Infection of the Grasshopper, *Rhammatocerus schistocercoides* Rehn by the Entomopathogenic Fungus, *Metarhizium flavoviride* Gams & Rozsypal

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Infecção do Gafanhoto *Rhammatocerus schistocercoides* Rehn pelo Fungo Entomopatogênico *Metarhizium flavoviride* Gams & Rozsypal

RESUMO - O processo infectivo de *Metarhizium flavoviride Gams & Rozsypal* contra *Rhammatocerus schistocercoides* Rehn foi investigado. Conídios germinaram 12 h após a inoculação de ninfas de 5° ínstar. Seguindo o desenvolvimento de hifas e apressórios, o fungo penetrou a cutícula do inseto *e* desenvolveu-se então dentro do hemocele, com formação de células conidiogênicas típicas e conídios três dias após a inoculação. Conídios produzidos internamente germinaram formando hifas que eventualmente saíram através da cutícula, atingindo o exterior. A conidiogênese sobre o inseto (cadáver) ocorreu entre cinco a seis dias após a inoculação.

PALAVRAS-CHAVE: Insecta, processo infectivo, controle biológico.

ABSTRACT - The infection process of *Metarhizium flavoviride* Gams & Rozsypal against *Rhammatocerus schistocercoides* Rehn was investigated. Conidia germinated 12 h after inoculation on 5th instar nymphs. Following hyphal and appressorial development, the fungus penetrated the insect cuticle and developed inside the hemocoel, with formation of typical conidiogeneous cells and conidia three days after inoculation. Conidia produced internally germinated forming hyphae that reached the exterior via the insect cuticle. The fungus sporulated externally five to six days after inoculation.

KEY WORDS: Insecta, infective process, biological control.

The grasshopper *Rhammatocerus schistocercoides* Rehn (Orthoptera: Acrididae) has been a major problem in Brazil causing severe agricultural losses since 1984 (Cosenza *et al.* 1990, 1994). In some years, more than two million ha are invaded by this pest in Central Brazil. As a result of the high costs of chemical control and environmental pollution, non-chemical alternatives are required. The use of entomopathogens as bioinsecticides against grasshoppers is under investigation as one of these alternatives (Greathead 1992). It has been well accepted that some species of entomopathogenic fungi can minimize or even replace the use of chemicals in a grasshopper integrated pest management program (Prior & Greathead 1989, Johnson *et al.* 1992).

The high virulence of two Brazilian isolates (CG 087 and CG 423) of the entomopathogenic fungus *Metarhizium flavoviride*

Gams & Rozsypal against *R. schis-tocercoides* is comparable to a Nigerian isolate (IMI 330189) (B.P. Magalhães, M. Faria, M. Tigano and B. Sobral, unpublished), which is being developed as a microbial insecticide against *Schistocerca gregaria* Forscål in Africa (Prior *et al.* 1992). The efficacy of these strains as mycoinsecticides and selection of new isolates depends on a good understanding of the mechanisms of fungal pathogenesis, including formation of appressoria. The infection processes of *M. anisopliae* (Metschn.) Sorokin on *S. gregaria* (Dillon & Charnley 1986, Gunnarsson 1988) and *M. flavoviride* on *Zonocerus variegatus* L. (Prior & Greathead 1989) were described previously. However, there is no published information on *M. flavoviride* mode of action against *R. schistocercoides*. The objective of this paper was to describe the sequence of events related to the infection of *R. schistocercoides* by *M. flavoviride*.

Material and Methods

Insects and Fungal Culture. Fifth instar nymphs of *R. schistocercoides* were field-collected in Campo Novo dos Parecis, MT, and fed with a mixture of cereals (oat, bran and wheat germ; 1:1:1) and fresh sugarcane leaves. This diet was replaced each other day. Insects were maintained in cages (up to 150 individuals/cage of 53 x 55 x 70 cm) at 26-28° C and 12:12 (light:dark) photoperiod.

   M. flavoviride (CG 423), isolated from *Schistocerca pallens* Thunb., an important grasshopper in Northeast Brazil, was stored in liquid nitrogen. Fresh cultures were initiated on complete medium (Ponte-corvo *et al.* 1953) at 30°C. Conidia were produced on rice (30°C), harvested 10 days after inoculation, formulated in soybean oil (Olvego, Pires do Rio, GO, Brazil) and 5% kerosene (Labareda, Goiânia, GO, Brazil), and stored at 6-8°C for up to eight days before use.

Insect Inoculation. Nymphs were inoculated topically in the pleural region, between the pro and mesothorax with 3 μl of a suspension

containing 10^7 conidia/ml. Insects were then kept in plastic boxes (11 x 11 x 3,5 cm) at 27°C and 12:12 (light:dark) photoperiod during 10 days and fed as described above.

Scanning Electron Microscopy. Three insects were killed daily by freezing and dissected to remove a piece (1 cm^2) of the inoculated cuticle. The material was then fixed with Karnovisk for 12 hours. Fixation and dehydration was performed using 2% paraformaldehyde, 3% glutaraldehyde, and 0,1% picric acid in 0,05M cacodylic acid buffer, at pH 7,3 and 4°C for 12 hours (Erlandsen *et al.* 1989, Silveira 1989). Samples were dried using a Balzers critical point dryer (CPD 030) and carbon dioxide as drying fluid. Observations were made using a scanning electron microscope (JEOL, JSM 840A). Photographs were taken with Neopan 120S film.

Light Microscopy. Samples were embedded in resin-spur and 100 sections were cut with a Sorval, MT 2B microtome. Observations were made in a Zeiss III RS microscope. Photographs were taken with TMAX P100 film.

Results and Discussion

Conidial Germination and Appressorial Formation. Conidia of *M. flavoviride* germinated 12 h post inoculation on *R. schistocercoides* cuticle (Fig. 1). Appressoria were observed at 12-18 h post inoculation before penetration, and were always formed close to the conidia (less than 6μ) (Fig. 2).

Penetration. The fungus penetrated the host cuticle without differentiating into appressoria, as germ tubes were also observed growing on the host cuticle before reaching the body cavity via spiracles and inter-segmental membranes. Similar results were obtained with *M. anisopliae* on *S. gregaria* (Gunnarsson 1988) and *M. anisopliae* on the termite *Nasutitermes exitiosus* Hill (Hänel 1982). These authors also observed germi-
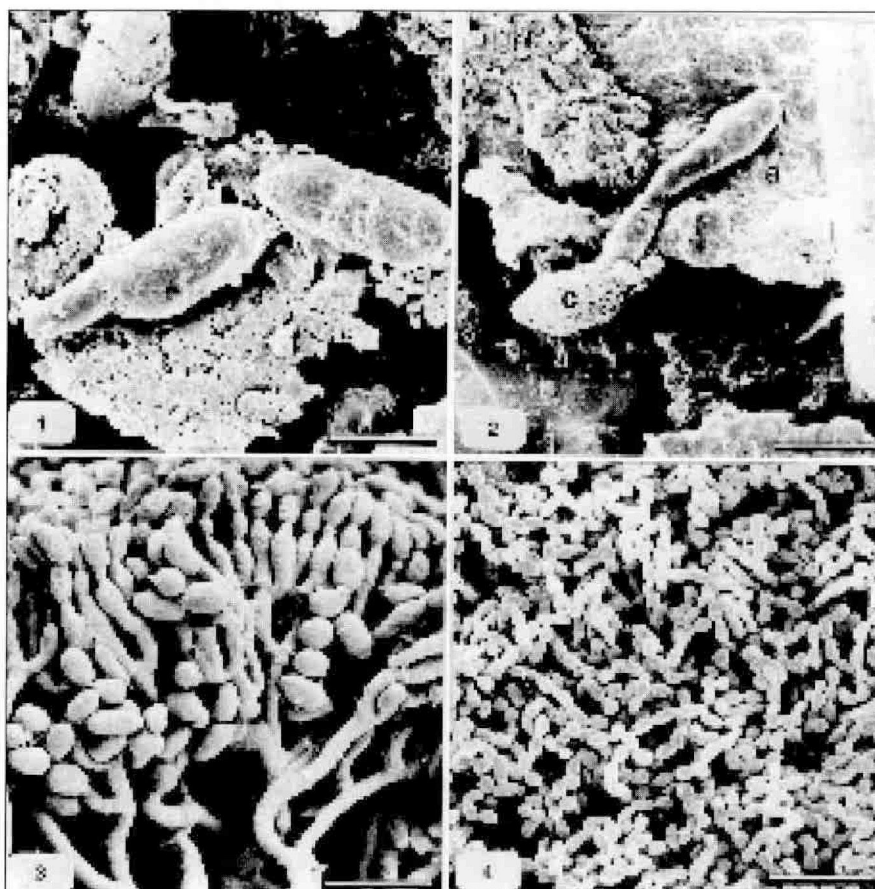
Figures 1-4. Invasion and development of *Metarhizium flavoviride* on *Rhammatocerus schistocercoides*: 1, Germinated conidia 12 h post inoculation (barr = 4μ); 2, Germinated conidium (c) and appressorium (a) (barr = 8μ); 3, Conidiophore structures of *M. flavoviride* formed in the hemocoel (barr = 8μ); 4, Conidiogenous cells bearing chains of conidia inside the hemocoel (barr = 16μ).

nation and penetration 12-18 h post inoculation.

The changes in the color of host cuticle around the penetration site recorded by Hänel (1982) were not observed in this study. However, when the infection was in an advanced stage, but before sporulation, the infected individuals presented a reddish color throughout the body surface. This probably occurred due to an increasing melanin concentration in the host tissues as a defense response to the fungal infection. We use these changes in pigmentation to confirm infection in laboratory and field bioassays.

Fungal Growth in the Hemocoel. Three to four days after inoculation, *M. flavoviride* invaded host tissues occupying the body cavity and formed conidiophores (Fig. 3), with conidiogenous cells bearing chains of conidia inside the hemocoel (Fig. 4). Some grass- hoppers were still alive at this time
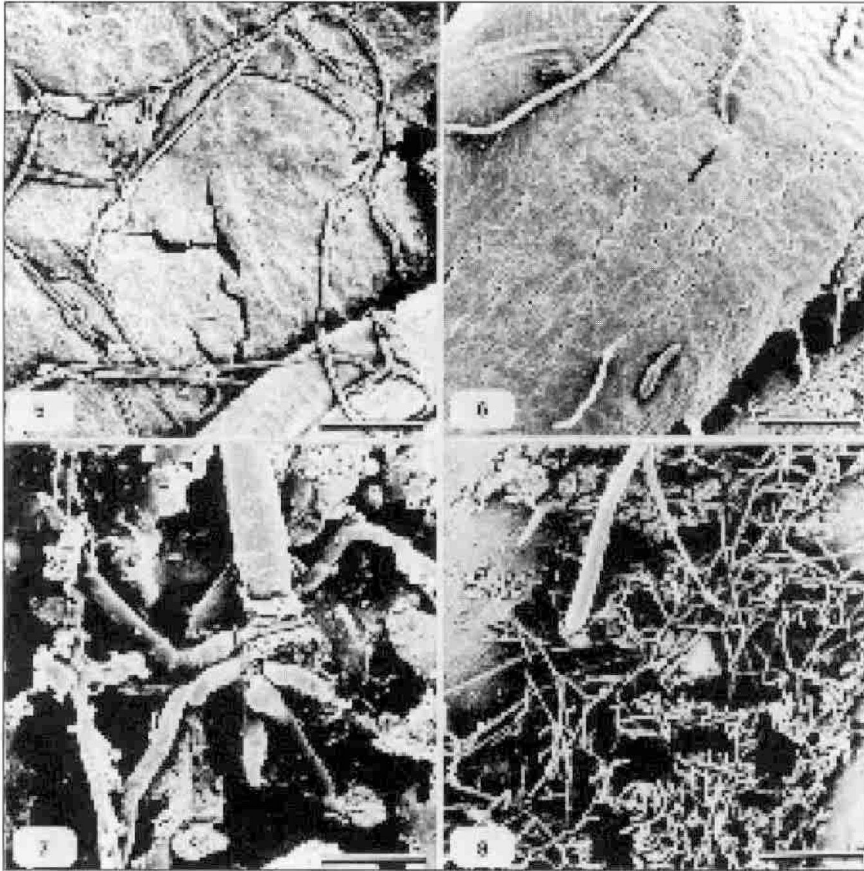
Figures 5-8. Development of *Metarhizium flavoviride* on *Rhammatocerus schistocercoides*: 5, Internal mycelia formed by germination of conidia produced in the hemocoel (barr = 25μ); 6, Hypha penetrating the host cuticle (arrow) inversely 3-4 days after inoculation (barr = 33μ); 7, Emerged hyphae from insect cuticle 4-5 days after inoculation (barr = 10μ); 8, External hyphal growth 5-6 days after inoculation (barr = 30μ).

but there was not external mycelial growth. The conidial formation in the hemocoel was also observed by Prior & Greathead (1989) in the grasshopper *Z. variegatus* in Africa.

The fast development, including internal sporulation, presented by *M. flavoviride* on *R. schistocercoides* may be related to the host nutrition and humidity. The capability of *M. flavoviride* to produce conidia in liquid environment in different nutritional concentrations was already demonstrated (Jenkins & Prior, 1993). In the same investigation, they showed that *M. aniso-*

pliae produces only blastospores and hyphal bodies. In another study, *M. flavoviride* produced 14 times more conidia when infected adults of *R. schistocercoides* were maintained at high humidity (saturated environment) as compared to low humidity (< 30% RH)(B.P. Magalhães, G. Gama & M.S. Goettel, unpublished).

Inverse Penetration and External Growth.
The infection progressed with mature conidia being released externally. These conidia germi-

nated forming hyphae (Fig. 5) to penetrate to the external host cuticle (Fig. 6) 3-4 days after inoculation. The fungus emerged from the insect cuticle 4-5 days after inoculation (Fig. 7) and grew (Fig. 8) forming a green-olive powdery mass of hyphae, conidiogeneous cells, and conidia covering the whole insect 5-6 days after inoculation.

The time needed by *M. flavoviride* (CG 423) to complete the whole cycle on *R. schistocercoides* was between five and six days after inoculation at 30°C. Similar timing (seven days) was recorded for *M. anisopliae* on *N. exitiosus* at 30°C (Hänel 1982). We observed the presence of a large number of structures similar to granulomas in the hemocoel during hyphal invasion, as recorded by Gunnarsson (1988). The pathogen also formed clamydospores in the tissues. In addition, there was an extrusion of intestinal contents by the host, possibly caused by the pathogen as a strategy to avoid opportunistic microorganisms.

The characteristics of the infection cycle of *M. flavoviride* in insects may favor the pathogen dissemination, even under dry conditions. Infected insects may act as sources of the fungus either by physical contact with other insects or by wind/rain action. However, high activity (jumping or flying) of infected individuals was scarcely observed.

Acknowledgements

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Errata. As figuras do trabalho de Vicentini e Magalhães — *An. Soc. Entomol. Brasil,* 25 (2): 309-314, 1996 — foram re-impressas. Os interessados devem solicitá-las aos autores.

Erratum. The figures of Vicentini and Magalhães — *An. Soc. Entomol. Brasil* 25 (2): 309-314, 1996 — were reprinted and, they can be requested directly from the authors.