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Authors: Hernández-Velázquez, Víctor M., Hunter, David M., Barrientos-lozano, Ludivina, Lezama-Gutiérrez, Roberto, and Reyes-Villanueva, Filiberto

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Susceptibility of *Schistocerca piceifrons* (Orthoptera: Acrididae) to *Metarhizium anisopliae* var. *acridum* (Deuteromycotina: Hyphomycetes): laboratory and field trials

VÍCTOR M. HERNÁNDEZ-VELÁZQUEZ, DAVID M. HUNTER, LUDIVINA BARRIENTOS-LOZANO, ROBERTO LEZAMA-GUTIÉRREZ AND FILIBERTO REYES-VILLANUEVA

(VMH-V) Centro Nacional de Referencia de Control Biológico. SAGARPA-SENASICA-DGSV. Km 1.5, Carretera Tecomán-Estación de FFCC, C. P. 28120, Tecomán, Colima, México. Email: jdepto@volcan.ucol.mx

(DMH) Australian Plague Locust Commission. Department of Agriculture, Fisheries and Forestry. GPO Box 858, Canberra ACT 2601, Australia. E-mail: david.hunter@affa.gov.au

(LB-L) Instituto Tecnológico de Cd. Victoria, Boulevard E. Portes Gil No. 1301, Cd. Victoria, Tamaulipas, México. E-mail: ludibinab@prodigy.net.mx

(RL-G) Facultad de Ciencias Biológicas y Agropecuarias. Universidad de Colima, Apartado Postal 36, C. P. 28100, Tecomán, Colima, México. Email: rlezama@tecoman.ucol.mx

(FR-V) Facultad de Ciencias Biológicas. Universidad Autónoma de Nuevo León. Laboratorio de Entomología Médica. Apdo. Postal 109-F, 66450, San Nicolás de los Garza, N.L. Email: freyesv@rt.nl

Abstract

The entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* Metsch. Sor. (Deuteromycotina: Hyphomycetes), was tested under laboratory and field conditions against the Central American locust *Schistocerca piceifrons* Walker (Orthoptera: Acrididae). Adults of *S. piceifrons* treated under laboratory conditions with the isolate MaPL40, 1.5×10^3 conidia/insect, showed 97% mortality 12 d after inoculation with a conidia mineral oil formulation and 59% mortality within the same period, when treated with a conidia-water suspension. Field trials against bands of *S. piceifrons* were undertaken with the isolates MaPL32 and MaPL40. Formulations of both isolates were applied as ULV at a dose of 50g conidia (2.5×10^{12})/litre citrolina oil/ha. Bands were followed for 13 d after treatment in treated and untreated blocks, to assess decline every other day. Bands that remained in the treated area for the full period showed 99% decline when treated with the MaPL32 isolate, while those treated with the MaPL40 isolate declined 85% within the same period. Bands that left the treated area a few days after treatment declined 94% by day 13 when treated with the MaPL32 isolate, and 84% when treated with the isolate MaPL40. Results suggest that *M. anisopliae* var. *acridum* may substantially reduce nymphal populations of *S. piceifrons* under field conditions, though further studies are necessary for the operational use of *M. anisopliae* var. *acridum* as part of a locust integrated management program in Mexico.

Key words

Metarhizium anisopliae var. *acridum*, mycoinsecticide, biological control, locust, *Schistocerca piceifrons*, México

Introduction

Control of locusts and grasshoppers has traditionally relied on synthetic insecticides. At present there is a growing awareness of the environmental issues associated with acridid control that are leading to an increased demand for biological methods (Lomer *et al.* 2001).

The Central American Locust, *Schistocerca piceifrons* (Walker 1870) (Orthoptera: Acrididae), often becomes a major pest of basic and industrial crops in Mexico and Central America. In 1993, laboratory and field studies were initiated to develop a biological control strategy for locusts and grasshoppers. The National Centre for Biological Control (Centro Nacional de Referencia de Control

Biológico-CNRCB) has in its entomopathogenic fungi collection 35 isolates of *Metarhizium* spp. (*M. anisopliae* and *M. anisopliae* var. *acridum*) (Deuteromycotina: Hyphomycetes), obtained from *S. piceifrons*. Our laboratory studies have shown that the isolates MaPL32 and MaPL40 are two of the most virulent against *S. piceifrons* (Barrientos *et al.* 2002).

The recent development of effective oil formulations of *Metarhizium* spores in Africa, Australia and Brazil opens new possibilities for environmentally safe control operations (Lomer *et al.* 2001). The mycoinsecticide *M. anisopliae* var. *acridum*, based on an oil-formulation of the fungal pathogen, acts through direct contact and can be applied using the standard equipment and procedures already used for application of chemical insecticides (Bateman 1997). Ultra Low Volume (ULV) application has allowed the use of a lower dose of insecticides and enables fungi to be used at very low levels of relative humidity (Prior *et al.* 1988, Bateman *et al.* 1993). Seyoum *et al.* (1994) report that adults of *S. gregaria* Forskål infected with *M. anisopliae* var. *acridum* had reduced flight capability. Hunter *et al.* (2001) demonstrated that ULV oil conidial formulations of *M. anisopliae* var. *acridum* could suppress populations of the Australian Plague Locust *Chortoicetes terminifera* (Walker). Under field conditions, mortality evaluation presents difficulties because substantial movement of locusts allows the mixing of treated and untreated individuals (Hunter *et al.* 2001). By following hopper bands in the field, declines have been demonstrated for *Rhammatocerus schistocercoides* (Rehn) in Brazil (Magalhães *et al.* 2000, Faria *et al.* 2002), *Schistocerca gregaria* (Forskål) in Africa (Langewald *et al.* 1997), *Locusta migratoria* and *Chortoicetes terminifera* in Australia (Hunter *et al.* 1999, 2001). The present work evaluates the effect of oil and water formulations of the fungus *M. anisopliae* var. *acridum* on the mortality of *S. piceifrons* in the laboratory and under field conditions.

Materials and methods

Laboratory trials

Locust.— First instar nymphs of *S. piceifrons* were collected in Tizimin, Yucatán, Mexico. Locusts were kept in 1 × 1 × 1 m-cages inside a controlled environment (CE) room and fed on a diet of maize leaves and rolled oats. The relative humidity in the CE room was 70±5%; the average temperature was 27°C with a photoperiod of 12:12 h (L:D).

Fungus.— *M. anisopliae* var. *acidum* was isolated from *S. piceifrons* from Socorro Island, Colima, Mexico (Hernández *et al.* 1997). This isolate (MaPL40) is the most virulent of several tested against *S. piceifrons* (Hernández & Berlanga unpub. data). A second isolate, MaPL32, was obtained from *S. piceifrons* collected from Colima, México and used in the field because it had been shown to develop at higher temperatures, possibly important in a tropical area like the Yucatán Península (Barrientos & Milner 2000).

Fungus culture.— Conidia of the fungus were produced using a diphasic method. In the first phase, the fungus was cultured in a liquid medium based on sucrose and yeast extract (Jenkins & Prior 1993). Fungal biomass was transferred to rice in a plastic bag (250 g/bag) containing a cotton filter for further growth and sporulation. After 14 d the bags were opened in the CE room and the fungus allowed to dry to approximately 50% moisture content. Conidia were separated from the rice by sieving through a 300µ mesh. The resulting conidia powder was then resieved through a 90µ-mesh to remove any remaining rice-dust particles.

Bioassay.— Two formulations of *M. anisopliae* var. *acidum* isolate MaPL40 were used: a) conidial suspension in citrolina (a mineral oil derived from petroleum for agricultural use in México), and b) aqueous suspension (a mixture of conidia plus water and dispersant Agral Plus). Control treatments consisted of either citrolina or water plus dispersant without conidia. Formulations were prepared by mixing 1 g of conidial powder in 100 ml of either citrolina or water plus dispersant. After agitation, the suspensions were adjusted to 1.5 × 10⁷ conidia/ml. Concentrations of conidia were estimated using a hemocytometer. Each treatment (conidia and citrolina or conidia and water) consisted of 20 adult locusts, 10 d postfledging (Prior *et al.* 1995) receiving 2µl of the formulation (1.5 × 10³ conidia/insect) applied to the pronotum via a SGE Hamilton syringe (Fargues *et al.* 1997). Control groups received 2 µl of either citrolina or water plus dispersant without conidia. Following inoculation, locusts were placed in subgroups of 5 insects in plastic containers 13 cm long, 10.5 cm in diameter and covered with muslin. Containers were maintained at 28°C and 70±5% RH. Insects were fed with maize leaves changed every 3 d. Each treatment was replicated 4 times.

Mortality was recorded daily for 12 d; dead insects were placed individually in Petri dishes on moistened filter paper at 25°C for 4 d to allow growth of surface mycelia. Mortality data were transformed using angular transformation and subjected to analysis of variance (ANOVA). Means were separated by the Tukey test ($P < 0.05$) (SAS Institute 1988).

Field trials

Fungus isolates.— The *M. anisopliae* var. *acidum* isolates MaPL40 and MaPL32 were used for field trials. Isolates were produced in diphasic culture, and formulated conidia were refrigerated at 6°C until used. Germination tests were carried out before application (Stathers *et al.* 1993); germination levels were 80 and 93% for the MaPL40 and MaPL32 isolates, respectively.

Field sites.— An infestation of early instar bands of *S. piceifrons* was located at Rancho San Isidro, 200 km east of Mérida near Tizimín, Yucatán, Mexico. A 5-ha block in open grassland with few trees was treated with the MaPL40 isolate. A second 12-ha block with many short trees was treated with the isolate MaPL32. The week following the application was mainly sunny in the morning but it clouded over by midday with nearly full cloud and scattered showers most afternoons. The second week was mainly sunny. Minimum temperatures °C were in the low 20's and maxima in the mid 30's.

Fungus application.— Dry conidial powder mixed with citrolina oil was applied at a rate of 50 g/l (about 2.5 × 10¹² conidia/ha). Both areas were treated with Knapsack sprayers at 8-m intervals at a nominal rate of 1 l/ha. There was a crosswind of 2 to 5 m/s during application and temperatures were 25 to 32°C.

Evaluation of mortality.— Mortality data were assessed by following the bands in the field. Each band was marked and its length and depth measured by pacing (1 pace = 0.7 m), which allowed the area of the band to be calculated (Hunter *et al.* 1999). Every 1 to 2 d each band was marked and its area and the distance it moved measured. The total area of band remaining at intervals after treatment was used as a measure of efficacy.

Results

Laboratory bioassay.— For locusts treated with conidia suspended in oil, mortality began by day 6, reaching 97% by day 12 (Fig. 1). Mortality caused by conidia formulated in water was significantly less, 52.5% (Fig. 1, Table 1).

Field trials.— Before the application of *M. anisopliae* var. *acidum*, the bands of *S. piceifrons* were dense and visible from a distance of 10 to 15 m, particularly in the morning when the nymphs rested in the upper part of the grass. While the untreated bands remained dense and visible during the experiment, the treated bands were not visible after about day 6, when they began to disintegrate. About

Table 1. Accumulated percent mortality of adults 12 d after being inoculated with *M. anisopliae* var. *acidum* isolate MaPL40, in the laboratory.

Treatments	n	% Mortality (SD)	*
Conidia + citrolina	80	97.50 (± 2.50)	A
Conidia + water + dispersant	80	52.50 (± 4.78)	B
Citrolina + dispersant	80	13.75 (± 3.75)	C
Water + dispersant	80	5.00 (± 2.04)	C

* Those with a different letter are significantly different for Tukey test ($P < 0.01$, ANOVA C.V. = 17.81)

Fig 1. Accumulated percent mortality of adult *S. piceifrons* treated with conidia of *M. anisopliae* var. *acridum* MaPL40 isolate, suspended in mineral oil or water in the laboratory.

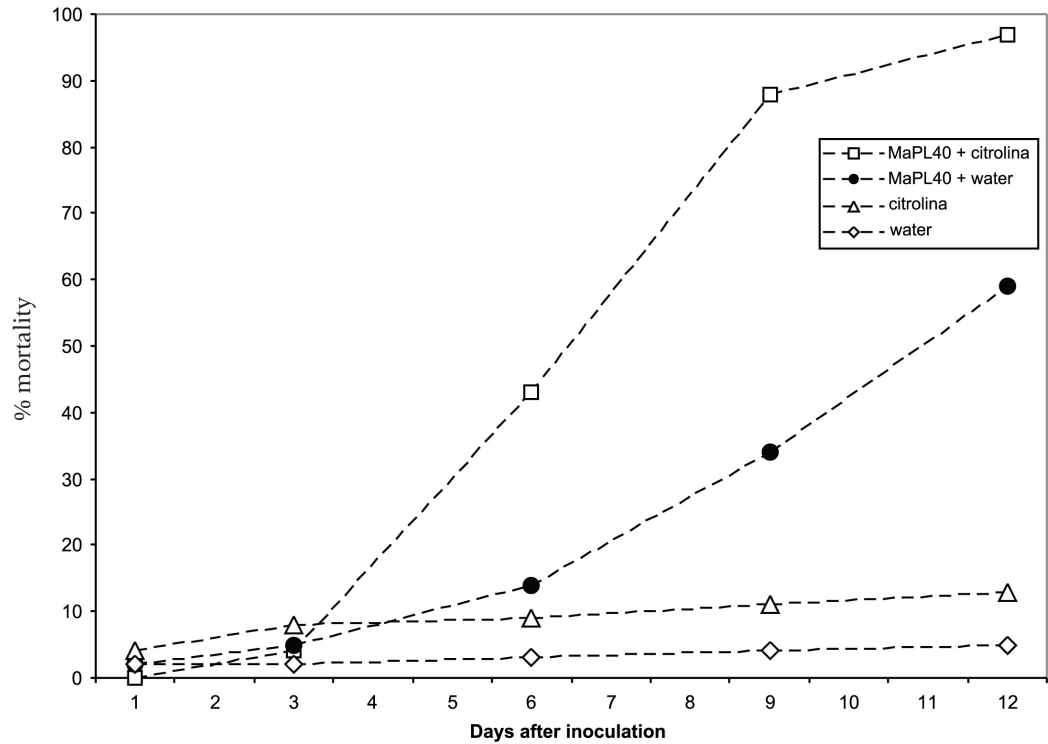
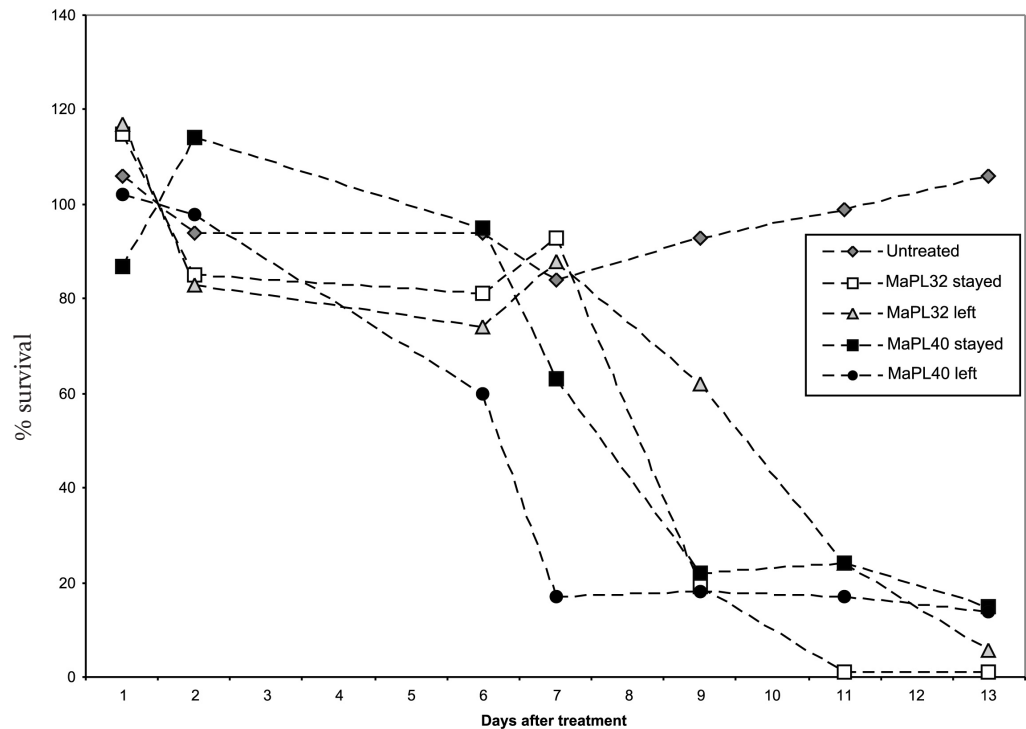


Fig. 2. Survival of early instar nymphs of *S. piceifrons* after ULV ground application of isolates MaPL32 and MaPL40 of *M. anisopliae* var. *acridum*. Comparisons were made of survival of bands that remained in the treated area with those that left.



the same time, their displacement decreased: treated bands moved 14 ± 2 m/d the first 4 d, declining to 10 ± 1 , 6 ± 1 and 4 ± 2 m/d by 6 to 7, 9 and 11 d after treatment, respectively.

The dispersal and reduction in movement of the treated bands was associated with a rapid decrease in band size. The bands that remained in the treated area diminished 84% by 13 d after the application of MaPL40 and more than 95% after application of

MaPL32 (Fig. 2). Bands that left the treated area within 2 to 3 days after treatment, declined 84% by day 13 when treated with the MaPL40 isolate and 94% when treated with the isolate MaPL32 (Fig. 2).

Discussion

Mortality of *S. piceifrons* was high when treated with *M. anisopliae* var. *acidum*, either in the field or the laboratory. The speed of mortality is related to the dose of *Metarhizium* (Moore *et al.* 1992, Prior *et al.* 1995, Bateman *et al.* 1996) but instead of applying the very high dose of 750,000 conidia/insect that is used to select highly virulent isolates (Prior *et al.* 1995), we used a moderate dose that extends mortality for 5 to 9 d (Moore *et al.* 1992) and more clearly determines any differences in mortality between strains or formulations. The higher, or at least more rapid, mortality in the laboratory when conidia were formulated in oil meant that oil formulations were used in the field in México, as they have been in Africa (Kooyman & Godonou 1997, Langewald *et al.* 1997), Brazil (Magalhães *et al.* 2000, Faria *et al.* 2002) and Australia (Hunter *et al.* 1999, 2001). In the field, mortality was high with both isolates, indicating that they are able to control *S. piceifrons*. The lesser control of bands that left the treated area soon after treatment is consistent with locusts normally acquiring a dose both directly and from the vegetation (Hunter *et al.* 2001). During the hot weather with afternoon cloud and some showers, conditions would have been ideal (warm temperatures and high humidity) for rapid development of the fungus. The resulting mortality of *S. piceifrons* beginning 6 to 9 d after treatment is very similar to that reported with *S. gregaria* (Kooyman & Godonou 1997) and *Locusta migratoria* (Hunter *et al.* 1999) under similar conditions. Though both isolates seem promising, further studies are necessary with lower doses and under a variety of environmental conditions, as part of moving towards the operational use of *M. anisopliae* var. *acidum* against *S. piceifrons*.

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