SCIENTIFIC NOTE

Infectivity of *Metarhizium flavoviride* Gams & Rozsypal (Deuteromycotina: Hyphomycetes) Against the Grasshopper *Schistocerca pallens* (Thunberg) (Orthoptera: Acrididae) in the Laboratory

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Infectividade de *Metarhizium flavoviride* Gams & Rozsypal (Deuteromycotina: Hyphomycetes) ao Gafanhoto *Schistocerca pallens* (Thunberg) (Orthoptera: Acrididae) em Laboratório

RESUMO - O gafanhoto *Schistocerca pallens* (Thunberg) (Orthoptera: Acrididae) tem causado prejuízos em diversas culturas no Brasil e seu controle tem sido à base de inseticidas químicos, o que, freqüentemente, resulta em efeitos indesejáveis, trazendo sérios prejuízos ambientais e econômicos. O fungo entomopatogênico *Metarhizium flavoviride* (Gams & Rozsypal), candidato potencial ao controle de acridídeos em vários países, foi isolado de *S. pallens* no Nordeste do Brasil. Desde então, o patógeno tem sido estudado visando ao seu desenvolvimento como bioinseticida contra *Rhammatocerus schistocercoides* (Rehn), *S. pallens* e *Stiphra robusta* Mello-Leitão, que são os principais gafanhotos-praga do Brasil. Em testes realizados em condições de laboratório, aplicações tópicas de *M. flavoviride* (isolado CG 423), formulado em suspensão oleosa com diferentes concentrações de conídios (9.000 – 21.000 conídios/inseto), causaram elevada mortalidade (≥ 86%) em adultos de *S. pallens*. Não houve mortalidade no grupo testemunha. Dentre as doses de conídios utilizadas, não houve diferença significativa quanto ao tempo médio de sobrevivência dos insetos (6,2 a 6,9 dias). Esses resultados evidenciaram que *M. flavoviride* apresenta alta virulência a esse hospedeiro, o que estimula a intensificação de pesquisas visando a sua utilização para o controle biológico de *S. pallens*.

PALAVRAS-CHAVE: Insecta, patogenicidade, controle microbiano, fungo entomopatogênico.

The pallid grasshopper, *Schistocerca pallens* (Thunberg), is a serious pest of rice, corn and sugar cane in the Northeast Brazil mainly in the States of Pernambuco, Paraíba, Rio Grande do Norte and Alagoas. Monitoring in different locations of Rio Grande do

Norte showed that this insect has one generation per year and that its biology is affected by climatic conditions. It goes into diapause in the adult stage during periods of high temperatures, low humidity and scarcity of food (Chagas *et al*. 1995). Untill now, *S. pallens* has been observed in a solitary phase, but there is a possibility that this species may also have a gregarious phase (Cosenza *et al.* 1994). This would make it a much more destructive pest. The control of grasshoppers has been based exclusively on chemical insecticides. These products are non specific for acridids and they have caused harm to the environment, reducing population of beneficial organisms and causing intoxication in human populations nearby the application areas (Milner 1997, Prior & Streett 1997). For these reasons, the implementation of alternative control measures is highly desirable.

The main natural enemies of grasshoppers are birds, parasitoids, nematodes and microorganisms. Some entomopathogens are able to cause natural epizootics in grasshoppers and are amenable to mass production and application. Among these microorganisms, the hyphomycetous fungi are promising candidates for grasshoppers control, because they penetrate directly through the host cuticle and can be applied in the same way as chemical insecticides (Prior & Greathead 1989, Goettel *et al.* 1995). The entomopathogenic fungus *Metarhizium flavoviride* Gams & Rozsypal has been studied as a potential agent for the control of grasshoppers in Africa and Australia, with good results (Moore *et al*. 1992, Prior *et al*. 1992, Bateman *et al*. 1992, 1993, Milner 1997). This fungus was isolated from *S. pallens* in Rio Grande do Norte, Brazil, in 1992 (Moreira *et al.* 1996). Ever since, there has been an increased interest in exploiting it as a bioinsecticide against grasshoppers, and studies involving cytology, growth and the invasion process have already been performed in Brazil (Xavier-Santos 1995, Vicentini & Magalhães 1996, Magalhães *et al.* 1996). We report here on the capacity of *M. flavoviride* to cause infection in *S. pallens*.

The isolate CG 423 of *M. flavoviride* used in this study was obtained from the Embrapa-Cenargen Collection of Entomopathogenic Fungi and its viability was assayed previously (85% germination, 12 h after seeding and incubation at 28ºC). Adults of *S. pallens* were obtained from a laboratory colony at Embrapa-Cenargen. Insects were kept in nylon cages (17x25x21cm) at 28°C and photophase of 12 h, and were fed grass (*Andropogon* sp.), rabbit food and oats flakes (Quaker®). Cages were cleaned daily. Under these conditions, an adaptation time of three days was adopted before running the experiment. Each insect (n = 15, three replicates/treatment) was topically inoculated in the pronotum region with 3 ml of a conidial formulation (95% soybean oil Liza®, 5% kerosene Bandeirante®) at the following doses: 9,000, 12,000, 15,000, 18,000 and 21,000 conidia/insect. Insects used as control received 3 ml of the formulation containing only soybean oil and kerosene. To confirm infection, dead insects were collected daily and transferred to humid chambers. After sporulation, conidial samples were examined using an optical microscope to confirm the presence of *M. flavoviride*. For the statistics, only the dead insects exhibiting external sporulation were considered. Statistics were performed using Polo-PC (Le Ora Software), SigmaStat™ and SigmaPlot™ (Jandel Scientific, Corte Madera, CA, USA). The average values between groups of treatments were compared using Kruskall-Wallis ANOVA on ranks.

M. flavoviride was able to infect *S. pallens*, producing high levels of mortality (≥ 86%) in all treatments (Fig. 1). Mortality started at the 6 th day after inoculation. The average survival time varied from 6.1 to 6.7 days and there were no significant differences between the doses tested (Table 1). The mean lethal dose (LD_{50}), at nine days was 4,765 conidia/insect. Another isolate of *M. flavoviride* (FI 985, ARSEF/Austrália) was studied by Milner *et al*. (1996) against the grasshopper *Locusta migratoria* (L.) with similar results (LD_{50} = 4,363 conidia/insect). However, *Austracris guttulosa* (Walker) was more susceptible to

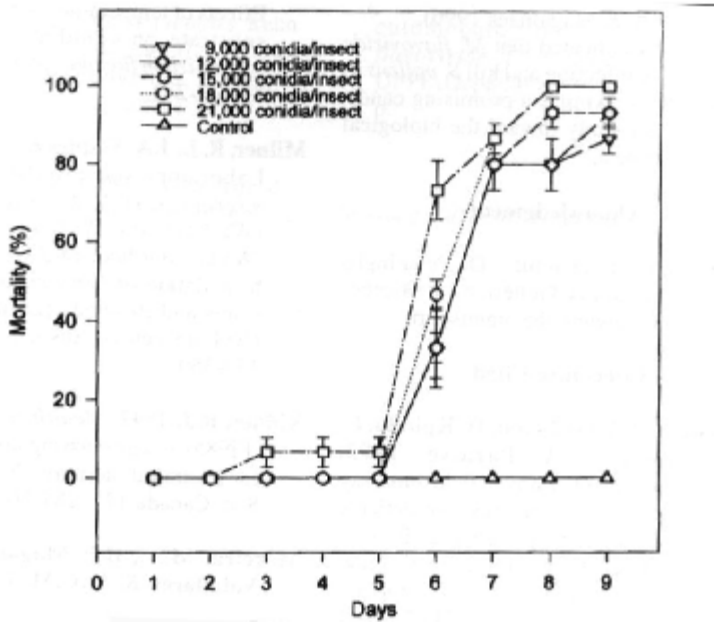

Figure 1. Cummulative daily mortality of *S. pallens* adults treated with different doses of an oil formulation containing conidia of *M. flavoviride* (isolate CG 423).

the fungus with a LD_{50} of 413 conidia/insect at nine days. In the adult stage, *S. pallens* shows a typical brownish color. However, following its death caused by *M. flavoviride*, its body exhibits a reddish color. This was also observed in *Rhammatocerus schistocercoides*

Table 1. Mortality and survival time (average ± SE) of *S. pallens* adults treated with conidia of *M. flavoviride* (isolate CG 423) formulated in soybean oil containing 5 % kerosene.

Treatment (conidia/insect)	Mortality with confirmed infection (%)	Survival time (days)
9,000	86.0 ± 3.8	6.5 ± 0.3
12,000	93.3 ± 3.8	6.7 ± 0.4
15,000	93.3 ± 3.8	6.5 ± 0.3
18,000	100	6.1 ± 0.5
21,000	100	6.1 ± 0,3
Control	0	-

There was no significant difference between treatments in terms of mortality (P=0.97) and survival time (P=0.43), according to Kruskall-Wallis ANOVA on Ranks. Data represent a mean of three experiments.

Rehn (Vicentini & Magalhães 1996).

The results indicated that *M. flavoviride* is able to cause infection and kill *S. pallens* in the laboratory making it a promising candidate for further study toward the biological control of this pest.

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