Metarhizium anisopliae and Beauveria bassiana (Hypocreales: Clavicipitaceae) are Compatible with Cotesia flavipes (Hymenoptera: Braconidae)

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METARHIZIUM ANISOPLIAE AND *BEAUVERIA BASSIANA* (HYPOCREALES: CLAVICIPITACEAE) ARE COMPATIBLE WITH *COTESIA FLAVIPES* (HYMENOPTERA: BRACONIDAE)

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Abstract

The aim of this study was to evaluate the effects of commercially available bioinsecticides based on *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, i.e., Biometha WP Plus® (*M. anisopliae*), Biovéria G® (*B bassiana*), Boverril WP® (*B. bassiana*), Metarril WP® (*M. anisopliae*), and Metiê WP® (*M. anisopliae*) on the pupae and adults of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) at concentrations of 1 × 10^9, 5 × 10^9, and 10 × 10^9 conidia mL^{-1}. This braconid is released to control the sugarcane borer, *Diatraea saccharalis.* In the completely randomized first experiment with each commercial product, 10 *C. flavipes* female adults were held individually in disposable cups, which contained a 9-cm^2 sugarcane leaf that had been treated with the one of the entomopathogenic fungal products. The mortality of *C. flavipes* females was assessed at 24, 48, 72, 96, and 120 h after treatment. In the second experiment, the same treatments were applied to *C. flavipes* pupae, because the latter can be exposed when the fungal products are applied to sugarcane to control various pests. In the second experiment we assessed the emergence of adults from treated pupae, the capacity of these adults to parasitize *Diatraea saccharalis* caterpillars, numbers of progeny of these *C. flavipes*, longevity of *C. flavipes* males and females, total adults emerged, and the percent emergence and longevity of males and females of the F1 generation. The mortality levels of *C. flavipes* pupae and adults were not affected by the 2 Entomopathogenic fungi. Therefore the use of *Beauveria bassiana* and *M. anisopliae* to protect sugarcane is compatible with the use of *C. flavipes* to suppress *D. saccharalis*.

Key Words: biological control, entomopathogen, inter-specific interaction, larval parasitoid

Resumo

O objetivo deste estudo foi avaliar o efeito de bioinseticidas comercialmente disponíveis com base em *Metarhizium anisopliae* (Metschnikoff) Sorokin e *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) Biometha WP Plus ® (*M. anisopliae*), Biovéria G ® (*B bassiana*), Boverril WP ® (*B. bassiana*), Metarril WP ® (*M. anisopliae*), e Metiê WP ® (*M. anisopliae*) em pupas e adultos de *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) nas concentrações de 1 × 10^9, 5 × 10^9 e 10 × 10^9 con.mL^{-1}. No primeiro experimento, para cada produto comercial, dez fêmeas de *C. flavipes* foram individualizadas em copos descartáveis e sua superfície de contato dentro (9 cm^2 folha de cana de açúcar) foram tratados com o produto. O delineamento experimental foi inteiramente casualizado (DIC), com 16 tratamentos e cinco repetições, cada repetição, incluindo 10 fêmeas. A mortalidade das fêmeas de *C. flavipes* foi avaliada depois de 24, 48, 72, 96, e 120 horas após o tratamento com os produtos. No segundo experimento DIC, os bioinseticidas e as concentrações foram mantidas as mesmas que no primeiro experimento, com 16 tratamentos e 10 repetições, cada repetição usando um número de pupas com um potencial de emergência de 50 *C. flavipes* adultos. A emergência, o parasitismo, a progênie, a longevidade de machos e fêmeas, o total de adultos emergidos, e a longevidade de machos e fêmeas da geração filial (F1) deste parasitoide foram avaliados.

A mortalidade de pupas e adultos de *C. flavipes* não foi afetada pelos fungos. *B. bassiana* e *M. anisopliae* são compatíveis com o parasitoide *C. flavipes*, permitindo assim a associação dessas duas formas de controle.

Palavras Chave: controle biológico, entomopatógenos, interação interespecífica, parasitoide larval

Brazil is the world's largest sugarcane producer, and the major pests of this crop are *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) and *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) (Tiago et al. 2011; Tiago et al. 2012; Vacari et al. 2012; Simões et al. 2012). Caterpillars of *D. saccharalis* can cause direct losses in the cane stem by inducing biomass losses and the death of apical buds (White et al. 2008; Rossato et al. 2013). Chemical insecticides have low efficiency against this pest, because its third instar remains hidden inside the sugarcane stalk (Cruz et al. 2011; Rodrigues et al. 2013).

Microbial control agents such as entomopathogenic fungus can regulate insect populations through inundative and inoculative applications (Kurtti & Keyhani 2008; Mahdavi et al. 2013). These fungi can cause disease in up to 80% of the insects of a population, and they offer advantages of high genetic variability, infection at different development stages of the host, penetration through the integument, and high capacities of dispersal in the field (Destéfano et al. 2004).

Diatraea saccharalis is susceptible to *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae), and contact with these entomopathogenic fungi can affect the biological characteristics of this insect (Williams et al. 2013). In Brazil, the entomopathogenic fungus *M. anisopliae* is produced on rice and then applied to the sugarcane crop to reduce populations of *M. fimbriolata* (Loureiro et al. 2005).

Entomopathogenic fungi and *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) are used in sugarcane crops, and *M. anisopliae* may impact beneficial parasitoids and other non-target insects (Roy & Cottrell 2008) synergistically or antagonistically (Fuentes-Contreras & Niemeyer 2000; Stolz et al. 2002; Delpuech & Delahaye 2013). Thus host-parasitoid-entomopathogen interactions in agricultural systems can be harmful to populations of beneficial arthropods and ecological communities (Meyling et al. 2009; Meyling et al. 2011). Clearly, it is important to know the mortality patterns and interactions between fungi and natural enemies involved in integrated pest management programs (Santos Jr. et al. 2006).

In sugarcane fields in Brazil, *M. anisopliae* is applied to suppress *Mahanarva fimbriolata* , and *B. bassiana* is applied against termites, *Metamasius hemipterus* (Coleoptera: Curculionidae) and *Sphenophorus levis* (Coleoptera: Curculionidae). These treatments typically occur after *C. flavipes* has been released to suppress *D. saccharalis*. The parasitoid searches for and parasitizes *D. saccharalis* caterpillars inside and outside of the sugarcane stalks. After the parasitoid larva emerges from the caterpillar, they pupate. Because *C. flavipes* pupae are exposed, they may come in contact with *M. anisopliae* and *B. bassiana*.

The objective of this study was to measure mortality of *C. flavipes* after exposure to commercial products containing *M. anisopliae* and *B. bassiana* that are used for controlling pests in sugarcane fields.

Materials and Methods

The experiments were performed in the Laboratories of Entomology/Biological Control (LECOBIOL) of the Faculdade de Ciências Agrárias (FCA), Microbiology and Entomology of the Faculdade de Ciências Biológicas e Ambientais (FCBA) of Universidade Federal da Grande Dourados (UFGD), Dourados, Mato Grosso do Sul State, Brazil, as detailed below.

Obtaining Commercial Formulations Based on *Metarhizium anisopliae* and *Beauveria bassiana*

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

Rearing of *Diatraea saccharalis*

Eggs of *D. saccharalis* were obtained by rearing this species in the LECOBIOL. Eggs were placed in glass jars (8.5-cm diam × 13-cm high) with artificial diet based on wheat germ, soybean, and the phagostimulant, sugarcane yeast (Saccharomyces cerevisiae Meyen ex E.C. Hansen; Saccaromycetales: Saccaromycetaceae) to provide food for neonates and 2nd, and 3rd instars. Fourth instars were transferred to disposable Petri dishes (6.5-cm diam × 2.5-cm high) and fed the same diet until they reached the pupal stage. Pupae were selected depending on their morphological characteristics and each held individually in a plastic pot covered with a screen until they

reached the adult stage. The adults were separated in groups of 20 males and 30 females per cage of polyvinyl chloride (PVC) tubes (10-cm diam × 22-cm high). These cages were closed with bond paper and elastic and internally lined with paper sheets to aid oviposition. Eggs of *D. saccharalis* were collected daily, washed with a solution of copper sulfate, and then stored in a climatic chamber at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D, i.e., methodology adapted from Parra (2007).

Rearing the Parasitoid *Cotesia flavipes*

Fourth instar *D. saccharalis* caterpillars were individually exposed to a mated 24-h-old *C. flavipes* female. After being parasitized, the fourth instars were placed in disposable Petri dishes (6.5-cm diam × 2.5-cm high) and provided with artificial diet. These disposable Petri dishes were placed at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D until *C. flavipes* pupae formed. Pupae were 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).

Experiment I, Quantification of Mortalities of *C. flavipes* Female Adults Exposed to Various Doses of *M. anisopliae* and *B. bassiana*

The objective of Experiment I was to quantify the mortality of adult females of *C. flavipes* when exposed to various doses of *M. anisopliae* and *B. bassiana*. Newly emerged *C. flavipes* females were exposed to commercial formulations of *M. anisopliae* and *B. bassiana* at the concentrations of 1×10^9, 5×10^9, and 10×10^9 conidia mL^{-1}. These concentrations of *M. anisopliae* are recommended for the control of *M. fimbriolata.* Doses of *B. bassiana* were identical. Ten *C. flavipes* females were enclosed per 100 mL disposable cup with a lid and with one droplet of honey inside. The surface of the sugarcane leaf was disinfected with a hypochlorite solution (0.02%). A disinfected sugarcane leaf (9 cm^2; 3-cm long × 3-cm wide) was introduced into each cup. The surface contacting the insect was treated with 1 mL of each standardized suspension of the biopesticide in a Neubauer® chamber (Alves & Leucona 1998). The contact surfaces were treated with the aid of a micropipette and placed on paper towels to dry (Cardoso et al. 2007). The experiment was developed in a completely randomized design (CRD) with 16 treatments and 5 replications each using 10 parasitoid females, and totaling to 50 females per treatment.

The mortality of *C. flavipes* female adults was assessed after 24, 48, 72, 96, and 120 h of contact with the fungal suspensions. Each dead female was transferred to a graduated Eppendorf® microtube (1.5-mL volumetric capacity) and capped with moistened cotton wool with sterile distilled water. These microtubes were kept in a climatic chamber at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D to observe the growth of the fungus and to confirm the death of the insect.

Cumulative mortality of *C. flavipes* data were subjected to analysis of variance at 5% probability and to regression analysis. The choice of the equation that best fit the data was obtained with the linear model, based on the coefficient of determination (R^2) and the significance of the regression by F test (up to 5% probability).

Experiment II, Quantification of Mortalities and Other Effects When *C. flavipes* Pupae Were Exposed to Various Doses of *M. anisopliae* and *B. bassiana*

The objective of Experiment II was to determine the effects of various doses of *M. anisopliae* and *B. bassiana*, when applied on *C. flavipes* pupae. Masses (size between 2.35 ± 0.83) of newly formed *C. flavipes* pupae were treated with 1 mL suspension of the insecticides based on *M. anisopliae* and *B. bassiana* at the concentrations of 1×10^9 conidia mL^{-1}, 5×10^9 conidia mL^{-1}, and 1×10^9 conidia mL^{-1} using an automatic pipette. These masses were then placed on absorbent paper to dry and each mass was held individually in 100 mL disposable cups with lids. These cups were placed in chambers at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D until the emergence of the adult parasitoids(Folegatti et al. 1990).

The experiment was developed in a CRD with 16 treatments and 10 replications, each replication included a mass of pupae with the potential for 50 *C. flavipes* adults to emerge. The data were subjected to analysis of variance (F test) and the means were compared by the Scott-Knott test at 5% probability.

Twenty females and 20 males of newly emerged *C. flavipes* were used per 1.5 mL Eppendorf® microtube, fed a droplet of honey, and capped with cotton wool to evaluate their longevity (days). The microtubes with insects were placed in a climatic chamber temperature at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D.

The percentage of parasitism (based on the number of parasitized larvae per treatment, discounting the natural mortality of the host) was evaluated with 5 *D. saccharalis* fourth-instars each exposed to five 24-h-old *C. flavipes* females. The experiment was performed with 10 replications, each replication included 5 parasitized caterpillars, totaling 50 caterpillars per treatment. Each caterpillar was parasitized by a female *C. flavipes* immediately when she found it. The caterpillars were placed in disposable Petri dishes (6-cm diam), fed with the above-mentioned artificial diet, and then transferred to an air conditioned room at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D.

The percentage of *C. flavipes* that emerged from parasitized larvae (filial generation - F1), the progeny (total individuals emerged), and the longevity (days) of this parasitoid were evaluated. The data were subjected to analysis of variance (F test) and the means were compared by Scott-Knott test at 5% probability.

Results

Experiment I, Quantification of Mortalities of *C. flavipes* Female Adults Exposed to Various Doses of *M. anisopliae* and *B. bassiana*.

Cumulative mortality of *C. flavipes* exposed to *M. anisopliae* commercial products was similar to the control at 24 h of exposure (HAE) (Table 1). Mortality at 24, 48, and 72 HAE was zero with Metarril WP® at a concentration of 1×10^9 conidia mL^{-1} (Table 1). Linear regression analysis showing mortality as a function of time was 80% at 96 HAE with Metarril WP® at 1×10^9 conidia mL^{-1} (Fig. 1B). Metiê WP® at 5×10^9 conidia mL^{-1} and 10×10^9 conidia mL^{-1} at 96 HAE caused cumulative mortalities of this parasitoid of 42% and 48%, respectively. Biovéria G®(10×10^9 conidia mL^{-1}) caused the least cumulative mortality (12%) after 24, 48, and 72 HAE (Fig. 1C). The cumulative mortalities of *C. flavipes* adults exposed to *M. anisopliae* products were similarly great among treatments after 96 and 120 h of the exposure to the fungi (Table 1).

Experiment II, Quantification of Mortalities and Other Effects When *C. flavipes* Pupae Were Exposed to Various Doses of *M. anisopliae* and *B. bassiana*

Parental Generation. The number of *C. flavipes* progeny (males and females) that emerged was similar among treatments with both entomopathogens (Table 2). Longevities (days) of females and males that emerged from pupae treated with Biometha WP Plus® (10×10^9 conidia mL^{-1}), Metiê WP® (5×10^9 conidia mL^{-1}) and Metarril WP® (1×10^9 conidia mL^{-1}) were not significantly different from the control. The levels of parasitism induced in *D. saccharalis* by *C. flavipes* females that had emerged from pupae treated with *M. anisopliae*-based commercial products did not differ among treatments (Table 2).

The number of progeny (males and females) of *C. flavipes* that emerged from pupae treated with *B. bassiana*-based commercial products was similar among treatments (Table 2). The longevities of both female and male *C. flavipes* that emerged from pupae treated with all *B. bassiana* formulations were significantly shorter than the control. The longevities of females and males treated with Boverril WP® at 1×10^9 $con.mL^{-1}$ and 10×10^9 $con.mL^{-1}$ were also significantly shorter than the control (Table 2). The levels of parasitism induced in *D. saccharalis* by *C. flavipes* females that had emerged from pupae treated with *B. bassiana*-based products did not differ significantly among treatments and the control with the exception of Boverril WP® at the 1×10^9 conidia mL^{-1} treatment (Table 2).

F1 Generation. The emergence of F1 adults was not significantly different from the control in the following *M. anisopliae* treatments: Biometha WP Plus® at 1×10^9 $con.mL^{-1}$ and 5×10^9 $con.mL^{-1}$, Metiê WP® at 10×10^9 $con.mL^{-1}$) and Metarril WP® at 1×10^9 $con.mL^{-1}$. However in the remaining *M. anisopliae* treatments the emergence of F1 adults was significantly lower than in the control; and the lowest rate of emergence occurred with the Metarril WP® treatment at 5×10^9 $con.mL^{-1}$ and 10×10^9 $con.mL^{-1}$ (Fig. 2A).

The longevity of F1 *C. flavipes* female adults did not differ significantly from the control for the following *M. anisopliae* treatments: Biometha WP Plus® at 5×10^9 $con.mL^{-1}$ and 10×109 $con.mL^{-1}$ and Metiê WP® at 1×10^9 $con.mL^{-1}$), but female longevity was significantly reduced in all remaining *M. anisopliae* treatments (Table 3). The longevity of males did not differ significantly from that of the control except it was lower with Metiê WP® 10×10^9 $con.mL^{-1}$ (1.65 days) (Table 3).

The percent emergence of F1 adults from *D. saccharalis* larvae parasitized by *C. flavipes* females in treatments with *B. bassiana* was not significantly different from the control in the following treatments: Biovéria G® at 1×10^9 $con.mL^{-1}$, Boverril WP® at 1×10^9 $con.mL^{-1}$ and Boverril WP® at 10×10^9 $con.mL^{-1}$. However the percent emergence of F1 adults was significantly lower than the control in the following treatments: Biovéria G® at 5×10^9 $con.mL^{-1}$, Biovéria G® at 10×10^9 $con.mL^{-1}$ and Boverril WP® at 5×10^9 $con.mL^{-1}$ (Fig. 2B).

The longevity of *C. flavipes* F1 females was not significantly different from the control (3.20 days) for the treatments Biovéria G® at 1×10^9 $con.mL^{-1}$ (3.05 days), Biovéria G® at 5×10^9 $con.mL^{-1}$ (3.00 days) and Boverril WP® at 1×10^9 $con.mL^{-1}$ (2.70 days). However the longevity of F1 females emerged was significantly shorter than the control for the following treatments: Biovéria G® at 10×10^9 $con.mL^{-1}$ (2.00 days), Boverril WP® at 5×10^9 $con.mL^{-1}$ (2.15 days) and Boverril WP® at 10×10^9 $con.mL^{-1}$ (2.40 days). The longevity of F1 males was significantly different from the control only for the following 2 treatments: Biovéria G® at 10×10^9 $con.mL^{-1}$ (1.90 days) and Boverril WP® at 5×10^9 $con.mL^{-1}$ (1.80 days).

Discussion

The very low 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 the adult of this parasitoid is approximate-

TABLE 1. PERCENT CUMULATIVE MORTALITY OF *COTESIA FLAVIPES* (HYMENOPTERA: BRACONIDAE) ADULTS AFTER CONTINUOUS EXPOSURE TO VARIOUS COMMERCIAL PRODUCTS BASED ON *METARHIZIUM ANISOPLIAE* (A) AND *BEAUVERIA BASSIANA* (B) IN THE LABORATORY ON SUGARCANE LEAVES FOR 24 TO 120 HOURS (HAE) AT 25 ± 2 ºC, 70 ± 10% RH AND 14:10 H L:D.

	24 HAE	48 HAE	72 HAE	96 HAE	120 HAE	(n)
Treatments			*Metarhizium anisopliae* (A)			
Control (untreated)	2.00 ± 2.00 a	8.00 ± 3.00 c	38.00 ± 2.29 b	94.00 ± 2.25 a	100.00 ± 0.00 a	50
Biometha WP Plus®(1 x 10^9 con.mL^{-1})	0.00 ± 0.00 a	42.00 ± 2.70 b	96.00 ± 1.25 a	100.00 ± 0.00 a	100.00 ± 0.00 a	50
Biometha WP Plus®(5 x 10^9 con.mL^{-1})	0.00 ± 0.00 a	94.00 ± 4.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	50
Biometha WP Plus®(10 x 10^9 con.mL^{-1})	2.00 ± 2.00 a	50.00 ± 2.76 b	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	50
Metiê WP®(1 x 10^9 con.mL^{-1})	8.00 ± 2.83 a	18.00 ± 3.16 c	36.00 ± 1.68 b	88.00 ± 1.16 a	100.00 ± 0.00 a	50
Metiê WP®(5 x 10^9 con.mL^{-1})	8.00 ± 2.83 a	10.00 ± 2.25 c	10.00 ± 1.75 c	42.00 ± 2.41 c	100.00 ± 0.00 a	50
Metiê WP®(10 x 10^9 con.mL^{-1})	2.00 ± 2.00 a	2.00 ± 2.00 c	4.00 ± 2.44 c	48.00 ± 2.36 c	100.00 ± 0.00 a	50
Metarril WP® (1 x 10^9 con.mL^{-1})	0.00 ± 0.00 a	0.00 ± 0.00 c	0.00 ± 0.00 c	72.00 ± 2.96 b	100.00 ± 0.00 a	50
Metarril WP® (5 x 10^9 con.mL^{-1})	0.00 ± 0.00 a	4.00 ± 2.44 c	90.00 ± 2.33 a	100.00 ± 0.00 a	100.00 ± 0.00 a	50
Metarril WP® (10 x 10^9 con.mL^{-1})	4.00 ± 2.44 a	6.00 ± 2.66 c	52.00 ± 1.57 b	100.00 ± 0.00 a	100.00 ± 0.00 a	50
CV	—	87.27	35.72	23.58	—	—
Treatments			*Beauveria bassiana* (B)			
Control (untreated)	2.00 ± 2.00 b	8.00 ± 3.00 c	38.00 ± 2.29 b	94.00 ± 2.25 a	100.00 ± 0.00 a	50
Biovéria G® (1 x 10^9 con.mL^{-1})	0.00 ± 0.00 b	4.00 ± 1.44 c	96.00 ± 1.50 a	98.00 ± 2.00 a	100.00 ± 0.00 a	50
Biovéria G® (5 x 10^9 con.mL^{-1})	0.00 ± 0.00 b	2.00 ± 2.00 c	60.00 ± 1.95 b	96.00 ± 1.88 a	100.00 ± 0.00 a	50
Biovéria G® (10 x10^9 con.mL^{-1})	2.00 ± 2.00 b	2.00 ± 2.00 c	12.00 ± 1.84 c	100.00 ± 0.00 a	100.00 ± 0.00 a	50
Boverril WP® (1 x 10^9 con.mL^{-1})	0.00 ± 0.00 b	56.00 ± 1.69 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	50
Boverril WP® (5 x 10^9 con.mL^{-1})	0.00 ± 0.00 b	28.00 ± 1.95 b	98.00 ± 2.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	50
Boverril WP® (10 x 10^9 con.mL^{-1})	8.00 ± 2.74 a	28.00 ± 2.72 b	96.00 ± 1.78 a	100.00 ± 0.00 a	100.00 ± 0.00 a	50
CV	131.23	84.22	26.83	—	—	—

Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability; CV- Coeficient of variation; HAE- Hours after exposition; (n) - Number of individuals per treatment.

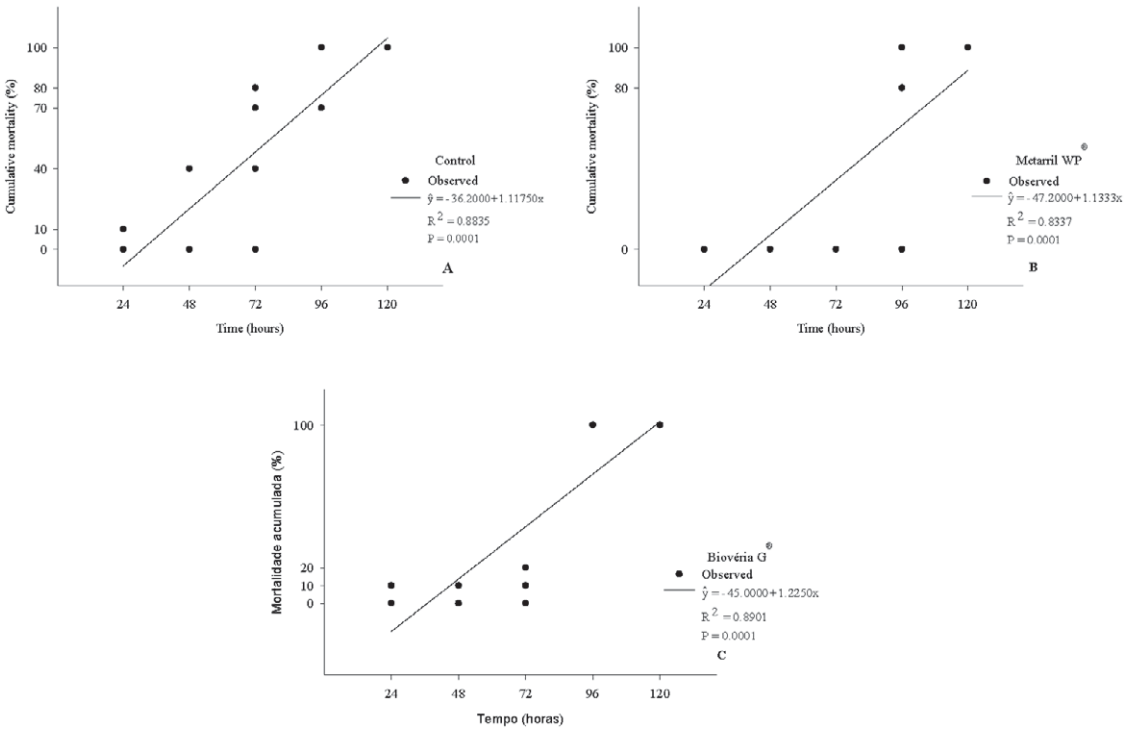

Fig. 1. Mortality of *Cotesia flavipes* (Hymenoptera: Braconidae) adults in the control (A) and after exposure to Metarril WP® (*Metarhizium anisopliae*) (B) at the 1×10^9 conidia $mL^{-1}$and Biovéria G® (*Beauveria bassiana*) (C) at 10×10^9 conidia mL^{-1} inoculated on leaves of cane sugar at 25 ± 2 °C, 70 ± 10% RH and14:10 h L:D.

ly 24 h in the laboratory at 24 ± 2 °C (Simões et al. 2012). 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).

Differences in the cumulative mortality of *C. flavipes* with the commercial products based on *B. bassiana* and *M. anisopliae* after 72 HAE and increased mortality in the control (38%) suggests the compatibility of this parasitoid with these fungi, as reported for the selectivity of *B. bassiana* to immature *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) (Mahdavi et al. 2013). The strain IPA 139E of *M. anisopliae* did not reduce egg parasitism of *D. saccharalis* by *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae), which demonstrates the safety of this entomopathogenic fungus to this parasitoid (Broglio-Micheletti et al. 2006).

A similar longevity of *C. flavipes* females with the products based on *M. anisopliae* and *B. bassiana* corroborate the results with *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) and *B. bassiana* Esalq 447 and *M. anisopliae* E9 at the concentration of 10^7 conidia $mL^{-1.}$ These entomopathogenic fungi did not reduce the longevity, but *B. bassiana* caused higher mortality of this parasitoid (21%) than did *M. anisopliae* (9%) (Santos et al. 2006). However, *B. bassiana* is rarely used in sugarcane crops, which can contribute for the efficiency of *C. flavipes* in controlling *D. saccharalis* larvae.

The high mortality of *C. flavipes* at 72 HAE in the control and the treatments with the entomopathogens may not reduce parasitism by *C. flavipes,* whose adults start to parasitize their hosts approximately 24 h after emergence (Simões et al. 2012). This suggests that the 2 methods of biological control may act synergistically in the management of *D. saccharalis* in sugarcane crops.

Experiment II

Parent Generation. The emergence of similar numbers of individuals and similar proportions of males and females per parasitized *C. flavipes* pupae treated either with *M. anisopliae* or *B. bassiana* showed that these bioinsecticides did not affect the development of immature parasitoid. This may be because they are in the pupal stage, which is resistant to penetration and infection by entomopathogens (Armitage & Siva-Jothy 2005; Lemaitre & Hoffmann 2007; Mahdavi et al. 2013).

The shorter longevity of *C. flavipes* females with the bioinsecticides Metarril WP®(*M. aniso-*

TABLE 2. PROGENY, NUMBER AND LONGEVITY OF FEMALES AND MALES IN THE PARENTAL GENERATION (G: P) OF *COTESIA FLAVIPES* THAT EMERGED FROM PUPAE TREATED WITH *METARHIZIUM ANISOPLIAE* (A) AND *BEAUVERIA BASSIANA* (B) AND THEIR CAPACITIES TO PARASITIZE *DIATRAEA SACCHARALIS* AT 25± 2 °C, 70 ± 10% RH AND14:10 H L:D.

	Progeny	Females*	Males*	(n^1)	L. Fem. (days)	L. Male (days)	(n^2)	Parasit.%	(n^3)
Treatments					*Metarhizium anisopliae* (A)				
Control (untreated)	82.00 ± 6.67 a	46.40 ± 0.88 a	28.70 ± 0.67 a	10	2.25 ± 0.10 a	2.10 ± 0.10 a	40	94.00 ± 3.05 a	50
§B. Plus®($1x10^9$ con.mL^{-1})	63.70 ± 5.26 a	28.20 ± 0.74 a	35.50 ± 0.81 a	10	1.65 ± 0.15 b	1.70 ± 0.14 b	40	80.00 ± 3.43 a	50
B. Plus®($5x10^9$ con.mL^{-1})	52.80 ± 4.71 a	29.40 ± 0.93 a	23.40 ± 0.56 a	10	1.90 ± 0.19 b	1.65 ± 0.18 b	40	88.00 ± 3.68 a	50
B. Plus®($10x10^9$ con.mL^{-1})	67.30 ± 7.85 a	32.90 ± 0.95 a	34.40 ± 0.70 a	10	2.05 ± 0.15 a	2.00 ± 0.16 a	40	80.00 ± 3.05 a	50
Metiê WP®($1x10^9$ con.mL^{-1})	60.90 ± 5.13 a	25.70 ± 0.85 a	35.20 ± 0.86 a	10	1.55 ± 0.13 b	1.65 ± 0.11 b	40	78.00 ± 3.52 a	50
Metiê WP®($5x10^9$ con.mL^{-1})	51.90 ± 6.46 a	27.60 ± 0.63 a	24.30 ± 0.59 a	10	2.20 ± 0.20 a	2.15 ± 0.17 a	40	92.00 ± 3.43 a	50
Metiê WP®($10x10^9$ con.mL^{-1})	55.40 ± 4.81 a	30.60 ± 0.92 a	24.80 ± 0.73 a	10	1.80 ± 0.17 b	1.90 ± 0.16 b	40	88.00 ± 3.26 a	50
Metarril WP® ($1x10^9$ con.mL^{-1})	61.60 ± 3.47 a	20.70 ± 0.81 a	40.90 ± 0.77 a	10	2.45 ± 0.19 a	1.85 ± 0.18 b	40	92.00 ± 3.42 a	50
Metarril WP® ($5x10^9$ con.mL^{-1})	43.70 ± 1.92 a	19.40 ± 0.87 a	24.30 ± 0.56 a	10	1.50 ± 0.16 b	1.90 ± 0.19 b	40	78.00 ± 8.01 a	50
Metarril WP® ($10x10^9$ con.mL^{-1})	68.41 ± 6.41 a	14.10 ± 0.69 a	59.50 ± 0.96 a	10	1.25 ± 0.09 c	1.65 ± 0.23 b	40	82.00 ± 6.13 a	50
CV	—	—	—	—	40.49	41.25	—	—	—
Treatments					*Beauveria bassiana* (B)				
Control (untreated)	82.00 ± 6.67 a	46.40 ± 0.88 a	28.70 ± 0.67 a	10	2.25 ± 0.10 a	2.10 ± 0.10 a	40	94.00 ± 3.05 a	50
Biovéria G® ($1x10^9$ con.mL^{-1})	74.40 ± 3.05 a	35.40 ± 0.95 a	14.90 ± 0.64 a	10	1.60 ± 0.10 b	1.75 ± 0.22 b	40	96.00 ± 3.01 a	50
Biovéria G® ($5x10^9$ con.mL^{-1})	71.20 ± 3.19 a	19.80 ± 0.83 a	41.10 ± 0.67 a	10	1.75 ± 0.16 b	1.85 ± 0.17 b	40	80.00 ± 5.88 a	50
Biovéria G® ($10x10^9$ con.mL^{-1})	83.50 ± 3.81 a	37.40 ± 0.98 a	33.00 ± 0.78 a	10	1.80 ± 0.18 b	1.45 ± 0.12 b	40	92.00 ± 4.42 a	50
Boverril WP® ($1x10^9$ con.mL^{-1})	73.10 ± 5.38 a	29.30 ± 1.04 a	25.30 ± 0.85 a	10	1.10 ± 0.05 c	1.15 ± 0.05 c	40	58.00 ± 5.57 b	50
Boverril WP® ($5x10^9$ con.mL^{-1})	78.50 ± 4.26 a	33.50 ± 0.83 a	36.40 ± 0.69 a	10	1.50 ± 0.12 b	1.60 ± 0.15 b	40	86.00 ± 5.33 a	50
Boverril WP® ($10x10^9$ con.mL^{-1})	67. 30 ± 3.27 a	24.00 ± 1.02 a	32.50 ± 0.58 a	10	1.20 ± 0.14 c	1.05 ± 0.05 c	40	84.00 ± 5.68 a	50
CV	—	—	—	—	40.49	41.25	—	24.35	—

*Number that emerged from treated pupae.

§Biometha WP Plus®. CV- Coefficient of variation; Means followed by the same letter do not differ by the Scott-Knott test at 5% probability. n^1- Mass number of pupae of *Cotesia flavipes* treated with bioinsecticides; n^2- Number of adults used to assess the longevity of *Cotesia flavipes*. n^3- Number of *Cotesia flavipes* adults and *Diatraea saccharalis* caterpillars used to evaluate parasitism; Control (untreated) (Test.); Biometha WP Plus ® (B.Plus).

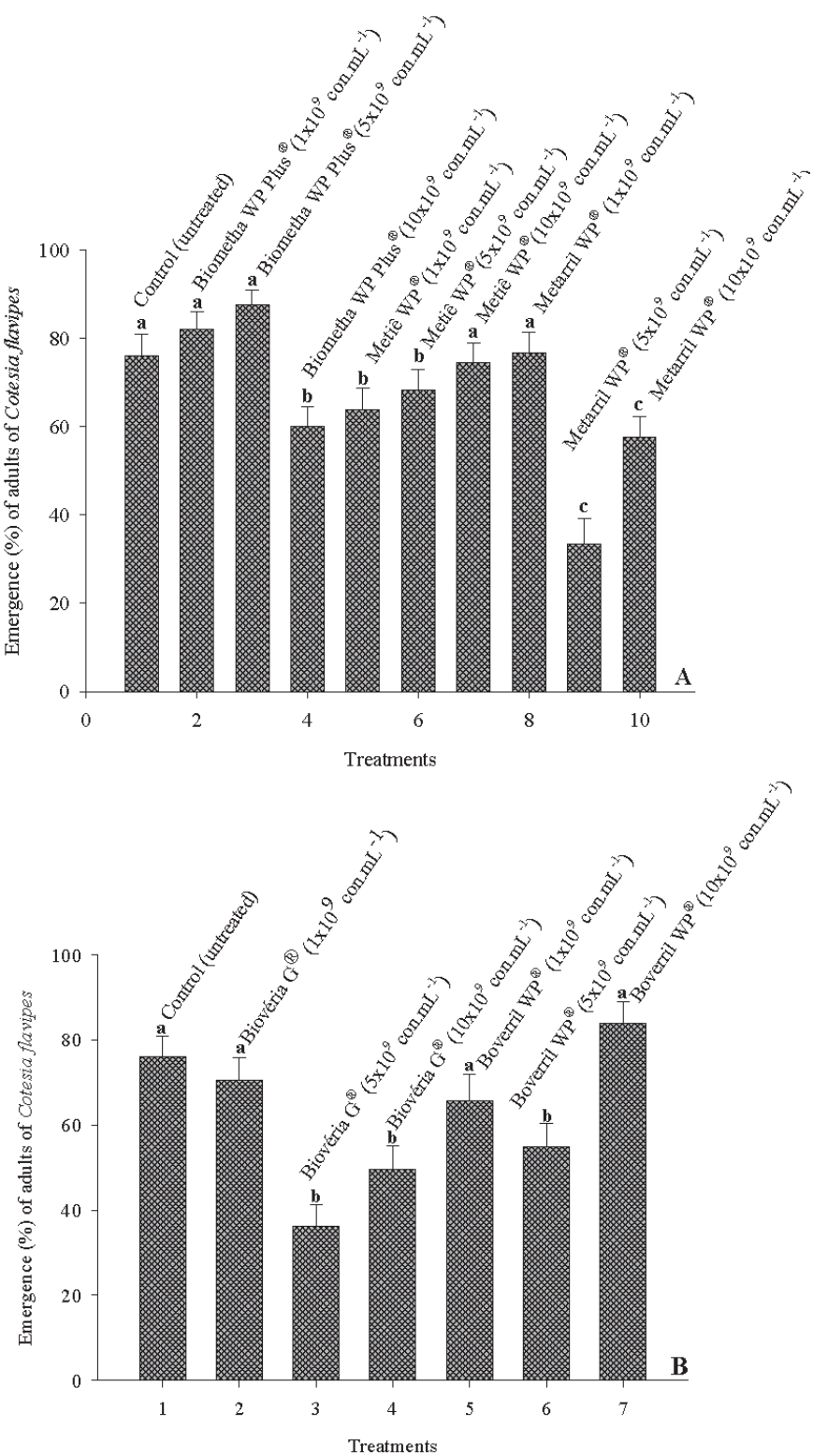

Fig. 2. Emergence (%) of adults in the F1 generation of *Cotesia flavipes* (Hymenoptera: Brachonidae) after exposure to the fungi *Metarhizium anisopliae* (A) and *Beauveria bassiana* (B) (Hypocreales: Clavicipitaceae) at 25 ± 2 °C, 70 ± 10% RH and 14:10 h L:D

TABLE 3. LONGEVITY OF FEMALES (LONG. FEMALES) AND MALES (LONG. MALES) OF *COTESIA FLAVIPES* (HYMENOPTERA: BRACHONIDAE) IN THE F1 GENERATION AFTER EXPOSURE TO *METARHIZIUM ANISOPLIAE* (A) AND *BEAUVERIA BASSIANA* (B) AT 25 ± 2 °C, 70 ± 10% RH AND 14:10 H L:D.

	Long. Females (days)	Long. Males (days)	
Treatments	*Metarhizium anisopliae* (A)		(n)
Control (untreated)	3.20 ± 0.19 a	3.00 ± 0.19 a	40
Biometha WP Plus® (1 x 10^9 con.mL^{-1})	2.55 ± 0.15 b	2.40 ± 0.24 a	40
Biometha WP Plus® (5 x 10^9 con.mL^{-1})	3.40 ± 0.24 a	2.30 ± 0.20 a	40
Biometha WP Plus® (10 x 10^9 con.mL^{-})	2.75 ± 0.19 a	2.25 ± 0.19 a	40
Metiê WP®(1 x 10^9 con.mL^{-1})	3.25 ± 0.21 a	2.70 ± 0.21 a	40
Metiê WP®(5 x 10^9 con.mL^{-1})	2.20 ± 0.23 b	2.45 ± 0.26 a	40
Metiê WP®(10 x 10^9 con.mL^{-1})	2.30 ± 0.16 b	1.65 ± 0.15 b	40
Metarril WP® (1 x 10^9 con.mL^{-1})	2.45 ± 0.19 b	2.25 ± 0.20 a	40
Metarril WP® (5 x 10^9 con.mL^{-1})	3.05 ± 0.25 a	2.50 ± 0.17 a	40
Metarril WP® (10 x 10^9 con.mL^{-1})	2.25 ± 0.16 b	2.10 ± 0.15 a	40
CV	33. 95	38.06	-
Treatments	*Beauveria bassiana* (B)		(n)
Control (untreated)	3.20 ± 0.19 a	3.00 ± 0.19 a	40
Biovéria G® (1 x 10^9 con.mL^{-1})	3.05 ± 0.24 a	2.30 ± 0.25 a	40
Biovéria G® (5 x 10^9 con.mL^{-1})	3.00 ± 0.25 a	2.15 ± 0.16 a	40
Biovéria G® (10 x 10^9 con.mL^{-1})	2.00 ± 0.20 b	1.90 ± 0.19 b	40
Boverril WP® (1 x 10^9 con.mL^{-1})	2.70 ± 0.14 a	2.20 ± 0.26 a	40
Boverril WP® (5 x 10^9 con.mL^{-1})	2.15 ± 0.15 b	1.80 ± 0.15 b	40
Boverril WP® (10 x 10^9 con.mL^{-1})	2.40 ± 0.15 b	2.30 ± 0.14 a	40
CV	33. 95	38. 06	-

CV- coefficient of variation; Means followed by the same letter do not differ by the Scott-Knott test at 5% probability; n- number of adults to evaluate *Cotesia flavipes* longevity.

pliae) at 10 × 10^9 con.mL^{-1} (1.25 days) and Boverril WP® (*B. bassiana*) at 1 × 10^9 con.mL^{-1} and 10 × 10^9 con.mL^{-1} by 1.10 days and 1.20 days, respectively, can be explained by the contact of *C. flapives* pupae with these entomopathogens. This result agrees with the premature death of other parasitoids species infected with these fungi (Rashki et al. 2009).

The reduction of parasitism by *C. flavipes* females that emerged from pupae treated with *B. bassiana*-based commercial products may indicate their weakening by this fungi (Emana 2007). On the other hand, similar parasitism in other treatments is due to the fact *C. flavipes* parasitizes its host almost immediately and even lives for a few days longer (Potting et al. 1997), which reinforces the safety of this fungus to its natural enemy.

The decrease in the longevity of *C. flavipes* males emerged from pupae treated with Boverril WP® (*B. bassiana*) at concentrations of 1 × 10^9 and 10 × 10^9 con.mL^{-1}, 1.15 and 1.05 days, respectively, was half of the longevity of males in the control. However, this decrease differed from that obtained for *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) that emerged from *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) treated with *B. bassiana* and *M. anisopliae* (Potrich et al. 2009). The reduction in *C. flavipes* male longevity may be due to the infection by these fungi. These fungi may have only minor deleterious effects, because *C. flavipes* copulates in the first hour of its life and thus the reduction on its longevity may not affect this parameter (Sagarra et al. 2000a, 2000b; Chichera et al. 2012).

F1 generation. The lower emergence of *C. flavipes* adults in the F1 generation from *D. saccharalis* caterpillars after contact with *M. anisopliae* was also observed for *Trichogramma galloi* Zucchi with *D. saccharalis* eggs treated with isolated IPA 159E (*M. anisopliae*) (Broglio-Micheletti et al. 2006). However, the smallest percent emergence and longevity of *C. flavipes* males and females with *B. bassiana* differs from that reported for *Trichogramma atopovirilia* Oatman & Platner (Hymenoptera: Trichogrammatidae) (Polanczyk et al. 2010) exposed to *M. anisopliae* and *B. bassiana*. The infection by entomopathogenics fungi on more advanced stages of immaturity may not reduce parasitoid emergence (Mesquita & Lacey 2001; Rashki et al. 2009), as observed in the parental generation. However, the exposure to this fungus may compromise other biological

characteristics of *C. flavipes* females by producing offspring with lower parasitic capacity and development in *D. saccharalis* caterpillars.

Most formulations based on the entomopathogenic fungi *B. bassiana* and *M. anisopliae* reduced the longevity of *C. flavipes* males and females, but the percent emergence, number of progeny, and percent parasitism of *D. saccharalis* caterpillars were less affected. The contact with the entomopathogenic fungi with *C. flavipes* pupae did not affect parasitism by this parasitoid, and, thus, they are compatible.

Conclusion

The mortality of *C. flavipes* pupae and adults was not influenced by *B. bassiana* and *M. anisopliae* at the concentrations of 1×10^9, 5×10^9, and 10×10^9 con.mL^{-1}. Thus *B. bassiana* and *M. anisopliae*, at the concentrations of 1×10^9, 5×10^9, and 10×10^9 con.mL^{-1}, were compatible with the use of the parasitoid *C. flavipes* for biological control of sugarcane pests.

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