AGRONOMY (AGRONOMIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.15, n.4, e7689, 2020
Recife, PE, UFRPE. www.agraria.pro.br
DOI:10.5039/agraria.v15i4a7689
Protocol 7689 - 08/09/2019 • Approved in 10/08/2020

Biological aspects and populational preference of *Cotesia flavipes* between *Diatraea saccharalis* and *Diatraea flavipennella*

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ABSTRACT: Biological aspects of wild and laboratory-reared populations of *Cotesia flavipes* was assessed parasitizing *Diatraea saccharalis* and *Diatraea flavipennella*. The experiments were conducted using a climate-controlled room adjusted to 26 ± 2 °C, relative humidity of 70 ± 10%, and 12:12 h (Light: Dark) photoperiod. The choice and no-choice tests were run using arenas holding infested pieces of stalks with parasitoid release inside the arena and allowed a searching time of 6 h. After this period, the stalks were opened and the larvae single reared until molting to pupa or forming parasitoid pupae. An experimental design completely randomized in a 2 × 2 factorial arrangement was performed. The wild population of *C. flavipes* exhibited preference and better performance parasitizing *D. flavipennella*. Research to evaluate the parasitism efficiency and quality of the parasitoids crossing wild and laboratory populations of *C. flavipes* is necessary to enhance field release success.

Key words: biological control; Braconidae; sugarcane

Aspectos biológicos e preferência de populações de *Cotesia flavipes* entre *Diatraea saccharalis* e *Diatraea flavipennella*

RESUMO: Aspectos biológicos de populações de campo e laboratório de *Cotesia flavipes* foram avaliados em função do parasitismo de *Diatraea saccharalis* e *Diatraea flavipennella*. Os experimentos foram conduzidos em sala climatizada ajustada à temperatura de 26 ± 2 °C, umidade relativa de 70 ± 10% e fotoperíodo de 12:12 (Luz: Escuro). Os tratamentos foram representados por arenas contendo colmos infestados, com posterior liberação do parasitoide, com e sem chance de escolha e tempo de busca de 6 h. Após esse período os colmos foram abertos e as larvas individualizadas e alimentadas até a formação das pupas da praga ou da massa de pupas do parasitoide. Utilizou-se o delineamento experimental inteiramente casualizado em arranjo fatorial 2 × 2. Os resultados mostram que a população de campo de *C. flavipes* exibe preferência e parasitismo de *D. flavipennella*. Pesquisas que avaliem a eficiência de parasitismo e qualidade dos parasitoides oriundos do cruzamento entre as populações de campo e de laboratório de *C. flavipes* são recomendadas para que a liberação massal deste parasitoide seja eficiente.

Palavras-chave: controle biológico; Braconidae; cana-de-açúcar

Introduction

The sugarcane borer complex belonging to the genus *Diatraea* (Lepidoptera: Crambidae), represents one of the main entomological problems of the aforementioned crop. *Diatraea saccharalis* Fabricius, 1794 is a severe pest of American sugarcane crops (Mazzonetto et al., 2013; Valente et al., 2014), which causes losses due to damage caused to sugarcane stalks. The drilling activity due to the presence of *D. saccharalis* and *Diatraea flavipennella* Box, 1931 according to Box (1931) is associated with stalks infection with fungal spores *Fusarium moniliforme* and *Colletotrichum falcatum,* which are later responsible for the sucrose inversion resulting in a decrease in both purity and an increase in contamination process of alcoholic fermentation (Macedo et al., 1983).

In Brazil, two species predominate, *D. saccharalis*, which is widely distributed throughout Brazil, and *D. flavipennella*, which is restricted to seven Brazilian states (Mendonça, 1996; Freitas et al., 2006) being considered the main sugarcane borer species in the Northeastern.

Control of *D. saccharalis* by means of chemical insecticides is not very effective and raises production costs (Erler & Nakano, 2011). Historically, the most used and viable *D. saccharalis* management method is the biological control with the use of larval parasitoid *Cotesia flavipes* Cameron, 1891 (Silva et al., 2014). The efficacy of this parasitoid is proved by a set of samples using visuals and olfactory cues (Silva et al., 2012), search capability in several ages (Santos & Mihsfeldt, 2014), and selectivity (Rossoni et al., 2014), leading to greater susceptibility to *Bacillus thuringiensis* action (Mahmoud et al., 2011). However, this Braconidae wasp has not been sufficient for *D. flavipennella* control, being its combined use with entomopathogenic fungi recommended to control this pest (Valente et al., 2014).

In regions where there is a wide variation of temperature and humidity, it is expected that populations of insect present variability, occurring populations tolerant to local conditions. Therefore, the effectiveness of biological control programs is closely related to the adaptation of populations of natural enemies to different climatic conditions (Messenger & van den Bosch, 1971; Hopper et al., 1993). Parasitoids of different origin from the place where the biological control will be applied may present differences in development, reproductive performance and survival when exposed to local environmental conditions (Liu et al., 2002). This way, the objective of this study was to evaluate the parasitism of two *C. flavipes* populations parasitizing *D. saccharalis* and *D. flavipennella* species, as well as to verify this parasitism influence on larvae morphological aspects.

Materials and Methods

All the bioassays were performed with insects reared and kept at a temperature of 26 ± 2 °C, relative humidity of 70 ± 10%, and 12h light/12h dark photoperiod at the Laboratory of Invertebrate Zoology (LABZOO), located in Campus II, Areia, PB.

Rearing insect

Immature stages of *D. saccharalis* and *D. flavipennella* were obtained from colonies established, respectively, at the Camaratuba Experimental Station - PB and at the Laboratory of Entomology of the Universidade Federal Rural de Pernambuco - UFRPE. The larvae were raised on an artificial diet by Hensley & Hammond (1968), with the following dietary modifications to 70 flat bottom (8.0 × 2.0 cm) test tubes: *D. flavipennella* [Ascorbic acid (2.5 g); Sorbic acid (1.5 g); Carrageenan-Agar (20.0 g); Distilled water (1200.0 mL); Crushed sugarcane culm (40.0 g); Choline Chloride (0.5 g); Soybean meal (52.5 g); formaldehyde (1.5 mL); Wheat germ (15.0 g); Methylparaben (2.25 g); Sucrose (67.5 g); Wesson salts (5.0 g); Vitamin Solution (15.0 mL); VitaGold (0.50 mL); Tetracycline (2.5 mL)], and *D. saccharalis* [Ascorbic acid (2.5 g); Carrageenan- Agar (16.0 g); Distilled water (1200.0 mL); Choline Chloride (0.5 g); Soybean meal (52.5 g); formaldehyde (1.0 mL); Wheat germ (40.0 g); Methylparaben (2.5 g); Sucrose (70.0 g); Wesson salts (10.0 g); Vitamin Solution (15.0 mL); VitaGold (0.50 mL) Tetracycline (2.5 mL)].

The feeding diet used in a tray (40 × 26 × 26 cm): *D. flavipennella* [Ascorbic acid (2.5 g); Sorbic acid (1.5 g); Carrageenan-Agar (25.0 g); Distilled water (1200.0 mL); Crushed sugarcane culm (40.0 g); Choline Chloride (0.5 g); Soybean meal (52.5 g); formaldehyde (1.5 mL); Wheat germ (20.0 g); Methylparaben (2.25 g); Sucrose (67.5 g); Wesson salts (5.0 g); Vitamin Solution (15.0 mL); VitaGold (0.50 mL); Tetracycline (2.5 mL)] and *D. saccharalis* [Ascorbic acid (0.75 g); Carrageenan-Agar (13.5 g); Distilled water (975.0 mL); Choline Chloride (0.4 g); Soybean meal (73.0 g); formaldehyde (2.0 mL); Wheat germ (15.0 g); Nipagin (3.0 g); Sucrose (52.5 g); Wesson's salts (7.5 g); Vitamin Solution (15.0 mL); VitaGold (0.4 mL) Tetracycline (0.5 mL)] being used at their origin laboratories.

Feeding diet was poured into test tubes and freshly hatched larvae were inoculated. When they reached the pupal stage, they were transferred to plastic containers (17 × 8 cm), containing filter paper and moistened cotton at the bottom, until adult emergence. These adults were reared in PVC cages (20 × 22 cm) with inner surface covered with paper as substrate for oviposition. The adults were fed 5% honey-water solution offered in cotton.

The egg mass collected were sterilized three trays by dipping them into water solution prepared with hypochlorite (5.0 mL), formaldehyde (40 mL) and copper sulfate (1g) for 1 min. After, the egg clutches were transferred to Petri dishes lined with filter paper for approximately five days when occurred eclosion of larvae.

Regarding the parasitoids, the field population was obtained during collections run at Fazenda Engenho Macaíba, municipality of Alagoa Nova - PB, where there are no records of mass release of parasitoids, being in this work called wild population maintained for three generations after field collections using *D. flavipennella* as hosts. Insects from laboratory population were obtained at the Experimental Station of Camaratuba - PB, where it has been reared and

named as long-term laboratory-reared population. Rearing of both *C. flavipes* populations were carried out using *D. saccharalis* as the standard host.

Experiment 1

For the evaluation *C. flavipes* preference between the two species of *Diatraea,* were used females from both wild population and long-term laboratory-reared population, with approximately 24h of age, mated and without previous contact with the hosts. The experiment consisted of: *C. flavipes* wild population, with choice and no-choice between larvae of *D. saccharalis* and *D. flavipennella*; and *C. flavipes* long-term laboratory-reared population, with choice and no-choice between the two *Diatraea* species.

The larvae were submitted to preference test in polyvinyl chloride cylindrical (PVC) arenas (20 × 25 cm). For free choice test, each arena held two fragments of sugarcane stalks with 23 cm in length, variety RB 579 infested with one larva of each *Diatraea* species per stalk, 24 hours before releasing *C. flavipes*. This procedure provided conditions close to those found in the field and the maximum parasitism capacity (Santos & Mihsfeldt, 2014). Each treatment consisted of 10 replicates, being each one represented by a cage having their ends sealed after the parasitoid female release. The release time was in the early hours in the morning, remaining in the cages for a period of 6 hours (Viel, 2012). After this period, the stalks were opened longitudinally and the larvae individualized in Petri dishes and fed with artificial diet.

For no-choice test, just one stalk fragment was infested with one larvae of each species separately. Each treatment consisted of 10 replicates, totaling 20 larvae per *C. flavipes* population.

In order to evaluate *C. flavipes* biological characteristics, after parasitoid larvae emergence from host body and its pupation, their pupae masses were placed in Petri dishes (5 × 2 cm), where they remained until adult parasitoid emergence and death, being tallied: larval and pupal period, number total of parasitoids number that was obtained by the sum of internal and external host larvae, pupae number, emerged insects number, pupal mass weight, pupal viability, males and females number, sex ratio, and adults longevity.

Experimental design and statistical analysis for experiment 1

The experimental was a completely randomized design. A generalized linear model with binomial distribution was used for the parasitism rate data analysis as a dependent variable, and parasitoid hosts and lineages as fixed effects factors. Data for choice possibility and no-choice tests were analyzed separately. Adjustments quality verification was done using a Half-normal probability plots with simulated envelope (Demétrio et al., 2014). When there was a significant difference in the variance analysis the averages were separated by chi-square test ($p < 0.05$) by means of the function "glth" package "multcomp" (Hothorn et al., 2008), on the statistical software R (R Development Core Team, 2018).

For the other biological parameters, a generalized linear model with quasipoisson distribution was used for total number of larvae, pupae, adults (males and females), mean duration of the larval and pupal stages, male and female longevity. A generalized linear model with quasibinomial distribution was used for sex ratio. The verification of the quality of the adjustments of the quasipoisson and quasibinomial models was performed using a half-normal probability plot with the envelope using the Half-Normal Plots package (Moral et al., 2017). When there were significant differences in the analysis of variance, the means were compared by the F test ($p < 0.05$), using the "glth" function of the "multcomp" package (Hothorn et al., 2008) using the statistical program "R" (R Development Core Team, 2018).

Regarding the mass weight parameter of *C. flavipes* pupae, data were verified according to ANOVA assumptions, homogeneity of variances and normality of data were performed using the Bartlett and Shapiro-Wilk test. The Gaussian model was the one that best fitted the data. When there were significant differences in the analysis of variance, the means were compared using the Tukey HSD test ($p < 0.05$), using the "aov" function of the "agricolae" package (Mendiburu, 2016) using the statistical program "R" (R Development Core Team, 2018).

Experiment 2

Hosts biological characteristics observations were also performed by evaluations every two days in all larvae (parasitized and no-parasitized), by treatment for larvae thorax width, anterior width, posterior width, and total length using digital caliper 150 mm.

Experimental design and statistical analysis of experiment 2

The experiment was run using a completely randomized design and the data used for analysis were related only to the 10th-day after the beginning of the experiment, due to the pupal formation of both the parasitoid and the hosts submitted to binomial LOGISTIC regression analysis, version 9.4 (SAS Institute, 2010).

Results and Discussion

Experiment 1

Lack of interaction was observed between *C. flavipes* populations and the species of *Diatraea* regarding the parasitism. In the free-choice test, there was no significant difference between the host species for the wild population (Table 1). On the other hand, significant differences were observed in the long-term laboratory reared females, in which parasitism ranged from 10 to 90%. In the no-choice test, there was no significant difference between the hosts *D. saccaralis* and *D. flavipennella*. Volatiles that were concentrated in the arena may have affected parasitoid behavior, especially in the area where host frass concentration is high, then attracting it more effectively according to the percentages of 50% (Table 1).

Table 1. *Cotesia flavipes* parasitism rate on larvae of *Diatraea saccharalis* and *Diatraea flavipennella* in choice and no no-choice test.

Hosts	*Cotesia flavipes*	
	Wild Population	Lab reared population
	Choice	
D. saccharalis	50.0 ± 16.6 a	90.0 ± 10.0 a
D. flavipennella	50.0 ± 16.6 a	10.0 ± 10.0 b
Df	1	1
P-value	1.000	0.016
	No-choice	
D. saccharalis	80.0 ± 13.3 a	80.0 ± 13.3 a
D. flavipennella	90.0 ± 10.0 a	50.0 ± 16.6 a
Df	1	1
P-value	0.926	0.515

Means followed by the same lowercase letter in the column do not differ from each other (GLM with binomial distribution, followed by posthoc chisquare test, p > 0.05).

Table 2. *Cotesia flavipes* mean duration (days ± SE)[1] of the larval and pupal stages from *Diatraea saccharalis* and *Diatraea flavipennella*.

Hosts	*Cotesia flavipes*	
	Wild population	Lab-reared population
	Larval stage	
D. saccharalis	11.4 ± 0.4 bA	9.8 ± 0.1 bB
D. flavipennella	17.1 ± 0.1 aA	16.7 ± 0.1 aA
Df	1	1
P-value	<0.001	<0.001
	Pupal stage	
D. saccharalis	5.2 ± 0.0 aA	5.2 ± 0.1 aA
D. flavipennella	5.0 ± 0.0 aB	6.0 ± 0.0 bA
Df	1	1
P-value	0.494	>0.001

Means followed by the same lowercase letter in the column and upper case in the row do not differ by (GLM with quasipoisson distribution, followed by posthoc F test, p > 0.05).
[1]SE: mean standard error.

Similarly to behavior, new strategies and unique adaptations are developed by the parasitoid to be able to explore its host, thus allowing the female to locate and parasitize the host (Godfray, 1994). Females of *C. flavipes* exhibited aggressive behavior toward *D. flavipennella* larvae when compared to *D. saccharalis*. Furthermore, laboratory-reared females of *C. flavipes* presented difficulty to introduce its ovipositor in *D. flavipennella*. It was evidenced by the searching for different regions of the cuticle penetration or requiring adequate parasitoid adaptations, such as greater speed and strength on parasitism. These are characteristics observed in the wild population. Also about the behavior of *C. flavipes* parasitizing *D. saccharalis* and *D. flavipennella*, it was observed that the females performed parasitism in both free and no-choice tests, showing that the testing arena did not hinder the search for hosts.

For *C. flavipes* biological characteristics, the larval period differed for laboratory-reared wasps lasting 16.7 days when reared on *D. flavipennella* and only 9.8 days when reared on *D. saccharalis*. For the wild population, a longer larval period of 17.1 days was also observed with the host *D. flavipennella.* When analyzing the data about the required period for larval development with the same host, *D. saccharalis*, a significant difference was observed among the populations of *C. flavipes*, 9.8 and 11.4 days for laboratory-reared wasps and wild population, respectively. On the other hand, it was observed lack of difference for parasitoids developing on *D. flavipennella* (Table 2). A nutritionally suitable host results in shorter development time, higher survival rate, larger body size, and higher reproduction rate (Stamp, 1991). Data from this work confirm that *D. saccharalis* furnished the necessary nutritional requirements to both parasitoid populations.

The pupal stage differed for wasps reared in the laboratory with a duration of 6.0 days when reared with *D. flavipennella* and 5.2 days with *D. saccharalis*. For the wild population, there was no difference when comparing the hosts. The parasitoid pupal stage for parasitoid developed on *D. saccharalis* was similar. However, when the laboratory-reared population performed the parasitism, a longer duration was observed for parasitoid developed parasitizing *D. flavipennella* (Table 2). Though, longer periods for *C. flavipes* developing on *D. saccharalis* can occur such as 6.2 days at temperature of 26 ± 1 °C (Trevisan, 2014), 19.8 days at temperature 27 ± 2 °C (Fonseca et al., 2015), and slightly longer than 20 days at temperature 27 ± 1 ºC (Silva et al., 2014).

The number of parasitoid larvae per host larvae in the two *C. flavipes* populations was similar (Table 3). When *D. saccharalis* was the host there was also no significant difference between the populations of parasitoids (76.8 larvae) and (79.3 larvae) when parasitized by the population laboratory-reared females and wild parasitoid females, respectively.

The number of pupae formed regarding the different parasitoid populations did not differ when the host was *D.*

Table 3. Total number of larvae, pupae and adults (± SE)[1] of *Cotesia flavipes* from *Diatraea saccharalis* and *Diatraea flavipennella*.

Hosts	*Cotesia flavipes*	
	Wild Population	Lab-reared population
	Larvae	
D. saccharalis	79.3 ± 7.9 a	76.8 ± 7.4 a
D. flavipennella	64.6 ± 4.6 a	68.4 ± 9.1 a
Df	1	1
P-value	0.914	0.435
	Pupae	
D. saccharalis	71.5 ± 7.9 a	67.1 ± 7.3 a
D. flavipennella	56.4 ± 4.6 a	46.4 ± 8.1 a
Df	1	1
P-value	0.405	0.349
	Adults	
D. saccharalis	70.2 ± 7.9 aA	66.0 ± 7.3 aA
D. flavipennella	43.3 ± 4.4 bA	35.2 ± 7.5 aA
Df	1	1
P-value	0.023	0.070

Means followed by the same lowercase letter in the column and upper case in the row do not differ by (GLM with quasipoisson distribution, followed by posthoc F test, p > 0.05).
[1]SE: mean standard error.

saccharalis. However, it was superior when the wild females parasitized *D. flavipennella* (Table 4).

The number of emerged parasitoids was equal when reared on *D. saccharalis* with 70.2 adults for wild population and 66 adults for long-term laboratory-reared population (Table 3). However, differences were observed between the wild population (43.3 adults) and long-term laboratory-reared population (35.2 adults) when *D. flavipennella* was used as a host. As observed in the behavior of *C. flavipes* parasitizing *D. saccharalis* and *D. flavipennella*, the parasitoid ability to recognize volatile host was demonstrated, showing preference or not when reared in their natal host (Silva, 2009).

The pupae weight differed statistically in the long-term laboratory-reared population, being higher (49.0 mg) when parasitizing *D. saccharalis* (Table 4). In the wild population, there was no significant difference, being the mass weight 57.8 and 53.6 mg, originating *D. saccharalis* and *D. flavipennella*, respectively. When comparing the two *C. flavipes* populations, no statistical difference was observed when *D. saccharalis* was the host. However, the largest mass weight was observed in the wild population (53.6 mg) compared to long-term laboratory-reared population (19.9 mg) in *D. flavipennella* as a host (Table 4).

In studies with *D. flavipennella*, *C. flavipes* pupae had body mass of 41.1 mg (Fonseca et al., 2015), as well as, 56.0 and 51.0 mg for pupal mass F1 and F2, respectively (De Bortoli et al., 2015). Rearing quality of *C. flavipes* is considered better when the pupae are large (Vacari et al., 2012). One of the parameters to determine *C. flavipes* parasitoid quality is the weight of pupae (Parra, 2002). Among the characteristics observed, a large number of larvae originating from *D. flavipennella* when parasitized by the long-term laboratory-reared population were unable to perform a proper formation of pupa, individuals were dispersed and consequently unprotected, not forming the mass, since the silk that surrounds pupae were not properly produced.

For the larval viability parameter, the outcome resulted in viability greater than 70% regardless of host and parasitoid population. The viabilities were 89.5 and 85.9% when using *D. saccharalis* as a host by the wild population and long-term laboratory-reared wasps, respectively. When using *D. flavipennella* as a host, the larval viabilities were 86.7 and 71.8%. For pupal viability parameter, the highest percentage was observed in *D. saccharalis*. The two parasitoid populations, the wild population (96.1%) and the long-term laboratory-reared population (96.6%); while in *D. flavipennella* the wild population (93.8%) and the long-term laboratory-reared population (77.9%).

Differently from those values found in the present work, there are records in the literature of 72 and 77% of viability for *C. flavipes* parasitizing *D. saccharalis* in two sugarcane varieties SP - 71 1081 and SP - 71 3146, respectively (Boiça Júnior et al., 1997). In addition, pupal viability values were recorded between 70 and 80% (Trevisan, 2014), around 91.6% (De Bortoli et al., 2015), and 94% (Vacari et al., 2012).

Low viability values are obtained when the host is nutritionally unable to provide sufficient proteins and carbohydrates to the parasitoid affecting insect development (De Bortoli et al., 2015). During evaluations, a smaller amount of the pupae mass of the long-term laboratory-reared population was observed, originating from *D. flavipennella*. These pupae individually were poorly shaped and dispersed, having no silk, as a result, leaving it fully exposed, a factor that caused a decrease in viability.

The number of adults produced by the wild population and the laboratory-reared population was statistically the same for the two species of hosts (Table 5). On the other hand, the host usage affected the number of emerged females with lower value when using *D. flavipennella* and the long-term laboratory-reared population, and only a partial significance with the wild population ($p < 0.06$) (Table 5).

A higher proportion of females is observed in progeny of the long-term laboratory-reared population when they were reared with *D. saccharalis*, compared to *D. flavipennella*. For wild parasitoid progeny the sex ratio was similar between both hosts. Variation on production of male in the progeny of *C. flavipes* takes place when female uses a host that is not suitable for progeny development. It is considered as a strategy to guarantee the greatest number of mating and

Table 4. *Cotesia flavipes* pupae mass weight (mg ± SE)[1] from *Diatraea saccharalis* and *Diatraea flavipennella*.

Hosts	*Cotesia flavipes*	
	Wild Population	Lab-reared population
D. saccharalis	57.8 ± 1.2 a	49.0 ± 1.2 a
D. flavipennella	53.6 ± 1.2 a	19.9 ± 1.2 b
CV (%)	44.83	

Means followed by the same lowercase letter in the column do not differ by Tukey HSD test ($p > 0.05$); [1] SE: mean standard error.

Table 5. *Cotesia flavipes* total number of males and females (± SE)[1] from *Diatraea saccharalis* and *Diatraea flavipennella*.

Hosts	*Cotesia flavipes*	
	Wild Population	Lab-reared Population
	Males[2]	
D. saccharalis	19.76 ± 2.7 a	21.18 ± 2.9 a
D. flavipennella	20.46 ± 2.1 a	21.40 ± 3.3 a
df	1	1
P-value	0.998	0.989
	Females[2]	
D. saccharalis	50.6 ± 6.2 aA	47.4 ± 5.5 aA
D. flavipennella	31.1 ± 4.3 aA	17.8 ± 3.8 bA
df	1	1
P-value	0.053	0.015
	Sex ratio[3]	
D. saccharalis	0.72 ± 0.03 aA	0.68 ± 0.04 aA
D. flavipennella	0.58 ± 0.04 aA	0.44 ± 0.02 bA
df	1	1
P-value	0.084	0.008

[2]Means followed by the same lowercase letter in the column and upper case in the row do not differ by (GLM with quasipoisson distribution, followed by posthoc F test, $p > 0.05$). [3] Means followed by the same lowercase letter in the column do not differ from each other (GLM with quasibinomial distribution, followed by posthoc F test, $p > 0.05$). [1] SE: mean standard error.

thus a greater number of descendants (Campos-Farinha et al., 2000). Results obtained in this work corroborate with 0.7 for sex ratio on the records for F1 generation of *C. flavipes* parasitizing *D. saccharalis* (Trevisan, 2014), as well as other studies performed for the evaluation of *C. flavipes* sex ratio, presenting values of 0.76 (De Bortoli et al., 2015) and 0.70 (Vacari et al., 2012; Silva et al., 2014). It is observed in these cases that sex ratio of *C. flavipes* is directed to a greater number of females, being useful to the biological control *D. saccharalis* (Vacari et al., 2012).

Adult males from the long-term laboratory-reared population, independently of host species, exhibited similar longevity (4.1 and 4.0 days for *D. saccharalis* and *D. flavipennella*, respectively) (Table 6). However, in the wild population, greater longevity was observed for the male parasitoids originated from *D. saccharalis* (4.0 days). Comparing the two parasitoids populations, no statistical differences were observed when they were reared on *D. saccharalis*, but in *D. flavipennella* there was increased longevity for males in the long-term laboratory-reared population.

Longevity of females differed between the hosts when parasitized by the wild population, but lack difference when the parasitoid was the long-term laboratory-reared, and longevity of female in the host *D. saccharalis* was higher in both cases: 4.6 days for long-term laboratory-reared females and 4.0 days for wild females. Larvae *D. flavipennella*, when parasitized by wild *C. flavipes*, differed from those reported in the literature, from 2.8 days for males and 3.5 days for females (De Bortoli et al., 2015), or 3.74 days for males and 2.92 for females (Lohmann, 2011). These differences can be justified by the differences in temperature, humidity, and different lineages of the parasitoid. In addition, pre-tests performed in the laboratory suggest that the longevity of adults can be twice as long when they feed in 5% honey solution.

Table 6. *Cotesia flavipes* male and female longevity (days ± SE)[1] reared with *Diatraea saccharalis* and *Diatraea flavipennella*.

Hosts	*Cotesia flavipes*	
	Wild population	Lab-reared population
	Males	
D. saccharalis	4.0 ± 0.0 aA	4.1 ± 0.1 aA
D. flavipennella	3.6 ± 0.1 bB	4.0 ± 0.0 aA
df	1	1
P-value	0.018	0.895
	Females	
D. saccharalis	4.0 ± 0.1 aA	4.6 ± 0.1 aA
D. flavipennella	3.7 ± 0.1 bA	4.0 ± 0.0 aA
df	1	1
P-value	0.017	0.895

Means followed by the same lowercase letter in the column and upper case in the row do not differ by (GLM with quasipoisson distribution, followed by posthoc F test, p > 0.05).[1] SE: mean standard error.

Experiment 2

For the relation Morphometric data × Parasitism, the analyses there were similarities for median width, anterior width and posterior width for both hosts. The total length in *D. saccharalis*, however, presented a significant difference when parasitized or not by *C. flavipes* (Figure 1). According to the results, it is possible to infer that after 10 days on artificial diet, *D. saccharalis* larvae with 22.5 mm in length are highly likely to be parasitized. The parasitoid presents an ability to adapt to the host in order to have its development needs fulfilled, manipulating its physiology, altering growth and development, as a strategy to reach nourishment at the time of parasitism (Pennacchio & Strand, 2006). The parasitized *D. saccharalis* extend their development to the pre-pupae stage without undergoing metamorphosis, being thus affected in its growth and development (Lopes, 2008).

The presence of the parasitoid causes changes in the hemolymph and fatty body of the *D. saccharalis* larvae (Passos et al., 2019), and also to the size of the larvae. Our data showed only the total length of the widths as a morphometric parameter that can be used to differentiate parasitized and non-parasitized larvae.

In general, the characteristics evaluated in this study showed a relationship between the groups of parasitoids of *C. flavipes* and the two species of *Diatraea*, with both serving as hosts for the parasitoid. Despite that, the greatest parasitism was observed by the parasitoid long-term laboratory-reared population upon *D. saccharalis*. The number of larvae, pupae and adults of this host, as well as the high rates of viability, characterize the effective action of the parasitoid-host relationship after successive generations in the laboratory. Otherwise, it was observed a defensive behavior of *D. flavipennella*, which attack the adult parasitoid before being parasitized. This behavior and a variation in the quality of the host plant, hence bringing variation into the nutritional quality as a host for the parasitoid may affect the parasitism differently from *D. saccharalis* continuously reared using a high performance artificial diet.

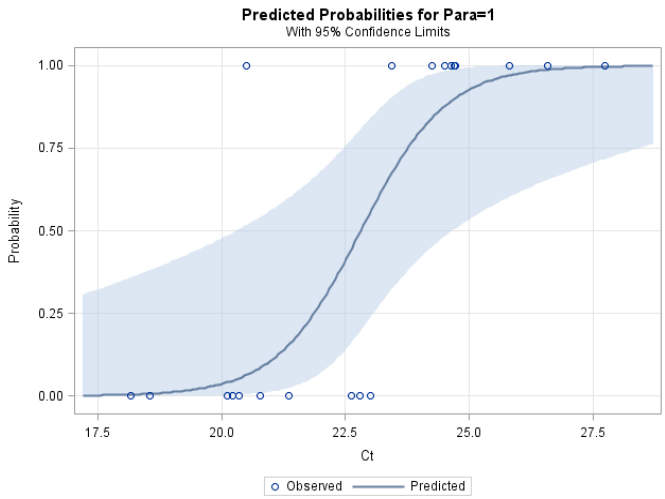

Figure 1. Total length schematic representation (Ct) of *Diatraea saccharalis* with its parasitism by *Cotesia flavipes*.

Conclusions

The wild population of *C. flavipes* exhibits preference and better performance parasitizing *D. flavipennella*.

Research to evaluate the parasitism efficiency and quality of the parasitoids crossing wild and laboratory populations of *C. flavipes* is necessary to enhance the chance for success of releasing this parasitoid.

Acknowledgments

The authors thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for granting scholarships (Finance Code 001).

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