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Advances in biological control of locusts and grasshoppers in Mexico

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Abstract

A project to develop a biological control strategy for locusts and grasshoppers is being conducted in Mexico. Major activities include: surveys of entomopathogenic fungi, laboratory screening of isolates, testing of methods for mass production, and formulation and field evaluation of virulent strains.

The Centro Nacional de Referencia de Control Biológico (CNRCB) has, in its entomopathogen collection, 40 isolates of *Metarhizium* spp. obtained from the Central American locust *(Schistocerca piceifrons piceifrons*). The isolates MaaPL16, MaaPL25 and MaPL40 are among the most virulent.

A comparative analysis on Random Amplified Polymorphic DNA patterns between two Mexican isolates of *Metarhizium*, MaPL40 and MaPL32, and an Australian isolate of *Metarhizum anisopliae var. acridum* (FI-985), showed that the Mexican isolates and the Australian isolate have similar DNA fingerprints, suggesting they may belong to the same variety.

Field trials using oil formulations of the MaPL40 and MaPL32 isolates against hopper bands of *S. piceifrons piceifrons*, applied at a rate of 50g of conidia in oil, 1 l ha^{-1}, provided >90% reduction in the hopper bands 10 d after treatment. Comparative studies between the Mexican and the Australian isolates were conducted over a range of temperatures: results are reported in detail.

Key words

Schistocerca piceifrons, biological control, *Metarhizium*, entomopathogen

Introduction

Locusts and grasshoppers are major pests of agriculture in Mexico. The Central American locust, *Schistocerca piceifrons piecifrons* Walker,, is the only true locust capable of forming dense bands and large adult swarms that migrate long distances (Barrientos *et al*. 1992). This species is widely distributed from northern Mexico to Costa Rica, and at least 50,000 ha are treated every year to protect crops and pasture from it. Chemical insecticides are the main control products. It is estimated that the cost of control, including chemicals and application costs, is over US$500,000 per y. Other locusts such as *Schistocerca pallens* (Thunberg), *Schistocerca nitens* (Thunberg) or *Rhammatocerus viatorious* (Saussure) cause less damage and are considered secondary pests. Two species of grasshoppers are of major concern: *Boopedon nubilum* Say and *Mermiria bivittata* Serville. These species have become more serious in recent years with populations of up to 70 to 80 nymphs-adults per m^2 being recorded .

In 1993, laboratory and field studies were initiated to develop a biological control strategy for locusts and grasshoppers. Major activities of this program include: a Mexico-wide survey of entomopathogenic fungi, laboratory screening of isolates to identify suitably virulent strains, testing methods for mass production of the virulent strains, and formulation and field evaluation of these strains. The Centro Nacional de Referencia de Control Biológico (CNRCB), El Instituto Tecnológico de Cd. Victoria, in collaboration with the Australian Plague Locust Commission (APLC) and Commonwealth Scientific and Industrial Research Organization (CSIRO) Australia, and El Colegio de Posgraduados, among others, have contributed with research work aiming to develop a biopesticide and to establish the use of *Metarhizium* sp. as part of an Integrated Control Programme. At present *M.anisopliae var. acridum* is being produced and formulated on a small scale for field trials on locust and grasshoppers. The initial results have proved very promising and more extensive field trials with isolates MaPL32 and MaPL40 are planned from October 2001 onwards.

Local surveying of entomopathogenic fungi and laboratory screening of isolates.— The CNRCB coordinates surveying of entomopathogenic fungi. At present it has in its entomopathogen collection 40 isolates of *Metarhizium* spp. (*M. anisopliae*, *M. anisopliae var. acridum* and *M. flavoviride*) (Table 1), obtained from the Central American locust (*S. piceifrons piceifrons*) in the States of Colima, Michoacán, Chiapas and Revillagigedo Island of the Pacific Coast (Hernández Velázquez *et al.*1997, 2000, 2001). In the State of Tamaulipas, northern Mexico, other fungi such as *Beauveria bassiana* and *Entomophaga* spp. have been obtained from locusts and grasshoppers. In 1998, a major epizootic of *Entomophaga* spp. controlled dense infestations of *M. bivittata* and *B. nubilum* in South Tamaulipas (Barrientos 1998).

Pathogenicity and virulence of *B. bassiana*, *Paecilomyces* spp. and *Metarhizium* spp. isolates have been evaluated under laboratory conditions. *Metarhizium* isolates showed the highest virulence: mortality begins by day 3 after treatment, reaching >90% by day 8 (Fig. 1). Virulence and pathogenicity were assessed by considering medium lethal times (MLT) which varied from 5.0 to 7.0 d for *Metarhizium* spp. (Table 2). The isolates MaaPL16, MaaPL25 and MaPL40 are among the most virulent, with medium lethal times (MLTs) of 5.5, 5.6 and 5.0 d, respectively. The insects were inoculated with 2 µl (1 x 10^8) conidia ml^{-1} and maintained at 27± 1°C.

Comparative studies on Mexican strains of M.anisopliae and an Australian isolate of M. anisopliae var. acridum.— A series of comparative studies on two Mexican strains of *M. anisopliae* (MaPL40 and MaPL32) and an Australian isolate of *M. anisopliae var. acridum* (FI-985) were conducted in the laboratory. These included morphology and rate of growth, Random Amplified Polymorphic DNA patterns, effect of dose and temperature on virulence of the isolates over a range of temperatures and susceptibility of a grasshopper species to different doses of the isolates (Milner *et al*. 2001).

Morphology, rate of growth and RAPDs.— The morphology of the strains indicates that FI-985 has larger conidia than the two Mexican isolates, which showed only small differences from each other in conidial size and colony morphology (Table 3). RAPD patterns were similar to the Australian isolate (Driver *et al.* 2000; Barrientos & Milner 2000a,b; Milner *et al.* 2001) suggesting they belong to the same variety. The three isolates had similar growth profiles on agar plates, though MaPL40 did not grow at 36°C; however the other two isolates grew slowly at this temperature.

The effect of dose and temperature on virulence and susceptibility of wingless grasshoppers to the three isolates.— Two bioassays were undertaken with field-collected 5th instars and adults of *P. vittatum*; the grasshoppers were inoculated on the mouth parts with 0.2 µl of the oil-formulated conidia. In a first bioassay, three doses per isolate (100,000; 10,000 and 1000 spores per insect) plus a control were compared at five constant temperatures: 15, 20, 25, 30, 35°C. In a second bioassay, five doses were studied per isolate (48,000; 9,600; 2000; 380; 80 spores per insect) at 30°C ± 1°C and probit analysis used to compute the LD_{50} values.

The results of the first bioassay are shown in Table 4. Temperature was a key factor: all 3 isolates were highly virulent at 30°C, with a 100% mortality at high and intermediate doses 5 to 6 d after treatment, while low dosage caused 100% mortality 9 to 10 d after inoculation. At 17 d after treatment, MaPL32 gave >90% mortality at temperatures of 20 to 35°C, but mortality was much reduced at 15°C; MaPL40 gave high mortalities between 20 and 30°C, but mortality was much lower at the extremes of the temperature range, 15 and 35°C. The Australian isolate FI-985 gave >90% mortality at 20 to 35°C and 50% mortality at 15°C, while isolates MaPL40 and MaPL32 at 15°C gave 30 and 20% mortality respectively.

In the second bioassay the LD_{50} for MaPL32 was 248 spores per insect, while the LD_{50} for isolates MaPL40 and FI-985 was 410 and 501 spores per insect, respectively (Table 5). These differences between the isolates were not significant ($p > 0.05$).

Formulation and field trials.— The two Mexican isolates have been formulated in mineral and vegetable oils as well as aqueous suspension. Citroline mineral oil (a derivative of petroleum) provided the highest viability of *M. anisopliae* conidia following storage for 40 d at 7 and 27°C. This formulation caused a 100% mortality 7 d after inoculation with 2 µl (1 X 10^6) conidia ml^{-1} when incubated at 25 to 30°C (Hernández-Velázquez *et al*. 2000).

During September 2000, about forty 3rd and 4th instar bands of *S. piceifrons piceifrons* near Tizimin, Yucatán, were treated with MaPL40 and MaPL32 (Hernández-Velázquez *et al*. 2001). Experimental sites were chosen near the edge of the infestation to minimize the invasion of untreated bands. A control plot, untreated, was chosen at approximately 50m from the treated area. The application was with knapsack sprayers at a dosage of 50g *Metarhizium* in 1l of citroline oil ha^{-1} and after treatment mortality was assessed by following the bands and estimating the band size and density daily in the field. Weather was usually sunny in the morning, with cloud and showers during most afternoons; temperatures ranged from 20 to 25°C at night to 32 to 35°C during the day.

Bands that remained in the treated area began to decline 6 d after spraying, with the population decline reaching 86% by days 11 to 13 for bands treated with strain MaPL40 and >95% for bands treated with MaPL32 (Fig. 2). Bands that left the treated areas within 2 to 3 d of treatment had less opportunity to pick up *Metarhizium* from the vegetation, but declined by about 80% by 12 d after spraying (Fig. 2).

The rapid decline beginning 6 to 9 d after spraying seen in this field trial, is similar to the rapid decline of *Chortoicetes terminifera* Walker bands treated with *M. anisopliae var. acridum* in Australia during hot weather (Hunter *et al*. 2001). Both *Metarhizium* strains (MaPL40 and MaPL32) worked well, and it is hoped that future trials on a larger scale will lead to the operational use of *Metarhizium* for locust control in Mexico.

Discussion and conclusion

Temperature is a key factor for virulence of *M. anisopliae var. acridum* isolates, with the optimum temperature range being 25 to 30°C. At extreme temperatures of 15°C they were much less virulent (Table 4). However the isolates FI-985 and MaPL32 were very virulent at 35°C. These results agree with those reported by Hunter *et al.* (2001) who indicate that aerial treatment of bands of *C. terminifera* with *M. anisopliae var. acridum* in the spring (temperature maxima 22 to 30°C) reached >90% mortality 14 d after spraying, while bands treated in the summer (maxima 36 to 42°C), declined 7 to 10 d after spraying.

FI-985 appears to be more consistent and perform better at lower temperatures: at 15°C it provided 50% mortality regardless of dose used (Table 4). This may be an advantage under field conditions, since it has been demonstrated that high temperatures (>40°C) and thermoregulation can adversely affect mycosis of grasshoppers by inhibiting the disease (Inglis *et al*. 1996, Jaronsky & Goettel 1997).

Dose is also important for *M. anisopliae var. acridum* activity: low doses are slower to kill, even at the preferred temperatures. At low temperatures (below 20°C) mortality is also slow, even at high doses (this paper, Bateman *et al*. 1996, Hernández-Velázquez *et al*. 2001).

Isolate MaPL32 provided the lowest LD_{50} and performed well at 35°C. Preliminary field trials with this isolate showed satisfactory results, suggesting that it may be promising for locust control in a tropical country like Mexico, though the results also suggest that FI-985 would be very effective. More extensive field trials using the isolates MaPL32 and MaPL40 of *M. anisopliae var. acridum* are planned starting October 2001.

Table 1. Entomopathogenic fungi of locusts (*S. piceifrons piceifrons)* and grasshoppers in the Centro Nacional de Referencia de Control Biológico collection. CNRCB-DGSV-CONASAG-Mexico.

State	Species	Number of isolates
Colima y Michoacan	*Metarhizium anisopliae var. acridum*	31
	M. anisopliae	4
	Beauveria bassiana	8
	Paecilomyces fumosoroseus	6
Isla Socorro	*M. flavoviride*	4
Tamaulipas	*B. bassiana*	1
	Entomophaga sp.	1
Chiapas	*M. anisopliae*	1
	B. bassiana	10

Table 2. Medium lethal time (MLT), variation coefficients (VC) and percentage mortality by day 7 of *M.anisopliae* (Ma) and *M. anisopliae var. acridum* (Maa) isolates on adults of the Central American Locust (*S. piceifrons piceifrons*).

Isolate	MLT	VC	% Mortality Day 7
MaaPL16	5.5	0.2436	92
MaaPL29	6.1	0.1703	91
MaaPL21	6.7	n.d.	62
MaaPL25	5.6	0.1491	87
MaaPL29	5.9	0.1724	81
MaaPL34	5.86	n.d.	75
MaaPL39	7.03	n.d.	50
MaPL35	6.27	0.1980	68
MaPL40	5.0	n.d.	100
control			7

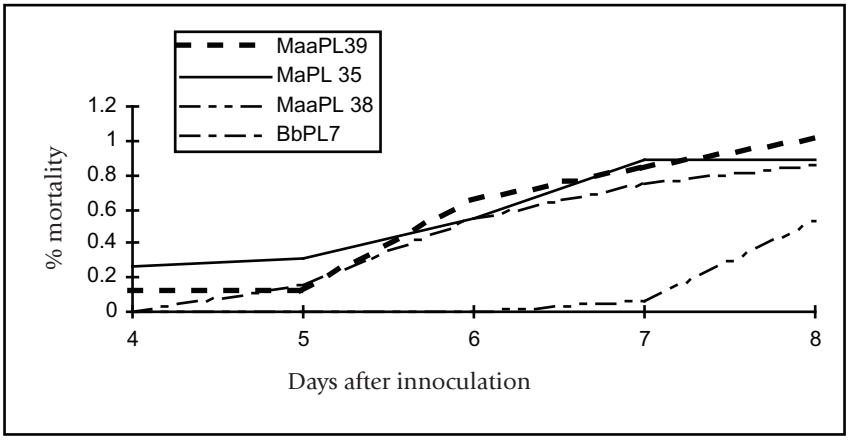

Fig. 1. Cumulated, corrected percentage mortality caused by *M. anisopliae* (Ma), *M. anisopliae var. acridium* (Maa) and *B. bassiana* (Bb) on adults of the Central American Locust (*S. piceifrons piceifrons*).

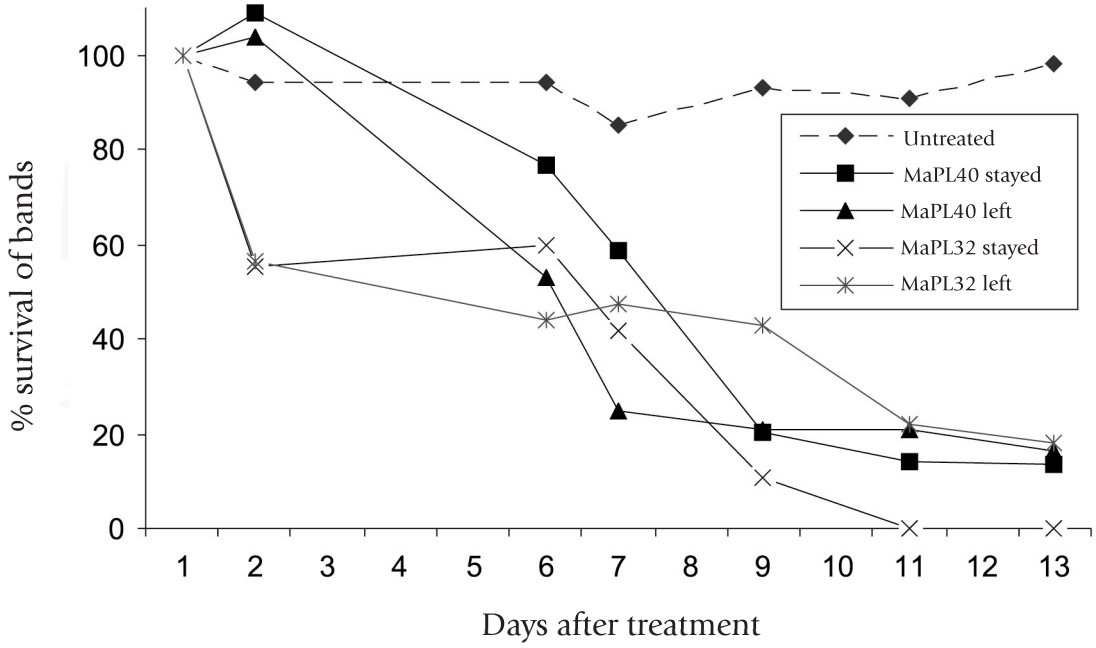

Fig. 2. Percent survival of untreated bands of *S. p. piceifrons* and those treated with *M. anisopliae* strains MaPL40 and MaPL32.

Table 3. Summary of isolates studied and conidial size.

Isolate	Source cost	Host	Source	Date isolate	Conidial size ± $s_{\bar{x}}$
Fl-985	ARSEF 324[1]	*A. guttulosa*	Australia	1979	7.6 ± 0.78 x 2.9 ± 0.37
QF-001	MaPL40[1]	*S. p. piceifrons*	Mexico	1996	4.5 ± 0.51 x 3.2 ± 0.38
QF-002	MaPL32[2]	*S. p. piceifrons*	Mexico	1996	4.9 ± 0.46 x 3.6 ± 0.57

[1] USDA-ARS Collection of Entomopathogenic Fungal Cultures, Ithaca, New York, USA.
[2]Centro Nacional de Referencia de Control Biológico (CNRCB), Tecomán, Colima, México.

Table 4. Effect of isolate, temperature and dose on mortality, % sporulation (in parens) of the wingless grasshopper, *P. vittatum*, at 17 d.

Dose (conidia/insect)	Temperature(°C)	Isolate		
		Fl-985	MaPL40	MaPL32
100,000	35	100 (20)	100 (24)	100 (70)
	30	100 (48)	100 (76)	100 (84)
	25	100 (69)	100 (91)	100 (84)
	20	100 (60)	100 (77)	100 (78)
	15	53 (29)	33 (10)	20 (27)
10,000	35	100 (18)	57 (7)	97 (77)
	30	100 (38)	100 (87)	100 (83)
	25	100 (45)	100 (81)	100 (90)
	20	100 (70)	100 (76)	100 (87)
	15	50 (29)	23 (4)	17 (6)
1,000	35	97 (3)	37 (3)	73 (0)
	30	100 (27)	100 (64)	100 (72)
	25	90 (48)	97 (79)	90 (78)
	20	50 (14)	63 (65)	50 (48)
	15	47 (0)	33 (0)	23 (0)

Table 5. Probit analysis of bioassay of 3 isolates of *M. anisopliae var. acridum*, against final instars and adults of *P. vittatum*, based on mortality after 17 d incubation at 30°C.

Isolate	LD_{50} conidia/ insect	95% confidence limits	common slope
Fl-985	501	18 - 4511	0.706
MaPL40	410	21 - 3442	
MaPL32	248	10 - 1785	

Literature cited

Barrientos-Lozano L. 1998. Control Biológico de Acridoideos del Noreste de México. IV Simposio de Ciencia y Tecnología. SEP-CONACYT. Mexico, pp. 68.

Barrientos-Lozano L., Milner R.J. 2000a. Comparison of the pathogenicity of three isolates of *Metarhizium anisopliae var. acridum*; effect of dose and temperature. XXI International Congress of Entomology. Foz de Iguacu, Brazil. Abstracts Book 1, pp. 513.

Barrientos-Lozano L, Milner R.J. 2000b. Uso potencial de *Metarhizium anisopliae var acridum* para el control biológico de langosta y saltamontes en Latino América. Memorias XXIII Congreso Nacional de Control Biológico. Guanajuato, Mexico, pp. 189-191.

Barrientos-Lozano L., Astacio-Cabrera O., Alvarez-Bonilla F.J., Poot-Martínez O. 1992. Manual Técnico Sobre Langosta Voladora (*Schistocerca piceifrons piceifrons,* Walker) y Otros Acridoideos de Centro América y Sureste de México. FAO/OIRSA. San Salvador, El Salvador, C.A., pp. 200.

Bateman R., Carey M., Batt D., Prior C., Abraham Y., Moore D., Jenkins N.E., Fenlon J. 1996. Screening for virulent isolates of entomopathogenic fungi against the desert locust, *Schistocerca gregaria* (Forskal). Biocontrol Science and Technology 6: 549-560.

Driver F., Milner R.J., Trueman W.H.H. 2000. A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. Mycological Research 104:134-150.

Hernández-Velázquez, V.M., Berlanga-Padilla A.M., Barrientos-Lozano L. 2000. Vegetable and mineral oil formulations of *Metarhizium anisopliae var. acridum* to control the Central American Locust (*Schistocerca piceifrons piceifrons* Walker) (Orthoptera:Acrididae). Journal of Orthoptera Research 9: 223-227.

Hernández-Velázquez, V.M., Hunter D.M., Barrientos-Lozano L., Lezama-Gutiérrez R. 2001. Evaluación de *Metarhizium anisopliae var. acridum* para el control de langosta voladora *Schistocerca piceifrons* (Orthoptera: Acrididae). In preparation.

Hernández-Velázquez V.M., Berlanga-Padilla A.M., Garza G.E. 1997. Detección de *Metarhizium flavoviride* sobre *Schistocerca piceifrons piceifrons* (Orthoptera: Acrididae) en la Isla Socorro, Archipiélago de Revillagigedo, México. Vedalia 4: 45-46.

Hunter D.M., Milner R.J., Spurgin P.A. 2001. Aerial Treatment of the Australian Plague locust, *Chortoicetes terminifera* (Orthoptera:Acrididdae) 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 7:131-39.

Jaronski S.T., Goettel M.S. 1997. Development of *Beauveria bassiana* for control of grasshoppers and locusts. Memoirs of the Entomological Society of Canada. 171: 225-237.

Milner R.J., Barrientos-Lozano L., Driver F., Hunter D.M. 2001. A comparative study of two Mexican isolates with an Australian isolate *of Metarhizium anisopliae var. acridum*-strain characterization, temperature profile and pathogenicity for wingless grasshopper, *Phaulacridium vittatum*. In preparation.