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Selectivity of *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) on Adults of *Cotesia flavipes* (Hymenoptera: Braconidae)*

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Understanding mortality patterns and interactions between entomopathogenic fungi and parasitoids is important to improve insect biological control programs. The aim of this study was to evaluate the effect of *Metarhizium anisopliae* (Metschnikoff, 1879) Sorokin, 1833 and *Beauveria bassiana* (Balsamo) Vuillemin, 1912 (Hypocreales: Clavicipitaceae) on adults of *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae) with biological insecticides Biometha WP Plus® (*M. anisopliae*), Biovéria G® (*B. bassiana*), Boverril WP® (*B. bassiana*), Metarril WP® (*M. anisopliae*), and Metię WP ® (*M. anisopliae*) at concentrations of 1×10^9 conidia (con).mL^{-1}, 5×10^9 con.ml^{-1}, and 10×10^9 con.ml^{-1}. In the experimental, 10 females of *C. flavipes* were packed in disposable cups capped with a contact surface (filter paper, 9 cm^2) treated with commercial product. The experimental design was completely randomized, with 16 treatments and five replicates of 10 females each. Mortality was assessed at 24, 48, 72, 96, and 120 hours after exposition (HAE) of the products. In general, *B. bassiana* and *M. anisopliae* in the concentrations of 1×10^9 con.ml^{-1}, 5×10^9 con.ml^{-1}, and 10×10^9 con.ml^{-1} can't affect *C. flavipes* females because the peak of mortality in treatments with bioinsecticides was similar to the control and this demonstrated the selectivity of fungi *B. bassiana* and *M. anisopliae* on *C. flavipes* females.

Key words: Biological control, *Diatraea saccharalis*, entomopathogenic fungus, *Saccharum* spp., larval parasitoid.

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The sugarcane industry is one of the most important sectors of agribusiness of the world, and, currently, 8 million hectares are cultivated in Brazil (CONAB 2012). The growth areas for the cultivation of sugarcane may favor the incidence of insect pest populations, especially the spittlebug of root *Mahanarva fimbriolata* (Stål, 1854) (Hemiptera: Cercopidae) and borer *Diatraea saccharalis* Fabricius, 1794 (Lepidoptera: Crambidae) (WHITE *et al*. 2008; KASSAB *et al.* 2012; TIAGO *et al*. 2011; VACARI *et al*. 2012).

Diatraea saccharalis is one of the most important pests of sugarcane in Brazil (CRUZ *et al*. 2011; DINARDO-MIRANDA *et al.* 2011; RODRIGUES *et al*. 2013; COSTA *et al*. 2014). Feed of the sugarcane stem result in direct damage, whereas indirect

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losses occur because of microorganism growth, which in turn results in decrease of sugar and alcohol production (BOTELHO & MONTEIRO 2011; ROSSATO *et al*. 2013).

Chemical insecticides have low efficiency against sugarcane borer, because its larvae develop in galleries within the sugarcane stem, and biological control can help reduce populations of *D. saccharalis* (CRUZ *et al*. 2011; RODRIGUES *et al*. 2013). Among natural enemies, the fungi *Metarhizium anisopliae* (Metschnikoff, 1879) Sorokin, 1833 (Hypocreales: Clavicipitaceae), *Beauveria bassiana* (Balsamo) Vuillemin, 1912 (Hypocreales: Clavicipitaceae), and the parasitoid *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae) (OLIVEIRA *et al.* 2008; VACARI & DE BORTOLI 2010; WILLIAMS *et al*. 2013; HAYASHIDA *et al*. 2014) are prominent. In Brazil, *C. flavipes* is the most efficient means of controlling *D. saccharalis* because of its ability to locate the host inside the stalk of sugarcane and easily rearing a massive natural enemy (ZAPPELINI *et al.* 2010).

On the other hand, the mechanization of harvesting areas for the cultivation of sugarcane causes accretion of straw on the soil and crop residues, associated with high temperature, and humidity favored the development and increase of populations of *M. fimbriolata* (DINARDO-MIRANDA *et al.* 2003, 2007; TIAGO *et al.* 2012). *Mahanarva fimbriolata* can cause injuries of sugarcane and promote significant losses in yield and quality of plants (DINARDO-MIRANDA *et al.* 2000; DINARDO--MIRANDA *et al.* 2002, 2014; MADALENO *et al.* 2008; BARBOSA *et al.* 2011; KASSAB *et al*. 2012; KASSAB *et al*. 2014).

Metarhizium anisopliae is also used to control *M. fimbriolata*, which reduces the pest population at levels below the economic injury (LOUREIRO *et al.* 2005; DINARDO-MIRANDA *et al.* 2006; ALMEIDA *et al.* 2007; ALVES & LOPES 2008; LOUREIRO *et al.* 2012).

Studies related to the interaction of the control agents like fungi and parasitoids require research to clarify the coexistence of both in the same environment and to define whether their interaction is synergistic (ALVES 1998; SANTOS JR. *et al.* 2006a; ROY & COTTRELL 2008). The success of biological control programs depends on the broadest possible understanding of the agents involved, both natural enemies and their hosts, taking into account other factors that can affect the development of these agents so that their use is efficient (MAGALHÃES *et al.* 1998; FUENTES-CONTRERAS & NIEMEYER 2000; STOLZ *et al*. 2002; PEREIRA *et al.* 2010; DELPUECH & DELAHAYE 2013).

The use of biological control agents has been common for the management of populations of insect pest in sugarcane (GOEBEL & SALLAM 2011). *B. bassiana* and *M. anisopliae* have been isolated from species of parasitoids microhymenopteran to emphasize the need to assess the possible negative effects of these entomopathogens on these natural enemies (DE LA ROSA *et al.* 2001; OLIVEIRA *et al.* 2008). The aim of this research was to evaluate the mortality of females of *C. flavipes* when exposed to *M. anisopliae* and *B. bassiana*, which are entomopathogenic fungi most commonly used to control pests in sugarcane.

Material and Methods

The experiment was conducted at the laboratories "Entomologia/Controle Biológico (LECOBIOL)" and "Microbiologia" of the "Universidade Federal da Grande Dourados (UFGD)" in Dourados, Mato Grosso do Sul, Brazil.

Obtaining commercial formulations of *M. anisopliae* and *B. bassiana*

Commercial formulations used were Biometha WP Plus® (*M. anisopliae*), Biovéria G® (*M. anisopliae*), Metarril WP® (*M. anisopliae*), Boverril WP® (*B. bassiana*) e Metiê WP® (*M. anisopliae*) of the companies "Biotech Controle Biológico Ltda.," "Itaforte BioProdutos," and "Ballagro Agro Tecnologia," respectively. All commercial formulations showed over 95% viable spores.

Rearing of *D. saccharalis*

Eggs of *D. saccharalis* were from the LECOBIOL. Newly hatched first instar larvae were placed in glass vials with an artificial diet until they reached the pupa stage. Pupae were then collected, sexed, and groups of 50 pupae (20 males and 30 females) were placed in polyvinyl chloride (PVC) cages (10 cm diameter × 22 cm height). Each cage was wrapped with moistened bond paper and contained a Petri dish lined with filter paper as an oviposition substrate. Each PVC cage was closed with bond paper and rubber bands. Emerging adults were fed with a 10% water and honey solution supplied through a cotton wick inserted into plastic containers (3 cm diameter × 4 cm height), modified according to PARRA (2007).

Rearing of *C. flavipes*

Fourth instar *D. saccharalis* caterpillars were individually exposed to a mated female of *C. flavipes* with 24-h-old. After parasitism, four larvae were transferred in each disposable Petri dish (6.5 cm diameter × 2.5 cm height) with a diet feedback portion. These plates were placed in temperature-

controlled room with a temperature of 25 ± 1°C, 70 ± 10% relative humidity (RH), and 14:10 h L:D until the formation of *C. flavipes* pupae. These pupae were each held individually in disposable cups with lids (100 ml) using a drop of honey to feed the adults at 25 ± 2°C, 70 ± 10% RH, and 14:10 h L:D until emergence of parasitoids (GARCIA *et al.* 2009).

Experimental Design

Females of newly emerged *C. flavipes* were exposed to commercial formulations Biometha WP Plus®, Metarril WP®, and Metiê WP® the basis of *M. anisopliae* and products Bioveria G® and Boverril WP®, the base of *B. bassiana*, both with three concentrations of 1×10^9 conídia (con).ml^{-1}, 5×10^9 con.ml^{-1}, 10×10^9 con.ml^{-1}. The concentrations of products based on *M. anisopliae* were recommended by manufacturers for the control of *M. fimbriolata*, and these doses were standardized for the formulations of *B. bassiana* used in the experiments.

Ten females of *C. flavipes* were packed in disposable cups with lid (capacity of 100 ml) containing a drop of honey inside. Each disposable cup received a contact surface, represented by a square with area of 9 cm^2. The contact surfaces represented by filter paper were treated using a micropipette with 1 ml of bioinsecticides containing standardized suspensions at different concentrations with the aid of chamber Neubauer® (ALVES & LECUONA 1998). Once treated, the contact surfaces were placed on paper towels for about an hour to dry (CARDOSO *et al.* 2007; DO CARMO *et al.* 2010). The bioassay included 16 treatments with 5 replicates of 10 parasitoid females, totaling 50 females per treatment in a completely randomized design.

Mortality was assessed after 24, 48, 72, 96, and 120 h in both experiments. Each dead insect was transferred to graduated microtubes Eppendorf® (capacity 1.5 ml) capped with cotton wool moistened with sterile distilled water. The microtubes were kept in climatic chambers at 25 ± 1°C, 70 ± 10% RH, and 14:10 h L:D for confirmation of the death of the insect.

The data accumulated mortality of *C. flavipes* was subjected to analysis of variance at 5% probability. The control and treatments Metarril WP® (1×10^9 conídia con.ml^{-1}) and Biovéria G (10×10^9 conídia con.ml^{-1}) was subjected to analysis of variance and regression. The equation that best fitted the data was chosen from the model with all the significant parameters based on the determination coefficient (R^2), the significance of the regression coefficients (βi) and the F test.

Results

No significant differences were found between the values of cumulative mortality of *C. flavipes* in treatments with *M. anisopliae* in 24 hours after exposition (HAE). In the evaluation of 48 HAE treatments, Metiê WP® at concentrations of 5×10^9 con.ml^{-1} and 10×10^9 con.ml^{-1}, the lowest values of cumulative mortality did not differ statistically between them.

The biological insecticide Metarril WP® (1×10^9 con.ml^{-1}) caused low mortality to adults of *C. flavipes* (10%), which did not differ significantly from the values of biopesticide Metiê WP® (10×10^9 con.ml^{-1}) at 72 HAE. At 96 HAE evaluation, the Metarril WP® (1×10^9 con.ml^{-1}) was 56% of cumulative mortality, and at 120 HAE, no statistical difference between the values of the treatments were found (Table 1).

The product Metarril WP® (1×10^9 con.ml^{-1}) of the base *M. anisopliae* caused 80% mortality in adult *C. flavipes* at 96 HAE, and until 72 HAE, low mortality was at 10% (Fig. 1B).

The cumulative mortality of commercial products based on *B. bassiana* comparing different doses of 24, 96, and 120 HAE did not differ significantly among the treatments. Biovéria G® (10×10^9 con.ml^{-1}) differed from the other insecticides tested and did not differ from the control values causing lower mortality (0.00 and 26%) at 48 and 72 HAE, respectively (Table 1). The Biovéria G® (10×10^9 con.ml^{-1}) of *B. bassiana* after 96 HAE obtained 100% mortality (Fig. 1 C).

Discussion

The lowest of mortality of *C. flavipes* females at 24 and 48 HAE with the products *M. anisopliae* and *B. bassiana* is important, because the life period of *C. flavipes* is short (SIMÕES *et al*. 2012) and this indicates that fungi did not influence mortality of this parasitoid. However, the similar selectivity of *B. bassiana* and *M. anisopliae* to the parasitoid *C. flavipes* should not be generalized, because this natural enemy was susceptible to other isolates of *M. anisopliae* and *B. bassiana* (FOLEGATTI *et al*. 1990). Similar observations were made by BROGLIO-MICHELETTI *et al.* (2006), who tested the action of some biological insectides on adults of *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae) and showed that the 159E strain of *M. anisopliae* reduced parasitism of eggs of *D. saccharalis* at 78.26%, while the IPA 139E strain did not differ from the control, thus showing that both the species, as the strain of the fungus,

Table 1

Mortality of adults of *Cotesia flavipes* (Hymenoptera: Braconidae) after exposure to different commercial products based on *Metarhizium anisopliae* (A) and *Beauveria bassiana* (B) (Hypocreales: Clavicipitaceae). Temperature of 25°C ± 1°C, 70 ± 10% RH, and 14:10 h L:D

Treatments	*Metarhizium anisopliae* (A)					
	24 HAE^{ns}	48 HAE	72 HAE	96 HAE	120 HAE^{ns}	(n)
Control (untreated)	0.00±00.0^{a}	6.00±4.00^{b}	52.00±2.17^{b}	78.00±2.00^{b}	100.00±0.00^{a}	50
Biometha WP Plus®(1x10^{9}con.m/l.$^{-1}$)	4.00±2.44^{a}	52.00±3.32^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Biometha WP Plus®(5x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	32.00±4.16^{a}	98.00±1.66^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Biometha WP Plus®(10x10^{9}con.m/l.$^{-1}$)	4.00±2.44^{a}	46.00±1.79^{a}	94.00±3.40^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Metié WP® (1x10^{9}con.m/l.$^{-1}$)	2.00±2.00^{a}	60.00±3.20^{a}	88.00±4.40^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Metié WP® (5x10^{9}con.m/l.$^{-1}$)	4.00±2.44^{a}	6.00±2.65^{b}	44.00±3.32^{b}	96.00±3.33^{a}	100.00±0.00^{a}	50
Metié WP® (10x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	0.00±00.0^{b}	20.00±3.54^{c}	92.00±3.66^{a}	100.00±0.00^{a}	50
Metarril WP® (1x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	0.00±00.0^{b}	10.00±2.45^{c}	56.00±1.45^{c}	100.00±0.00^{a}	50
Metarril WP® (5x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	0.00±00.0^{b}	80.00±3.32^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Metarril WP® (10x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	0.00±00.0^{b}	90.00±2.34^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
CV	–	86.17	31.24	13.05	–	–
Treatments	*Beauveria bassiana* (B)					
	24 HAE^{ns}	48 HAE	72 HAE	96 HAE	120 HAE^{ns}	(n)
Control (untreated)	0.00±00.0^{a}	6.00±4.00^{b}	52.00±2.17^{b}	78.00±2.07^{b}	100.00±0.00^{a}	50
Biovéria G® (1x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	12.00±2.00^{b}	90.00±4.72^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Biovéria G® (5x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	38.00±3.78^{a}	92.00±4.78^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Biovéria G® (10x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	0.00±00.0^{b}	26.00±4.35^{b}	100.00±0.00^{a}	100.00±0.00^{a}	50
Bioveril WP® (1x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	38.00±3.78^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Bioveril WP® (5x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	0.00±00.0^{b}	90.00±3.47^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
Bioveril WP® (10x10^{9}con.m/l.$^{-1}$)	0.00±00.0^{a}	54.00±3.68^{a}	96.00±4.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	50
CV	–	53.34	29.21	10.24	–	–

ns – not significant data; means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability; CV – coefficient of variation; HAE – hours after exposure; (n) – number of insectsused in treatment.

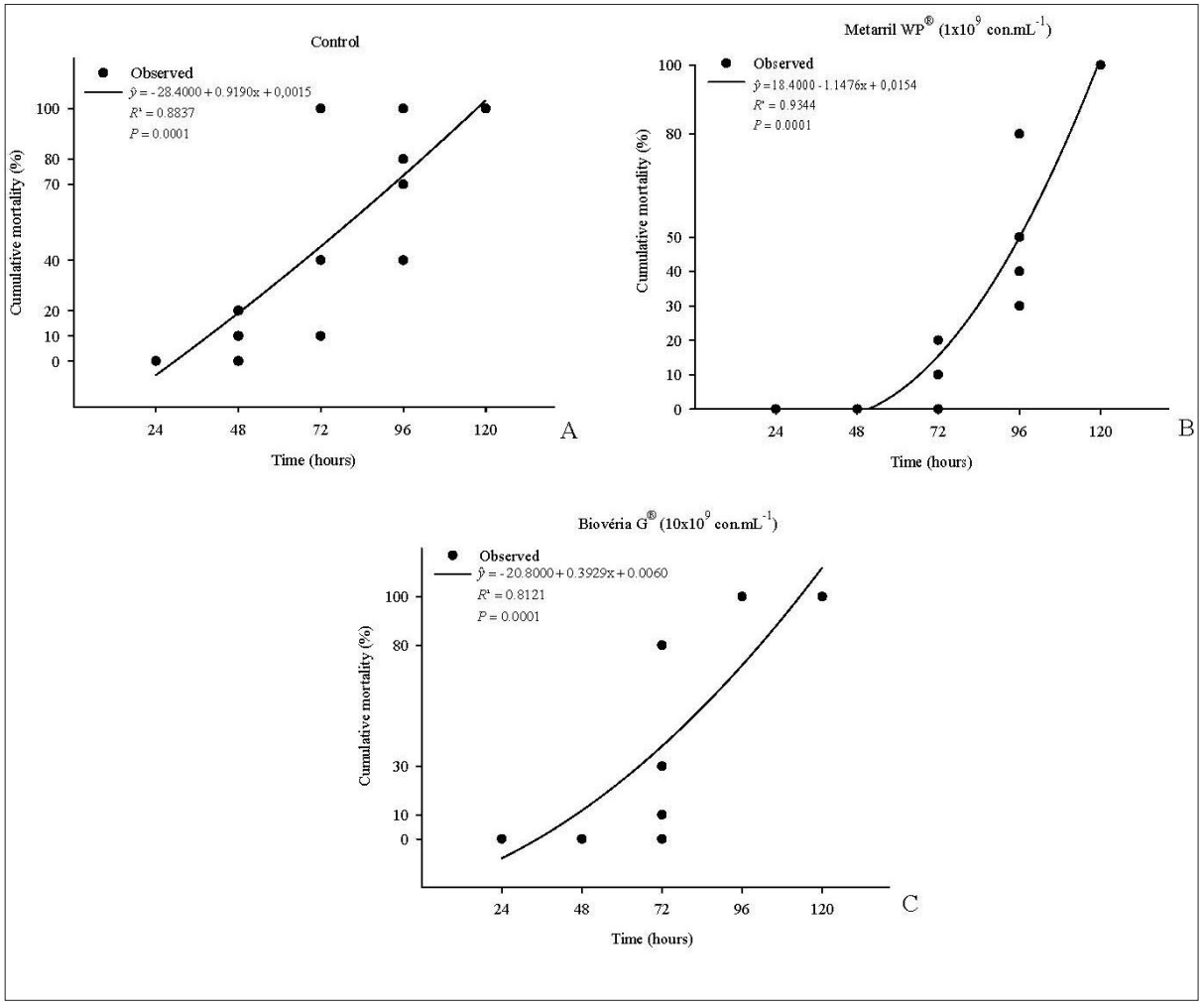

Fig 1. Mortality of adults of *Cotesia flavipes* in the control (A) and after exposure to a concentration of Metarril WP® [*Metarhizium anisopliae*] (B) and Biovéria G® [*Beauveria bassiana*] (C), inoculated filter paper. Temperature of 25 ± 1°C, 70 ± 10% RH, and 14:10 h L:D.

may affect parasitism. On the other hand, the susceptibility of adult *Aphelinus asychis* (Walker, 1839) (Hymenoptera: Aphelinidae) to the fungus *Paecilomyces fumosoroseus* (Wise) Brown & Smith (Deuteromycotina: Hyphomycetes) was observed just under conditions of high relative humidity (MESQUITA *et al.* 1999).

The results obtained in this study corroborate with that of SANTOS JR *et al.* (2006b) for the parasitoid *Oomyzus sokolowskii* (Kurdjumov, 1912) (Hymenoptera: Eulophidae) using isolates of *B. bassiana* (Esalq 447) and *M. anisopliae* (E9) at a concentration of 10^7 conídia mL^{-1}, which found that both entomopathogenic fungi tested did not reduce the average longevity of the parasitoid, but *B. bassiana* showed higher confirmed mortality (21%) as compared to *M. anisopliae* (9%), and this was less harmful to the parasitoid in the tested conditions.

Other studies, such as that by VÁZQUEZ (2002), also demonstrated the pathogenic *B. bassiana* to parasitoids. This study with *Cotesia americanus* (Lepeletier, 1825) (Hymenoptera: Braconidae) reported that *B. bassiana* caused mortality confirming parasitoid of 84.5% when applied to the mass formed pupae and 82.3% when the adults were in contact with the surface, represented by a rectangle of filter paper treated with this entomopathogenic fungus.

Considering that the average survival of adult *C. flavipes* at a temperature of 24 ± 2°C is 48 to 72 h and the fact that this natural enemy locates its host through soluble substances present in the feces of larvae of *D. saccharalis*, this parasitoid performs parasitism in a period of 3-6 days, undergoing a pre-oviposition 24 h (BENNET 1977; BOTELHO & MACEDO 2011).

Beauveria bassiana and *M. anisopliae* concentrations of 1×10^9 con.ml^{-1}, 5×10^9 con.ml^{-1}, and 10×10^9 con.ml^{-1} did not affect the adults of *C. flavipes* to 72 HAE, allowing the parasitoid females to survive long enough to develop parasitism with *D. saccharalis*. Thus, fungi *B. bassiana*

and *M. anisopliae* demonstrated selectivity of *C. flavipes* females, because the peak of mortality in treatments with bioinsecticides was similar to the control.

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