Comunicação Científica

Occurrence of *Metarhizium flavoviride* Gams & Rozsypal (Hyphomycetes) on *Schistocerca pallens* (Thunberg) (Orthoptera: Acrididae) in Rio Grande do Norte, Brazil

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Ocorrência de *Metarhizium flavoviride* Gams & Rozsyopal (Hyphomycetes) em *Schistocerca pallens* (Thunberg) (Orthoptera: Acrididae) no Rio Grande do Norte, Brasil

RESUMO - *Metarhizium flavoviride* Gams & Rozsypal (Hyphomycetes) foi isolado de cadáveres do gafanhoto nordestino, *Schistocerca pallens* (Thunberg) (Orthoptera: Acrididae), importante praga na região, coletados em Jandaíra e Vera Cruz (RN). Este primeiro registro de *M. flavoviride* no Brasil é resultado de levantamentos periódicos de entomopatógenos visando ao desenvolvimento de bioinseticidas para o controle dessa praga. O material isolado foi registrado na coleção de fungos entomopatogênicos do CENARGEN/EMBRAPA sob o código CG 423. Análise de isoenzimas (α e β-esterase) demonstrou que o iso- lado brasileiro é distinto do isolado nigeriano IMI 330189.

PALAVRAS-CHAVE: Insecta, fungo entompatogênico, gafanhoto nordestino, controle microbiano.

The grasshopper *Schistocerca pallens* (Thunberg) is a serious pest in Northeast Brazil. Outbreaks are cyclic and usually occur following alternating raining and dry years, mainly in the State of Rio Grande do Norte, Paraiba, Pernambuco and Alagoas. Because of its high biotic potential and adaptative behavior to different habitats (sertão, agreste, and coast areas), *S. pallens is* becoming a major pest of beans, corn, cotton, sugarcane, cashew, and native and cultivated pastures. In 1992, estimated losses in these crops of 60% were attributed to *S. pallens*. In Pedro Avelino and Jandaira (RN) there were 8-10 insects/m^2, a population density considered very high.

The use of chemical insecticides is the only control measure adopted so far. However, this strategy has clearly been ineffective. In addition, it has caused problems with

the non-target populations of beneficial insects such pollinators and predators. The possible adoption of alternative strategies such as the biological control with entomopathogens is very attractive. With this in mind, we conducted systematic surveys to find pathogens of *S. pallens* in Rio Grande do Norte during the years of 1992 and 1994.

Different agroecosystems were visited and inspected for the presence of sick or dead insects showing some symptoms of disease. In Jandaira, several cadavers of *S. pallens* bearing profuse fungal conidiation were collected in a pasture cultivated with *Andropogon* sp. The insects were brought to the laboratory (CENARGEN/EMBRAPA) for fungal isolation. The pathogen was identified as *Metarhizium flavoviride* Gams & Rozsypal using arbitrarily primed PCR, although conidial morphology was intermediate between *M. flavoviride* and *M. anisopliae* (Metschnikov) Sorokin (B. P. Magalhães, M. R. Faria, M. S. Tigano & B. W. S. Sobral, unpublished).

Conidia are mostly ovoid measuring ca. 5.3 x 3.0 µm (Xavier-Santos 1995) and according to Rombach *et al.* (1996), these dimensions are typical of *M. flavoviride* var. *minus*. The colony surface on SDAY (1% soytone, 2% dextrose, 1.5% agar, and 1% yeast extract) is dark green after 5-6 days incubation at 28° C. The fungus was stored in liquid nitrogen and registered in the collection of entomopathogenic fungi (CE- NARGEN/ EMBRAPA) as isolate CG 423. Two years later (1994), during the rainy season, the same fungus was found causing high level of disease on *S. pallens* nymphs in Vera Cruz (RN) in a 20 ha area cultivated with citrus.

Isoenzyme analysis was used to distinguish the Brazilian isolate CG 423 from the *M. flavoviride* standard, the Nigerian isolate IMI 330189, which is being developed as a mycoinsecticide in Africa (Bridge *et al.* 1993). The conidia were inoculated in a complete medium prior to protein extraction. The fungus was cultivated for 48h at 28 C and 200 rpm. The mycelium was harvested by filtration, washed with sterile distilled water and broken with liquid nitrogen. The material was then resuspended in 0.5ml extraction buffer (Tris-HCl 0.5M, pH 6.8) and centrifuged at 3000 rpm for 10 minutes. The proteins were separated electrophoretically in a polyacrylamide gel (10%) using a discontinuous system. The protein extract was mixed (1:1) with sample buffer (Tris-HCl 0.5M, pH 6.8, glycerol, bromophenol blue), and 100µl of this mixture used in each electrophoresis run. Gels were run for 4h at 100 Volts at 7 C and stained for α and β -esterases according to Paccola-Meirelles *et al.* (1988). A clear variation was observed in the and -sterase profile of *M. flavoviride* isolates CG 423 and IMI 330189 (Fig. 1).

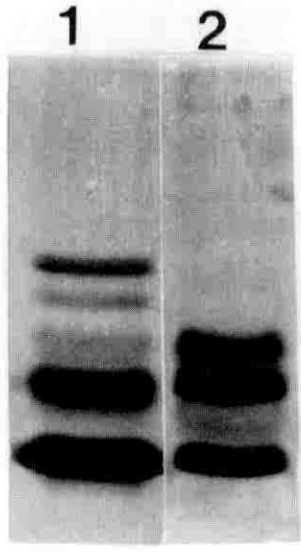

Figure 1. Variation of α and β -sterase in the isolates CG 423 (1) and IMI 330189 (2) of *Metarhizium flavoviride*.

M. flavoviride has been shown to infect *Rhammatocerus schistocercoides* (Rehn) and *Stiphra robusta* Mello-Leitão, and sporulate internally (Fig. 2) as previously observed (unpublished). Scanning electron micrograph

Figure 2. Conidiophore structures and conidia of *Metarhizium flavoviride* formed in the hemocoel of *Rhammatocerus schistocercoides* (bar = 8 μm).

was taken. The pathogenicity of *M. flavoviride* has also been tested in field conditions against $3^{rd}/4^{th}$ instar nymphs of *S. robusta* (M. Moreira, B. P. Magalhães, M. C. Chagas & M. F. Barreto, unpublished). They recorded 65% mortality and confirmed infection 13 days after treatment. These encouraging results should stimulate the search for new isolates of *M. flavoviride* associated with grasshoppers in Brazil. However, the development of this pathogen as a bioinsecticide relies on more in depth studies so that the final product can offer high virulence, stability, and persistence under field conditions.

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References Cited

Bridge, P.D., M.A.J. Williams, C. Prior & R.R.M. Paterson. 1993. Morphological, biochemical and molecular characteristics of *Metarhizium anisopliae* and *M. flavoviride*. J. Gen. Microbiol. 139: 1163--1169.

Paccola-Meireles, L.D., M.J. Valarini, J.L. Azevedo & A.C. Afenas. 1988. Manual de técnicas eletroforéticas em microrganismos. Piracicaba, SP, Ed. FEALQ, 54p.

Rombach, M.C., R.A. Humber & D.W. Roberts. 1986. *Metarhizium flavoviride* var. *minus*, var. nov., a pathogen of plant- and leafhoppers on rice in the Philippines and Solomon Islands. Mycotaxon 27: 87-92.

Xavier-Santos, S. 1995. Aspectos do comportamento cultural, citológico e entomopatogênico de *Metarhizium flavoviride*. Tese de mestrado, UFPE, Recife, 108p.

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