in a wide variety of vegetation and weather conditions. The decline of locust populations was slower where vegetation was taller and denser. In the aerial spray treatment, the final percent survival of locusts was lowered to 10% at 11 and 14 days in the field cage and open field locusts, respectively. Furthermore, the *M. anisopliae* oil miscible suspension formulation did not appear to harm natural enemies of locusts in the field.



## 1. Introduction

The oriental Migratory locust, *Locusta migratoria manilensis* (Meyen), found in much of northern China, has been listed as one of the major pests of crops and pastures in China since 707 B.C. (Wu, 1951). During the last two decades, most outbreaks of *L. migratoria manilensis* likely developed because of drought resulting from global warming (Lei and Wen, 2004). To limit damage to crops and pastures, treatment is required virtually every year, usually with large amounts of broad-spectrum chemical pesticides that pollute the environment and cause health and safety issues as well as exacerbating locust problems due to the loss of natural enemies (Zhu, 1999). In order to reduce both locust damage and insecticide input, it is important to develop alternative biological control agents as part of an integrated pest management (IPM) program.

One of the most promising biological agents for controlling locusts and grasshoppers is the Acridid-specific fungal pathogen *Metarhizium anisopliae* var. *acridum* (formerly *Metarhizium flavoviride*) (Ascomycota: Hypocreales) (Driver et al., 2000; Lomer et al., 1993; Hooper et al., 1995; Kpindou et al., 1997; Langewald et al., 1997; Hunter et al., 1999, 2001). In the past 10 years, several virulent isolates of *M. anisopliae* var. *acridum* active against locusts and grasshoppers have been found separately in South Africa (Lomer et al., 1993), Queensland (Hooper et al., 1995), Sudan (Kooyman and Abdalla, 1998) and China (Wang et al., 2003). The use of oil-based formulations and ultra-low volume (ULV) application techniques remarkably enhanced virulence of conidia and produced very promising Acridid control in the field (Symmon, 1992; Bateman, 1997). The LUBILOSA (Lutte Biologique contre les Locustes et les Sauteriaux) program in Africa (Lomer et al., 1993; Langewald et al., 1997) and the Commonwealth Scientific and Industrial Research Organization Program in Australia (Hooper et al., 1995; Milner et al., 1997) demonstrated that this fungus causes high mortality of locusts and grasshoppers in the field.

In China, strain CQMa102, isolated from *Ceracris Kiangsu* (Orthoptera: Acrididae) in Chongqing, was identificated as *M. anisopliae* var. *acridum* by The Institute of Microbiology, Chinese Academy of Sciences and stored in the China General Microbiological Culture Collection Center (Strain number: CGMCC No. 0877). In the laboratory, the biological characteristics of strain

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CQMa102 have been studied and compared with those of strain IMI330189 which has been applied successfully to control the desert locust, *Schistocerca gregaria*, in Africa (Wang et al., 2003). There are no significant differences in heat resistance and ultraviolet resistance of conidia between the two strains, but CQMa102 was significantly more virulent than IMI330189 against *L. migratoria manilensis*. CQMa102 is pathogenic for the main species of locust in China including *L. migratoria manilensis*, *Chondracris rosea* (de Geer), *Ceracris nigricornis* (Walker), *Oxya chinensis* (Thunbery) and *Acrida chinensis* (Westwood). This strain is specific for locusts and does not infect non-target insects such as *Bombyx mori* Linnaeus, *Apis cerana*, *Tetramorium* sp. It also sporulates well on rice and wheat bran and thus shows potential for commercial production. Additionally, CQMa102, having been isolated locally, is likely to be more compatible with Chinese habitats and have intrinsically higher bio-safety than exotic strains.

The present research aimed to determine the field efficacy of an oil miscible suspension formulation of local strain CQMa102 against populations of *L. migratoria manilensis* in a wide variety of locust habitats in China. We also obtained the data required for registration and application to ensure that a commercially viable product of *Metarhizium* could be used as a significant strategy within an IPM program for the oriental migratory locusts in China.

## 2. Materials and methods

### 2.1. The conidia of M. anisopliae production

Conidia of strain CQMa102 of *M. anisopliae* var. *acridum* used in the field trials were provided by the Genetic Engineering Research Center, College of Bioengineering at Chongqing University, China. In a two-phase fermentation process, mycelia were first produced in a liquid fermentation reactor and then inoculated into autoclaved rice with 40–50% water in compound plastic bags, this permitted gas exchange but prevented microbial contamination. After 15 days, the conidia were harvested, dried and formulated in soybean salad oil. The concentration of the *Metarhizium* oil formulation contained ca. $1\times10^{13}$ conidia $l^{-1}$ determined by serial dilution of the formulation and counting on a Petroff–Hausser counting slide. The formulated conidia of strain CQMa102 were stored in a sealed vessel at room temperature until use. For application, a *Metarhizium* oil miscible suspension formulation was made by mixing the soybean *Metarhizium* oil formulation to the required dilution with a mixture comprising 40% water, 56% soybean salad oil and 4% mixed emulsifiers (Span-80 and Tween-20) mixed well at high speed in a beater.

### 2.2. Application of M. anisopliae in oil miscible suspension formulation

The formulation was applied to blocks infested at 1 l $ha^{-1}$ by ground and aircraft ULV spray. In ground spray trials, the oil miscible suspension formulation was applied by local authorities at 10-m track spacing, using backpack ULV sprayers fitted with a restrictor, by which the flow rate was adjusted. In order to achieve even coverage, each sprayer was directed by two signalers holding red flags at plot ends during spraying.

The spray agricultural utility aircraft 'Yun-5', fitted with six 3# nozzles with rotary cage atomizers (50° blade angle), was used to treat locust plots. The formulation was applied from a 5 to 10 m height at 50-m track spacing. A Micronair monitor logged flow rates and a DGPS was used to set and record track spacing. To ensure that the upwind edge of the block was treated, the first spray run was 200 m upwind of the block. Treatment was carried out when winds were consistently 2–5 m $s^{-1}$ to ensure even coverage.

### 2.3. Dosage experiments

Dosage experiments were conducted by ground spray in four provinces of Northern China during summer 2002–2003 (Table 1). Test blocks, about 600 ha in total, were in main ecologic areas where the Oriental migratory locusts occur in China, including the Yellow River flooding areas (Zhongmu county in Henan province; Dongying in Shandong province), Bohai Beach (Dagang in Tianjin) and Lake shore areas (Pingshan county in Hebei province). Each trial consisted of a control (non-treatment) and three treatments (test formulation, which was obtained by mixing *Metarhizium* oil formulation and diluent of mycoinsecticide with the ratio of 1:3, 1:2 and 1:1, containing $2.5\times10^{12}$, $3.3\times10^{12}$ and $5.0\times10^{12}$ conidia $l^{-1}$, respectively) with each treatment having four replications. The trial blocks were infested with 2–4 instar nymphs (5–50 nymphs $m^{-2}$) and covered 20–50% by 0.2–0.5-m tall vegetation (partly interspersed with open woodland). Sixteen 9–10 ha plots, 100 m apart, were arranged randomly in the test blocks.

In the field, treated locusts often mixed with the untreated individuals due to the movement of the locusts during the 7–10 days lag period between treatment and death, which makes it difficult to clearly demonstrate population declines in the field. This phenomenon has been described previously (Lomer et al., 1993; Langewald et al., 1997). Consequently, mortality was always determined form locusts kept in field cages (see also Kooyman and Godonou, 1997; Price et al., 1997).

During the dosage experiments, the cage tests were synchronously carried out using a method modified from Hunter et al. (1999). At 1 day after treatment, about 50 locusts from each treated and untreated plot were placed in separate $1\times1\times1$ $m^3$ field cages made of 10-mesh plastic cloths (20–30% shade). The field cages were located in corresponding plots with adequate grass. Because of cannibalism (Kooyman and Godonou, 1997), cages were checked every day for mortality and any dead locusts were counted. The dead or dying locusts, both in the field cages and in field, were collected and a proportion of them (ca. 10%) were kept at 26 °C and high humidity for 5 days to check for sporulation.

### 2.4. Large-scale field trials by ground spray

During 2004, the large-scale field trials, covering ca. 1500 ha in total, were conducted against nymph infestations (instars 1–5) using the *Metarhizium* test formulation (mixing *Metarhizium* oil formulation and diluent of mycoinsecticide at a ratio of 1:2, containing approximately $3.3\times10^{12}$ conidia $l^{-1}$) in 24 blocks which were sited in seven provinces of Northern China (Table 2). Ground cover rate, height of vegetation and temperatures were measured. In the neighborhood of each treated site, about 15–20 ha was left untreated as control. The ground cover rate was assessed by percent shading of vegetation. During each trial, an additional 50–100 m strip around each plot was treated to reduce the chance of invasion by untreated locusts.

The temperature and relative humidity were measured from 9 am to 4 pm each day when the locusts were marching. The wet and dry bulb thermometers were hung on a 0.5 m tall flag inserted in corresponding plot.

**Table 1**
Mean percent mortality (±S.E.) of *L. migratoria* (Meyen) treated with various doses of *M. anisopliae* after treatment in the field cages (2002–2003)

| Site and date | Doses (conidia $ha^{-1}$) | Days after treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 | | 7 | | 9 | | 11 | | 13 | |
| Dagang in Tianjin (2002) | $5.0\times10^{12}$ | 31.9±3.9 | a | 55.4±2.8 | a | 92.0±2.2 | a | 95.2±1.7 | a | 96.5±2.4 | a |
| | $3.3\times10^{12}$ | 34.1±5.8 | a | 48.6±10.6 | a | 85.7±7.1 | a | 90.4±4.4 | a | 93.5±1.9 | a |
| | $2.5\times10^{12}$ | 15.4±2.4 | b | 26.7±2.3 | b | 59.3±8.8 | b | 64.6±9.5 | b | 81.8±8.0 | b |
| | Untreated | 2.0±1.6 | c | 2.0±1.6 | c | 2.0±1.6 | c | 2.0±1.6 | c | 2.0±1.6 | c |
| Pingshan county in Hebei (2002) | $5.0\times10^{12}$ | 23.2±2.3 | a | 53.1±5.6 | a | 90.5±1.5 | a | 95.3±2.6 | a | 96.8±2.3 | a |
| | $3.3\times10^{12}$ | 20.7±3.4 | a | 52.2±6.9 | ab | 87.6±6.4 | a | 90.4±3.2 | a | 90.9±3.2 | a |
| | $2.5\times10^{12}$ | 14.1±10.2 | b | 34.3±10.1 | b | 64.1±5.9 | b | 69.0±8.9 | b | 76.1±14.6 | b |
| | Untreated | 1.5±1.0 | c | 1.5±1.0 | c | 1.5±1.0 | c | 2.5±1.0 | c | 2.5±1.0 | c |
| Zhongmu county in Henan (2003) | $5.0\times10^{12}$ | 33.4±7.7 | a | 68.8±7.0 | a | 86.5±6.8 | a | 90.1±2.1 | a | 95.9±3.2 | a |
| | $3.3\times10^{12}$ | 27.4±8.6 | ab | 54.4±5.2 | a | 81.4±7.6 | a | 87.2±6.7 | a | 91.9±2.6 | a |
| | $2.5\times10^{12}$ | 23.4±3.1 | b | 37.7±10.9 | b | 54.0±9.8 | b | 65.2±6.2 | b | 74.8±12.3 | b |
| | Untreated | 3.0±2.5 | c | 3.0±2.5 | c | 3.0±2.5 | c | 3.0±2.5 | c | 3.0±2.5 | c |
| Dongying in Shandong (2003) | $5.0\times10^{12}$ | 28.3±8.1 | a | 59.3±10.6 | a | 82.5±4.6 | a | 88.3±3.5 | a | 92.6±3.2 | a |
| | $3.3\times10^{12}$ | 27.2±11.9 | ab | 49.8±9.1 | ab | 80.8±6.8 | a | 88.1±4.0 | a | 90.6±2.5 | a |
| | $2.5\times10^{12}$ | 21.7±13.3 | b | 44.5±7.3 | b | 64.7±11.6 | b | 69.0±7.2 | b | 79.3±9.8 | b |
| | Untreated | 2.5±1.9 | c | 2.5±1.9 | c | 3.0±1.2 | c | 3.0±1.2 | c | 3.0±1.2 | c |

Mean mortalities followed by different letters in the same column at each trial site were significantly different ($P<0.05$), according to ANOVA and Tukey's multiple range test.

### 2.5. Aerial spray experiments

Eight blocks infested by mainly 2–4 instar nymphs at 4–60 nymphs $m^{-2}$, covering 3000 ha in total, were treated with *Metarhizium* test formulation (mixing *Metarhizium* oil formulation and diluent of mycoinsecticide with 1:2, containing approximately $3.3\times10^{12}$ conidia $l^{-1}$) in three trial locations during 2005 and 2006. In the vicinity of each *Metarhizium*-treated block, an equivalent block was treated with chemical pesticide (40% malathion, an organophosphate widely used for acridid control, at 1.5 l $ha^{-1}$) and an untreated area about 30–40 ha served as a control. All blocks in the adjacent location were separated by at least 5 km. The trials blocks were in Huanghua county in Hebei province (June 2005, three blocks), Mengzhou county in Henan province (June 2006, three blocks) and Dongying in Shandong province (July 2006, two blocks). These trial locations adjacent to the Yellow River were covered with thick grass which is about 0.2–0.6 m tall and averaged 25–60% ground cover, interspersed with open woodland. The field cage tests were performed synchronously by the method described in the dosage experiment. During the aerial spray experiments, the number of locust natural enemies was recorded at each point where locust density was estimated.

### 2.6. Investigation and analysis

Population decline rate in the field was used as the primary test of efficacy. Post-treatment bands in treated and untreated blocks were selected following techniques adapted from Hunter et al. (1999). All blocks were intensively searched for 2–4 h to locate every band that initially traversed at 100 m intervals, and these original bands were followed. Each day after treatment, the band size was determined from band length estimated by pacing the band front and placing flags every 30–40 m along its length border, and band width estimated by walking at right angles to the main band front at each of the flags. The size and average density of locusts were used to estimate the total number of locusts in each band. Average density estimates were made by counting locusts in a $1\times1\times0.3\,m^3$ sampling cage thrown randomly in the central part of each block, for 20–30 times. Mortality or survival was estimated from changes in the mean (±standard error) density of locusts in each band originally in a block. The correct efficacy in treatment block was calculated by comparisons to untreated control by formulation, that is Correct efficacy = [$Survival_{(control)}$−$Survival_{(treatment)}$]/$Survival_{(control)}$. Statistical analyses (Tukey's multiple range test) were carried out using DPS software (Tang and Feng, 2002).

## 3. Results

### 3.1. The dosage experiments

Because locusts aggregated and moved long distances due to food scarcity and higher temperature and the plot area in dosage trials was small, a few bands from untreated areas mixed with treated bands at partly trial plots at 1–3 days after treatment during dosage experiments. The mean mortality was assessed using the remaining unmixed plots (Fig. 1). *Metarhizium* in oil miscible suspension formulation at high dosage ($5.0\times10^{12}$ conidia $ha^{-1}$) and moderate dosage ($3.3\times10^{12}$ conidia $ha^{-1}$) resulted in $a>90\%$ mortality at 11–15 days in a variety of environments infested by oriental migratory locusts during 2002–2003. However the mortality of the oriental migratory locust at low doses was apparently lower, with the final mortality being <80%.

The field cage results of dosage experiments against the oriental migratory locust are presented in Table 1. In four trials, there were high mortality at the $5.0\times10^{12}$ and $3.3\times10^{12}$ conidia $ha^{-1}$ doses and low mortality at $2.5\times10^{12}$ conidia $ha^{-1}$ doses, but mortalities at controls (untreated) were lower than 3%. The locusts infected with *M. anisoplia* began dying at 5 days after treatment, and the mean percent mortality of the oriental migratory locust rapidly increased in the following 6–7 days. At doses of $5.0\times10^{12}$ and $3.3\times10^{12}$ conidia $ha^{-1}$, the mortalities of locusts were over 90%, respectively, at 9–13 days and 11–13 days after treatment in all four trials. However, there was no significant

**Table 2**
Results of the ground spray trial against *L. migratoria manilensis* (Meyen), in the field during summer, 2004

| Locust stage treated | Area treated (ha) | Day temperature and relatively humidity each day | Ground cover rate and height of vegetation | Survival (%) and efficacy (%, mean ± S.E.) after treatment | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 11 d | | 13 d | | 15 d | |
| *Mengzhou county in Henan province, June 2004 (3 blocks)* | | | | | | | | | |
| 2–3 instar nymphs | 105 | 34–41 °C, 35–42% | 20–30%, 0.2–0.3 m | 12.5 | 91.6 ± 1.7 | 10.3 | 94.2 ± 2.0 | 8.3 | 95.5 ± 1.4 |
| 3–4 instar nymphs | 90 | 35–41 °C, 37–43% | | 8.5 | | 4.6 | | 2.4 | |
| 3–5 instar nymphs | 73 | 36–42 °C, 35–40% | | 7.4 | | 6.5 | | 5.5 | |
| Untreated | 20 | 35–39 °C, 36–40% | | 112.1 | – | 123.2 | – | 120.7 | – |
| *Dagang in Tianjin, July 2004 (3 blocks)* | | | | | | | | | |
| 2–4 instar nymphs | 113 | 34–43 °C, 45–55% | 25–40%, 0.1–0.3 m | 10.1 | 91.1 ± 2.6 | 9.3 | 94.2 ± 4.3 | 6.7 | 94.6 ± 0.9 |
| 3–4 instar nymphs | 89 | 35–43 °C, 35–50% | | 9.2 | | 6.6 | | 5.3 | |
| 3–4 instar nymphs | 59 | 34–42 °C, 35–50% | | 6.4 | | 4.1 | | 4.4 | |
| Untreated | 20 | 35–43 °C, 50–60% | | 95.7 | – | 110.2 | – | 102.4 | – |
| *Pingshan county in Hebei province,[a] June 2004 (3 blocks)* | | | | | | | | | |
| 2–4 instars nymphs | 67 | 29–39 °C, 35–70% | 30–50%, 0.2–0.4 m | 13.9 | 85.6 ± 1.1 | 9.2 | 91.1 ± 1.0 | 7.9 | 93.8 ± 0.6 |
| 2–4 instars nymphs | 86 | 28–40 °C, 35–68% | | 15.1 | | 10.8 | | 5.6 | |
| 3–5 instars nymphs | 69 | 30–41 °C, 35–65% | | 17.8 | | 12.1 | | 7.7 | |
| Untreated | 20 | 29–41 °C, 36–40% | | 108.2 | – | 119.8 | – | 114.6 | – |
| *Dongying in Shandong province, July 2004 (4 blocks)* | | | | | | | | | |
| 2–4 instar nymphs | 93 | 34–41 °C, 31–36% | 40–60%, 0.4–0.6 m | 17.4 | 80.9 ± 1.9 | 13.1 | 87.7 ± 1.2 | 9.4 | 91.2 ± 0.7 |
| 2–4 instar nymphs | 85 | 34–40 °C, 31–35% | | 21.1 | | 15.1 | | 8.4 | |
| 2–4 instar nymphs | 94 | 34–40 °C, 32–40% | | 15.9 | | 10.7 | | 6.2 | |
| 3–5 instar nymphs | 131 | 34–40 °C, 32–38% | | 19.1 | | 11.3 | | 9.3 | |
| Untreated | 20 | 34–41 °C, 36–40% | | 96.4 | – | 102.6 | – | 98.1 | – |
| *Hancheng county in Shanxi province, July 2004 (3 blocks)* | | | | | | | | | |
| 2–5 instar nymphs | 97 | 35–41 °C, 31–36% | 50–70%, 0.3–0.6 m | 25.3 | 75.1 ± 2.6 | 16.1 | 81.6 ± 2.1 | 9.1 | 90.0 ± 1.4 |
| 3–5 instar nymphs | 107 | 35–41 °C, 31–36% | | 27.1 | | 21.3 | | 11.1 | |
| 2–4 instar nymphs | 86 | 34–40 °C, 31–36% | | 19.7 | | 14.7 | | 14.5 | |
| Untreated | 20 | 35–41 °C, 36–40% | | 119.1 | – | 112.3 | – | 102.4 | – |

[a] There was rain 4–5 days after treatment.

difference in mortalities between high and medium doses in each trial. The mortalities, at doses of $5.0 \times 10^{12}$ and $3.3 \times 10^{12}$ conidia $ha^{-1}$, were significantly higher than that at the lowest dose ($2.5 \times 10^{12}$ conidia $ha^{-1}$), in which mortality was lower than 80%, at 9–13 days after treatment. The dead locusts collected from the field, which were kept at 26 °C and moisture for 7–10 days, produced hypha and conidia of *M. anisopliae*.

Although time to >90% in the field cages was shortened by 1–2 days, the results from the field cages were consistent with those in the field. The mortality of locusts in field cages and field did not start till 5–7 days after treatment, but gathered pace rapidly. These results showed that the efficacy of *M. anisopliae* in oil miscible suspension formulation against the oriental migratory locust in field cages can reflect that in the field.

### 3.2. The field large-scale trials by ground spray

At a dose of $3.3 \times 10^{12}$ conidia $ha^{-1}$, there was <10% survival of locusts 11–15 days post-application in treated areas, where there were high mean temperatures, low relative humidity, 20–60% ground cover rate and 0.1–0.6 m tall vegetation, sited in Mengzhou county in Henan province, Dagang in Tianjin, Pingshan county in Hebei province, Dongying in Shandong province and Hancheng county in Shanxi province at 11–15 days after treatment (Table 2). However the number of locusts in some control plots actually increased. Results of the ground spray trial in the field also showed that the efficacy of *M. anisopliae* against *L. manilensis* was affected by ground cover rate, height of vegetation and weather conditions. The efficacy of 90% was delayed 3–5 days where vegetation is denser and taller in our trials. Optimum (⩾90%) efficacy occurred 15 days after treatment in Hancheng Shanxi province and Dongying Shandong province where there was 40–60% ground cover and vegetation was 0.3–0.6 m tall, and 11 days after treatment in Mengzhou county in Henan province and Dagang in Tianjin where there was 20–40% ground cover and vegetation was 0.1–0.3 m tall. However, due to low mean temperature and high relative humidity in Pingshan county in Hebei province where the ground cover rate and

vegetation height was similar to Hancheng county in Shanxi province and Dongying Shandong province, over 90% mortality was achieved at 13 days after treatment. This indicated that temperature and relative humidity were two important factors affecting efficacy.

### 3.3. The aerial spray trial

The results of treating locusts during 2005–2006 by aerial spray *M. anisopliae* at a dose of $3.3 \times 10^{12}$ conidia $ha^{-1}$ are shown in Fig. 2. New nymphs were discovered in some control and treated plots at 1–4 days after treatment. As a result, the mean numbers of locusts in control blocks increased about 20%. However a slow but prolonged reduction in locusts was observed in the *M. anisopliae* plots. Mean percent survival declined slowly 1–6 days after treatment followed by a rapid decline 7–13 days and the final percent survivals was $<10\%$ 14–15 days after treatment. This indicated that the locusts could be indirectly infected by *M. anisopliae* isolate CQMa102 in oil miscible suspension formulation on the vegetation and soil at 1–4 days after treatment.

The achievement of a $<10\%$ survival in the field was delayed 2–4 days in comparison with that in field cages, but the rate of mortality was comparable. This also showed that the efficacy in the field could be indicated by that in corresponding field cages.

Treatment with malathion caused a dramatic decline in population density within 1 day of spraying. However, in these plots recolonization was relatively swift, and from 12 days after spraying onwards, there was no significant difference ($F = 7.4$; df = 1, 8; $P = 0.37$) in locust percent survival between malathion-treated and *M. anisopliae*-treated plots in field.

### 3.4. Effect on enemies of locusts

During the aerial spray experiments, three types of locust natural enemies were observed, including: ants (Hymenoptera: Formicidae), walking beetles (Coleoptera: Carabidae) and wolf spiders (Araneida: Lycosidae) in the chemical-treated, *Metarhizium*-treated and untreated blocks (Table 3). Populations of the natural enemies in *Metarhizium*-treated and untreated blocks increased constantly after trials and there was no significant difference between treatments. This showed that *M. anisopliae* in oil miscible suspension formulation did not poison natural enemies and contaminate environment. However these natural enemies in malathion-treated blocks rapidly declined over 90% 1–15 days after treatment.

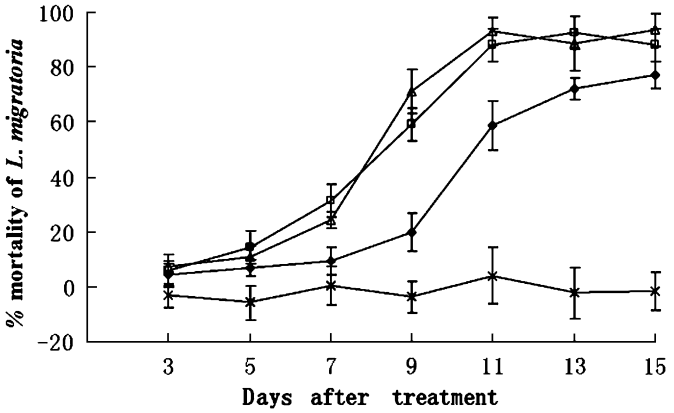


**Fig. 1.** Mean percentage mortality (±S.E.) of the oriental migratory locust treated with various doses of *M. anisopliae* in the field during June 2002–2003. (△), At a dose of $5.0 \times 10^{12}$ conidia $ha^{-1}$ (13 plots); (□) at a dose of $3.3 \times 10^{12}$ conidia $ha^{-1}$ (10 plots); (◆), four plots at a dose of $2.5 \times 10^{12}$ conidia $ha^{-1}$ (10 plots) ; (×), untreated (8 plots).

## 4. Discussion

In field trails, our results showed that there was high mortality of the oriental migratory locusts at a dose of $3.3 \times 10^{12}$ conidia of *M. anisopliae* (isolate CQMa102) per hectare in a wide variety of locust habitats in China. The dose of $3.3 \times 10^{12}$ conidia/ha is lower than the dose of $5 \times 10^{12}$ conidia/ha of the African isolate applied to grasshoppers in northwest China (Li et al., 2000). Although the Australian isolate gave high mortality to the oriental migratory locusts at the dose of $2.0 \times 10^{12}$ conidia/ha in China (Zhang and Hunter, 2005), these experiments were conduced in laboratory and small field trials using backup ULV spray equipment in few sites. At dose of $3.3 \times 10^{12}$ conidia/ha, the *M. anisopliae* var. *acridum* (strain CQMa102) in oil miscible suspension formulated is competitive with some insecticide in price.

Because plot size was small and distance moved by locust nymphs was longer in some trial blocks due to environmental differences, the field efficacy of the dose experiments against locusts in those trial plots was not assessed. This showed that

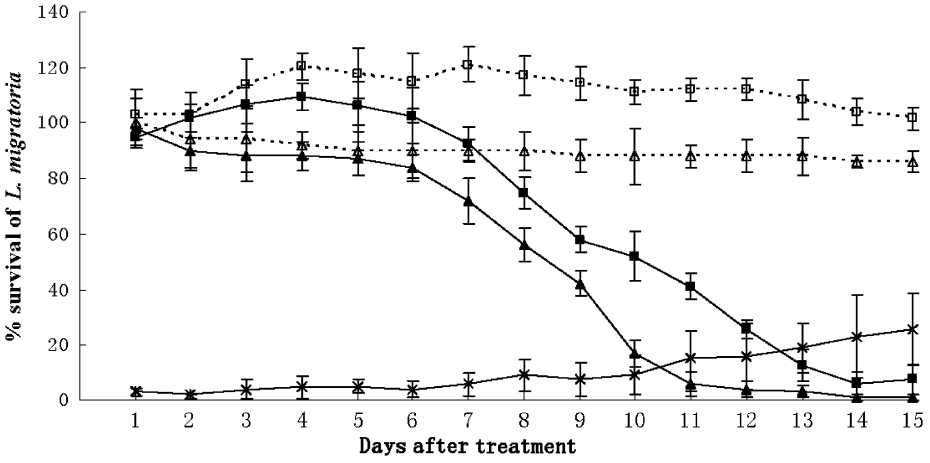


**Fig. 2.** Mean percentage mortality (±S.E.) of locust by aerial spraying *M. anisopliae* at a dose of $3.3 \times 10^{12}$ conidia $ha^{-1}$ during 2005–2006 (daytime mean temperatures 34–37 °C). Data are from eight treatment bands (■); eight untreated bands (□); eight treatment cages (▲); eight untreated cages (△) and eight chemical pesticide bands (*).

**Table 3**
Percent change of locust natural enemies after treatment by aerial spay during 2005– 2006

| Locust natural enemies | | Percent change (mean±S.E.) of natural enemies over time (days), after treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | | 8 | | 15 | |
| Ants | Malathion-treated | −86.2±1.3 | a | −83.4±1.45 | a | −80.9±1.9 | a |
| | *Metarhizium*-treated | +3.2±2.1 | b | +12.8±1.45 | b | +21.5±2.9 | b |
| | Untreated | +4.8±1.3 | b | +14.7±3.9 | b | +17.6±4.6 | b |
| Walking beetle | Malathion-treated | −98.8±0.8 | a | −96.5±1.8 | a | −94.8±1.5 | a |
| | *Metarhizium*-treated | −5.1±1.4 | b | −11.6±2.8 | b | −17.5±3.9 | b |
| | Untreated | −6.4±1.52 | b | −14.8±3.1 | b | −28.7±4.1 | b |
| Wolf spider | Malathion-treated | −95.8±1.8 | a | −91.5±2.8 | a | −90.8±2.4 | a |
| | *Metarhizium*-treated | +5.0±2.7 | b | −4.0±3.1 | b | −10.00±4.8 | b |
| | Untreated | +3.5±1.8 | b | −5.6±2.3 | b | −13.67±2.3 | b |

Mean mortalities followed by different letters in the same column were significantly different ($P<0.05$) according to ANOVA and Tukey's multiple range test.

demonstration of field efficacy against locusts is difficult, confirming previous results (Langewald et al., 1997; Price et al., 1997). This problem was solved by treating relatively large areas (see also Peveling et al., 1999) and setting cages in the field (Hunter et al., 1999). Otherwise, these experiments showed that the field efficacy against locusts could be assessed by selecting trial areas with higher vegetation cover rate, lower maximum air temperature and lower locust population densities. Thus, our results indicated that the efficacy of *M. anisopliae* against oriental migratory locusts in field cages can reflect the efficacy in the field.

The density and height of vegetation affected the efficacy of *M. anisopliae* against locusts. The conidia of *M. anisopliae* var. *acridum* infected the locusts by landing directly on the surface of locust after spraying and by secondary pickup from the vegetation for some days after application (as reported also by Thomas et al., 1997; Hunter et al., 2001). In the dense vegetation blocks, more locusts were infected by *Metarhizium* conidia on vegetation surface. Death of these locusts infected by conidia on the vegetation was deferred accordingly. In addition, the decline of the infectivity of *Metarhizium* conidia on vegetation surface also decreased efficacy (Hunter et al., 2001). The same result was observed in our aircraft spray test bands. Although the number of locusts was increased about 20% due to further hatching 1–4 days after treatment, the percent survival of the locusts in both field and field cages declined gradually and finally efficacy reached >90%. The high final efficacy in dense vegetation when new locusts hatched out shows that conidia of *Metarhizium* CQMa102 strain in oil miscible suspension formulation remained highly infective for at least 4 days in the vegetation. The same result was obtained in *Metarhizium* FI-985 strain by Hunter et al. (1999, 2001).

In the field trial using aircraft spray, achievement of <10% survival of treated locusts was delayed 2–4 days compared with that in field cages. The main reason was new locusts hatched out at 1–4 days after treatment. In addition, the >90% mortality of the locusts in field was only 1–2 days later than in corresponding field cages in dose trial plots where no new locusts hatched out. There was another explanation for more rapid mortality of caged locusts. Locusts in cages were likely exposed to reduced levels of solar radiation, which might have translated to lower body temperatures and, thus, more rapid fungal development (Inglis et al., 1996; Blanford and Thomas 2000a; Blanford et al., 2000b).

Weather conditions were an important factor restricting the efficacy of *M. anisopliae*. Development of *M. anisopliae* is most rapid at 25–35 °C (Milner et al., 1997). In most of the day during trials, the temperature on the ground surface was above 35 °C from late morning to late afternoon. Result showed that the conidia of *M. anisopliae* var. *acridum* strain CQMa102 in oil miscible suspension formulation remains highly infective against the oriental migratory locust at high temperatures. In addition, the oil miscible suspension formulation, which become w/o emulsified mixture and remained similar characteristic with oil formulation, reduced the dependence of germination of the conidia of *M. anisopliae* var. *acridum* on high humidity.

Previous studies have shown that *M. anisopliae* var. *acridum* has low toxicity to the Hymenoptera, Coleoptera, Homoptera and epigeal arthropods (Ball et al., 1994; Peveling and Demba, 1997; Prior, 1997; Arthurs et al., 2003). Likewise, *M. anisopliae* var. *acridum* strain CQMa102 did not infect the Hymenoptera, Coleoptera and Araneida natural enemies of locusts. This demonstrated that *M. anisopliae* var. *acridum* was safe for non-targets.

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. *Metarhizium* CQMa102 strain in oil miscible suspension formulation at moderate dose resulted in high mortality in 11–15 days in large-scale field trials and did not reduce the number of natural enemies. When mixed with vegetable oils (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. The preliminary results showed that the >85% germination rate was maintained over a year for conidia stored in soybean oil at 25 °C. These results show that *Metarhizium* CQMa102 strain, in oil miscible suspension formulation at moderate dose, has the capability of being a commercial product as a significant part of a program of IPM of locusts in China.

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