Reproductive potential and biological characteristics of the parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) in *Diatraea saccharalis* (Lepidoptera: Crambidae) depending on parasitoid-host ratio

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Abstract

This study assessed the biological characteristics of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) with different densities of female parasitoids with *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) caterpillars. Third instar caterpillars of *D. saccharalis* were exposed to *C. flavipes* females at parasitoid to host ratios of 1:1, 3:1, 6:1, 9:1, and 12:1, with 5 replications. The parasitism of *C. flavipes* was 90% at 3:1 ratio, and the emergence was 100% for all densities. The life cycle of *C. flavipes* ranged from 18.17 ± 0.26 to 18.93 ± 0.50 d with the densities of 12:1 and 1:1 parasitoid to host. The higher progeny of *C. flavipes* (87.38 ± 2.07 and 67.18 ± 2.57 individuals per *D. saccharalis* caterpillar) were obtained at the densities of 3:1 and 6:1, respectively. The sex ratio of parasitoid per *D. saccharalis* caterpillar ranged from 0.12 ± 0.05 to 0.66 ± 0.02 between the parasitoid to host densities of 12:1 and 3:1, respectively. The density of 3:1 *C. flavipes* females per *D. saccharalis* caterpillar was found to be optimal for propagation of the parasitoid.

Key Words: biological control; larval parasitoid; sugarcane borer

Resumen

Este estudo avaliou as características biológicas de *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) com diferentes densidades de fêmeas de *C. flavipes*, com lagartas de *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae). Lagartas do terceiro ínstar de *D. saccharalis* foram expostas às fêmeas de *C. flavipes* na seguinte proporção (parasitoide para hospedeiro): 1:1, 3:1, 6:1, 9:1, e 12:1, com 5 repetições. O parasitismo de *C. flavipes* foi de 90% na densidade de 3:1, e a emergência foi de 100% para todas as densidades. O ciclo de vida de *C. flavipes* variou de 18,17 ± 0,26 a 18,93 ± 0,50 dias, com as densidades de 12:1 e 1:1 parasitóide:hospedeiro. A maior progênie de *C. flavipes* (87,38 ± 2,07 e 67,18 ± 2,57 indivíduos por lagarta *D. saccharalis*) foi obtida nas densidades de 3:1 e 6:1, respectivamente. A razão sexual deste parasitoide por lagarta de *D. saccharalis* variou de 0,12 ± 0,05 a 0,66 ± 0,02 entre as densidades parasitoide para hospedeiro. A densidade de fêmeas de *C. flavipes* 3:1 por lagarta de *D. saccharalis* foi considerada ideal para a multiplicação propagação desse parasitoide.

Palabras Clave: controle biológico; parasitoide de larvas; broca da cana-de-açúcar

Diatraea saccharalis (Fabricius) (Lepidoptera: Crambidae) is a major pest of sugar cane in North, Central, and South America (White & Wilson 2012; Dinardo-Miranda et al. 2012; Svedese et al. 2013). Direct injuries by *D. saccharalis* may cause losses of biomass and death of apical buds of plants (Rossato et al. 2013). Damage is cause by the reduction in production of sugar and alcohol by microorganisms present in the damaged stems (Dinardo-Miranda et al. 2012; Simões et al. 2012).

Chemical insecticides usually present low efficacy in first instar *D. saccharalis* because the insect feeds on the leaves of the plant cartridge, then migrates to the stalk of sugar cane (Antigo et al. 2013). Consequently, the importance of parasitoids for biological control of *D. saccharalis* is increased (Rodrigues et al. 2013). Use of the endoparasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) is the most efficient method of controlling *D. saccharalis* (Silva et al. 2012). In Brazil, *C. flavipes* is released into about 3 million ha of sugar cane fields annually to control *D. saccharalis* (Vacari et al. 2012).

Mass rearing is important for biological control programs (Pastori et al. 2013; Pereira et al. 2013), and the density of female parasitoids

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per host may affect parasitism (Sampaio et al. 2001), progeny sex ratio (Chong & Oetting 2006, 2007), life cycle (Pereira et al. 2010), and the longevity of descendants (Favero et al. 2013; Pastori et al. 2013). *Cotesia flavipes* reared with *D. saccharalis* presented the appropriate biological characteristics, but the ideal proportion of the females of this natural enemy per caterpillar host needs to be better understood to increase the biological effectiveness of *C. flavipes*, and to improve the multiplication of this natural enemy. Therefore, the objective of this work was to evaluate the biological characteristics of *C. flavipes* with *D. saccharalis* caterpillars in different densities using adult parasitoid females.

Materials and Methods

MULTIPLICATION OF *DIATRAEA SACCHARALIS*

Eggs of *D. saccharalis* were obtained from the rearing facility of the Laboratory of Biological Insect Control, Dourados, Mato Grosso do Sul, Brazil, and placed in glass vials (8.5 cm diam × 13 cm high) with an artificial diet based on wheat germ, soybean, and sugar cane yeast for newly hatched to the fourth instar caterpillars (Hensley & Hammond 1968). At this stage, the caterpillars were transferred to disposable Petri dishes (6.5 cm diam × 2.5 cm high) and fed with a soybean meal and sugar cane yeast diet until they reached the pupal stage (Parra 2007). A group of 5% of pupae were randomly selected without deformation. Pupae were placed in plastic pots covered with a screen until they reached the adult stage. The adults were separated into groups of 20 males and 30 females per cage of polyvinyl chloride (PVC) tubes (10 cm diam × 22 cm high). These cages were closed using bond paper and elastic and lined internally with paper sheets as the oviposition site. Eggs of *D. saccharalis* were collected daily, washed with a copper sulfate solution, and stored in a climate chamber at 25 ± 2 °C,70 ± 10% RH, and a 14:10 h (L:D) photoperiod, per Parra (2007).

MULTIPLICATION OF PARASITOID *COTESIA FLAVIPES*

Every fourth instar *D. saccharalis* caterpillar was individually exposed to a mated 24 h old *C. flavipes* female. After parasitism, the third instar larvae were placed in disposable Petri dishes with artificial diet semisolid (6.5 cm diam × 2.5 cm high). These plates were placed in a room at 25 ± 2 °C, 70 ± 10% RH, and a 14:8 h (L: D) photoperiod until *C. flavipes* pupae were formed. These pupae were placed individually in disposable cups with lids (100 mL) (Topform Plastics, São Paulo, São Paulo, Brazil) until the emergence of parasitoids. After emergence, a drop of honey was used to feed the parasitoids (Garcia et al. 2009).

EXPERIMENTAL DESIGN

Third instar *D. saccharalis* caterpillars were placed in plastic cups (100 mL) with artificial diet (Parra 2007) and exposed to 24 h old *C. flavipes* females. They were placed in a room at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h (L:D) photoperiod for 24 h, after which time the parasitoid females were removed from the cups. Each treatment consisted in 10 caterpillars and were replicated 5 times. Treatments were represented by densities of 1, 3, 6, 9, and 12 *C. flavipes* females per 1 *D. saccharalis* (1:1, 3:1, 6:1, 9:1, 12:1) (parasitoid to host). A completely randomized design was used.

The duration of the life cycle and percentage of parasitism (natural mortality of the host) (Abbott 1925), percentage of emergence of the progeny produced per female (*rf* = number of female progeny divided by number of females density), sex ratio (*rs* = number of females divided by number of adults), longevity 20 males and 20 females, and body length (mm) of female *C. flavipes.* The progeny ratio of females per female, sex ratio, and the body size (mm) of *C. flavipes* females emerged per *D. saccharalis* caterpillar were subjected to the analysis of variance and regression. Data of the number of males and females, parasitism, and emergence were subjected to the analysis of variance and, when significant, at 5% probability by the Scott-Knott test. The statistical software used for data analysis was the free version SigmaPlot (Systat Software Inc, San Jose, California, USA).

Results

The percentage of parasitism of *D. saccharalis* caterpillars varied with the density of *C. flavipes* females from 34.33% to 90% (Fig. 1). However, the percentage of caterpillars with emergence of this parasitoid was 100% for all densities (Table 1).

The life cycle of *C. flavipes* was 18.93 ± 0.50, 18.26 ± 0.05, 18.45 ± 0.23, 18.34 ± 0.17, and 18.17 ± 0.26 d from egg to adult at the densities of 1:1, 3:1, 6:1, 9:1, and 12:1 parasitoid to host, respectively. The density of *C. flavipes* female per *D. saccharalis* caterpillar affected its progeny ($\hat{y} = 9.9335 + 40.2034x - 6.5109x^2 + 0.2775x^3$; $F = 6.6438$; $P = 0.0008$; $R^2 = 0.55$), and ranged from 32.94 ± 2.37 to 87.38 ± 2.07 individuals of *C. flavipes* per *D. saccharalis* caterpillar (Fig. 2A). The number of male and female *C. flavipes* per *D. saccharalis* caterpillar was higher at the densities of 3:1 (parasitoids per caterpillar) and 6:1 (parasitoids per caterpillar) parasitoid to host (Table 1).

The sex ratio of *C. flavipes* emerged per *D. saccharalis* caterpillar varied ($\hat{y} = 0.7203 - 0.0019x - 0.0023x^2$; $F = 9.1467$; $P = 0.0004$; $R^2 = 0.52$) with the parasitoid to host densities (Fig. 2B). The rate of female produced per female parasitoid ($\hat{y} = 35.7941 - 6.2973x + 0.2913x^2$; $F = 48.4844$; $P = 0.0001$; $R^2 = 0.82$) ranged from 1.82 ± 0.43 to 29.44 ± 3.86 at 12:1 to 1:1 parasitoid to host ratio (Fig. 3A).

The longevity of *C. flavipes* females ranged from 2.10 ± 0.64 to 1.51 ± 0.47 d at 1:1 and 12:1 parasitoid to host density. The longevity of males ranged from 1.84 ± 1.32 to 1.67 ± 1.07 d between densities.

The body length of the *C. flavipes* females was 2.27 ± 0.31 mm to 2.09 ± 0.52 mm ($\hat{y} = 2.5428 - 0.3317x + 0.0607x^2 - 0.0030x^3$) and for males ranged from 1.89 ± 0.27 mm to 1.83 ± 0.18 mm ($F = 34.1565$; $P = 0.0001$; $R^2 = 0.83$) (Fig. 3B).

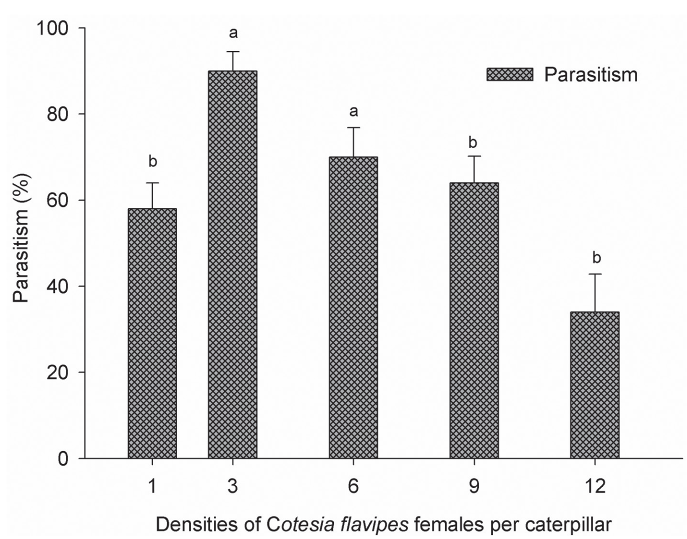

Fig. 1. Parasitism percentage of *Diatraea saccharalis* depending on *Cotesia flavipes* density: 1:1, 3:1, 6:1, 9:1, 12:1 (parasitoids to host) at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h (L:D) photoperiod. *P* = 0.05 (significance level).

Table 1. Number of female, males, and emergence percentage of *Cotesia flavipes* (Hymenoptera: Braconidae) (mean ± standard error) with 1, 3, 6, 9, and 12 female parasitoids per *Diatraea saccharalis* (Lepidoptera: Crambidae) caterpillar at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h (L:D) photoperiod.

Parasitoid to host	Females	Males	(*n*)	Emergence (%)
1:1	27.82 ± 2.24 b	12.62 ± 1.87 b	50	100 ± 0.00 a
3:1	57.16 ± 1.97 a	25.56 ± 1.97 a	50	100 ± 0.00 a
6:1	40.92 ± 2.35 a	26.42 ± 2.67 a	50	100 ± 0.00 a
9:1	34.74 ± 3.31 b	18.34 ± 2.13 b	50	100 ± 0.00 a
12:1	19.50 ± 2.89 b	13.44 ± 2.22 b	50	100 ± 0.00 a
CV	39.21	41.48	—	—

Means followed by the same letter per column do not differ ($P \leq 0.05$; Scott-Knott).

Discussion

This study indicated that parasitism, progeny, sex ratio, and longevity of *C. flavipes* females are directly affected by the density of parasitoid to host. Based in our results, *D. saccharalis* should be multiplied with a ratio of 3:1 (parasitoid to host). Chong and Oetting (2007) men-

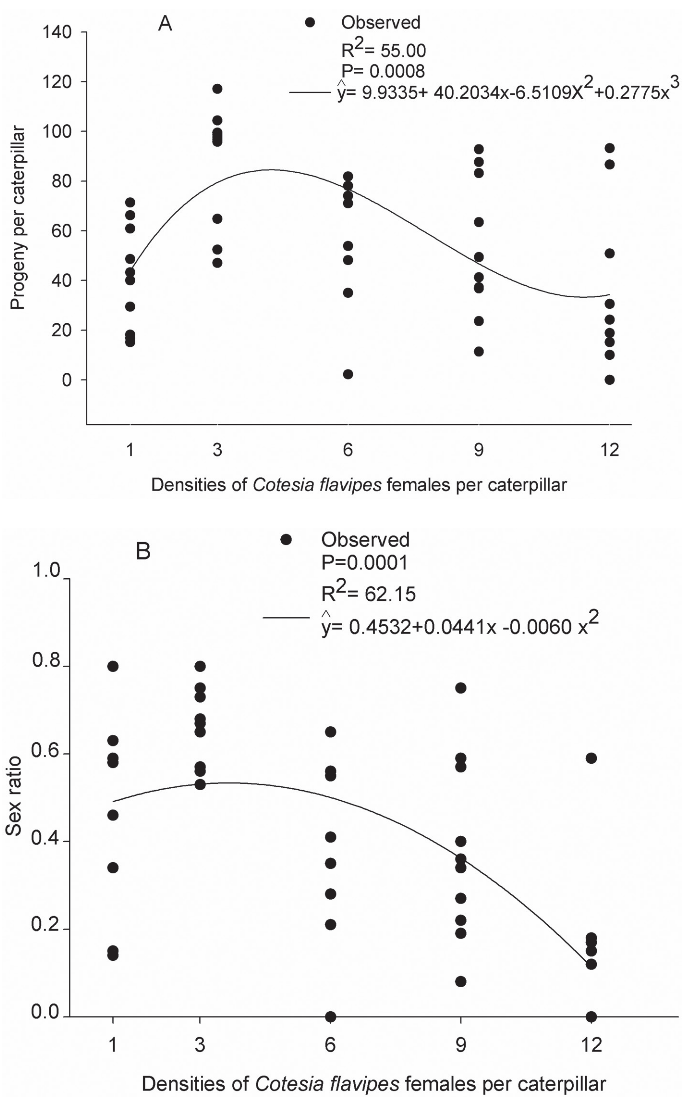

Fig. 2. Progeny (A) and sex ratio (B) of *Cotesia flavipes* density in *Diatraea saccharalis*: 1:1, 3:1, 6:1, 9:1, 12:1 (parasitoids to host) at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h (L:D) photoperiod.

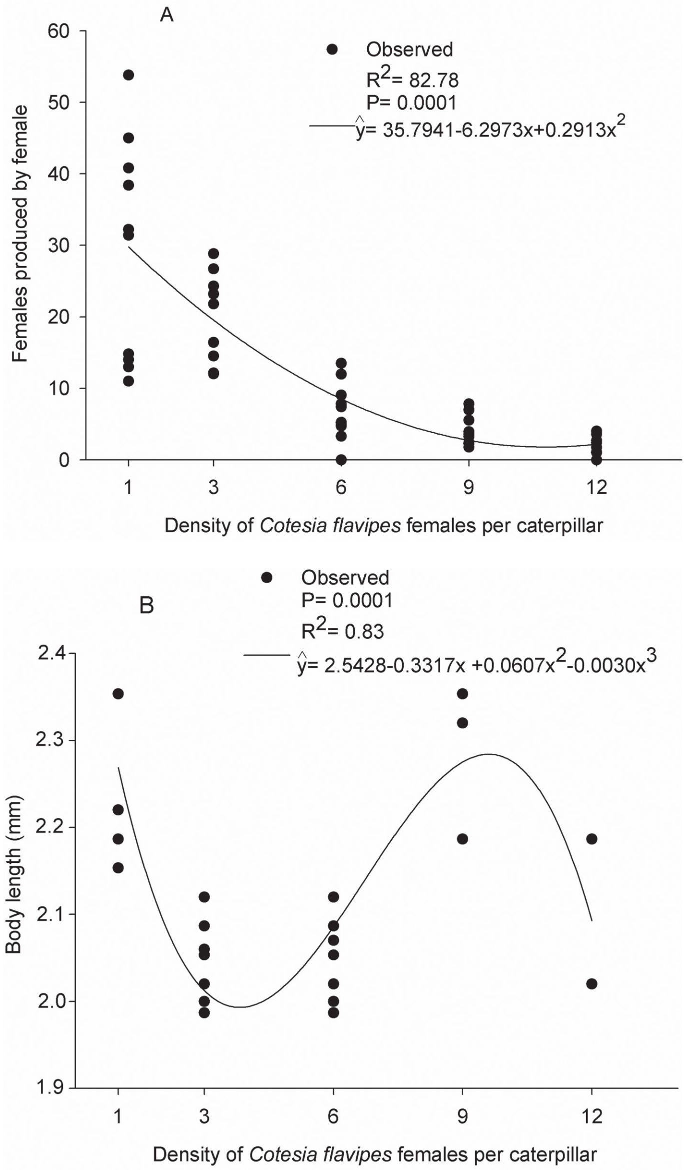

Fig. 3. Ratio of female produced by female (A) and body length (mm) (B) of *Cotesia flavipes* density in *Diatraea saccharalis*: 1:1, 3:1, 6:1, 9:1, 12:1 (parasitoids to host) at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h (L:D) photoperiod.

tioned that the density of parasitoid females per host can reduce the fertility and efficiency of mass rearing systems, principally due to the increased competition between immature parasitoids. Furthermore, Braconidae present the mechanisms to manipulate the immune response of their hosts (Strand & Pech 1995) with polydnavirus to facilitate the development of its larvae (Dupas et al. 2006, 2008).

The lowest parasitism rates of *C. flavipes* females on *D. saccharalis* caterpillars at the proportions of 1:1 to 12:1 parasitoid to host suggested the presence of defense mechanisms controlling immature parasitoids. Pennacchio and Strand (2006) suggested that hosts could present cellular defenses and reactions involving encapsulation and melanization of endoparasitoid eggs. On the other hand, parasitism by several females can increase the survival of immature parasitoids by overcoming the host immune response (Hood et al. 2012; Mahmoud et al. 2012). However, the increased competition for resources may hinder the development of immature parasitoids and the emergence of adults, as reported with the density of 28 *Trichospilus diatraeae* (Cherian & Margabandhu) (Hymenoptera: Eulophidae) females per *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae (Favero et al. 2013).

The reduced progeny with the higher densities (9:1 to 12:1) (parasitoid to host) suggested high proportions of parasitoid females per host result in greater competition and death of immature parasitoids. Similar results were found by Pereira et al. (2010) when evaluating how the density of female *Palmistichus elaeisis* Delvare and LaSalle (Hymenoptera: Eulophidae) affects their reproductive performance on pupae of *Bombyx mori* L. (Lepidoptera: Bombycidae).

The small size of the progenies found with 9:1 and 12:1 (parasitoid to host) ratio may also be explained by the lower amount of resources available per host, which limits the parasitoid development. However, Pereira et al. (2017) reported insect hosts that already have been parasitized are considered a low quality resource, which may affect the number of ovipositions made by other parasitoids. Because the number of eggs laid affects the host immune response, the offspring survivorship also may be affected. Pupae of *Diaphania hyalinata* L. (Lepidoptera: Crambidae) received 1 to 5 parasitoid *P. elaeisis* ovipositions. *Palmistichus elaeisis* developmental time decreased with increased oviposition density and 3 ovipositions provided higher offspring numbers, particularly female production, and optimal larval fitness. Progeny body mass and sex ratio were not affected by oviposition density. Females and males survived longer with 1 oviposition of the female parasitoid. Parasitoid emergence increased with the number of parasitoid ovipositions, and 100% parasitism and corresponding 100% host pupal mortality were achieved with all oviposition densities. An increased number of ovipositions decreased the number of total hemocytes and granulocytes, plasmatocytes, and prohemocytes in the circulating host hemolymph. Oenocytes and espherulocytes were not affect by the number of parasitoid ovipositions in the host. Superparasitism is a strategy of *P. elaeisis* for optimal progeny fitness, balancing optimal progeny performance with amelioration of host immune response (Pereira et al. 2017).

The high sex ratio of *C. flavipes* per *D. saccharalis* caterpillar at the densities of 3:1 and 6:1 parasitoid to host is important because the female parasitoids are responsible for parasitism and progeny production (Amalin et al. 2005; Rodrigues et al. 2013). Thus, the reduction of sex ratio may compromise the efficiency of parasitism of *C. flavipes* due to the lower number of females produced (Pereira et al. 2009; 2010). However, the variation in the sex ratio per caterpillar of this host may be related to the resource availability, as reported for *T. diatraeae* with *D. saccharalis* and *T. molitor* pupae (Chichera et al. 2012; Favero et al. 2013) and *P. elaeisis* with *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), *Bombyx mori*, and *D. saccharalis* pupae (Pereira et al. 2010, 2013; Chichera et al. 2012).

The inverse relation in the number of parasitoid females produced per *C. flavipes* female with the total number of offspring confirms the fact that superparasitism may decrease the number of females produced (Soares et al. 2009; Andrade et al. 2010). The number of females laying eggs determines the sex ratio; as this number increases, the number of fertilized eggs decreases (Andrade et al. 2012).

The variations in size of *C. flavipes* adults with different parasitoid female densities can be explained by the resources available per host pupae with the increasing number of larvae developing in its interior (Tian et al. 2008; Harvey et al. 2013). This can reduce the efficiency of biological control because the body size is positively correlated with the indicators of parasitoid quality, such as higher longevity, fecundity, progeny emergence, and sex ratio (Pereira et al. 2010).

The use of 3 *C. flavipes* females to each *D. saccharalis* caterpillar may increase the genetic variability of the offspring, improve propagation, and reduce the production costs of this parasitoid. This ratio also is the most suitable density for the propagation of this natural enemy due to its higher parasitism, progeny, sex ratio, and longevity of females, in addition to contributing to the increase in the genetic variability of the offspring.

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