Reduction of Feeding by Schistocerca piceifrons piceifrons (Orthoptera: Acrididae), Following Infection by Metarhizium anisopliae Var. acridum

Authors: Victor Manuel Hernández-Velázquez, Angélica Berlanga-Padilla, and Conchita Toriello

Source: Florida Entomologist, 90(4) : 786-789

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2007)90[786:ROFBSP]2.0.CO;2

REDUCTION OF FEEDING BY *SCHISTOCERCA PICEIFRONS PICEIFRONS* (ORTHOPTERA: ACRIDIDAE), FOLLOWING INFECTION BY *METARHIZIUM ANISOPLIAE* VAR. *ACRIDUM*

VICTOR MANUEL HERNÁNDEZ-VELÁZQUEZ[1], ANGÉLICA BERLANGA-PADILLA[2] AND CONCHITA TORIELLO[3]
[1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, México

[2]Centro Nacional de Referencia en Control Biológico (CNRCB), Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA), Tecomán, Colima, México

[3]Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Mexico City, México

The Central American locust, *Schistocerca piceifrons* ssp. *piceifrons* (Walker), often becomes a major pest of staple and cash crops in Mexico and Central America (Barrientos-Lozano et al. 2002). In 1993, laboratory and field studies were initiated to develop a biological control strategy for *S. piceifrons*. The National Centre for Biological Control (Centro Nacional de Referencia de Control Biológico-CNRCB) has in its entomopathogen collection 35 isolates of *Metarhizium anisopliae* var. *acridum* (*M. a. acridum*) from *S. piceifrons* in Mexico. Our laboratory studies have shown that the isolates MaPL32 and MaPL40 are 2 of the most virulent against *S. piceifrons* (Barrientos-Lozano et al. 2002).

Field trials with *M. a. acridum* oil-formulated spores have demonstrated effective control of *S. gregaria* Forskal in Africa (Langewald et al. 1997), *Locusta migratoria* (L.) and *Chortoicetes terminifera* (Walker) in Australia (Hunter et al. 1999, 2001), *Rammatocerus schistocercoides* (Rehn) in Brazil (Magalhaes et al. 2001) and *S. piceifrons* in Mexico (Hernández-Velázquez et al. 2003). One of the perceived disadvantages, evident in these trials, is the length of time (13 to 14 d) that the target insect takes to die after the application. However, in practical terms, successful control of locust is reflected by a reduction in food consumption; a locust that has ceased to feed due to infection is no longer a significant pest (Moore et al. 1992). Studies by Moore et al. (1992) and Seyoum et al. (1994) both reported significant reductions in feeding by *S. gregaria* following infection with *M. a. acridum*; similar results were also observed with the grasshopper *Zonocerus variegatus* (L.) (Thomas et al. 1997). All of these reported studies were carried out using a high dose of the pathogen, in some cases with few insects per treatment, without controls, and a mixture of instars. The aim of the present work was to examine the effect of a conidia low dose (6×10^4) per *S. piceifrons* adult on both feeding rate and feces production.

First instars of *S. piceifrons* locusts were collected in Tizimín, Yucatán, Mexico. Locusts were kept in $1 \times 1 \times 1$ m cages inside a controlled environment (CE) room and fed on a diet of leaves of the grass *Cynodon dactylon* (L.) and rolled oats. The mean daily temperature and relative humidity (RH) in the CE room was 27°C and 70 ± 5% respectively, with a photoperiod of 12:12 h (L:D).

The fungal isolate was a monospore isolate (EH-502/8) of *M. a. acridum* MaPL40 from *S. piceifrons* (Hernández-Velázquez et al. 1997). Fungal conidia were produced by a diphasic method. In the first phase, the fungus was cultured in a liquid medium of 2% sucrose and 2% yeast extract (Jenkins & Prior 1993). Fungal biomass was transferred to rice in a plastic bag (250 g/bag) with a cotton filter for further growth and sporulation. After 14 d the bags were opened in the CE room which allowed the fungus to dry to a moisture content of approximately 9%. Conidia were separated from the rice by sieving through a 300-µm mesh. The resulting conidia powder was then re-sieved through a 90-µm mesh to remove any remaining rice-dust particles.

Conidial suspensions were formulated in citroline (a mineral oil derived from petroleum for agricultural use in Mexico) by mixing 1 g of conidial powder in 100 mL with a magnetic stirrer. It was then adjusted to 3×10^7 conidia/mL with use of a Neubauer haemocytometer. Germination rates were determined by randomly examining 100 conidia per plate with a compound microscope (400×). A conidium was considered to have germinated if the germ tube was at least as long as the width of the conidium. Germination of conidia was 95-100% immediately after formulation and just before locust infection.

Each treatment consisted of 20 adult locusts, 7 d post-fledging, each receiving 2 µL of the formulation applied on the pronotum via a Hamilton SGE syringe. Control groups received 2 µL of either citroline or sterile distilled water with Tween 80 (0.1%, v/v, Difco™) without conidia. Following treatment, locusts were placed in subgroups of 4 insects in 13×10.5 cm plastic containers, covered with muslin. Containers were maintained at 28°C and 70 ± 5% RH. The experiment was replicated 4 times. Mortality was recorded daily for 12 d; dead insects were placed individually in Petri dishes on moist filter paper at 27°C for 4 d to allow growth

of mycelia on the insect surface. Each day, between 1.945 to 2.557 g of fresh *C. dactylon* seedling leaf was given to each subgroup of 4 locusts. At the beginning of the experiment, 4 samples of fresh *C. dactylon* seedling were dried for 24 h at 90°C to determine the seedling dry weight percentage so food supply could be calculated. Every 24 h the uneaten seedlings were removed from each container, and dried at 90°C for 24 h until reaching a constant weight. In addition, feeding was assessed indirectly by monitoring feces production. Feces were collected every 24 h. Individual samples were dried at 90°C for 48 h until reaching a constant weight.

Cumulative mortality until d 12 of the experiment, among the treated and negative control groups was analyzed by analysis of variance (ANOVA, $\alpha = 0.05$). Multiple comparisons were used to determine significant differences between means of infected and citroline negative control locusts at $P < 0.05$ (Tukey test). All data from food consumption and feces production between infected and citroline negative control locusts were analyses by a *t*-test ($\alpha = 0.05$) for each day until d 7. Since there was no difference in food consumption and feces production between both negative controls (citroline and Tween 80), Tween 80 was excluded from the statistical analysis.

The cumulative percent mortality of *S. piceifrons* inoculated with fungal conidia reached 97.5 ± 2.5% at d 12. Significant differences ($P < 0.01$) in mortality were observed between the treated and control groups since d 6 and thereafter until d 12 of the experiment (data not shown). Mortality did not exceed 10% in either control group throughout the 12 d of the experiment.

The result of food consumption in milligrams of dry weight/insect between the treated and citroline control groups is shown in Table 1. A significant ($P < 0.05$) reduction in food consumption was observed between treated and citroline control groups from d 4 to 7, although a reduction was evident by d 3 ($P = 0.07$). During the first 24 h of the experiment, food intake was low in both groups; 67.9% lower than the second day. After 7 d, on a per insect basis food consumption was reduced by 82.24%.

Table 2 presents the result of feces production in milligrams of dry weight/insect between the treated and citroline control groups. A significant ($P < 0.05$) reduction of feces production was observed between the treated and citroline control groups at d 3. The mean feces production from the control group was 64.01 mg, and during the first 24 h of the experiment, the mean of both groups was 51.62 mg. After 7 d, on a per insect basis, feces production was reduced by 76.45%.

This study provides further evidence that infection by *M. a. acridum* causes a significant reduction in *S. piceifrons* feeding by both direct (food consumption) and indirect (feces production) evaluation. Feces production showed a significant reduction (d 3), 24 h before food consumption (d 4). During the first 24 h before insect treatment, food consumption was very low, probably due to stress induced in locusts during manipulation, as already noted by Moore et al. (1992) in *S. gregaria*.

The observed effect on food consumption and feces production at d 3 and 4 after infection agrees with findings by Moore et al. (1992). They noted a reduction in the desert locust feeding 24 h after application of 8×10^5 conidia/insect of *M. a. acridum,* and at d 3 with 8×10^4 conidia/insect. The effect on feeding, movement, and flying abilities of the locust is related to the development and colonization of the fungus, rather than toxin production (Freimoser et al. 2003). Seyoum et al. (1994)

TABLE 1. MEAN FOOD CONSUMPTION IN MILLIGRAMS OF DRY WEIGHT BY *SCHISTOCERCA PICEIFRONS* TREATED WITH 6×10^4 CONIDIA OF *M. ANISOPLIAE* VAR. *ACRIDUM* AND CITROLINE NEGATIVE CONTROL LOCUSTS OVER A 7-D PERIOD.

	Citroline negative control locusts			Infected locusts		
Days	Ni	Nf	Food/insect* (mg dry weight)	Ni	Nf	Food/insect* (mg dry weight)
1	80	80	34.48 ± 3.63 a**	80	80	33.22 ± 3.33 a
2	80	78	74.84 ± 6.67 a	80	80	68.30 ± 6.41 a
3	78	75	78.91 ± 2.67 a	80	80	61.83 ± 7.88 a
4	75	74	82.56 ± 4.93 a	80	78	55.28 ± 2.99 b
5	74	73	75.35 ± 2.39 a	78	74	32.13 ± 5.89 b
6	73	72	78.25 ± 1.16 a	74	62	9.61 ± 4.73 b
7	72	72	64.22 ± 2.52 a	62	44	11.40 ± 5.67 b

N_I = number of surviving insects that initiates the corresponding day.
N_F = number of surviving insects at the end of that day (24 h).
*Means ± SE; **Mean followed by the same letter are not significantly different.
Significant differences ($\alpha = 0.05$) in food consumption were observed at d 4 ($t = 3.471$; $df = 6$; $P = 0.0033$), 5 ($t = 6.49$; $df = 6$; $P = 0.0005$), 6 ($t = 15.435$; $df = 6$; $P < 0.0001$), and 7 ($t = 9.534$; $df = 6$; $P = 0.0001$) between citroline negative control and infected locusts.

TABLE 2. MEAN FECES PRODUCTION IN MILLIGRAMS OF DRY WEIGHT BY *SCHISTOCERCA PICEIFRONS* TREATED WITH 6 $\times 10^4$ CONIDIA OF *M. ANISOPLIAE* VAR. *ACRIDUM* AND CITROLINE NEGATIVE CONTROL LOCUSTS DURING 7 D.

	Citroline negative control locusts			Infected locusts		
Days	Ni	Nf	Feces/insect* (mg dry weight)	Ni	Nf	Feces/insect* (mg dry weight)
1	80	80	52.61 ± 5.85 a**	80	80	49.72 ± 2.15 a
2	80	78	57.87 ± 5.04 a	80	80	46.56 ± 2.37 a
3	78	75	61.63 ± 2.94 a	80	80	47.91 ± 3.61 b
4	75	74	69.59 ± 3.78 a	80	78	45.21 ± 1.48 b
5	74	73	68.06 ± 3.93 a	78	74	33.94 ± 0.96 b
6	73	72	67.68 ± 1.36 a	74	62	21.57 ± 3.30 b
7	72	72	70.66 ± 5.14 a	62	44	16.64 ± 3.81 b

N_I = number of surviving insects that initiates the corresponding day.
N_F = number of surviving insects at the end of that day (24 h).
*Means ± SE; **Mean followed by the same letter are not significantly different.
Significant differences ($\alpha = 0.05$) in feces production were observed at d 3 ($t = 2.943$; $df = 6$; $P = 0.0259$), 4 ($t = 5.991$; $df = 6$; $P = 0.0010$), 5 ($t = 8.416$; $df = 6$; $P = 0.0002$), 6 ($t = 12.903$; $df = 6$; $P < 0.0001$), and 7 ($t = 8.597$; $df = 6$; $P = 0.0001$) between citroline negative control and infected locusts.

observed flight impairment in the desert locust, *S. gregaria*, after 3-4 d of *M. a. acridum* infection, and attributed it to a reduced availability of metabolizable fuel and physical damage to muscles and nerves caused by fungal colonization. In practical terms, the observed feeding reduction in our study would reflect a non-significant pest very early after *Metarhizium* infection.

This study was financed by grant G-31451-B of Consejo Nacional de Ciencia y Tecnología (CONACYT) of Mexico. We also thank Dr. Julio Bernal (Texas A&M University) for the review of the manuscript, and Mrs. Ingrid Masher for editorial assistance.

SUMMARY

This study examined the effects of Mexican strain (MaPL40) *Metarhizium anisopliae* var. *acridum* on feeding and fecal production of adult Central American locusts, *Schistocerca piceifrons* ssp. *piceifrons*. Locusts infected with 6×10^4 *M. anisopliae* conidia per insect showed 97.5% mortality in contrast to 13% in negative control locusts after 12 d of the infection. A significant ($P < 0.01$) reduction in feeding was observed, as indicated by *Cynodon dactylon* leaf consumption and feces production at 4 and 3 d, respectively. The data reflect a non-significant pest very early after *Metarhizium* infection.

REFERENCES CITED

BARRIENTOS-LOZANO L., V. M. HERNÁNDEZ-VELÁZQUEZ, R. J. MILNER, AND D. M. HUNTER. 2002. Advances in biological control of locusts and grasshoppers in Mexico. J. Orthoptera Res. 11: 77-82.

FREIMOSER F. M., S. SCREEN, B. SAVITA, G. HU, AND R. J. ST. LEGER. 2003. Expressed sequence tag (EST) analysis of two subspecies of *Metarhizium anisopliae* reveals a plethora of secreted proteins with potential activity in insect hosts. Microbiol. 149: 239-247.

HERNÁNDEZ-VELÁZQUEZ V. M., A. M. BERLANGA-PADILLA, AND E. GARZA-GONZÁLEZ. 1997. Detección de *Metarhizium flavoviride* sobre *Schistocerca piceifrons piceifrons* (Orthoptera: Acrididae) en la Isla Socorro, Archipiélago de Revillagigedo, México. Vedalia 4: 45-46.

HERNÁNDEZ-VELÁZQUEZ V. M., D. M. HUNTER, L. BARRIENTOS-LOZANO, R. LEZAMA-GUTÍERREZ, AND F. REYES-VILLANUEVA. 2003. Susceptibility of *Schistocerca piceifrons* (Orthoptera: Acrididae) to *Metarhizium anisopliae* var. *acridum* (Deuteromycotina: Hyphomycetes): laboratory and field trials. J. Orthoptera Res.12: 89-92.

HUNTER D. M., R. J. MILNER, J. C. SCANLAN, AND P. A. SPURGIN. 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., R. J. MILNER, AND P. A. SPURGIN. 2001. Aerial treatment of the Australian plague locust, *Chortoicetes terminifera* (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). Bull. Entomol. Res. 91: 93-99.

JENKINS, N. E., AND C. PRIOR. 1993. Growth and formation of true conidia by *Metarhizium flavoviride* in a simple liquid medium. Mycological Res. 97: 1489-1494.

LANGEWALD, J., C. KOOYMAN, O. DOURO-KPINDOU, C. J. LOMER, A. O. DAHMOUD, AND H. O. MOHAMED. 1997. Field treatment of desert locust (*Schistocerca gregaria* Forskål) hoppers in Mauritania using an oil formulation of the entomopathogenic fungus *Metarhizium flavoviride*. Biocontrol Sci. Technol. 7: 603-611.

MAGALHAES B. P., M. LECOQ, M. FARIA, F. G. V. SCHMIDT, AND W. D. GUERRA. 2000. Field trial with the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* against bands of the grasshopper *Rhammatocerus schistocercoides* in Brazil. Biocontrol Sci.Technol. 10: 427-441.

MOORE D., M. REED, G. LE PATOUREL, Y. J. ABRAHAM, AND C. PRIOR. 1992. Reduction of feeding by desert locust, *Schistocerca gregaria*, after infection with *Metarhizium flavoviride*. J. Invertebrate Pathol. 60: 304-307.

SEYOUM E. D., D. MOORE, AND A. K. CHARNLEY. 1994. Reduction in flight activity and food consumption by the desert locust, *Schistocerca gregaria* Forskål Orth., Cyrtacanthacrinae, after infection with *Metarhizium flavoviride*. J. Applied Entomol. 118: 310-315.

THOMAS M. B., S. BLANFORD, AND C. J. LOMER. 1997. Reduction of feeding by the variegated grasshopper, *Zonocerus variegatus*, following infection by the fungal pathogen, *Metarhizium flavoviride*. Biocontrol Sci. Technol. 7: 327-334.