

Nomuraea rileyi (Hypocreales: Clavicipitaceae) in *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae in Brazil

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Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is a prominent polyphagous pest in many global agricultural systems. The larvae of this insect feed on different types of crops such as tomato, soybean, corn, and cotton (Tay et al. 2013). Due to its high reproductive rate, high voracity, high dispersal rate, and resistance development against insecticides (Yang et al. 2013), *H. armigera* causes economic and environmental problems that have been estimated to result in a loss of more than \$2 billion annually worldwide (Tay et al. 2013). In Brazil, *H. armigera* was recently reported for the first time in the Bahia, Goiás, and Mato Grosso States on cotton and soybean crops (Czepak et al. 2013; Specht et al. 2013). Monitoring by pheromone traps and applying insecticides, natural enemies, and resistant cultivars expressing *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Bt) proteins may help prevent outbreaks of this pest (Czepak et al. 2013).

Resistance of *H. armigera* to the major insecticides and cultivars expressing Bt proteins used for its management has been reported previously (Yang et al. 2013). This resistance reduces the efficacy of these technologies and can lead to pest resurgence, reduced populations of natural enemies, and increased infestation of crops (Vianna et al. 2009; Castro et al. 2012; Menezes et al. 2013). Entomopathogenic fungi can control pests, and *Beauveria* spp., *Metarhizium* spp., and *Nomuraea* spp. (Hypocreales: Clavicipitaceae) are considered worldwide for control of *H. armigera* (Nguyen et al. 2007; Hatting 2012; Wakil et al. 2013). The integrated management of *H. armigera* should include identification of natural enemies in the field. The objective of this study was to report the occurrence of an entomopathogenic fungus as a natural enemy of *H. armigera* in Brazil.

In Apr 2014, 589 larvae were collected from cotton crops in the municipality of Luís Eduardo Magalhães in Bahia, Brazil (12°06'39"S, 45°50'08"W, and 760 m altitude), and transported to the laboratory of biological control of Embrapa Milho e Sorgo in Sete Lagoas, Minas Gerais, Brazil. These larvae were placed in plastic cups (50 mL volume) containing artificial diet (5 × 5 × 1 cm) based on white beans (Greene et al. 1976), held in an acclimatized room (temperature: 25 ± 2 °C, relative humidity: 70 ± 10%, photoperiod: 12:12 h L:D), and monitored daily.

During 38 d of observation in the laboratory, 320 collected larvae died; of these, 203 died from unknown factors (63.4%), 106 due to fungal diseases (33.1%), and 11 due to parasitism (3.4%). Deaths due to unknown factors were attributed to natural mortality, transport, adaptation to the laboratory, and poisoning by insecticides that had been applied before the collection. Larvae with fungal disease symptoms were placed in Petri dishes lined with filter paper moistened daily to facilitate pathogen sporu-

lation (Fig. 1) (Tang et al. 1999). After sporulation, spores were sampled from the dead larvae's bodies with a sterile needle and observed under a microscope for preliminary identification of the fungus. Another portion of the spores were spread on Petri dishes containing solid Sabouraud maltose medium (10 g maltose, 2.5 g peptone, 2.5 g yeast extract, and 4 g agar added to 250 mL distilled water). Inoculated Petri dishes were incubated at 25 °C, 75% relative humidity, and a photoperiod of 16:8 h L:D for 21 d. Upon sporulation, the entomopathogenic fungus was identified as *Nomuraea rileyi* (Farl.) Samson (Hypocreales: Clavicipitaceae) based on symptomatology, spores, and conidiophores by Dr. Rogerio Biaggioni Lopes of Embrapa Recursos Genéticos e Biotecnologia (Cenargen), Brazil.

Nomuraea rileyi is known to infect and cause mortality in insects (Hatting 2012; Wakil et al. 2013), with several *N. rileyi*-lepidopteran associations reported from the South American continent. These include *Anticarsia gemmatilis* Hübner, *Trichoplusia ni* Hübner, and *Alabama argillacea* Hübner (Lepidoptera: Noctuidae) (Corrêa & Smith 1975; Alves et al. 1978; Villani et al. 1984; Sujii et al. 2002). However, this is the first report on the natural occurrence in Brazil of *N. rileyi* infecting larvae of *H. armigera*, which is an exotic pest introduced in South America. Control methods based on entomopathogenic fungi were not previously used in the collection area. Thus, the high humidity recorded in the days preceding the collections may have favored the development of fungal infections naturally present in the environment. Tests to evaluate the biological activity of this *N. rileyi* isolate in healthy larvae are being developed to determine its pathogenicity. This fungus may have potential to be used for the control of *H. armigera* in Brazil.

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Summary

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is one of the most important polyphagous pests globally. It was reported in Brazil at the end of the 2012/2013 crop season. The aim of this study was to report the occurrence of an entomopathogenic fungus

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Fig. 1. *Helicoverpa armigera* larvae collected from cotton plantations in Bahia, Brazil. A: Larva on cotton bud; B, C: signs caused by *Nomuraea rileyi* on *H. armigera* larvae; D: light micrograph of the *N. rileyi* spores observed at 400-fold magnification.

on *H. armigera* larvae in Brazil. We collected 589 larvae from cotton plantations in Bahia State, Brazil, and transported them to the laboratory of biological control of Embrapa Milho e Sorgo in Sete Lagoas, Minas Gerais State, Brazil. Of the 320 dead larvae, 106 were infected by *Nomuraea rileyi* (Farl.) Samson (Hypocreales: Clavicipitaceae), causing 33.1% of the total mortality. This is the first report on the natural occurrence of the fungus *N. rileyi* infecting *H. armigera* larvae in Brazil.

Key Words: biological control; cotton; damage; entomopathogenic fungus

Sumário

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) é uma das pragas polífaras mais importantes do mundo e foi registrada no Brasil no final da safra 2012/2013. O objetivo foi relatar a ocorrência de um

fungo entomopatogênico em lagartas de *H. armigera* no Brasil. Um total de 589 lagartas foi coletado em plantios de algodoeiro no estado da Bahia, Brasil e transportadas para o laboratório de Controle Biológico da EMBRAPA Milho e Sorgo em Sete Lagoas, estado de Minas Gerais, Brasil. Das 320 lagartas mortas, 106 foram infectadas por *Nomuraea rileyi* (Farl.) Samson (Hypocreales: Clavicipitaceae), representando 33,1% da mortalidade total. Este é o primeiro relato da ocorrência natural do fungo *N. rileyi* em lagartas de *H. armigera* no Brasil.

Palavras Chave: algodão; controle biológico; dano; fungo entomopatogênico

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