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

Cinthia Conceição Matias da Silva, Edmilson Jacinto Marques*, José Vargas Oliveira and Ellen Carine Neves Valente

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.

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 26°C ± 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, 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...
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) (4SE) | Statistics  |
|--------------------|-----------------------|-------------|
| D. saccharalis | 655.8 ± 69.25 | t = 0.0518; p = 0.2310 |
| D. flavipennella | 694.5 ± 116.17 | t = 0.0512; p = 0.7228 |
| D. saccharalis | 132.30 | t = 0.0512; p = 0.7228 |
| D. flavipennella | 116.17 | t = 0.0512; p = 0.7228 |
| D. flavipennella | 69.25 | t = 0.0512; p = 0.7228 |
| D. saccharalis | 133.81 | t = 0.0512; p = 0.7228 |
| D. flavipennella | 854.1 ± 133.81 | t = 0.0512; p = 0.7228 |

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 (F2, 30 = 38.48; p < 0.0001; F2, 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 (F2, 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 (F2, 45 = 41.37; p < 0.0001) (Table 2).
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 host in which they were reared. This result is similar to that found by Jembere et al. (2003), who tested the preference for *C. flavipes* when the combination of the host and controls varied, using a total of 30 females. Temp.: 26 ± 2°C, RH 70 ± 10% and a 12-h photophase.

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 for *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.

**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) | Statistics |
|-----------|-----------------------|-----------------|------------|
| *D. saccharalis* | 20 | 308.8 ± 30.38 a | F2.38 = 38.48; p < 0.0001 |
| Control A | 6 | 22.0 ± 13.90 b | |
| Control B | 8 | 41.4 ± 17.91 b | |
| *D. flavipennella* | 20 | 325.6 ± 34.18 a | F2.46 = 50.38; p < 0.0001 |
| Control A | 14 | 19.6 ± 5.69 b | |
| Control B | 12 | 18.6 ± 5.02 b | |
| *D. saccharalis* | 20 | 259.9 ± 29.07 a | F2.46 = 15.08; p < 0.0011 |
| Control A | 16 | 152.2 ± 29.98 b | |
| Average residence time of female *D. flavipennella* | 20 | 403.8 ± 30.00 a | F2.36 = 63.03; p < 0.0001 |
| Control A | 6 | 22.0 ± 8.29 b | |
| Control B | 13 | 52.2 ± 11.25 b | |
| *D. flavipennella* | 20 | 377.5 ± 34.99 a | F2.36 = 33.30; p < 0.0001 |
| Control A | 8 | 40.5 ± 11.55 b | |
| Control B | 7 | 29.7 ± 14.04 b | |
| *D. saccharalis* | 20 | 332.7 ± 28.32 a | F2.46 = 41.37; p < 0.0001 |
| Control A | 14 | 150.4 ± 21.49 b | |
| Control A* | 12 | 15.7 ± 8.89 b | |

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 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](image-url)  
**Figure 1.** Choice frequency during 10 minutes for female *C. flavipes* from *D. saccharalis* when the combination of the host varied.

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