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SEARCHING AND PARASITISM OF *Diatraea saccharalis* (LEPIDOPTERA: CRAMBIDAE) BY *Trichospilus diatraeae* (HYMENOPTERA: EULOPHIDAE)

Búsqueda y parasitismo de *Diatraea saccharalis* (Lepidoptera: Crambidae) por *Trichospilus diatraeae* (Hymenoptera: Eulophidae)

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ABSTRACT

The ability of *Trichospilus diatraeae* Cherian and Margabandhu, 1942 (Hymenoptera: Eulophidae) to search and parasitize *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) pupae in sugarcane stalks was evaluated. To analyze the ability for search and parasitism were used stalks of sugarcane (20 cm) where it was introduced a pupa of *D. saccharalis* (T1); a pupa and a caterpillar (T2) or a pupa and fecal matter (T3). Each stalk was placed in a transparent plastic bottle with 21 females of *T. diatraeae*. These pupae were isolated, after 72 h, in glass tubes at 25 ± 2 °C, 70 ± 10 % relative humidity, 14:10 light/dark. The experiment was developed in an entirely casualized design with three treatments and 12 repetitions. Percentage of *D. saccharalis* pupa parasitized by *T. diatraeae* was 50 %, 83.33 % and 16.66 % in the T1, T2 and T3, respectively (χ^2 = 3.896, p= 0.04). The presence of *D. saccharalis* caterpillars favored searching and parasitism of this host.

Keywords: biological control, host location, parasitoid.

RESUMEN

La capacidad de *Trichospilus diatraeae* Cherian y Margabandhu, 1942 (Hymenoptera: Eulophidae) para buscar y parasitar las pupas de *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) en los tallos de la caña de azúcar fue estudiada. Para analizar la habilidad de búsqueda y parasitismo fueron utilizados tallos de la caña de azúcar (20 cm) donde se introdujo una pupa de *D. saccharalis* (T1); pupas y orugas (T2) o pupa y residuos fecales (T3). Cada tallo fue colocado en una botella plástica transparente con 21 hembras de *T. diatraeae*. Esas pupas fueron individualizadas, luego de 72 h, en tubos de vidrio a 25 ± 1 °C, 70 ± 10 % UR y 14 h de foto período. El experimento se desarrolló en un diseño completamente al azar, con tres tratamientos y 12 repeticiones. Los porcentajes de pupas de *D. saccharalis* parasitadas por *T. diatraeae* fueron del 50 %, 83,33 % y 16,66 % en el T1, T2 y T3, respectivamente (χ^2 = 3.896, p = 0,04). La presencia de *D. saccharalis* en los tallos de caña de azúcar favorecieron la búsqueda y parasitismo de su hospedero.

Palabras clave: control biológico, localización hospedero, parasitoide.

RESUMO

A capacidade de *Trichospilus diatraeae* Cherian e Margabandhu, 1942 (Hymenoptera: Eulophidae) para procurar e parasitar pupas de *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) em colmos de cana-de-açúcar foi avaliada. Para analisar a capacidade de busca e parasitismo foram usados colmos de cana-de-açúcar (20 cm), onde foi introduzida uma pupa de *D. saccharalis* (T1); uma pupa e uma lagarta (T2) or uma pupa e resíduo fecal (T3). Cada colmo foi colocado em uma garrafa plástica transparente com 21 fêmeas de *T. diatraeae*. Essas pupas foram retiradas dos colmos após 72 h, e colocadas em tubos de vidro a 25 ± 2 ° C, 70 ± 10 % de umidade relativa e fotofase de 14 h. O experimento foi desenvolvido em delineamento inteiramente casualizado, com três tratamentos e 12 repetições. A porcentagem de pupas de *D. saccharalis* parasitadas por *T. diatraeae* foi de 50 %, 83,33 % e 16,66 % em T1, T2 e T3, respectivamente (χ^2 = 3,896, p = 0,04). A presença de lagartas de *D. saccharalis* favoreceu a busca e parasitismo deste hospedeiro.

Palavras-chave: controle biológico, localização de hospedeiro, parasitoide.

INTRODUCTION

Adult female parasitoids respond to semiochemicals in the micro-habitat to find host pupae (Pinto *et al.*, 2007; Fontana *et al.*, 2011). These compounds are released from plants infested by herbivores and/or from fecal matter of these insects which are detected by parasitoid receptors (Fatouros *et al.*, 2007; Dicke *et al.*, 2009; Girling *et al.*, 2011; Hegde *et al.*, 2011). The efficiency of parasitoids depends on its ability to detect hosts in the field natural conditions (Silva-Torres *et al.*, 2009), although it lay be reduced in absence of hosts at right developmental stages for parasitism (Hausmann *et al.*, 2005). The capacity parasitoids to find endophytic pupa is important because they usually remain hidden which reduces emission of detectable volatiles (Bruinsma *et al.*, 2009). Caterpillars may build shelters as protection that reduce its parasitism (Rodovalho *et al.*, 2007).

Females of the parasitoid *Brachymeria intermedia* (Nees, 1834) (Hymenoptera: Chalcididae) can identify kairomones of *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Lymantriidae) after being exposed to pupa of this host (Cardé and Lee, 1989). This indicates that searching-behaviors may depend on acquired experience of parasitoids (Hoballah and Turlings, 2005; Peñaflor *et al.*, 2011). Contact with host before releasing may improve searching efficiency of laboratory reared parasitoids released in the field (González *et al.*, 2011).

Trichospilus diatraeae Cherian and Margabandhu, 1942 (Hymenoptera: Eulophidae) parasitizes insect pupa, mainly those of lepidopterans, and present potentially for biological control of agricultural and forest pests (Boucek, 1976; Zaché *et al.*, 2010; Melo *et al.*, 2011). The ability of this insect to find and parasitize *D. saccharalis* pupae inside sugarcane stalks are related with semiochemicals (Krugner *et al.*, 2008), vibrations (Fischer *et al.*, 2003), and/or chromatic and achromatic cues (Fischer *et al.*, 2004).

Diatraea saccharalis is an important pest of sugarcane, because the intensity of the attack and cause losses in sugar and alcohol productivity (Segato *et al.*, 2006; Dinardo-Miranda *et al.*, 2012). Controlling the sugarcane borer with chemicals is difficult, because this insect develops in a protected location, inside the stem of the plant (Vacari *et al.*, 2012). Therefore, biological control with parasitoids has been the most used way to combat this insect, so it is important to simulate the natural conditions before performing releases of *T. diatraeae* in the plantations of sugarcane, to check efficiency of parasitism in the field.

The present work evaluated capacity of *T. diatraeae* to search and parasitize *D. saccharalis* pupae in the presence of final instar caterpillars or fecal matter of this host.

MATERIALS AND METHODS

Rearing *D. saccharalis*

Diatraea saccharalis pupae were supplied by Empresa Agentes Biológicos BUG. This insect was reared with the following methodology: recently hatched caterpillars were maintained in glass tubes (8.5 x 2.5 cm) sealed with cotton and fed an artificial diet until pupa stage. Pupae were collected, sexed, and 20 males and 30 females were placed together for ovipositon in PVC cages (22 x 10 cm) lined with sheets of sulfite paper humidified with distilled water. This PVC cages were sealed with a voile-type fabric and elastic (Parra, 2007).

Rearing *T. diatraeae*

Trichospilus diatraeae adults were maintained in glass tubes (14 x 2.2 cm) sealed with cotton and fed with drops of pure honey. *D. saccharalis* pupae with 24 to 48 hours old were subsequently introduced into these tubes during 24 hours. Pupae were removed and individualized in glass tubes (14 x 2.2 cm) in a chamber with controlled conditions chamber (25 ± 2 °C, 70 ± 10 % relative humidity, under a 14:10 light/dark) until emergence (Pereira *et al.*, 2008).

Experimental Design

Sugarcane stalks were cut in 20 cm long segments. An orifice was made in each segment and a 24 hour-old *D. saccharalis* pupa (184.00 ± 0.01 mg) was placed in each cavity. After the fixed inside each cavity and the stalk segments placed individually in transparent plastic tubes (25 x 9 cm) with 21 *T. diatraeae* females for 72 hours (Chichera *et al.*, 2012). Pupae were then removed and individualized in glass tubes (14 x 2.2 cm) in a chamber with controlled conditions (25 ± 2 °C, 70 ± 10 % relative humidity, 14:10 light/dark) to evaluate parasitism and to observe the emergence of *T. diatraeae*. Controls consisted of *D. saccharalis* pupae fixed into sugarcane stalk segments under identical conditions, but without *T. diatraeae* females.

Treatments consisted of exposing the parasitoid to: a pupa

(T1); a pupa and a caterpillar (T2) or a pupa and the fecal matter (T3) of *D. saccharalis*. Treatment T2 had two holes in the stalks (one at each internode) with one pupa in one hole and a fourth or fifth instar caterpillar in the other one. Treatment T3 had one pupa in an orifice into the stalk with fecal matter of *D. saccharalis* around its external area.

Each parcel was exposed to 21 *T. diatraeae* females, with 12 replications in an entirely randomized design. It was evaluated the percentage of parasitism [(number of pupae of *D. saccharalis* with emergence of parasitoid + pupae without adult emergence of *D. saccharalis*) / (total number of pupae) × 100]; the emergence percentage [(number of pupae of *D. saccharalis* to adult emergence of parasitoids) / (number of parasitized pupae) × 100] and progeny per pupa (number of parasitoid emerged per pupa of *D. saccharalis*). The successful of search of the parasitoid was measured by the percentage of parasitism of pupae.

Percentages of *T. diatraeae* parasitism were analyzed with a general linear model with binomial distribution ($p \leq 0.05$) using the R Statistical System software package (Ihaka and Gentleman, 1996). This analysis was performed using the original non-parametric data in percentages to facilitate interpretation. Data of emergence of *T. diatraeae* progeny were submitted to an analysis of variance with the test F at 5 % significance.

RESULTS

The present of final instar caterpillars or fecal matter of *D. saccharalis* affected the search and percentage of parasitism of females *T. diatraeae*. Percentage of *D. saccharalis* pupa parasitized by *T. diatraeae* was 50 %, 83.33 % and 16.66 % in the T1, T2 and T3, respectively ($\chi^2 = 3.896$, $p = 0.04$) (Fig. 1). The presence of *D. saccharalis* caterpillars increased its shear-ching and parasitism of *T. diatraeae*.

Emergence of *T. diatraeae* adults from *D. saccharalis* pupa was similar between treatments with pupa (66 %) or pupa and caterpillars (70 %) ($p > 0.05$). This parasitoid did not emerged in the treatment with pupa and fecal matter of *D. saccharalis*.

Progeny of *T. diatraeae* from *D. saccharalis* pupae was similar between treatments with pupae and caterpillars or only pupae (Table 1) of this host.

DISCUSSION

The ability of *T. diatraeae* to find *D. saccharalis* pupae in the orifices in sugarcanes talks this species is important for biological control (Chichera *et al.*, 2012). High parasitism levels of *D. saccharalis* pupae indicated potential of *T. diatraeae* as a biological control agent of this lepidopteran.

The parasitism of *T. diatraeae* in pupae of *D. saccharalis* introduced in the stalks of sugarcane was 50 %. Chichera *et al.* (2012) also noted 56 % parasitism in the experiment where the pupae of *D. saccharalis* were introduced in stems and exposed to *T. diatraeae*. A total of 83.33 % of *D. saccharalis* pupae was parasitized in the presence of caterpillars that host, this indicates that *T. diatraeae* was stimulated in the presence of larvae.

Higher ability of *T. diatraeae* to search *D. saccharalis* pupae with host caterpillars present suggests that it can recognize substances released by them and that they were close to pupation. As *Cotesia kariyai* (Watanabe, 1937) (Hymenoptera: Braconidae) females can distinguish chemical from damage by *Pseudaletia separata* (Walker, 1865) (Lepidoptera: Noctuidae) caterpillars up to fourth instar, but not between

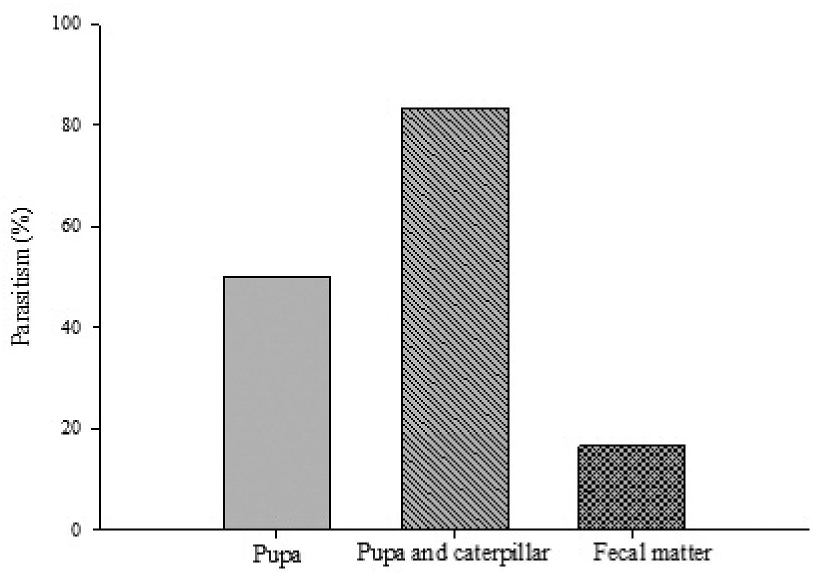

Figure 1. Percentage of parasitized pupae of *Diatraea saccharalis* (Lepidoptera: Crambidae) in sugarcane stalks by *Trichospilus diatraeae* (Hymenoptera: Eulophidae). Treatments represented by: pupa, pupa and caterpillar or pupa and fecal matter. 25 ± 2 °C, RH: 70 ± 10 % and 14 hours ($\chi^2 = 3.896$, $p = 0.04$).

Table 1. Progeny (mean ± standard error) of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) on pupae of *Diatraea saccharalis* (Lepidoptera: Crambidae) in sugarcane stalks. Treatments: pupa, pupa and a caterpillar or pupa and fecal matter 25 ± 2 ° C, RH: 70 ± 10 % and 14 hours.

Treatments	Progeny
Pupa	188.00 ± 55.50 a
Pupa and caterpillar	231.57 ± 45.64 a
Pupa fecal matter	-

Means followed by same letter do not differ by the F test ($p > 0.05$).
(-) No progeny was obtained.

later ones (Takabayashi *et al.*, 1995) – when they are not appropriate for parasitism.

The emergence of the progeny of *T. diatraeae* indicates that this parasitoid can search, parasitized and develop within *D. saccharalis* pupae in the orifices in sugarcane stalks – again demonstrating its potential for biological control (Keasar and Steinberg, 2008; Chichera *et al.*, 2012). The emergence of progeny favors establishment of this parasitoid in plantations (Bellows *et al.*, 2006) and may reduce the numbers of re-introductions and costs of producing and releasing this wasp (Gichini *et al.*, 2008).

Lower parasitism of *D. saccharalis* pupae by *T. diatraeae* with host fecal matter may be due to odors released these residues. Additionally, this fecal matter used in the experiment came from *D. saccharalis* caterpillars reared on artificial diet, and may have different composition in fecal matter of caterpillars fed on sugarcane plants in the natural environment. The searching behavior of the parasitoid *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae) is mediated by a water-soluble substance from the fecal matter of *D. saccharalis* caterpillars. The contact with this substance induces searching-behavior characterized by reduced locomotion and tapping the feces with its antenna (Van Leerdam *et al.*, 1985). On the other hand, semiochemicals may not be important for *Spathius agrili* Yang, 2005 (Hymenoptera: Braconidae) to find *Agrilus planipennis* Fairmaire, 1888 (Coleoptera: Buprestidae) by this parasitoid females rely on host-generated vibrations to find suitable hosts (Wang *et al.*, 2010).

Pupae and caterpillars of *D. saccharalis* together in sugarcane stalks favored parasitism of this pest by *T. diatraeae* by simulating naturally infested plants. Volatile compounds released from the sugarcane stalks due to *D. saccharalis* caterpillar-feeding may facilitated searching of pupae of this host by this natural enemy. Cabbage plants infested by *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) (Girling *et al.*, 2011) stimulated searching behavior of females of the larval parasitoid *Cotesia vestalis* (Haliday, 1834) (Hymenoptera: Braconidae). The parasitoid *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) was attracted by volatile compounds released by corn plants damaged by caterpillars of *Elasmopalpus lignosellus* (Zeller, 1848) (Lepidoptera: Pyralidae) (Xavier *et al.*, 2011).

The presence of *D. saccharalis* caterpillars in sugarcane stalks increased its searching and parasitism by *T. diatraeae* – which could be due to the perception and identification of substances released by the caterpillars or to odors from damaged sugarcane stalks. On the other hand, fecal matter from *D. saccharalis* reduced parasitism, which could be attributed to chemicals such us not identified by *T. diatraeae*. The origin and compositions of chemical substances that help this parasitoid to search host pupae need to be investigated to understand the efficiency of *T. diatraeae* to control *D. saccharalis* in the field.

In summary, *T. diatraeae* females searched and parasitized *D. saccharalis* pupae in sugarcane stalks in the laboratory. The presence of *D. saccharalis* caterpillars favored searching and increased parasitism of pupae of this pest in sugarcane stalks. Fecal matter from *D. saccharalis* caterpillars reduced parasitism by *T. diatraeae*.

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