

Influence of *Saccharum officinarum* (Poales: Poaceae) Variety on the Reproductive Behavior of *Diatraea flavipennella* (Lepidoptera: Crambidae) and on the Attraction of the Parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae)

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INFLUENCE OF *SACCHARUM OFFICINARUM* (POALES: POACEAE)
VARIETY ON THE REPRODUCTIVE BEHAVIOR OF *DIATRAEA*
FLAVIPENNELLA (LEPIDOPTERA: CRAMBIDAE) AND ON THE ATTRACTION
OF THE PARASITOID *COTESIA FLAVIPES* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

The aims of this laboratory-based study were: (1) to elucidate the reproductive behavior of *Diatraea flavipennella* Box in the presence of different sugarcane varieties, (2) to evaluate the capacities of *Cotesia flavipes* Cameron parasitoids raised on *Diatraea saccharalis* Fabricius and *D. flavipennella* to search for healthy and *D. flavipennella*-infested sugarcane varieties, and (3) to assess the response of the parasitoids towards volatiles released by healthy and *D. flavipennella*-infested sugarcane plants. There were no significant differences ($P \geq 0.05$) in calling, courtship, and copulation activities when *D. flavipennella* females, males, or couples were exposed to different sugarcane varieties. According to the number of egg masses and the total number of eggs oviposited, *D. flavipennella* females showed no preference for any sugarcane variety. The attraction of *C. flavipes* females to infested plants was significantly greater ($P < 0.001$) than to healthy plants, independent of the species of *Diatraea* on which the parasitoids had been reared. The number *C. flavipes* attracted to volatiles from infested sugarcane plants was significantly greater than those attracted to volatiles from healthy plants or to a hexane control ($P < 0.001$). It is concluded that none of the sugarcane varieties influenced the reproductive behavior of *D. flavipennella*, although the state of infestation of the host plants strongly influenced the response of *C. flavipes*.

Key Words: tritrophic interaction, sugarcane borer, volatile compounds, host location

RESUMO

Os objetivos deste estudo, conduzido em laboratório, foram: (1) elucidar o comportamento reprodutivo de *Diatraea flavipennella* Box na presença de diferentes variedades de plantas de cana-de-açúcar, (2) avaliar a capacidade do parasitóide *Cotesia flavipes* Cameron criadas em *D. saccharalis* Fabricius e *D. flavipennella* na busca de variedades de plantas cana de açúcar sadias e infestadas por *D. flavipennella* e (3) avaliar a resposta do parasitóide diante de compostos voláteis liberados por plantas de cana-de-açúcar sadias e infestadas por *D. flavipennella*. Não houve diferença significativa ($P \geq 0,05$) nas atividades de chamamento, de corte e cópula quando as fêmeas, machos ou casais de *D. flavipennella* foram expostos às diferentes variedades de cana-de-açúcar. De acordo com o número de posturas e o número total de ovos postados, fêmeas de *D. flavipennella* não mostraram nenhuma preferência por qualquer variedade de cana-de-açúcar. A atração de fêmeas de *C. flavipes* para plantas infestadas foi significativamente maior ($P < 0,001$) do que nas plantas sadias, independentemente da espécie de *Diatraea* em que os parasitóides foram criados. O número de *C. flavipes* atraídos pelos voláteis de plantas infestadas de cana-de-açúcar foi significativamente maior do que aqueles atraídos pelos voláteis de plantas sadias ou o controle, hexano ($P < 0,001$). Conclui-se que nenhuma das variedades de cana influenciou o comportamento reprodutivo de *D. flavipennella*, embora o estado de infestação das plantas hospedeiras tenha influenciado fortemente a resposta do inimigo natural de *D. flavipennella*.

Translation provided by the authors.

Sugarcane, *Saccharum officinarum* L. (Poaceae), is a giant perennial grass that originated in Asia but is currently cultivated through-

out the tropical and subtropical regions of the world. The recent upsurge of interest in the production of green fuels, such as ethanol, and in the

exploitation of renewable energy resources in general, has created a significant increase in the social and economic importance of sugarcane (UNICA 2009). In this context, Brazil has been a pioneer in the development of non-fossil fuels and is currently the largest producer of sugarcane with a harvest of more than 558.7 million tons recorded in 2007/2008 (CONAB 2008).

The productivity of sugarcane and the overall agricultural and industrial return that can be attained are dependent on numerous topographic, climatic, and environmental factors. From an entomological point of view, attack by pests such as those belonging to the genus *Diatraea* (Lepidoptera: Crambidae) represents one of the major challenges to sugarcane, and has the potential to create enormous economic losses (Mendonça 1996). Presently, 2 species of *Diatraea* predominate in Brazil, namely, *Diatraea saccharalis* Fabricius, 1974 (known as the sugarcane borer), which is distributed widely throughout the country, and *D. flavipennella* Box, 1931, which occurs in 7 states but chiefly in the north-eastern state of Alagoas (Mendonça 1996; Freitas et al. 2006).

Since chemical protection against *Diatraea* spp. is ineffective because of its stem boring habit, various strategies, including manual removal of larvae, introduction of genetically modified sugarcane varieties, and application of biological control are commonly used in combination as part of an integrated program to manage *D. saccharalis* (Baker et al. 1992; Arencibia et al. 1997; Setamou et al. 2002). In this regard, strains of *Cotesia flavipes* Cameron, 1891 (Hymenoptera: Braconidae) recovered from various species of stem borers that attack maize, sorghum, rice, sugarcane, and other crops have been successfully employed in the biological control of *Proceras sacchariphagus* (Betbeder-Matibet & Malinge 1967), *Chilo partellus* (Alam et al. 1972; Overholt et al. 1994) and *D. saccharalis* (Gifford & Mann 1967; Alam et al. 1971; Fuchs et al. 1979). Moreover, larvae of *C. flavipes* have been shown to be very efficient in minimizing the damage caused by *Diatraea flavipennella* (Freitas 2005).

It is known that *Cotesia flavipes*, *C. marginiventris*, *C. glomerata*, and *C. rubecula* are strongly attracted by the odors emitted by herbivore-damaged plants (Turlings et al. 1990; Dicke et al. 1990a,b; Turlings et al. 1991). The release of these signaling molecules is not restricted to the plant parts that are actually infested by the insect but is triggered systemically (Turlings & Tumlinson 1992).

Although detailed studies of the biology and reproductive behavior of *D. flavipennella* have been conducted under laboratory conditions (Freitas et al. 2007; Mendonça 2009), little is known concerning the mating activities of the insect pest in the presence of sugarcane plants, and there are no reports regarding the influence of sugarcane

variety on the reproduction of *D. flavipennella* or on the behavior of the parasitoid *C. flavipes*. The present paper reports the results of a comprehensive study aimed at elucidating (1) the reproductive behavior (i.e., female calling, male courtship, copula, and oviposition) of *D. flavipennella* in the presence of different sugarcane varieties under laboratory conditions; (2) the ability of *C. flavipes* females (reared on *D. saccharalis* or *D. flavipennella*) to search for healthy and infested sugarcane varieties; and (3) the response of *C. flavipes* females towards volatiles released by healthy and *D. flavipennella*-infested sugarcane plants.

MATERIALS AND METHODS

Insects and Parasitoids

Adults and larvae of the *Diatraea* spp. employed in the study originated from the insect collection of the Laboratório de Ecologia Química, Universidade Federal de Alagoas (UFAL). Insects were reared and maintained at $24 \pm 2^\circ\text{C}$, $70 \pm 10\%$ relative humidity and 12 h light/dark regime, and were supplied with an artificial diet (Freitas et al. 2007). A Wild Leica M3B stereomicroscope was employed in determining the gender of the pupae according to a previously published technique (Freitas et al. 2007). *Cotesia flavipes* parasitoids were reared on 9-20-d-old larvae of *D. flavipennella* according to the method of Freitas et al. (2007). *Cotesia flavipes* parasitoids that had been reared on *D. saccharalis* were provided by Assistência Fitossanitária e Controle Biológico Ltda (FITOSSAN), Jequiá da Praia, AL, Brazil.

Sugarcane Plants

Culms of 3 sugarcane varieties, SP791011, RB92579 and RB867515, together with dark fertile soil, filter cake, and ashes from sugarcane leaves were kindly provided by Usina Utinga Leão (Rio Largo, AL, Brazil). Culms were planted in labeled 3-L plastic bags and maintained under semi-natural conditions in a fenced area ($2.0 \times 2.0 \times 2.5$ m) annexed to the Laboratório de Ecologia Química, UFAL. Plants were watered daily and weeds removed manually until the shoots were 70 d old, at which stage they were transferred to 10-L plastic pots and maintained under similar conditions for a maximum of a further 50 d.

Behavior of *D. flavipennella* as a Function of Sugarcane Variety

Bioassays were carried out in the laboratory between the second and sixth hour of the scotophase with the experimental room maintained at $23 \pm 2^\circ\text{C}$ and $65 \pm 8\%$ relative humidity. Four sugarcane plants (>60 d old) of 1 variety were placed in a glass arena and positioned in the form

of a square with 0.5-m sides. Groups of *D. flavipennella*, comprising 5 virgin females or 5 virgin males or 5 couples, were released into the arena and their behavior was observed for 60 min under a 15-W red lamp. During this time, mating activities were recorded as follows: number of calling females, number of females with raised abdomen, number of females with exposed pheromone gland, number of males beating the wings, number of males with opened claspers, and number of copulas. The sequence of activities observed was observed and recorded in the presence of more than 1 researcher. The experiment was repeated 8 times for each of the sugarcane varieties, and new insects were employed in each assay.

Oviposition of *D. flavipennella* as a Function of Sugarcane Variety

Temperature and humidity conditions for this bioassay were similar to those described above. Three sugarcane plants (1 of each variety) were positioned in the form of a triangle and the experimental area so-formed was enclosed by a muslin net. Five *D. flavipennella* females (24-42 h after copulation) were released into the experimental area and allowed to oviposit during a 48-h period. After this time, the number of egg masses and the total number of eggs oviposited on each plant were assessed. The experiments were repeated 8 times.

Searching Behavior of *Cotesia flavipes* Females as a Function of Sugarcane Variety

Temperature and humidity conditions for this bioassay were similar to those described above. Sugarcane plants (60 d old) were infested with 5 *D. flavipennella* larvae (3rd instar) each during a period of 48 h, which was sufficient time for the larvae to penetrate the culms. Infested plants were then placed inside a glass chamber (30 × 45 × 60 cm) into which 10 adult *C. flavipes* females (1-2 d old) were introduced, and the behavior of the parasitoids was observed for 15 min. This procedure was repeated 10 times for each sugarcane variety, with infested plants and their healthy counterparts separately as control, in the presence of either *D. saccharalis*-reared or *D. flavipennella*-reared parasitoids. The levels of attraction of the parasitoids to the infested and healthy sugarcane plant varieties and to the sources of odors were assessed. For this purpose, the source of odor was considered to be the orifice made by the insect larvae on entering the plant culms.

Collection of Volatiles from Sugarcane Plants

Sugarcane plants (variety SP791011; 60 days old) were infested with 5 *D. flavipennella* larvae (3rd instar) each during a period of 48 h. Ten

plants presenting signs of infestation (presence of galleries made by the larvae or death of the apical gem), together with 10 healthy plants of a similar age, were used for the collection and analysis of the volatiles released by a modification of the method described by Stewart-Jones & Poppy (2006). The aerial part of each plant was introduced into a separate microwave-resistant polyester bag, thus forming an aeration compartment, while the culm of the plant was isolated with polytetrafluoroethylene (Teflon) tape commencing at a height of 2 cm above the substrate. The air stream from an aquarium aeration pump (flow rate 500 mL/min) was passed through a tube containing activated charcoal and then subdivided in order to supply 3 separate aeration compartments with inlet flows of approximately 170 mL/min each. The volatiles released by the plant material within the individual compartments were collected by passing the outlet flows through separate tubes containing activated charcoal. Aeration was continued for 24 h, after which the charcoal from each of the outlet tubes was washed with 6 mL of hexane and the resulting desorbed solution divided into 3 equal fractions and transferred to glass ampoules. These ampoules were closed with the help of a blowtorch and stored at -20°C until required for the olfactometer assay. In total, 20 extracts were obtained: 10 from healthy and 10 from infested sugarcane plants.

Olfactory Response of *Cotesia flavipes* to Sugarcane Volatiles

In this bioassay, the olfactory responses of *C. flavipes* females towards volatiles originating from healthy and *D. flavipennella*-infested sugarcane plants were assessed. Bioassays were carried out in the laboratory, maintained at $24 \pm 2^\circ\text{C}$ and $70 \pm 10\%$ relative humidity, between the eighth and eleventh hour of the photophase. The no-choice Y-tube olfactometer employed in the assay was constructed with perfect fitting ground-glass joints in order to ensure a constant air flow throughout the instrument (Fig. 1). Aliquots of volatile collections (10 μL) were absorbed onto filter paper discs and immediately introduced into the extract chamber located in the 2 arms of the olfactometer. The parasitoids were released into the insect chamber, which was connected to the sample compartments via glass tubes that narrowed midway in order to facilitate the counting of individual parasitoids as they moved in the direction of the odors. An air stream, induced by an aquarium air pump was introduced into the extract chamber and then allowed to pass through the olfactometer at a constant flow rate of 250 mL/min for a period of 20 min. Three tests were performed, each of them involved 2 different samples (treatments) as follows: (1) volatiles from an infested plant *versus* volatiles from a healthy

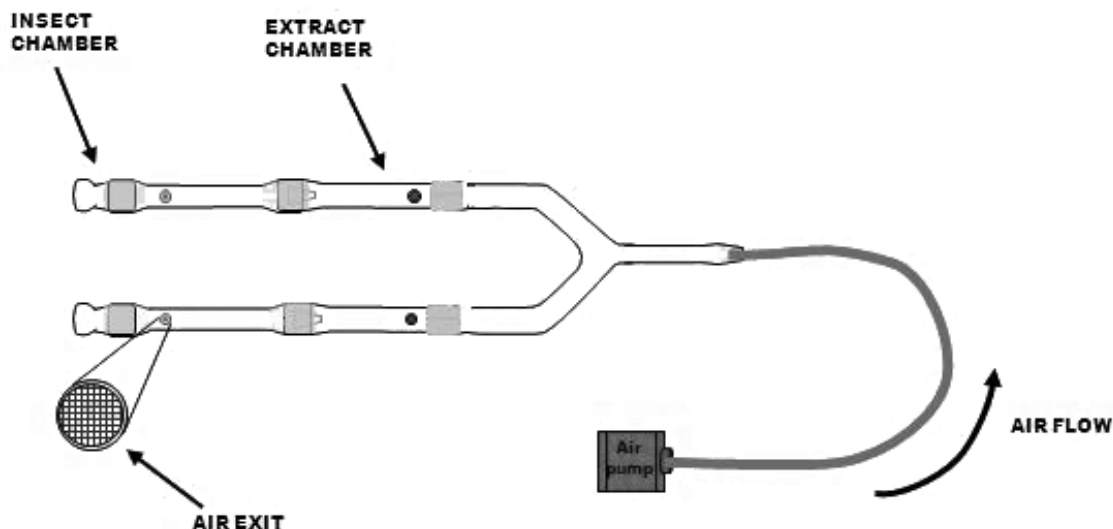


Fig. 1. The no-choice Y-tube olfactometer.

plant, (2) volatiles from an infested plant *versus* pure hexane (control), and (3) volatiles from a healthy plant *versus* pure hexane. In all assays, the positions of the volatile samples in the extract chamber were inverted in order to avoid the effect of habituation. Between each test, the olfactometer was washed with detergent and ethanol and heated to 100° for 1 h in order to prevent cross-contamination of the test materials. Each type of sample was tested 20 times and 10 *C. flavipes* females were used on each test (200 parasitoids in total).

Experimental Design and Statistical Analysis

The experiments were conducted according to a completely randomized design. Lilliefors test (Lilliefors 1967; Soest 1967) was applied in order to determine whether data were normally distributed, while the homogeneity of error variances was analyzed by Levene's test (Levene 1960). For parametric data, significant differences between mean values were established by analysis of variance (ANOVA) with the F Snedecor test ($P < 0.05$), and the Tukey Honestly Significant Difference (HSD) and the Student *t* tests. For non-parametric data, Wilcoxon, Kruskal-Wallis and Dunnett T3 tests were employed.

RESULTS

Influence of Sugarcane Variety on the Behavior of *D. flavipennella*

There were no significant differences ($P \geq 0.05$) regarding calling, courtship, or copula activities when groups of *D. flavipennella* females, males, or

couples were exposed to different sugarcane varieties (Table 1). Single females exhibited typical calling behavior by raising the abdomen in order to expose the pheromone gland in the presence of all 3 varieties of sugarcane, while single males exhibited no courtship behavior but rather remained motionless on the glass walls of the arena. In bioassays involving couples, the females were receptive to males (with females exhibiting calling behavior) while the males flew and opened their claspers in order to attempt copulation. Since similar behavior was observed in the presence of all sugarcane varieties, it is possible to state that variety did not influence the reproductive activities of *D. flavipennella*.

Influence of Sugarcane Variety on the Oviposition of *D. flavipennella*

There were no significant differences ($P \geq 0.05$) between the sugarcane varieties with respect to the number of egg masses and the total number of eggs oviposited by *D. flavipennella* (Table 2).

Influence of Sugarcane Variety on Searching Behavior of *Cotesia flavipes*

The numbers of *C. flavipes* females attracted to infested plants was similar for all 3 sugarcane varieties independent of the host (i.e., *D. saccharalis* or *D. flavipennella*) on which the parasitoids had been reared. Analogous results were obtained with respect to the different sources of odor (Table 3). Thus, there were no significant differences ($P \geq 0.05$) between the 3 infested sugarcane varieties regarding their influence on the searching behavior of *C. flavipes*. In contrast, the numbers of *C. fla-*

TABLE 1. REPRODUCTIVE BEHAVIOR EXHIBITED BY FEMALES, MALES, AND COUPLES OF *DIATRAEA FLAVIPENNELLA* IN THE PRESENCE OF DIFFERENT SUGARCANE VARIETIES. VALUES ARE EXPRESSED AS MEAN $\pm$ STANDARD DEVIATION. ACCORDING TO THE TUKEY HSD TEST ($P < 0.05$), THERE ARE NO STATISTICAL DIFFERENCES BETWEEN ANY OF THE MEAN VALUES.

Variable	Sugarcane variety			P value
	SP791011	RB867515	RB92579	
Number of calling females in female-exclusive assays	1.00 $\pm$ 1.07	1.25 $\pm$ 0.46	1.25 $\pm$ 0.46	0.771
Number of females with raised abdomen in female-exclusive assays	2.13 $\pm$ 0.35	2.25 $\pm$ 0.46	2.25 $\pm$ 0.46	0.831
Number of females with exposed pheromone gland in female-exclusive assays	1.50 $\pm$ 0.76	1.38 $\pm$ 0.52	1.25 $\pm$ 0.46	0.681
Number of calling females in assays with couples	1.50 $\pm$ 1.69	1.20 $\pm$ 1.07	1.50 $\pm$ 0.32	0.259
Number of females with raised abdomen in assays with couples	2.45 $\pm$ 0.35	2.05 $\pm$ 1.07	2.58 $\pm$ 0.61	0.294
Number of females with exposed pheromone gland in assays with couples	1.85 $\pm$ 0.46	1.53 $\pm$ 1.07	1.83 $\pm$ 0.54	0.617
Number of males beating their wings in assays with couples	2.75 $\pm$ 0.46	2.84 $\pm$ 0.80	2.79 $\pm$ 0.89	0.342
Number of males with opened claspers in assays with couples	1.00 $\pm$ 0.67	0.80 $\pm$ 1.07	1.20 $\pm$ 1.12	0.670
Number of copulas	0.84 $\pm$ 0.21	0.75 $\pm$ 0.12	0.93 $\pm$ 0.34	0.512

TABLE 2. OVIPOSITION BY *DIATRAEA FLAVIPENNELLA* IN THE PRESENCE OF DIFFERENT SUGARCANE VARIETIES. VALUES ARE EXPRESSED AS MEAN $\pm$ STANDARD DEVIATION. ACCORDING TO THE TUKEY HSD TEST ($P < 0.05$), THERE ARE NO STATISTICAL DIFFERENCES BETWEEN MEAN VALUES IN ROWS.

Variable	Sugarcane variety			P value
	SP791011	RB867515	RB92579	
Number of egg masses	0.88 $\pm$ 1.73	3.00 $\pm$ 3.74	2.88 $\pm$ 5.14	0.514
Total number of eggs	18.88 $\pm$ 42.71	35.88 $\pm$ 28.66	23.38 $\pm$ 26.34	0.573

vipes females attracted to infested plants was significantly greater than those attracted to healthy plants ($P < 0.001$; Table 4), independent of the host on which the parasitoids had been reared.

Olfactory Response of *Cotesia flavipes* to Sugarcane Volatiles

The mean number of *C. flavipes* parasitoids attracted to volatiles isolated from infested sugarcane plants (1.41 ± 0.50) was significantly greater than those of parasitoids attracted to volatiles isolated from healthy plants (1.22 ± 0.38) or to hexane control (1.25 ± 0.41) according to the Dunnett T3 test ($P = 0.008$ and 0.04 , respectively). In contrast, olfactory responses to volatiles isolated from healthy plants and to hexane control were similar ($P = 0.933$).

DISCUSSION

The calling behavior of *D. flavipennella* females observed in the present study was typical

of the Lepidoptera, and the mating activities were similar to those maintained under laboratory conditions but in the absence of plants (Mendonça 2009). Under such circumstances, the mating behavior of *D. flavipennella* males is characterized by a typical sequence of stereotypic behaviors exhibited by lepidopterans including vibration of the wings, the display of male claspers in front of the female, and continuous copulation for more than 1 h (Mendonça 2009). Silva (2003) reported that the sequence of events during courtship and copulation of male *D. saccharalis* does not depend on the presence of sugarcane plants. Our results show that, in the presence of the host plant, *D. flavipennella* males and females exhibit the typical reproductive behavior that has already been described for this species (Mendonça 2009), and also for *D. saccharalis* (Silva 2003), in the absence of sugarcane plants.

The search for sugarcane varieties that are less susceptible to pests and diseases is very important since the costs associated with the cultivation of plants with an improved genetic

TABLE 3. SEARCHING BEHAVIOR OF *COTESIA FLAVIPES* IN THE PRESENCE OF DIFFERENT VARIETIES OF SUGARCANE (*DIATRAEA FLAVIPENNELLA*-INFESTED PLANTS). VALUES ARE EXPRESSED AS MEAN ± STANDARD DEVIATION. ACCORDING TO THE WILCOXON AND KRUSKAL-WALLIS TESTS ($P \geq 0.05$) THERE ARE NO STATISTICAL DIFFERENCES BETWEEN ANY OF THE MEAN VALUES WITHIN THE SAME ROWS AND COLUMNS.

Sugarcane variety	Number of <i>C. flavipes</i> females (mean values)			
	Attracted by infested plants		Attracted by source of odor (entry orifice of larvae into plant culm)	
	<i>D. saccharalis</i> -reared*	<i>D. flavipennella</i> -reared*	<i>D. saccharalis</i> -reared*	<i>D. flavipennella</i> -reared *
SP791011	0.35	0.27	0.14	0.11
RB867515	0.44	0.32	0.16	0.13
RB92579	0.52	0.33	0.24	0.12
p value	0.06	0.62	0.15	0.91
				P value
				0.32
				0.37
				0.27
				—

*All residues were heterokedastic according to Levene test ($P < 0.05$).

makeup can be greatly reduced. As shown by the number of egg masses and the total number of eggs oviposited by *D. flavipennella*, the insect has no preference for any of the different sugarcane varieties studied. It is important to emphasize that the reproductive success of this pest depends essentially on the selection of a suitable oviposition substrate, hence females use a range of visual, olfactory, chemical, and mechanical clues to enhance the chances of carrying out efficient oviposition (Courtney & Kibota 1990; Chew & Renwick 1995; Stadler 2002). Results from the present study involving *D. flavipennella* are in agreement with those reported by Silva (2003), who did not observe significant differences ($P \geq 0.05$) in oviposition of *D. saccharalis* in the presence of different sugarcane varieties.

It is known that plants attacked by herbivores release volatiles as part of their indirect defense strategy (Dicke et al. 1990a; Turlings et al. 1990). Since such substances attract the natural enemies of the insect pests, and consequently favor the plant host, they are known as herbivore-induced synomones (Vet & Dicke 1992). However, parasitoids often exhibit an innate preference for odors from particular varieties of plants. For example, odors released by different varieties of cotton attract the parasitoid *Campoletis sonorensis* in diverse ways (Elzen et al. 1986). In contrast, Vaughn et al. (1996) demonstrated that in wind tunnel experiments, the parasitoid *Diaeretiella rapae* could not distinguish between 6 different grass varieties.

In this present study, the attraction of *C. flavipes* to healthy sugarcane plants was independent of variety. On the other hand, *D. flavipennella*-infested plants attracted a larger number of parasitoids, indicating that the mixture of volatiles released by these plants not only presented a composition that was different from that released by their healthy counterparts but was also more appealing to the parasitoids (Turlings et al. 1991; Steinberg et al. 1993; Agelopoulos et al. 1995). This results suggesting that the defense system of plants infested with *Diatraea* spp. is activated, leading to the production of compounds that are able to attract the parasitoid.

Results from the present study indicate that *C. flavipes* reared in different insect hosts (i.e., *D. saccharalis* or *D. flavipennella*) exhibit equal competence in recognizing larvae of *D. flavipennella* larvae. This finding contrasts with earlier reports that inexperienced parasitoids are oriented by stimuli associated with their original microhabitat and with insects from which they emerged (Vinson 1975, 1976).

In conclusion, it has been shown that the reproductive behavior of *D. flavipennella* adults follows a similar pattern independent of the presence and variety of the studied host plants, and that *D. flavipennella*-infested sugarcane produces

TABLE 4. SEARCHING BEHAVIOR OF *COTESIA FLAVIPES* (REARED IN DIFFERENT HOSTS) IN THE PRESENCE OF HEALTHY AND *DIATRAEA FLAVIPENNELLA*-INFESTED SUGARCANE PLANTS. WITHIN EACH ROW, MEAN VALUES BEARING DISSIMILAR LOWER CASE SUPERScript LETTERS ARE SIGNIFICANTLY DIFFERENT ACCORDING TO STUDENT'S T TEST ($P < 0.01$).

Source of <i>C. flavipes</i>	Mean number of <i>C. flavipes</i> attracted by		<i>P</i> value
	Healthy plants	Infested plants	
<i>D. flavipennella</i> -reared	1.38 ± 0.65 ^a	3.67 ± 1.31 ^b	0.001
<i>D. saccharalis</i> -reared	2.13 ± 1.45 ^a	5.00 ± 1.69 ^b	0.001

volatile compounds that facilitate localization by the parasitoids of the sites of insect penetration. The evidence presented here reinforces the idea that such chemical signals, once they are fully characterized, could be used in combination with *D. flavipennella* pheromone to attract the insect pest and its natural enemy *C. flavipes* concomitantly to appropriately placed traps. This type of strategy could be very valuable in the integrated pest management of sugarcane crops.

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