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Preference of the parasitoid *Cotesia flavipes* (Cam.) (Hymenoptera: Braconidae) for *Diatraea* (Lepidoptera: Crambidae)

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ABSTRACT. The sugarcane borer *Diatraea flavipennella* (Box.) (Lepidoptera: Crambidae), has ultimately predominated in the sugarcane fields of the Brazilian northeast region, despite the continual release of the parasitoid *Cotesia flavipes* (Cam.) (Hymenoptera: Braconidae). Questions have been raised about the efficiency of *Diatraea* spp. in controlling *D. flavipennella*. In this study, females reared in one of the borers were tested individually and as hosts with the larvae of either of the *Diatraea* species alone or the larvae of both species. In the first experiment, the females were released for 10 min within the experimental arena using only olfactory cues. In the second experiment, *C. flavipes* females were individually released for 30 min. within the arena, allowing direct contact with its host, either *D. saccharalis* or *D. flavipennella*, or both host species at the same time. The wasps showed no preference for either one of the hosts, thus being able to locate borers in general.

Keywords: parasitism, behavior, sugarcane borer.

Preferência do parasitoide *Cotesia flavipes* (Cam.) (Hymenoptera: Braconidae) a espécies de *Diatraea* (Lepidoptera: Crambidae)

RESUMO. A broca da cana-de-açúcar *Diatraea flavipennella* (Box.) (Lepidoptera: Crambidae), tem predominado nos canaviais nordestinos apesar de constantes liberações do parasitoide larval *Cotesia flavipes* (Cam.) (Hymenoptera: Braconidae). Apesar de ser utilizado no controle das brocas *Diatraea* spp. a sua eficiência de controle sobre *D. flavipennella* tem sido questionada. Objetivando elucidar esse problema, foram conduzidos testes de preferência, utilizando-se fêmeas de *C. flavipes* com 24h de idade, acasaladas e alimentadas oferecendo-se lagartas de *Diatraea saccharalis* (Fabr.) (Lepidoptera: Crambidae) e *D. flavipennella*. As fêmeas de *C. flavipes* foram criadas em uma das brocas, sendo testadas individualmente e tendo como hospedeiros lagartas de uma das espécies de *Diatraea*, ou ambas as espécies. No primeiro experimento, as fêmeas foram liberadas por 10 minutos em arena experimental utilizando apenas pistas olfativas, ou seja, as fêmeas não tinham contato direto com o hospedeiro. No segundo experimento, as fêmeas foram liberadas por 30 min. em uma arena que permite o contato direto das vespas com o hospedeiro, sendo liberadas individualmente na arena com larvas de *D. saccharalis* e *D. flavipennella*, simultaneamente. Os resultados dos testes mostraram que as vespas não tiveram preferência entre os dois hospedeiros. Assim, conclui-se que *C. flavipes* é capaz de localizar as duas espécies de *Diatraea*.

Palavras-chave: parasitismo, comportamento, broca da cana-de-açúcar.

Introduction

One of the major problems in sugarcane crops throughout the growing season is its susceptibility to infestations of the sugarcane borers *Diatraea saccharalis* (Fabr.) and *Diatraea flavipennella* (Box) (Lepidoptera: Crambidae). These infestations normally occur at a lower incidence when the sugarcane is young. The borers increase their attacks considerably as the plant develops, and in fact, the sugarcane stalk becomes more infested than the ratoon (FREITAS et al., 2006; MACEDO; BOTELHO, 1988). The biological control of these borers has been undertaken by inoculating them with the release of *Cotesia flavipes* (Cam) (Hymenoptera: Braconidae). This parasitoid (wasp) was introduced to Brazil from specimens brought from Trinidad in 1974 (MENDONÇA et al., 1977).

Successful control depends on *C. flavipes* finding an infested plant and subsequently finding the host, which is inside the stem of the plant (POTTING et al., 1997).

According to Botelho and Macedo (2002), the location of the host by the *C. flavipes* female is mediated by a water soluble substances present in the dried or rehydrated feces of the larvae of *D. saccharalis*. The parasitoid, when in contact with the feces, is induced to begin its searching behavior, which is characterized by reducing the rhythm of its motion and by touching the feces with its antennae. Studies show that in addition to larval feces, *C. flavipes* uses regurgitated material and the gallery system in the stem of the host plant to locate the host (POTTING et al., 1995).

Thus, it is believed that *C. flavipes* females use tactile and olfactory stimuli to locate plants infested by hosts. Given the consistent observation of the increased occurrence of *D. flavipennella* in relation to *D. saccharalis* and that *C. flavipes* is their major natural enemy, this study set out to investigate whether *C. flavipes* presents similar preference behaviors for these species of *Diatraea*.

Material and methods

The experiments were conducted at the Laboratory of Insect Pathology, Department of Agronomy, Universidade Federal Rural de Pernambuco. The rearing of *D. flavipennella*, *D. saccharalis* and *C. flavipes*, as well as the experiments using them, were performed at 26ºC ± 2, RH 70% ± 10 and with a 12-h photophase.

Borer Rearing. Larvae of *D. flavipennella* and *D. saccharalis* were reared on an artificial diet of Hensley and Hammond Jr. (1968), modified by Araújo et al. (1985). This diet is basically soybean meal, wheat germ, sugar, vitamin solution, Wesson salts, ascorbic acid, and water. When they reached the pupal stage, the larvae were transferred to plastic containers (26 x 17 x 8 cm) lined with filter paper with moistened cotton on the bottom until they emerged as adults. The adults were confined in PVC cages (20 x 22 cm), the inside of which was lined with sulfite paper as a substrate for oviposition. A 5% honey solution was added to feed the adults. The eggs collected were sterilized with formaldehyde (3%) and copper sulfate (1%) and then stored on Petri dishes (15 x 2 cm) lined with moistened filter paper for approximately five days, when they were distributed on the diet.

Rearing the parasitoid *C. flavipes*. Rearing was conducted using *D. saccharalis* larvae in the third instar as a standard host. To complete parasitism, 24h-old adults were confined in inoculation cages, i.e., plastic containers (5 x 7 cm). Each cage contained a hole in the lid through which the adult *C. flavipes* could come out. Subsequently, the larvae were placed next to the hole so that the parasitoids could deposit their eggs inside the bodies of the larvae (MACEDO et al., 1983). After being subjected to parasitism, the larvae were transferred to organizer-type plastic boxes with 19 compartments (30 x 18 x 4 cm) containing the artificial diet where they remained until the pupae of *C. flavipes* formed. The pupae were withdrawn and transferred back to the inoculation cage, where they remained until the adults' emergence.

Home host effect on the preference of *C. flavipes* using olfactory and visual cues. To test the preference of the parasitoid for either *D. flavipennella* larvae or *D. saccharalis* larvae, this experiment was conducted using female *C. flavipes* wasps that were about 24h old and were reared, fed and inexperienced (no previous contact with hosts). The experiment consisted of four treatments: (1) - wasps reared on *D. saccharalis* with the possibility of them choosing (preference) between the larvae of two species of *Diatraea*; (2) - the control using wasps reared on *D. saccharalis* and offering the same host, (3) - wasps reared on *D. flavipennella*, which had the possibility of choosing between the two species of *Diatraea*; and (4) - the control using wasps reared on *D. flavipennella* and offering the same host. Each treatment consisted of 20 replicates (females). The larvae were subjected to the preference test in transparent acrylic cages (30 x 40 x 40 cm), which had 10-cm diameter circular openings on each side and were covered with voile tissue to allow internal ventilation. The larvae of each species were offered pieces of sugar cane stalk, placed as feed on the Petri dish one day before the experiment. The females were released inside the cages for a period of 30 minutes and kept under continuous observation to record their first choice and the time spent. The observations were carried out with the naked eye, and the time was recorded in assessment tables. The data on the average time until the first choice were subjected to variance analysis, and the means between treatments without transformations were compared using the PROC T TEST from SAS (1999-2001). For the first choice analysis, the means were compared using the null hypothesis of choice drawn from PROC FREQ from SAS (1999-2001) and interpreted by the chi-squared test at 5% significance.

Home host effect on the preference of *C. flavipes* using only olfactory cues. To investigate the home host effect on the available host's mating behavior, larvae of *D. flavipennella* and *D. saccharalis* were used. The experiment was conducted in an arena that blocked the visualization of the host, using female *C. flavipes*, which were more than 24 hours old and were bred, fed and inexperienced (no previous contact with hosts). Six combinations of hosts and controls were investigated using the following treatments: (1) - females reared on *D. saccharalis* and exposed to the same host; (2) - females exposed to host *D. flavipennella*; (3) - females exposed to both hosts; (4) – females reared on *D. flavipennella* and exposed to the same home host; (5) - females exposed to host *D. saccharalis*; (6) - and females exposed to both hosts. For each combination of hosts, the preferences of 30 female *C. flavipes* were observed. The larvae of the hosts were fed with pieces of sugar cane stalk one day before the experiment. The study was conducted in an arena described in Silva-Torres et al. (2005). Briefly, this arena consists of an acrylic base (20 x 20 x 1.2 cm) and a 15-cm diameter glass Petri dish. The Petri dish acts as a cover, and it is placed on the base in a circular depression of the same diameter.

The supply of hosts was carried out in three rectangular depressions (1 x 2 x 1 cm) on the acrylic base, thus allowing a three-way choice. The hosts were offered along with pieces of cane and with their feces after a day of food. In each respective treatment, a 25-cm diameter filter was placed to cover the entire base to prevent the parasitoids from having visual contact with the hosts. Thereby, they could only use olfactory cues to locate the hosts. Through a preliminary test, it was observed that *C. flavipes* presented negative geotropism. Thus, two bases lifted the arena to a certain height, and a mirror was placed under it to view the wasps' preferences, as in Silva-Torres et al. (2005). After each group of three females was observed, the whole arena was cleaned with 70% alcohol, and the filter paper was exchanged. The females were released inside the arena for a period of 10 minutes, and a record was made of the mean residence time in the marked out area, which corresponded to the females' preference for the host selected and their first choice. The observations were performed with the naked eye, and the times were recorded in assessment tables. The data for the average time of residence were subjected to variance analysis, and the means between treatments were compared using ANOVA of SAS (1999-2001). The preference of females among hosts was compared using the absence of choice hypothesis from PROC FREQ of SAS (1999-2001), and it was interpreted by the chi-squared test at 5% significance.

Results and discussion

Home host effect on the preference of *C. flavipes* using olfactory and visual cues. The results provided support for the preference tests, which used clues mediated by volatile chemical substances. These tests showed that, regardless of whether the females were reared on larvae of *D. flavipennella* or *D. saccharalis,* they took the same length of time to choose between the two hosts, and there was no significant significant difference ($t = 0.0518$, $p = 0.2310$, $t = 0.0512$, $p = 0.7228$) (Table 1). Regarding the first choice, the females also presented no preference among hosts.

Table 1. Average time in seconds for the females' first choice of *C. flavipes* (bred, fed and inexperienced) from *D. flavipennella* and *D. saccharalis* when the host combination varied. Temp.: 26 ± 2°C, RH 70 ± 10% and a 12-h photophase.

Home host	Treatment	Average time (s) (±SE)	Statistics
D. saccharalis	*D. saccharalis*	655.8 ± 69.25	t = 0.0518; p = 0.2310
	D. flavipennella	804.6 ± 116.17	
D. flavipennella	*D. saccharalis*	922.8 ± 132.30	t = 0.0512; p = 0.7228
	D. flavipennella	854.1 ± 133.81	

Using a Y-type olfactometer, which provides visual and olfactory cues, Potting et al. (1997) studied the attraction of *C. flavipes* to volatiles emitted by the interaction between maize plants, sugar cane and sorghum and the hosts *Chilo partellus* (Swinh.) and *Sesamia calamistis* (Hamps.). The females that were placed initially to lay eggs in the host larvae, reared on plants or on artificial diets, showed that female *C. flavipes* had no preference for the hosts in which they were reared. These results are similar to those found in the experiment with visual and olfactory cues in relation to *C. flavipes* choosing between *D. flavipennella* and *D. saccharalis* when reared in one of these hosts. In addition to the use of the components of the host itself for host localization, Potting et al. (1995) observed that female *C. flavipes* were attracted by volatiles emitted due to the larvae feeding on the host plant (injuries and their residues) compared to artificial injuries, even in the absence of larvae.

Home host effect on the preference of *C. flavipes* using only olfactory cues. Female *C. flavipes* located the fields with the presence of the host, and they showed a greater preference for hosts in relation to the controls ($F_{2, 33} = 38.48$; $p < 0.0001$; $F_{2, 34} = 33.30$; $p < 0.0001$). This result confirms that the parasitoid females use olfactory stimuli to locate their hosts. Females reared on *D. saccharalis* differed in the time they resided in the two hosts, showing a preference for their home host ($F_{2, 68} = 15.08$; $p < 0.0011$). This behavior also occurred in female *D. flavipennella,* which spent most of their time in their host of origin ($F_{2, 45} = 41.37$; $p < 0.0001$) (Table 2).

Table 2. Average residence time of female *C. flavipes* (bred, fed and inexperienced) from *D. flavipennella* or *D. saccharalis* when the combination of host and controls varied, using a total of 30 females. Temp.: 26 ± 2°C, RH 70 ± 10% and a 12-h photophase.

Treatment	Females that responded	Average time (s)1	Statistics
	Home host	*D. saccharalis*	
D. saccharalis	20	368.8 ± 30.38 a	$F_{2, 33} = 38.48$; $p < 0.0001$
Control A[2]	6	22.0 ± 13.90 b	
Control B[3]	8	41.4 ± 17.91 b	
D. flavipennella	20	325.6 ± 34.18 a	$F_{2, 45} = 50.38$; $p < 0.0001$
Control A[2]	14	19.6 ± 5.69 b	
Control B[3]	12	18.6 ± 5.02 b	
D. saccharalis	28	259.9 ± 29.07 a	$F_{2, 68} = 15.08$; $p < 0.0011$
D. flavipennella	25	152.2 ± 29.98 b	
Control A[2]	16	30.2 ± 8.57 c	
	Home host	*D. flavipennella*	
D. saccharalis	20	403.8 ± 30.00 a	$F_{2, 38} = 63.03$; $p < 0.0001$
Control A[2]	6	22.0 ± 8.29 b	
Control B[3]	13	52.2 ± 11.25 b	
D. flavipennella	20	377.5 ± 34.99 a	$F_{2, 34} = 33.30$; $p < 0.0001$
Control A[2]	8	40.5 ± 11.55 b	
Control B[3]	7	29.7 ± 14.04 b	
D. flavipennella	20	332.7 ± 28.32 a	$F_{2, 45} = 41.37$; $p < 0.0001$
D. saccharalis	14	150.4 ± 21.49 b	
Control A[2]	12	21.5 ± 8.89 c	

[1]Averages (± SE) followed by the same letter in the column do not differ from each other at 5% by the Tukey test for each test. [2]Control using a piece of glass. [3]Control without any material.

In the results of the first choice test, female *D. flavipennella* showed no difference between the controls and hosts. However, female *D. saccharalis* more often chose the hosts. Thus, females reared on *D. saccharalis* were attracted by both host species (Figure 1). The results show that females, independently of the host in which they were reared, stayed longer and tolerated their first choice in the arena sites with the host in relation to the control. This behavior demonstrates the ability of this parasitoid to locate the presence of the host independently of the home host, a result that is certainly related to the fact that both hosts are naturally parasitized by *C. flavipes*. Silva-Torres et al. (2005), when studying the parasitoid *Melittobia digitata* (Dahms) using extracts from the hosts *Trypoxylon politum* (Say), *Megachile rotundata* (F.), and *Sarcophaga bullata* (Parker) as olfactory cues, observed that the *M. digitata* wasps spent significantly more time in the treatments than in the controls, but they showed a preference for the natural host, *M. rotundata*, in both the average time the parasitoid remained on the host and in making its first choice.

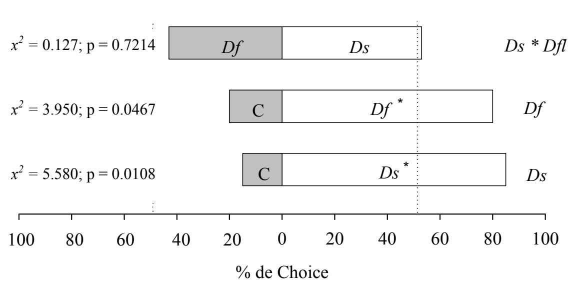

Figure 1. Choice frequency during 10 minutes for female *C. flavipes* from *D. saccharalis* when the combination of the host varied.
*5% significance. Ds - *D. saccharalis*; Df - *D. flavipennella*.

In the experiment where only olfactory cues were used, *C. flavipes*, when reared in the larvae of *D. flavipennella* and *D. saccharalis* and released into the arena, showed a preference for the host on which they were reared. This result is similar to that found by Jembere et al. (2003), who tested the preference of *C. flavipes* for the odors emitted by plants and natural and alternative hosts. The natural hosts used were *C.partellus* and *C. orichalcociliellus* (Strand.) and other alternative insects, such as *Galleria mellonella* (L.), *Charaxes cithaeron* (Felder), *Bombyx mori* (L.), and *Eldana saccharina* (Walker). All of these hosts were fed on different plants of the families Poaceae, Moraceae, Leguminosae, and Cyperaceae. The results showed that the parasitoid showed a preference for its natural host *C. partellus* fed on maize in which it occurs naturally, thus demonstrating the use of volatile chemical substances by the parasitoids to locate their hosts and their preference for their original host.

Conclusion

It is concluded that *C. flavipes* presented the ability to recognize their hosts' volatiles, showing a preference even when not reared on its original host. However, more detailed studies using an olfactometer will be necessary to clarify whether *C. flavipes* prefers its home host or, when released in the field, it displays a preference for one of the *Diatraea* species considered.

References

ARAÚJO, J. R.; BOTELHO, P. S. M.; ARAÚJO, S. M. S. S.; ALMEIDA, L. C.; DEGASPARI, N. Nova dieta artificial para criação da *Diatraea saccharalis* (Fabr.). ***Saccharum* APC**, n. 36, p. 45-48, 1985.

BOTELHO, P. S. M.; MACEDO, N. *Cotesia flavipes* para o controle *de Diatraea saccharalis*. In: PARRA, J. R. P.; BOTELHO, P. S. M.; CORRÊA-FERREIRA, B. S.; BENTO, J. M. S. (Ed.). **Controle biológico no Brasil**: parasitóides e predadores. São Paulo: Manole, 2002. p. 477-494.

FREITAS, M. R. T.; FONSECA, A. P. P.; SILVA, E. L.; MENDONÇA, A. L.; SILVA, C. E.; MENDONÇA, A. L.; NASCIMENTO, R. R.; SANT'ANA, A. E. G. The predominance of *Diatraea flavipennella* (Lepidoptera: Crambidae) in sugar cane fields in the state of Alagoas, Brazil. **Florida Entomologist**, v. 89, n. 4, p. 539-540, 2006.

HENSLEY, S. D.; HAMMOND JR., A. M. Laboratory technique for rearing the sugarcane borer on an artificial diet. **Journal of Economic Entomology**, v. 61, n. 6, p. 1742-1743, 1968.

JEMBERE, B.; NGI-SONG, A. J.; OVERKOLT, W. Olfactory responses of *Cotesia flavipes* (Hymenoptera: Braconidae) to target and non-target Lepidoptera and their host plants. **Biological Control**, v. 28, n. 3, p. 360-367, 2003.

MACEDO, N.; BOTELHO, P. S. M. Controle integrado da broca da cana-de-açúcar *Diatraea saccharalis* (Fabr., 1794) (Lepidoptera: Pyralidae). **Brasil Açucareiro**, v. 106, n. 2, p. 2-14, 1988.

MACEDO, N.; BOTELHO, P. S. M.; DEGASPARI, N.; ALMEIDA, L. C.; ARAÚJO, J. R.; MAGRINI, E. A. Controle biológico da broca da cana-de-açúcar. **Manual de instruções**. Piracicaba: IAA/Planalsucar, 1983.

MENDONÇA, A. F.; RISCO, S. H.; COSTA, J. M. B. Introduction and rearing of *Apanteles flavipes* Cameron (Hymenoptera: Braconidae) in Brazil. **Proceedings International Society of Sugarcane Technologists**, v. 1, p. 703-710, 1977.

POTTING, R. P. J.; OTTEN, H.; VET, L. E. M. Absence of odour learning in the stembores parasitoid *C. flavipes*. **Animal Behaviour**, v. 53, n. 6, p. 1211-1223, 1997.

POTTING, R. P. J.; VET, L. E. M.; DICKE, M. Host microhabitat location location by stemborer parasitoid *Cotesia flavipes:* the role of herbivore volatiles and locally and systemically induced plant volatiles. **Journal of Chemical Ecology**, v. 21, n. 5, p. 525-539, 1995.

SAS-Statistical Analisys System. **Statistical analisys system user's guide**: statistic. Version 8.02. Cary: Statistical Analysis System Institute, 1999-2001.

SILVA-TORRES, C. S. A.; MATTHEWS, R. W.; RUBERSON, J. R.; LEWIS, W. J. Olfactory cues finding by *Melittobia digitata* (Hymenoptera: Eulophidae). **Annals of the Entomological Society of America**, v. 98, n. 4, p. 595-600, 2005.

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