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Potential of Amazonian isolates of *Metarhizium* to control immatures of *Bactrocera carambolae* (Diptera: Tephritidae)

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The carambola fruit fly (*Bactrocera carambolae* Drew & Hancock; Diptera: Tephritidae) is a quarantine pest present in Brazil, with its distribution restricted to the states of Amapá and Roraima, located in the Brazilian Amazon. Since its detection in 1996, *B. carambolae* has been controlled officially by the Ministry of Agriculture, Livestock and Food Supply (Brazil 2013; Silva et al. 2015; Moraes et al. 2016). In Brazil, it is currently among the pests with the highest impact on the fruit industry and is considered the main phytosanitary barrier to fruit exportation (Godoy et al. 2011; Ferreira & Rangel 2015). The potential dispersion of *B. carambolae* into Brazilian regions where fruits are grown for exportation would bring considerable commercial losses, with negative effects to income generation, employment, and rural development within Brazilian agribusiness (Brazil 2007). The environmental impacts associated with the presence of this pest should also be considered, as the main current strategy to control *B. carambolae* is based on chemical insecticides, with proven harmful effects on natural resources and non-target organisms (Nascimento & Carvalho 2000; Godoy et al. 2011).

Prevalent methods for the control of *B. carambolae* include the use of toxic baits containing spinosad, as well as the male annihilation technique, which uses blocks of fiberboard dipped in a solution containing the parapheromone methyl eugenol and the organophosphate insecticide malathion (Godoy et al. 2011). Although these methods are efficient, it has become necessary to phase out the use of malathion, given the known environmental and toxicological impacts of organophosphates (Aktar et al. 2009; Lionetto et al. 2013).

Biological control using entomopathogenic fungi is an alternative for the integrated management of fruit flies (Yousef et al. 2013). This study presents the possibility of using *Metarhizium* Sorokin to control immature forms of *B. carambolae*. This is groundbreaking work, as there is no record in the literature of this pest species being controlled with this entomopathogenic fungus.

In this work, we used 2 isolates of *Metarhizium* obtained from the soil of urban orchards in the municipalities of Macapá (0.02°S, 51.1°W) and Oiapoque (3.83°N, 51.8°W), in the state of Amapá, Brazil. To obtain the isolates, 3rd-instar larvae of *B. carambolae* were used as bait. After isolation procedures and colony purification were complete, the isolates were identified through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF

MS) (Lopes et al. 2014). The isolate from Macapá was identified as *Metarhizium anisopliae* (Metsch.) Sorokin, and the isolate from Oiapoque was identified as *Metarhizium robertsii* J.F. Bisch., Rehner & Humber. Freeze-dried and cryopreserved samples of the isolates are stored in the Collection of Fungi of Invertebrates at Embrapa Recursos Genéticos e Biotecnologia, under the codes CG1313 (*M. anisopliae*) and CG1314 (*M. robertsii*).

To obtain conidia of *M. anisopliae* and *M. robertsii*, we used the culture media Sabouraud dextrose agar and potato dextrose agar, respectively. The isolates were cultivated for 28 d in a B.O.D. growth chamber at 26 ± 2 °C in darkness. After the growth period, 15 mL of sterilized distilled water with 0.1% TWEEN® 80 were added to each plate for conidia retrieval. The concentrations of the conidial suspensions were adjusted to 1×10^8 conidia per mL, estimated in a Neubauer Chamber. To the final suspension of each isolate, we added 0.1% TWEEN® 80 and 2% AGRAL® (spreading agent).

The study was performed on 2 substrates (sterile soil and vermiculite), each treatment was repeated 10 times, and each repetition included 5 third-instar larvae of *B. carambolae* reared at the Plant Protection Laboratory of Embrapa Amapá. The soil used was obtained from an urban orchard in Macapá, state of Amapá. The amounts of substrates used in each repetition were 160 g of sterile soil and 30 g of vermiculite. The substrates were poured into crystal polystyrene boxes ($11 \times 11 \times 3$ cm), in a layer approximately 1.5 cm thick.

The substrates were sprayed with 2.5 mL of the conidial suspension of 1×10^8 conidia per mL in sterile aqueous solution with 0.1% TWEEN® 80 and 2% AGRAL®. For the control treatments, the substrates were sprayed with 2.5 mL of sterile aqueous solution with 0.1% TWEEN® 80 and 2% AGRAL®. The larvae were subsequently placed on the substrates to allow for pupation. The substrates were moistened on a daily basis with sterile water to maintain a high water potential.

Assessments were based on counting the number of emerged adults of *B. carambolae*. Mortality was calculated as the difference between total number of larvae and total number of emerged adults. Arcsine square root transformation was used to calculate percentage of mortality. Analysis of variance and Tukey's test at 5% probability were performed on the data. The statistical analyses were performed using the R statistics software (R Core Team 2014).

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The isolate of *M. anisopliae* caused greater mortality of *B. carambolae* immatures than the isolate of *M. robertsii*, in both substrates (Table 1). Both isolates caused higher mortality when applied on vermiculite, possibly because this substrate retains more moisture when compared with soil, favoring survival and development of the entomopathogen. The present work did not include an assessment of the mortality of *B. carambolae* adults after emergence. However, preliminary observations in the laboratory indicated that adults emerged from substrates treated with the isolates of *Metarhizium* had an ephemeral life span (around 7 d) when compared with adults emerged from untreated substrates, whose mean longevity is more than 45 d (Morais et al. 2016). Some prematurely dead specimens were placed in a moist chamber and, after 7 d, were observed to be completely colonized externally by the entomopathogenic fungus. This finding indicates that mortality caused by the entomopathogen may be significantly higher than that reported here.

Yousef et al. (2013), in a study on the control of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) with the entomopathogenic fungus *Metarhizium brunneum* Petch (a synonym for *M. anisopliae*) in sterilized soil, obtained a significant effect on the mortality of the insects, as 82.3% of larvae in treated soil did not reach adulthood, whereas 64.5% of control group larvae emerged as adults. On the other hand, it should be noted that mortality of immatures in that study was high even in the control treatment (35.5%), which did not occur in our study (Table 1).

In a study conducted by Gul et al. (2015), *M. anisopliae* was also assessed for its potential to control larvae and pupae of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) in sterile soil. At a concentration of 1×10^8 conidia per mL, adult mortality was 5%. This mortality rate is lower than that obtained in this study, as our treatment involving *M. anisopliae* in sterile soil resulted in an adult mortality of 36% (Table 1).

Finally, the economic and environmental implications associated with the carambola fruit fly should be considered a good justification for any investment in alternatives to suppress populations of this pest. In that context, our results show the effectiveness of *M. anisopliae* as a measure to control immatures of *B. carambolae*, suggesting the potential use of this isolate to reduce the population of this pest by application of the fungus to the soil.

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Summary

This study shows the potential of Amazonian isolates of *Metarhizium* Sorokin to control immatures of *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae), a quarantine pest present in Brazil. Our

Table 1. Mean percentage of mortality of immatures of *Bactrocera carambolae* on substrates treated with *Metarhizium anisopliae* or *Metarhizium robertsii*.

Treatment	Substrate	
	Vermiculite (mean % \pm SD)	Sterile soil (mean % \pm SD)
Control	6.00 \pm 3.06 aC	10.00 \pm 2.98 aB
<i>M. anisopliae</i>	54.00 \pm 6.00 aA	36.00 \pm 7.18 bA
<i>M. robertsii</i>	30.00 \pm 6.15 aB	14.00 \pm 4.27 bB

Mean values followed by the same lowercase letter in the same row or the same uppercase letter in the same column do not differ significantly, based on Tukey's test at 5% probability.

Treatment: $F = 22.74$; $df = 2,54$; $P < 0.001$

Substrate: $F = 3.15$; $df = 1,54$; $P = 0.082$

Treatment*Substrate: $F = 3.59$; $df = 2,54$; $P = 0.034$

results reveal the effectiveness of an isolate of *Metarhizium anisopliae* (Metsch.) Sorokin to control immatures of *B. carambolae*, suggesting the potential for direct application of this isolate onto the soil to reduce populations of the pest.

Key Words: carambola fruit fly; quarantine pest; entomopathogenic fungi

Sumário

Este estudo mostra a potencialidade de isolados amazônicos de *Metarhizium* Sorokin para o controle de formas imaturas de *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae), praga quarentenária presente no Brasil. Nossos resultados mostraram a eficácia do isolado de *Metarhizium anisopliae* (Metsch.) Sorokin no controle dos imaturos de *B. carambolae*, sugerindo sua aplicação diretamente no solo para reduzir a população da praga.

Palavras Chave: mosca-da-carambola; praga quarentenária; fungos entomopatogênicos

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