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(DEUTEROMYCOTA: HYPHOMYCETES) AGAINST THE  
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(COLEOPTERA: CURCULIONIDAE) UNDER  
LABORATORY CONDITIONS**

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PATHOGENICITY OF *BEAUVERIA BASSIANA* (DEUTEROMYCOTA:  
HYPHOMYCETES) AGAINST THE CACTUS WEEVIL,  
*METAMASIVUS SPINOLAE* (COLEOPTERA: CURCULIONIDAE)  
UNDER LABORATORY CONDITIONS

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ABSTRACT

Three strains of the entomopathogenic fungus *Beauveria bassiana* Vuill. were tested for pathogenicity against adults of *Metamasius* (= *Cactophagus*) *spinolae* Gyllenhal. *M. spinolae* is an important pest of cactus plants (*Opuntia ficus indica*), which are used as a food crop and to avoid erosion in Mexico. After inoculation in a spray tower, *M. spinolae* adults were susceptible to *B. bassiana* at concentrations of  $1 \times 10^8$  conidia per milliliter. Female mortality was steadily higher than male mortality for all isolates. One of the three isolates caused significantly higher mortality (82%) in females, whereas male mortality was the same for all isolates. These results indicate for the first time the possible use of *B. bassiana* as biocontrol agent against this insect pest.

Key Words: *Beauveria bassiana*, *Cactophagus spinolae*, *Opuntia* spp., microbial control, *Opuntia* borer, cactus weevil, *Metamasius spinolae*.

RESUMEN

Tres aislamientos del hongo entomopatógeno *Beauveria bassiana* Vuill. fueron evaluados en su patogenicidad contra adultos de *Metamasius* (= *Cactophagus*) *spinolae*, Gyllenhal. *M. spinolae* es una importante plaga de plantas de nopal (*Opuntia ficus indica*), que son usadas como alimento y para evitar la erosión en México. Después de ser inoculados en una torre de aspersión, adultos de *M. spinolae* fueron susceptibles a concentraciones de *B. bassiana* de  $1 \times 10^8$  conidios por mililitro. La mortalidad en hembras fue consistentemente mayor que la mortalidad de machos para todos los aislamientos. Uno de los tres aislamientos causó significativamente mayor mortalidad (82%) en hembras, mientras que la mortalidad en machos fue la misma para todos los aislamientos. Estos resultados muestran por vez primera el posible uso de *B. bassiana* como agente de control biológico en contra de este insecto plaga.

Translation provided by the authors.

The cactus (*Opuntia ficus indica*) is a very important plant in Mexico, especially in semi-arid regions where few crops can be cultivated (Vigueras & Portillo 2001). The cactus weevil *Metamasius* (= *Cactophagus*) *spinolae* Gyllenhal is a limiting factor for commercial production of *Opuntia* spp. (Baddi & Flores 2001; Flores-Valdez 2001). The larvae, 25-35 mm long, tunnel into apparently healthy cactus pads, from joint to joint, where they cause disintegration of the cactus tissues (Granados & Castañeda 1991). Pupation takes place in the hollowed stem of the plant, which provides a protected environment for pupal overwintering. Adults can be found from May through September; they are relatively large weevils at 23-

36 mm in length. They feed on the margins of the young pads causing additional damage. Control strategies for *M. spinolae* rely to a large extent on the use of chemical insecticides (Borrego & Burgos 1986; Baddi & Flores 2001). However, a biologically based control strategy that can be used on *M. spinolae* would be highly desirable.

Microbial insect pathogens may offer a strategy for use as localized biopesticides, but little is known about natural microbial enemies of *M. spinolae*. Several products based on *Beauveria bassiana* Vuill. are available for managing adults of other pest insect species, such as *Hypothenemus hampei* Ferrary (coffee berry borer), and various species of Curculionidae (Adane et al. 1996;

de la Rosa et al. 1997; Rice & Cogburn 1999). So far, however, no entomopathogenic fungus has been evaluated for the control of *M. spinolae*. In this study we assess the susceptibility of adult *M. spinolae* to isolates of *B. bassiana* under laboratory conditions.

## MATERIALS AND METHODS

### Biological Material

Isolates from the Insect Pathology Collection (Colegio Postgraduados Texcoco, Mexico) were used in bioassays with *M. spinolae* adults (Table 1). All isolates were cultured on Sabouraud dextrose agar (SDA) with yeast extract (2g/l) (SDA-Bioxon, Mexico) and incubated at 27°C, 70% RH for 2-3 weeks until conidia were produced. For bioassays, conidia were harvested into sterile 0.01% Tween 80 solutions to a final concentration of  $1 \times 10^8$  conidia/ml. Conidia viability was determined by serial dilution plating onto SDA and colony forming units were counted six days after incubation at 27°C.

For bioassay experiments, *M. spinolae* adults were collected directly from cactus pads in Morelos, Mexico. They were sexed, held separately in screen cages (32 × 32 × 32 cm, 1.5 mm mesh size). Adults were stored in fresh cactus pads at 23 ± 1°C, 33 ± 5% RH, and a 12:12 (L:D) photoperiod, before use in bioassays.

### Laboratory Test

For inoculation, *M. spinolae* adults were placed into 90-mm plastic Petri dishes. Groups of 10 adults of the same sex were stored at 4°C for 15 minutes to anesthetize them. The adults were exposed to a *B. bassiana* isolate by applying 10 ml of a  $1 \times 10^8$  conidia/ml suspension in a spray tower (Altre et al. 1999) with constant pressure (0.7 kg/cm<sup>2</sup>). On average, each insect received  $1 \times 10^8$  conidia, while control adults received 0.1% Tween 80. The density of conidia on the insects was estimated by counting the number of conidia in a sample area on five agar disks (1 cm dia.) placed in the Petri dish during inoculation.

After application, the adults were placed in separate screen cages, fed with fresh untreated cactus pads and kept for 16 days at 23 ± 1°C, 33 ± 5% RH, and a photoperiod of 12:12 (L:D) h. The bioassays were repeated three times. The adults were examined for mortality every 48 h for 16 d. Dead insects were removed and incubated at 25°C and 90% relative humidity to check for *B. bassiana* infection by direct visual observation.

### Statistical Analyses

Analysis was performed on mean cumulative percentage mortality data. After correction for control mortality (Abbott 1925), percentage mortality and average time to death were subjected to a one-way analysis of variance (SAS Institute 1999). Treatment effects were tested by the Fisher protected least significant difference (LSD) test (Sokal & Rohlf 1995).

## RESULTS AND DISCUSSION

*B. bassiana* isolates were pathogenic to *M. spinolae* exposed to  $1 \times 10^8$  conidial suspensions (Table 2). However, the three *B. bassiana* isolates used for this report differed in their virulence to *M. spinolae* adults. For all three, the females were more susceptible to fungal infection than males ( $F = 20.49$ ;  $df = 1$ ;  $P = 0.00005$ ). *B. bassiana* isolate Bb88 was more virulent against females than Bb4 or Bb113 isolate ( $F = 5.55$ ;  $df = 2$ ;  $P = 0.04$ ). Fungal virulence of the three isolates were similar against males ( $F = 1.18$ ;  $df = 2$ ;  $P = 0.36$ ; Table 2). All females exposed to the fungi died within 8.5 to 10.6 days ( $F = 0.45$ ;  $df = 2$ ;  $P = 0.65$ ) and males died within 7.5 to 11 days ( $F = 1.06$ ;  $df = 2$ ;  $P = 0.40$ ). There were no significant differences in mean time to death (Table 2). Most cadavers supported fungal sporulation, indicating successful infection and the ability of the isolates to sporulate under low humidity. An increase in female mortality was noted by day four after inoculation and female cumulative mortality was steadily higher than male mortality for all isolates (Fig. 1).

TABLE 1. *BEAUVERIA BASSIANA* ISOLATES<sup>1</sup> TESTED AGAINST *METAMASIVUS SPINOLAE*, THEIR HOST INSECT, ORIGIN, AND YEAR ISOLATED.

Fungus	Host insect	Country of origin	Year isolated
Bb4	<i>Hypothenemus hampeii</i> (Col.: Scolytidae)	Ecuador	1987
Bb88	<i>H. hampeii</i>	Mexico, Oaxaca	1994
Bb113	<i>Metamasivus spinolae</i> (Col.: Curculionidae)	Mexico, Morelos	2003

<sup>1</sup>Deposited at the Colegio de Postgraduados (Texcoco, Mexico).

TABLE 2. MORTALITY OF *METAMASIVUS SPINOLAE* ADULTS AFTER TREATMENT WITH THREE *BEAUVERIA BASSIANA* ISO-LATES AT A CONCENTRATION OF  $10^8$  CONIDIA PER MILLILITER.

Treatment	Dosage <sup>1</sup>	Females		Males	
		% $\pm$ SE <sup>2</sup> mortality	Mean time $\pm$ SE to death (d)	% $\pm$ SE <sup>2</sup> mortality	Mean time $\pm$ SE to death (d)
Bb4	780 $\pm$ 43 a	46.4 $\pm$ 1.2 a	9.3 $\pm$ 1.4 a	10.0 $\pm$ 0.5 a	11.0 $\pm$ 2.8 a
Bb88	1187 b $\pm$ 51 b	82.1 $\pm$ 0.8 b	8.5 $\pm$ 0.2 a	20.0 $\pm$ 1.1 a	7.5 $\pm$ 0.2 a
Bb113	1341 b $\pm$ 86 b	42.8 $\pm$ 1.2 a	10.6 $\pm$ 2.3 a	26.6 $\pm$ 0.3 a	9.0 $\pm$ 0.4 a

Within columns, means followed by different letters are significantly different ( $P \leq 0.05$ ; LSD test).

<sup>1</sup>Number  $\pm$ SE of viable conidia per square millimeter. <sup>2</sup>Percentage of mortality corrected for control mortality of 0% in males and 6.6% in females (Abbott 1925).

Conidial dosages applied per  $\text{mm}^2$  ranged from 780  $\pm$  43 (Bb4) to 1341  $\pm$  86 (Bb113) (Table 2). The Bb4 dosage was lower than Bb88 and Bb113 dos-

ages ( $F = 21.07$ ;  $df = 2$ ;  $P = 0.0003$ ). Thus, the low virulence of the Bb4 strain could be related to the lower conidial dosage applied. However, previous work showed that the same strain was highly virulent to its original host *H. hampei* (de la Rosa et al. 1997). Hence, these two species appear to have differing susceptibility to Bb4. Similar comparisons for the *M. spinolae* isolate (Bb113) are not available.

The data in this report also demonstrate that it is feasible to contaminate adults by spraying conidia, and that most cadavers supported fungal sporulation. This may be important for any control strategy aimed at attracting beetles to fungus contaminated traps, and subsequent transfer to adults or larvae in cactus pads and tunnels. Fortunately, there is evidence of an aggregation pheromone (Tafoya et al. 2003) which can be used to attract the males to a contaminated trap with fungi and possibly transfer the infective conidia to adults or larvae in tunnels. Similar strategies of autodissemination have been developed for other insects (Furlong et al. 1995).

The results presented in this study demonstrate a pathogenic effect of *B. bassiana* on *M. spinolae* adults under laboratory conditions. To our knowledge, this is the first report on infection of this pest insect with an entomopathogenic fungus. Further research is necessary to determine the effectiveness of *B. bassiana* under field conditions and to examine its potential impact on non-target species.

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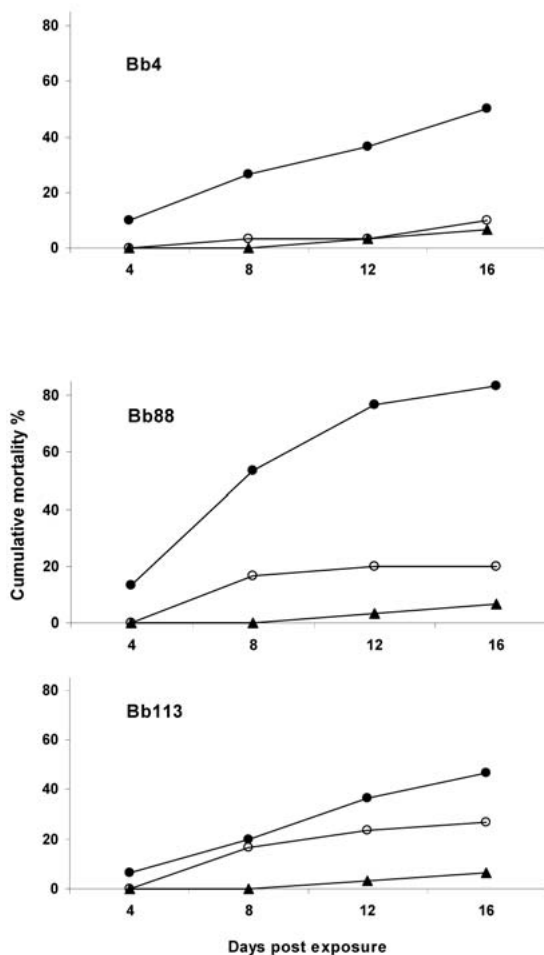


Fig. 1. Cumulative mortality % of adult *Metamasius spinolae* treated with three *Beauveria bassiana* strains under laboratory conditions. Females (○), males (●), and control (▲).

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