Semina: Ciências Agrárias
ISSN: 1676-546X
semina.agrarias@uel.br
Universidade Estadual de Londrina
Brasil

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Semina: Ciências Agrárias, vol. 37, núm. 1, enero-febrero, 2016, pp. 25-31
Universidade Estadual de Londrina
Londrina, Brasil

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DOI: 10.5433/1679-0359.2016v37n1p25

Effects of entomopathogenic fungi on different developmental stages of *Cotesia flavipes* (Cam.) a parasitoid of *Diatraea flavipennella* (Box) (Lepidoptera: Crambidae)

Efeito de fungos entomopatogênicos nas diferentes fases de desenvolvimento do parasitoide *Cotesia flavipes* (Cam.) em *Diatraea flavipennella* (Box) (Lepidoptera: Crambidae)

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Abstract

Biological control using the parasitoid *Cotesia flavipes* (Cam.) is one of the main components in the integrated management of the sugarcane moth borer *Diatraea* spp. Besides this parasitoid, the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill and *Metarhizium anisopliae* (Metsch.) Sorok. are used to control sugarcane pests, and they can be found naturally parasitizing caterpillars. This study aimed to evaluate the effects of *M. anisopliae* and *B. bassiana* on different developmental stages of the *C. flavipes* parasitoid on *Diatraea flavipennella* (Box). The experiments were carried at laboratory with isolates PL 43 of *M. anisopliae* and ESALQ 447 of *B. bassiana* were applied to the parasitoid at the immature and adult phases. No negative effects were observed on the larval development of *C. flavipes*, and it could complete its development on caterpillars of *D. flavipennella* treated with fungi. The fungi did not cause pupal mortality. However, *B. bassiana* caused high mortality in the adult parasitoid (76%). The fungi had negative effects on parasitoids when applied during certain developmental stages of *C. flavipes*.
Key words: Sugarcane moth borer, biological control, microbial control, endoparasitoid

Resumo

No manejo integrado das brocas da cana-de-açúcar *Diatraea* spp, o controle biológico com o parasitoide *Cotesia flavipes* (Cam.) é um dos principais componentes. Além do parasitoide, os fungos entomopatogênicos *Beauveria bassiana* (Bals.) Vuill. e *Metarhizium anisopliae* (Metsch.) Sorok. também são empregados no controle de pragas da cana-de-açúcar, podendo ser encontrados naturalmente parasitando lagartas na cultura. Assim, o objetivo do trabalho foi avaliar o efeito dos fungos *M. anisopliae* e *B. bassiana* nas diferentes fases de desenvolvimento do parasitoide *C. flavipes* em *Diatraea flavipennella* (Box). Os experimentos foram conduzidos em condições de laboratório com

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Recebido para publicação 23/09/14 Aprovado em 26/06/15

os isolados de *M. anisopliae* PL-43 e de *B. bassiana* ESALQ 447, aplicados sobre as fases imatura e adulta do parasitoide. Não foram observados efeitos negativos sobre o desenvolvimento larval de *C. flavipes*, que completou seu desenvolvimento em lagartas de *D. flavipennella*, tratadas com os fungos. Não foi observada mortalidade de pupas, porém, *B. bassiana* provocou elevada mortalidade nos adultos do parasitoide (76%). Foram evidenciados efeitos negativos dos fungos sobre o parasitoide, quando aplicados em determinado período de desenvolvimento de *C. flavipes*.
Palavras-chave: Broca da cana-de-açúcar, controle biológico, controle microbiano, endo-parasitoide

Introduction

Sugarcane is one of the main crops in Brazil, and its planting area is expanding every year, occupying approximately 9000 ha (COMPANHIA NACIONAL DE ABASTECIMENTO – CONAB (NATIONAL SUPPLY COMPANY), 2014). Pests are one of the main factors limiting sugarcane production. The species *Diatraea flavipennella*, known as the borer of yellow head, is considered an important pest causing injuries in sugarcane similar to those caused by *Diatraea saccharalis* (FREITAS et al., 2007). Its distribution, unlike *D. saccharalis* (which is widespread in Brazil), is restricted mainly to the northeastern states of the country (FREITAS et al., 2006). According to White et al. (2008), *D. saccharalis* caterpillars cause losses of sugar/ha up to 0.30% for each 1% of internodes that are attacked.

These borers are managed with inundative releases of *Cotesia flavipes*, a larval endoparasitoid (CAMERON, 1891) (Hymenoptera: Braconidae) (PINTO et al., 2006). Although this braconid is an effective control agent, it has not been effective in controlling *D. flavipennella*, and entomopathogenic fungi are an alternative to improve the management of this pest. According to Alves et al. (2008), *D. saccharalis* is susceptible to *Metarhizium anisopliae* (Metsch.) Sorok. and *Beauveria bassiana* (Bals.) Vuill., which infect about 10% of the caterpillars under natural conditions in northeastern Brazil.

Thus, mass release of *C. flavipes* and the application of *M. anisopliae* are commonly done by sugar and ethanol mills in Brazil; however, there is a need to evaluate the effects of the interactions among these biological agents for control optimization (ALVES; LOPES, 2008). Pathogens may or may not harm the natural enemies, depending on factors such as the pathogen species, isolate, concentration, and parasitoid species. However, the effects of pathogens on parasitoids are usually less severe than those of pesticides, which may or may not cause premature death of eggs, larvae, and adults of the parasitoid (ROSSI-ZALAF et al., 2008). This is due to the wide host range of entomopathogenic fungi and the possibility of direct infection on non-target organisms, which should be carefully evaluated before the large-scale use of these microorganisms (MAGALHÃES et al., 1998). Folegatti and Alves (1987) found that *C. flavipes* parasitizing *D. saccharalis* caterpillars had its development hampered by *M. anisopliae*.

This study aims to evaluate the effects of *B. bassiana* and *M. anisopliae* on the larval, pupal, and adult stages of *C. flavipes* parasitizing *D. flavipennella*.

Material and Methods

Effect of fungi on Parasitoid Larvae. Fourth instar *D. flavipennella* caterpillars were separated and sprayed with 1 mL of fungal suspension at a concentration of 10^7 conidia mL^{-1} in every treatment using a micro-atomizer. For parasitism, 24-hour-old adults were kept in plastic containers (5 × 7 cm) with a small hole in the lid that allowed adults to exit. Subsequently, caterpillars were placed close to the hole, enabling the deposition of parasitoid eggs. After observing the female parasitoid oviposition behavior and the fungal application, the caterpillars were transferred to plastic pots containing pieces of sugarcane and kept in climatic chambers at 27 ± 1 °C and 70 ± 10% relative humidity (RH). The

control was treated with ADE + A. The effects of natural enemies were evaluated daily by observing the parasitism and mortality of caterpillars, which were transferred to humid chambers and kept at a temperature of 26 ± 1 °C, 70 ± 10% RH and 12 h photophase for confirming the causal agent. The experiment was carried out in a completely randomized design consisting of 10 treatments; for every treatment, 8 replicates of 5 caterpillars were prepared, for a total of 40 caterpillars per treatment (Table 1).

Table 1. Treatments and days after inoculation with *C. flavipes* and fungi.

Treatments (Control Agents)	Days after inoculation
Metarhizium anisopliae	-
Beauveria bassiana	-
Cotesia flavipes	-
Cotesia flavipes + *Metarhizium anisopliae*	1
Cotesia flavipes + *Metarhizium anisopliae*	3
Cotesia flavipes + *Metarhizium anisopliae*	6
Cotesia flavipes + *Beauveria bassiana*	1
Cotesia flavipes + *Beauveria bassiana*	3
Cotesia flavipes + *Beauveria bassiana*	6
Control	-

The evaluated parameters were mortality caused by fungi and parasitoids, sex ratio, number of emerged adults, and egg-adult period. The data were submitted to analysis of variance using the SAS software (SAS, 1999-2001), and the means were compared by a Tukey test ($P \leq 0.05$).

Effect of Fungi on Parasitoid Pupae. D. flavipennella caterpillars in the fourth instar were parasitized by *C. flavipes* and subsequently transferred to plastic pots containing an artificial diet and kept in climatic chambers at 27 ± 1 °C and 70 ± 10% RH up to the formation of the parasitoid masses. These masses were separated and sprayed with 1 mL of fungal suspension at a concentration of 10^7 conidia mL^{-1} in every treatment, using a micro-atomizer. The control was treated with ADE + A. After fungal application, the masses were transferred and kept in climatic chambers at 27 ± 1 °C and 70 ± 10% RH. The control was treated with ADE+A. Evaluations were made daily until adults' emergence. The experiment was carried out in a completely randomized design, consisting of 3 treatments with 15 replicates, and every replicate consisted of a cocoon mass. The treatments were: (1) Masses sprayed only with the *M. anisopliae* fungus; (2) Masses sprayed only with *B. bassiana* fungus; and (3) Control.

The evaluated parameters were the pupal viability and the number of adults. The data were submitted to analysis of variance using the SAS software (SAS, 1999-2001), and the means were compared by a Tukey test ($P = 0.05$).

Effect of Fungi on Parasitoid Adults. Circular plastic cages (6.5 cm diameter × 7.5 cm height) with 2 side openings (2.5 cm diameter), sealed with 'voile' tissue, were internally sprayed with 2 mL of the fungal suspension at a concentration of 10^7 conidia mL^{-1} using a micro-atomizer and placed to dry in a laminar flow chamber. Then, 10 one-day-old parasitoid adults were released into every cage and fed with honey applied on the inner side of the cage. Then, the plastic cages were placed in a climate-controlled biological oxygen demand (BOD) chamber at 26 ± 1 °C, 12 h photoperiod, and 70 ± 10% RH. The control was treated with

ADE+A. Evaluations were made daily up to the death of adults. The dead insects were transferred to a humid chamber and kept at a temperature of 26 ± 1 °C, 70 ± 10% RH, and photophase of 12 hours to confirm the causal agent. The experiment was carried out in a completely randomized design with 3 treatments and 10 replicates; every replicate consisted of 10 adults. The treatments were: (1) Adults sprayed only with the *M. anisopliae* fungus; (2) Adults sprayed only with the *B. bassiana* fungus; and (3) Control.

The evaluated parameters were confirmed mortality, corresponding to mortality caused by fungal colonization, and adult longevity. The data were submitted to analysis of variance using the SAS software (SAS, 1999-2001), and the means were compared by a Tukey test (P = 0.05).

Results and Discussion

Effect of Fungi on Parasitoid Larvae. The average mortality of *D. flavipennella* caterpillars was 90% and 87.5% in treatments with only the application of PL 43 of *M. anisopliae* and ESALQ 447 of *B. bassiana*, respectively. In treatments in which the caterpillars were subjected only to the parasitoid, the mortality was 72.5%.

Greater mortality of *D. flavipennella* caterpillars was observed in treatments in which both fungi were used than in those in which these agents were applied alone. There was increased mortality in almost all treatments, except when *M. anisopliae* was applied 1 day after the parasitism, which prevented the development of *C. flavipes*. The mortality caused by fungi was reduced in the treatments of 1, 3, and 6 days after the parasitoid inoculation. However, mortality caused by the parasitoid increased over the course of days after spraying the fungi (Table 2). This suggests that the parasitoid and fungi have an inverse relationship over time when used together.

Table 2. Mortality (Mean ± SE) of *Diatraea flavipennella* caterpillars parasitized by *Cotesia flavipes* and sprayed with *Metarhizium anisopliae* and *Beauveria bassiana* fungi at a concentration of 10^7 conidia mL^{-1} in different developmental stages of the parasitoid.

Treatments [1]	Fungi	Parasitoid	Total
C. flavipes	-	72.5 ± 4.78 a	-
M. anisopliae	90.0 ± 5.77 a	-	-
B. bassiana	87.5 ± 4.78 ab	-	-
M. anisopliae (1 day)	92.5 ± 4.33 a	-	-
M. anisopliae (3 days)	90.0 ±4.08 ab	5.0 ± 2.88 c	95.0 ± 2.04 a
M. anisopliae (6 days)	82.5 ± 7.50 ab	12.5 ± 4.78 c	95.0 ± 2.04 a
B. bassiana (1 day)	85.0 ± 6.45 ab	5.0 ± 5.00 c	90.0 ± 3.74 ab
B. bassiana (3 days)	77.5 ± 11.08 ab	22.5 ± 6.29 bc	90.0 ± 3.74 ab
B. bassiana (6 days)	45.0 ± 5.00 b	55.0 ± 2.88 ab	92.5 ± 3.22 a
Control	-	-	-

[1]Means (±SE) followed by the same letter in a column do not differ among themselves at 5% by a Tukey test.

Folegatti and Alves (1987) also observed that, with increasing days after inoculation with *C. flavipes*, mortality caused by *M. anisopliae* on D. *saccharalis* decreased as the mortality caused by parasitoids increased. A similar result was obtained by Rashki et al. (2009) with *B. bassiana* and *Aphidius matricariae* Haliday, a parasitoid of *Myzus persicae* (Sulzer).

Folegatti and Alves (1987) also observed an additive effect from using the 2 agents together,

except in the treatment with fungus application one day after inoculation with the parasitoid, when the fungus did not allow the parasitoid to develop. Moreover, symptoms of *M. anisopliae* infection in *Apanteles flavipes* (Cam.) were reported, confirming the results found in this study, i.e., although the parasitoid has completed its development in the host caterpillar, symptoms of *B. bassiana* infection on *C. flavipes* adults were also found.

The mortality caused by *M. anisopliae* was not affected by the number of days after parasitism when the fungus was applied. *B. bassiana* caused decreasing mortality, i.e., with increasing days after parasitism, the mortality caused by the fungus was reduced, reaching 37.5%, thus enabling the parasitoid to develop (Table 3). Comparing the mortality caused by the different fungi on different days, it was observed that on the first day, there was no difference among treatments, but *M. anisopliae* had higher mortality rates of 90.0% on the third day and of 82.5% on the sixth day than *B. bassiana*.

Table 3. Mortality (Mean ± SE) of *Diatraea flavipennella* caterpillars parasitized by *Cotesia flavipes* and sprayed with *Metarhizium anisopliae* and *Beauveria bassiana* fungi at a concentration of 10^7 conidia mL^{-1} at different days after parasitism.

Days[1]	*M. anisopliae*	*B. bassiana*	Statistics
1 day	92.5 ± 2.50 a	85.0 ± 8.66 a	$0.83^{0.4584}$
3 days	90.0 ± 3.77 a	67.5 ± 3.65 a	$4.28^{0.0008}$
6 days	82.5 ± 4.53 a	37.5 ± 4.50 b	$7.02^{0.0001}$
Statistics	$F = 1.30^{0.3192}$	$F = 23.65^{0.0001}$	-

[1]Means (±SE) followed by the same letter in the column do not differ among themselves at 5% by a Tukey test.

The sex ratio was 0.69 in the treatment in which the caterpillars were submitted only to parasitism. In treatments in which the parasitoid was used with *B. bassiana*, it increased over time, with higher values when the fungus was applied after 3 (0.67) and 6 (0.68) days of inoculation. In the other treatments, the sex ratio was low, ranging from 0.11 to 0.35, with a negative effect of the fungi on the parasitoid. A similar effect could be observed in the number of adults in treatments in which the 2 agents were used together; most treatments had reductions, ranging from 6.12 to 36.75 adults, except when *B. bassiana* was applied 6 days after inoculation with the parasitoid (43.98) (Table 4).

Table 4. Sex ratio, number of emerged adults, and average length of egg-adult period (days ± SE) of *Cotesia flavipes* parasitizing *Diatraea flavipennella* caterpillars, which were also sprayed with *Metarhizium anisopliae* and *Beauveria bassiana* fungi at a concentration of 10^7 conidia mL^{-1} at different developmental stages of the parasitoid.

Treatments[1]	Sex ratio	Number of adults	Egg-adult period
C. flavipes	0.7 ± 0.02 a	47.7 ± 2.68 a	17.2 ± 1.30 a
M. anisopliae (3 days)	0.1 ± 0.01 b	6.1 ± 4.48 c	18.0 ± 2.0 ab
M. anisopliae (6 days)	0.3 ± 0.06 ab	15.2 ± 9.45 bc	17.0 ± 1.58 b
B. bassiana (1 day)	0.2 ± 0.11 b	8.5 ± 5.57 c	24.5 ± 0.50 a
B. bassiana (3 days)	0.7 ± 0.11 a	32.2 ± 10.20 abc	16.2 ± 1.22 b
B. bassiana (6 days)	0.7 ± 0.07 a	43.9 ± 3.74 ab	17.0 ± 0.68 b

[1]Means (±SE) followed by the same letter in a column do not differ among themselves at 5% by a Tukey test.

Regarding the egg-adult period, results showed that fungi could extend the parasitoid development. However, treatment with *B. bassiana* showed no negative effect on the parasitoid development when applied 6 days after parasitoid inoculation, allowing it to complete its cycle.

Effect of Fungi on Parasitoid Pupae. *M. anisopliae* and *B. bassiana* did not cause pupal mortality, confirming the results obtained by Folegatti et al. (1990) compared to *M. anisopliae*. However, a negative effect of fungi on the reduction of pupal viability was observed, mainly by application of *B. bassiana* (72.20%). The number of emerged adults was also affected by fungi, with averages of 29.80, 33.20, and 52.20 adults for *B. bassiana*, *M. anisopliae*, and the control, respectively (Table 5).

Table 5. Pupae viability and number of adults emerged from *Cotesia flavipes* pupae after exposure to *Metarhizium anisopliae* and *Beauveria bassiana* fungi at a concentration of 10^7 conidia mL^{-1}.

Treatments[1]	Pupal viability	Number of adults
B. bassiana	72.2 ± 11.54 b	29.8 ± 10.80 b
M. anisopliae	76.4 ± 9.91 ab	33.2 ± 9.10 b
Control	89.0 ± 3.16 a	52.2 ± 8.60 a
Statistics	$F = 4.75^{0.0303}$	$F = 7.89^{0.0065}$

[1]Means (±SE) followed by the same letter in a column do not differ among themselves at 5% by a Tukey test.

Effect of Fungi on Parasitoid Adults. *B. bassiana* was found to cause high mortality (76.0%), unlike *M. anisopliae*, which showed low adult mortality (9.0%) (Table 5). This suggests that *M. anisopliae* would be more suitable for using with *C. flavipes*, since it causes low mortality in endoparasitoid adults, favoring borer control. Folegatti et al. (1990), when evaluating the pathogenicity of *M. anisopliae* on *A. flavipes* adults, found that this fungus caused low mortality. Similar results were obtained by Hayashida et al. (2012), who found low pathogenicity when testing higher concentrations of *M. anisopliae* on *C. flavipes* adults.

Regarding the longevity of adults, no effect of fungi was observed, because the adult lifetimes of survivors in treatments with fungi did not differ from the control (Table 6).

Table 6. Mortality (Mean ± SE) and longevity of *Cotesia flavipes* adults after exposure to *Beauveria bassiana* and *Metarhizium anisopliae* fungi at a concentration of 10^7 conidia mL^{-1}.

Treatments[1]	Mortality	Longevity
B. bassiana	76.0 ± 2.91a	4.7 ± 0.83 a
M. anisopliae	9.0 ± 6.0 b	4.8 ± 0.44 a
Control	-	4.6 ± 1.08 a
Statistics	$F = 10.04^{<0.0001}$	$F = 0.07^{0.9307}$

[1]Means (±SE) followed by the same letter in a column do not differ among themselves at 5% by a Tukey test.

Conclusions

The ESALQ 447 and PL 43 isolates of *B. bassiana* and *M. anisopliae*, respectively, have no negative effect on the larval stage of *C. flavipes* parasitizing *D. flavipennella* caterpillars. They also are not pathogenic to *C. flavipes* pupae. However, *B. bassiana* cause high mortality in adults. Nevertheless, fungi can cause mortality when applied in certain stages of parasitoid development, necessitating further studies to determine the most appropriate time for using it in integrated pest management programs for sugarcane pests.

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