

SPORULATION OF *METARHIZIUM ANISOPLIAE* VAR. *ACRIDUM* AND *BEAUVERIA BASSIANA* ON *RHAMMATOCERUS SCHISTOCERCOIDES* UNDER HUMID AND DRY CONDITIONS

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SHORT COMMUNICATION

ABSTRACT

The sporulation of the fungi *Metarhizium anisopliae* var. *acidum* and *Beauveria bassiana* in cadavers of the grasshopper *Rhammatocerus schistocercoides* was studied in dry and humid environments. Both fungi were equally virulent against *R. schistocercoides*. However, internally, *M. anisopliae* produced more conidia than *B. bassiana* at 53% and 75% relative humidity. Externally, there was no sporulation at 53% and 75% RH, and *M. anisopliae* produced more conidia than *B. bassiana* at 100% RH.

Key words: entomopathogenic fungi, internal sporulation, grasshoppers, microbial control

Rhammatocerus schistocercoides is a serious pest of several crops and native pastures in Mato Grosso State, Brazil (8). The reduction or replacement of chemical insecticides by mycoinsecticides to control this insect is under investigation (5, 6). *Metarhizium anisopliae* var. *acidum* (= *M. flavoviride*; 1) is very infective to *R. schistocercoides* (6, 10), and *Beauveria bassiana* to *Melanoplus sanguinipes* (3). During previous research at Embrapa Genetic Resources and Biotechnology it was noted that *M. anisopliae* var. *acidum* sporulated profusely within dried cadavers of *R. schistocercoides* (10). We report here on a laboratory experiment devised to compare internal and external sporulation of *M. anisopliae* var. *acidum* and *B. bassiana* on *R. schistocercoides* cadavers kept in humid and dry conditions.

Insects. Insects used in this study were collected in the field (Silvânia, GO, Brazil) and maintained in 60 cm x 60 cm x 80 cm cages in the laboratory at 25°C and 12h photophase. They were fed sugar cane leaves, oats and wheat germ during 15 days before testing, without regard to gender.

Fungal isolates and culture. Isolates CG 423 (*M. anisopliae* var. *acidum*) and CG 425 (*B. bassiana*) were

provided by Embrapa Genetic Resources and Biotechnology, Collection of Entomopathogenic Fungi, Brasília, DF. The isolate CG 423 was first found infecting the pallid grasshopper *Schistocerca pallens* in Rio Grande do Norte State (7), and the isolate CG 425 infecting *R. schistocercoides* in Mato Grosso (5). The fungi were cultured in SDAY (1% neopeptone, 2% dextrose, 1.5% agar, and 1% yeast extract, completed to 1000 ml distilled water, pH calibrated to 6.2) after retrieval from storage in liquid nitrogen. For the bioassay, the fungi were culture in rice as described by Magalhães and Frazão (4).

Bioassay. Groups of 30 *R. schistocercoides* adults were tested with either *M. anisopliae* var. *acidum* (CG 423) or *B. bassiana* (CG 425). To perform the bioassays, insects were individually inoculated with 3 µl of a conidial suspension (5000 conidia/insect) applied on the right pleural region with a micropipette. Insects were maintained in 17 cm x 21 cm x 25 cm cages (10/cage) at 25°C and 12 photophase and were fed sugar cane leaves, oats and wheat germ. Dead adults were removed daily. One femur was dissected from each insect and a drop of hemolymph was extracted and observed under the microscope for presence of blastospores.

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Sporulation test. Desiccators containing saturated solutions of $Mg(NO_3)_2 \cdot 6H_2O$ and $NaCl$ to provide 53% and 75% relative humidity, respectively, were used for both fungi. Infected hoppers were then placed in 7 cm diameter Petri dishes left opened in the desiccators. The high humidity (100%) was obtained by placing a small Petri dish (3 cm) containing moistened cotton inside 90 cm diameter dishes and sealing them with plastic film. In all cases, *M. anisopliae* var. *acridum* was incubated at 30°C and *B. bassiana* at 25°C. This difference in temperature was due the better development of the *B. bassiana* isolate at 25°C (unpublished data). After 10 days, all hoppers were weighted to express conidial production by gram of insect. To estimate internal sporulation, cadavers were macerated with the aid of a 50 ml commercial blender (Waring, Dynamics Corporation of America, Connecticut, USA) working at high speed for 20 seconds. To estimate external sporulation, cadavers were washed in a 10 ml Tween 80 solution (1% in distilled water; v/v). Internal production of conidia by *M. anisopliae* var. *acridum* and *B. bassiana* was estimated by the colony forming units (CFU) method. The culture medium for *M. anisopliae* var. *acridum* was Oatmeal Dodine Agar (5.55g oat, 0.55g magnesium sulfate, 0.83 potassium phosphate, 0.55g sodium nitrate, 32.95g agar, 5ml violet crystal, 4g penicilin G, 10g streptomycin, completed to 1000 ml with distilled water). The same medium, except dodine, was used for *B. bassiana*. Dodine is a fungicide used to prepare selective media to grow specific fungi (1).

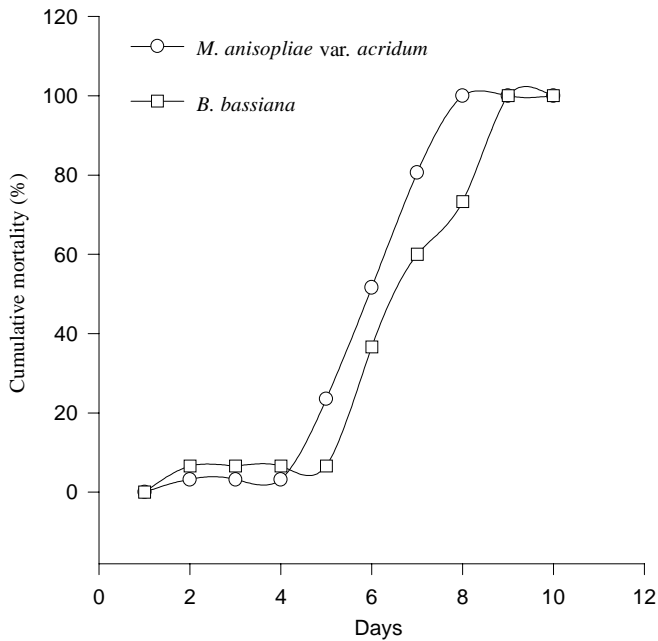


Figure 1. Mortality of *Rhammatocerus schistocercoides* adults infected with *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana*.

Both fungi were equally virulent against *R. schistocercoides* (Fig. 1), but *M. anisopliae* produced more conidia internally than *B. bassiana* at low humidity ($P < 0.03$; Fig. 2A). Externally, there was no sporulation when infected insects were incubated at 75% RH and *M. anisopliae* var. *acridum* produced significantly more conidia than *B. bassiana* at 100% RH ($P < 0.01$; Fig. 2B). Regarding total fungal production per insect at 100% RH, *M. anisopliae* var. *acridum* produced more conidia and CFU (7.1×10^8) than *B. bassiana* (4.1×10^8).

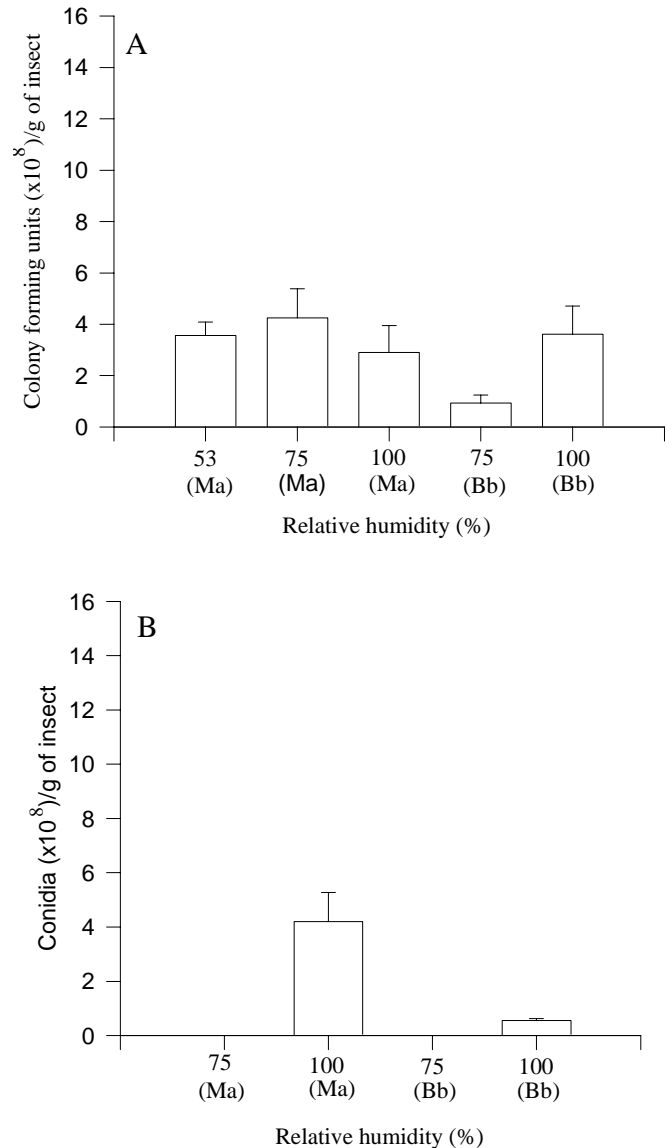


Figure 2. (A) Colony forming units produced by *Metarhizium anisopliae* var. *acridum* (Ma) and *Beauveria bassiana* (Bb) in the internal body cavity of *Rhammatocerus schistocercoides* at different humidities. (B) Conidial production by *M. anisopliae* var. *acridum* (Ma) and *B. bassiana* (Bb) on the external body of *R. schistocercoides* at different humidities.

Internal sporulation of *M. anisopliae* var. *acridum* in cadavers of the grasshopper *Zonocerus variegatus* was registered by Prior and Greathead (9). Since *M. anisopliae* var. *acridum* is being developed as mycoinsecticide against grasshoppers in Brazil, its ability to sporulate within cadavers under dry conditions would be important in the epizootiology of the disease, especially if applied in low humidity environments.

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RESUMO

Esporulação de *Metarhizium anisopliae* var. *acridum* e *Beauveria bassiana* em *Rhammatocerus schistocercoides* em ambientes secos e úmidos

A esporulação dos fungos *Metarhizium anisopliae* var. *acridum* e *Beauveria bassiana* em cadáveres do gafanhoto *Rhammatocerus schistocercoides* foi estudada em ambientes seco e úmido. As duas espécies de patógenos foram igualmente patogênicas a *R. schistocercoides*. Entretanto, internamente, *M. anisopliae* produziu mais conídios que *B. bassiana* a 53% e 75% de umidade relativa. Externamente, não houve esporulação a 53% e 75% de umidade relativa e *M. anisopliae* produziu mais conídios que *B. bassiana* a 100%.

Palavras-chave: Fungos entomopatogênicos, esporulação interna, gafanhotos, controle microbiano.

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