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Pathogenicity of *Beauveria bassiana* (Deuteromycota: Hyphomycetes) to the red cashew beetle *Crimissa cruralis* (Coleoptera: Chrysomelidae: Galerucinae: Blepharida-group) in laboratory conditions

Patogenicidad de *Beauveria bassiana* (Deuteromycota: Hyphomycetes) al escarabajo rojo anacardo (Coleoptera: Chrysomelidae: Galerucinae: grupo Blepharida) en condiciones de laboratorio

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Abstract: The increased pest actions of the red cashew beetle *Crimissa cruralis* (Coleoptera: Chrysomelidae in Brazilian populations of cashew *Anacardium occidentale* (Anarcardiaceae) is a cause for concern. The entomopathogenic fungus, *Beauveria bassiana*, was evaluated as a biocontrol of larvae. The percent mortalities of second and third instar larvae produced by *in vivo* application of a commercial formulation containing the fungal spores were evaluated. Treatment of larvae at concentrations of 1.2 x 10^6, 2.5 x 10^6, 3.7 x 10^7 e 5.0 x 10^7 conidia/mL resulted in 49, 56, 66 and 72 % mortality within eight days, respectively. Based on the finding, it is recommended that the *B. bassiana* formulation should be employed at the lowest concentrations (1.2 x 10^6 and 2.5 x 10^6 conidia/mL) with repeat application to avoid possible inactivation of the fungus by abiotic environmental factors. Cost-benefit assessments of the use of this biological agent will be important for determining the economic feasibility of its application in the field.

Keywords: Entomopathogenic fungus, controlled conditions, biological product, caju.

Resumen: El aumento de la frecuencia de lo escarabajo rojo de la anacardiácea *Crimissa cruralis* (Coleoptera: Chrysomelidae) en poblaciones de *Anacardium occidentale* (Anarcardiaceae) del Brasil es motivo de preocupación. Se evaluó la mortalidad (en larvas de segundo y tercer instar) producida por la aplicación *in vivo* de una formulación comercial con esporas del hongo entomopatógeno, *Beauveria bassiana*. El tratamiento de larvas con concentraciones de 1,2 x 10^6, 2,5 x 10^6, 3,7 x 10^7 e 5,0 x 10^7 conidia/Ml resultó en 49, 56, 66 y 72 % de mortalidad dentro de ocho días, respectivamente. Se recomienda emplear la formulación de *B. bassiana* en las concentraciones más bajas (1,2 x 10^6 y 2,5 x 10^6 conidia/mL) con aplicación repetida para evitar la posible inactivación de lo hongo por factores abióticos ambientales. Las evaluaciones de costo-beneficio del uso de este agente biológico serán importantes para determinar la viabilidad económica de su aplicación en el campo.

Palabras clave: Hongo entomopatógeno, condiciones controladas, producto biológico, cajú.

Introduction

In 2017, the cultivated area of cashews, *Anacardium occidentale* Linnaeus (Anacardiaceae), in Brazil was 575,779 ha with a production of 99,073 tons of cashew nut extraction. The northeastern of Ceará, Rio Grande do Norte and Piaui States together produced approximately 86,450 tons, which corresponds to 87.3 % of the national production (Instituto Brasileiro de Geografia e Estatística 2017) of Brazil.

The red cashew beetle, *Crimissa cruralis* Stal (Coleoptera: Chrysomelidae: Galerucinae: Blepharida-group), is considered to be a secondary pest in these cashew producing States and controlled naturally by the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) (Mesquita and Braga Sobrinho 2014). In recent years, the insect pest has often occurred in the municipality of Pacajus, State of Ceará. This is probably due to the low rainfall recorded which has been providing inadequate relative humidity conditions for the development of the entomopathogenic fungus in the soil (Alves 1998), thus resulting in a significant increase in the population of the insect.

In the field, *C. cruralis* mating occurs at any time of the day. Females oviposit in the cracks or holes in the trunk of the plants. Shortly after oviposition, the females secrete a substance that covers the entire surface of the eggs that remain in contact with the external environment outside the slit (Fig. 1A). After embryonic development, the larvae hatch and move toward the plant canopy in search of leaves for feeding (Fig. 1B). The larva starts feeding, causing a yield in the leaf production. Throughout its development, the larvae's feeding activity culminates with the defoliation of the plant (Fig. 1C). In its last larval instar, the larva descends through the trunk to the soil, where it becomes a pupa. The pupa will remain in the soil until the emergence of adults (Figs. 1D-1E).

The entomopathogenic fungus, *B. bassiana*, can be employed as a biological control agent for management of immature and adult forms of *C. cruralis* (Mesquita *et al.* 2009); however, no information is available regarding the effects of the commercial *B. bassiana*-based products on *C. cruralis*. Therefore, the aim of the present study was to

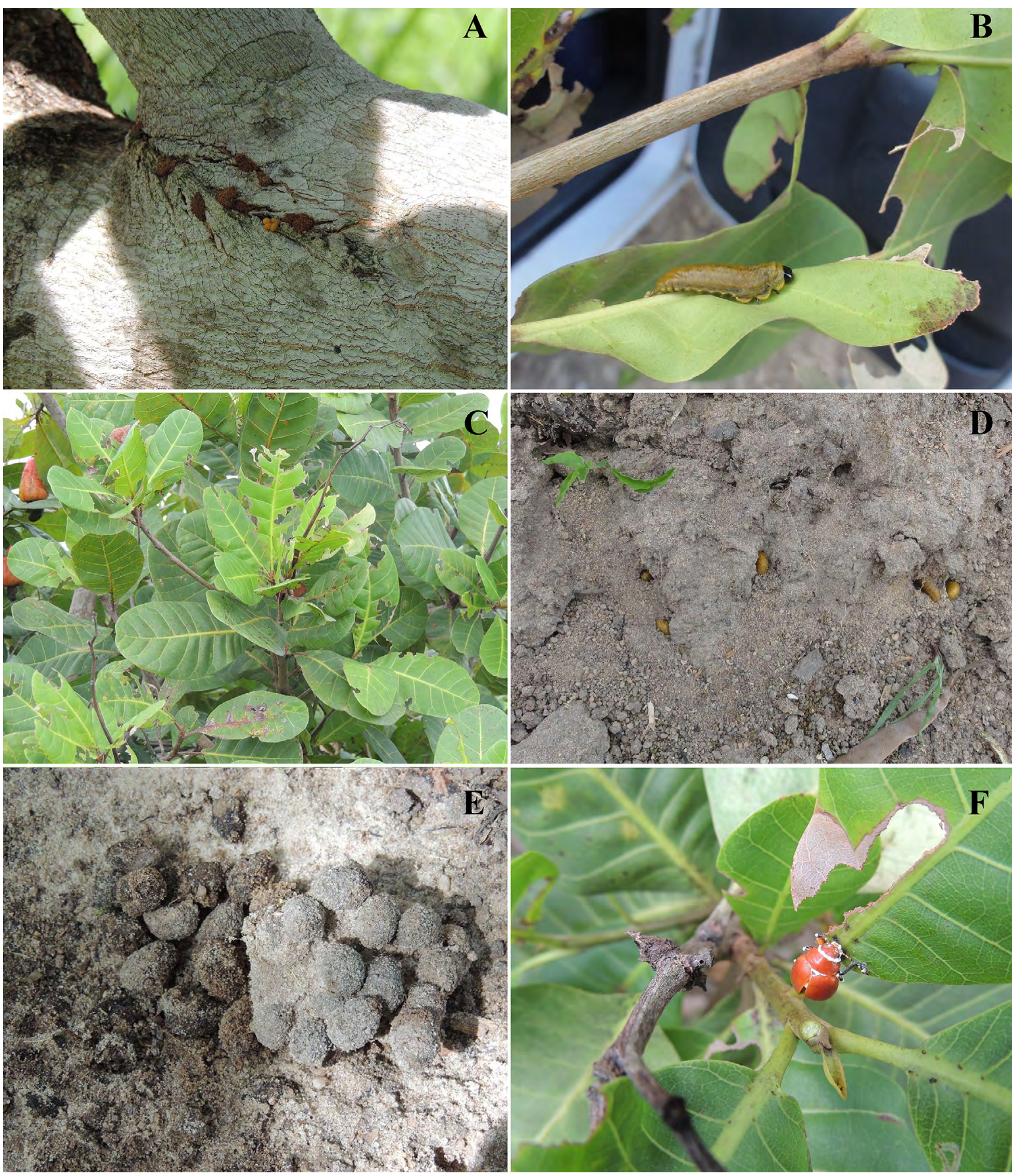

Figure 1. A. *Crimissa cruralis* eggs in dwarf cashew trunk cracks. **B.** Larvae of the *C. cruralis*. **C.** Defoliation in dwarf cashew plants. **D.** Pre-pupae along the dwarf cashew trunk in the soil. **E** and **F.** Pupae in the soil and adult of the *C. cruralis* infected by *Beauveria bassiana*.

evaluate the outcomes of *in vivo* applications of a commercial formulation of *B. bassiana* on larvae of *C. cruralis*.

Materials and methods

Larvae of *C. cruralis* were collected from cashew trees cultivated in the experimental field of "Embrapa Agroindústria Tropical" (Pacajus, CE, Brazil: 4°10'35"S, 38°28'19"W; altitude 79 m). Second and third instar larvae were separated based on their size (1 cm) and placed individually into glass tubes (diameter 8.5 x 2.5 cm) containing new cashew leaves. The rearing conditions for the larvae were 26 ± 2 °C, 50 ± 10 % relative humidity under a 12 h photophase and leaf material was replaced daily.

The commercial formulation Ballvéria® (Ballagro Agro Tecnologia, Bom Jesus dos Perdões, SP, Brazil) employed in the experiment is a wettable powder containing spores of *B. bassiana* strain IBCB66. Powdered bioinsecticide was diluted in distilled water (0.02, 0.1, 1.0 and 2.0 g/L) and a 1.0 mL dose was sprayed over 5 larvae with the help of a micro-paint air compressor Wimpel ® (15-25 PSI). Pure distilled water was used as the negative control. Following application of the treatments, larvae were transferred to Petri dishes (5 cm diameter) and maintained at 26 ± 2 °C and 50 ± 10 % relative humidity in a biochemical oxygen demand incubator.

Mortality was defined as the total immobilization of larvae and observations were carried out every 24 h over 8 consecutive days. In order to confirm the death was caused by *B. bassiana*, dead larvae were immersed in 70 % ethanol for 60 s to remove external contaminants and placed in sterile Petri dishes (5 cm diameter) lined with damp filter paper and closed with plastic film for eight days to allow fungal sporulation. Thus, the percentage of dead larvae in each treatment that presented *B. bassiana* spores in their tegument was considered to be the confirmed mortality rate.

The experimental design was fully randomized and consisted of five treatments and five replicate dishes each with five larvae/Petri dish. The confirmed percent mortality caused by each of the bioinsecticide concentrations was corrected using Abbott's formula (Abbott 1925), and the significant differences amongst treatments were determined using Kruskal-Wallis test ($\alpha = 0.05$). Statistical analyses were carried out using PAST software (Hammer *et al.* 2001).

Results and discussion

Treatment of larvae with the commercial formulation of *B. bassiana* at the lowest concentration tested (1.2×10^6 conidia/mL) resulted in 49 % mortality of *C. cruralis* over eight days. The confirmed mortality percentage increased progressively with greater concentration of the bioinsecticide and attained 56, 66 and 72 %, respectively, at 2.5×10^6, 3.7×10^7 e 5.0×10^7 conidia/mL, giving an overall increase in mortality of 1.46-fold between the lowest and highest concentrations employed (Table 1).

Table 1. Percentage confirmed mortality of second and third instar larvae of *C. cruralis* (Coleoptera: Chrysomelidae) caused by different concentrations of *B. bassiana*- based product (Ballvéria®).

Concentrations (Con./mL)	Mortality (%)		
	Total	Confirmed	Corrected
Control	18.0 ± 8.0 a	0.0 ± 0.0 a	-
1.2×10^6	55.0 ± 8.0 b	49.0 ± 8.0 b	37.6 ± 13.0 a
2.5×10^6	56.2 ± 7.0 b	56.2 ± 7.0 b	47.3 ± 11.0 a
3.7×10^7	66.0 ± 11.0 b	66.0 ± 11.0 b	60.3 ± 13.0 a
5.0×10^7	81.0 ± 9.0 c	72.0 ± 10.0 c	69.3 ± 9.0 a
P-value	0.009	0.009	0.46

Means followed by the same letter, in the column, do not differ among themselves by the Kruskal-Wallis test, at 5 % probability.

Associated with the main pests of the cashew tree there is a rich fauna of beneficial insects (predators and parasitoids) and entomopathogenic fungi. Among the entomopathogenic mitosporic fungi is *B. bassiana* which naturally infects *C. cruralis* (Mesquita and Braga Sobrinho 2014).

Despite the natural occurrence of this fungus, the introduction of commercial formulation of *B. bassiana* in the field would be interesting for achieving an effective control when populations of this pest reach higher levels. We suggest that the successful control of *C. cruralis* infestation on cashew trees could be carried out using the commercial formulation of *B. bassiana* at the lowest concentration tested (i.e. 1.2×10^6, 2.5×10^6 conidia/mL) but with repeated applications. Thus, when the biocontrol is introduced into the field and the pest comes into contact with the fungus, even in low concentration, there is a possibility of causing insect mortality. This strategy is based on the acceptable mortality rate (49 to 56 %) induced by the two concentrations of insecticide and the necessity to prevent inactivation of the agent by abiotic factors such as solar and ultraviolet radiation. Although a single application of insecticide at a high concentration could produce greater mortality, it is possible that the fungal spores would not survive exposure to the environmental conditions and on-going control of the pest would most likely fail (Oliveira *et al.* 2016). In this context, it is worth noting that the manufacturer of the insecticide recommends application of the product at 1.25 g /L p.c. (1.2×10^7 conidia/mL) for soybean plantations.

At the maximum concentration, the high mortality rate for the red cashew beetle larvae (72 %) by the *B. bassiana* formulation in this study was close to when the entomopathogenic fungus, *Ophiocordyceps melolonthae* (Ascomycota: Hypocreales: Ophiocordycipitaceae) was applied against larvae of the white grub *Dyscinetus gagates* Burmeister (75 %) (Coleoptera: Melolonthidae) (Salgado-Neto *et al.* 2017). In addition, tests per formed by Rezende *et al.* (2009) revealed that suspensions of *B. bassiana* containing 10^7 conidia/mL induced 95 and 62.5 % mortality, respectively, against larvae and adults of the litter beetle *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae). These findings suggest that larvae are more susceptible to various strains of *B. bassiana* in comparison with adult insects.

The present study has confirmed the efficacy of the *B. bassiana* product against *C. cruralis* larvae in laboratory conditions. However, alongside the health and environmental benefits that normally accrue from the use of biological agents in the control of crop pests, the strategy must be economically feasible. Based on our suggested protocol involving three treatments with *B. bassiana* product, each at a concentration of 2.5×10^6 conidia/mL and applied at a rate of 200 L/ha, we calculate that the cost of treating 1 ha of cashew orchard would be R$ 42.00 (~ 13.2 USD) given that the cost of the product is currently R$ 140.00 per 200 g. Although it appears that our recommended procedure would be advantageous economically, the exact cost of the treatment will vary depending on the density of trees in the plantation and the number of applications determined to be necessary.

Conclusions

Field studies involving cost-benefit evaluations will be required to enable cashew producers to determine whether the use of *B. bassiana* is economically feasible. Moreover, it will be important to convey comprehensive information about the formulation (what the product is, how it works and how it should be used) to farmers so that they can make knowledgeable decisions regarding pest control of this chrysomelid pest. It is important to emphasize that the production of "caju" (fruit of the cashew tree) comprises mixed systems, *i.e.* it ranges from high-tech farms that grow genetically improved varieties to extractive farms that exploit natural resources. Hence, the economic viability of using the *B. bassiana* product must be evaluated for each particular production system alongside all other biocontrol measures being used on the array of pathogens of cashew in this part of Brazil. Furthermore, application of the *B. bassiana* product in the field must be validated since cashew cultures are grown mainly in semi-arid regions that receive little precipitation and the effect of such conditions on the fungi are currently unknown. Thus, these two issues require further evaluation and we commend them for future research based on the promising results reported herein.

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