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Original Article

Parasitism rate of *Plutella xylostella* (Lepidoptera: Plutellidae) larvae in greenhouse by *Tetrastichus howardi* (Hymenoptera: Eulophidae) females at different densities

Taxa de parasitismo de lagartas *Plutella xylostella* (Lepidoptera: Plutellidae) em estufa por fêmeas de *Tetrastichus howardi* (Hymenoptera: Eulophidae) em diferentes densidades

H. C. Pereira[a]*, F. F. Pereira[b], W. C. Fernandes[b], Z. F. Carneiro[c], J. T. Lucchetta[b], G. S. Andrade[c] and J. C. Zanuncio[d]

[a]Universidade Federal da Grande Dourados – UFGD, Departamento de Ciências Agrárias, Dourados, MS, Brasil
[b]Universidade Federal da Grande Dourados – UFGD, Departamento de Ciências Biológicas e Ambientais, Dourados, MS, Brasil
[c]Universidade Tecnológica Federal do Paraná, Departamento de Ciências Agrárias, Pato Branco, PR, Brasil
[d]Universidade Federal de Viçosa – UFV, Instituto de Biotecnologia Aplicada à Agropecuária – BIOAGRO, Departamento de Entomologia, Viçosa, MG, Brasil

Abstract

Parasitoids control insect pests, but their number per host affects their efficiency. The objective of this work was to evaluate the best density of *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) individuals parasitizing fourth instar *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) larvae in greenhouse conditions. These larvae were exposed to parasitism by *T. howardi* females with 1:1, 3:1, 6:1, 9:1, 12:1, 15:1 and 18:1 parasitoid/host ratios with 10 replications during 96 hours. After this period the larvae were kept on host plants (*Brassica oleracea*) until pupa formation. *Tetrastichus howardi* parasitized and reproduced in *P. xylostella* larvae at all its densities tested, but with higher values, 84% and 10 ± 2.4 individuals, respectively, with 9:1 parasitoids/host. Nine *T. howardi* females per *P. xylostella* larvae are the adequate number to manage this insect pest.

Keywords: biological control, diamondback moth, parasitoids.

Resumo

Os parasitoides controlam os insetos pragas, mas o seu número por hospedeiro afeta a sua eficiência. O objetivo deste trabalho foi avaliar a melhor densidade de *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) parasitando o quarto instar de larvas de *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) em condições de estufa. Estas lagartas foram expostas ao parasitismo por fêmeas *T. howardi* com densidades 1:1, 3:1, 6:1, 9:1, 12:1, 15:1 e 18:1 de parasitoide/hospedeiro com 10 repetições durante 96 horas. Após este período, as lagartas foram mantidas em plantas hospedeiras (*Brassica oleracea*) até à formação de pupas. *Tetrastichus howardi* parasitaram e reproduziram em lagartas *P. xylostella* em todas as suas densidades testadas, mas com valores mais elevados de 84% e 10 ± 2.4 indivíduos, respectivamente, com 9:1 parasitoides/hospedeiro. Nove fêmeas de *T. howardi* por lagarta de *P. xylostella* são o número adequado para controlar está praga de inseto.

Palavras-chave: controle biológico, traça-diamante, parasitoides.

1. Introduction

The endoparasitoid *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) is a potential agent for the biological control of lepidopteran pests. Polyphagy, parasitism of agricultural and forest pests, gregarious, high number of individuals per generation, developing at different temperatures and dispersion favour the use of this natural enemy in the biological control (Favero et al. 2015; Kumar et al. 2016; Favoreto et al. 2021). *Tetrastichus howardi* developed and emerged from *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) larvae and pupae (Pereira et al., 2015; Rodrigues et al., 2021).

The diamondback moth, Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), is the main pest of cultivated species of the Brassicaceae family (Rezaei et al., 2018). A characteristic that contributes for this pest to have a preference for plants of the Brassicacea family, it is due to the presence of compounds formed from glucosinolates. These compounds are toxic to most insects, however, they

*e-mail: heltercp@gmail.com
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stimulate feeding and host plant selection by the insect (Van-Loon et al., 2002). The life cycle of this lepidopteran is short depending on the temperature with several generations per year. This cosmopolitan, polyphagous insect is found globally (Dancau et al., 2018). Its larvae damage leaves and inflorescences of Brassicaceae plants reducing the photosynthetic area with damage reaching up to 100% (Boiça-Júnior et al., 2015; Machekano et al., 2017).

The use of synthetic insecticides to control *P. xylostella* alters the metabolism of this insect selecting those resistant to the products used (Sarfraz and Keddie, 2005). The natural biological control is an alternative with reports of parasitoid and predator species on *P. xylostella* in Brassicaceae crops (Karindah et al., 2005; Dancau et al., 2019). In biological control programs which use parasitoid insects, hymenoptera stand out for their wide diversity (Silva et al., 2020) Using mass breeding techniques, evaluating their biological parameters and the release of these parasitoids (Barbosa et al., 2019; Ongaratto et al., 2020).

Studies on the female densities are necessary to increase the efficiency of natural enemies in pest control (Scheiner and Martin, 2020). The best density of parasitoid females to host individuals is important to estimate the number of insects released and that of releasing points of natural enemies in biological control programs (Barbosa et al., 2019).

Tetrastichus howardi naturally parasitized *P. xylostella* (Silva-Torres et al., 2010) and the objective was to determine the best density of its females parasiting larvae of this pest in greenhouse conditions.

2. Material and Methods

The experiments were carried out at the Laboratory for Biological Control of Insects (LECOBIOL) and in a greenhouse (22° 19' 80" S, 54° 93' 38" W) of the Faculty of Biological and Environmental Sciences at the Universidade Federal da Grande Dourados in Dourados, Mato Grosso do Sul state, Brazil.

2.1. Laboratory rearing Plutella xylostella

Plutella xylostella larvae were obtained from the LECOBIOL, where this insect is reared in an air-conditioned room, at the temperature of 25 ± 2 °C, 70 ± 10% relative humidity, and a photoperiod of 12 h. *Plutella xylostella* adults were confined in transparent circular plastic cages (30 cm high and 20 cm in diameter), with a side rectangular opening sealed with a fine nylon mesh, to circulate the air. Adults of this pest were fed a solution of 10% honey in a sponge (10 cm long x 2 cm wide) on the top of the cage. Leaf discs of *Brassica oleracea* cabbage var. acephala (6 cm in diameter) from local organic producers were distributed on filter paper moistened in distilled water as substrate for the *P. xylostella* to lay eggs. The egg masses on the leaf discs were removed and placed in a container with a fresh cabbage leaf as food for the newly emerged larvae. Fresh leaves were provided daily until the pupa stage, which were removed from the leaves with the aid of a brush and placed in mating cages (Barros et al., 2012).

The identification of the species *Plutella xylostella* was made with the aid of a taxonomic identification key.

2.2. Collecting and laboratory rearing T. howardi

Tetrastichus howardi was collected in 2010 at the Experimental Farm of the Universidade Federal da Grande Dourados (UFGD) (22º13'16" S, 54º48'2" W, 530 m) from *D. saccharalis* pupa in sugarcane stalks. The "voucher specimens" were deposited in the Entomological Collection of the Universidade Federal do Espírito Santo (UFES), Department of Biological Sciences at UFES, 514 Fernando Ferrari Avenue, Biological Sciences Building, Block A, room 216-215, zip code 29.075-919, Vitória, Espirito Santo, Brazil. Archive: 150792 to 150816 to 157187, data available online from Splink. The parasitoid was identified by Prof. Dr. Marcelo Teixeira Tavares (Vargas et al., 2011).

Adults of *T. howardi* were kept in glass tubes (15 × 2 cm) covered with cotton with a droplet of pure honey. Each *D. saccharalis* pupae, 24 to 48 h old, were exposed to parasitism by five *T. howardi* females for 24 h at 25 ± 2 °C, relative humidity (RH) of 70 ± 10% and photophase of 14 h in a climate-controlled chamber. Parasitoids emerged after 18 to 20 days from parasitized host pupae were fed with a droplet of honey and left for 24 hours for mating. Females were individualized after mating and fed to maintain its rearing and/or to perform the bioassays (Vargas et al., 2011).

2.3. Experiment development

Brassica oleracea seedlings, in the second phenological development stage and characterized by less than five true leaves, were acquired from a local retailer in Dourados, Mato Grosso do Sul, Brazil for the greenhouse bioassay. These seedlings were placed in 1L volume vases with a substrate composed of organic fertilizer and soil (dystroferric red oxisol) in the proportion of 1:2. The seedlings were placed in a greenhouse until the third phenological stage, with six to eight true leaves.

The experiment was carried out in a polyethylene greenhouse measuring 6 X 18 m^2, the vases were on 1.20 m high benches systems arranged randomly spaced at 15 cm, with average temperature and relative humidity of 27.67 ± 2.29 °C and 54.2 ± 4.45%. Four hundred vases were used, containing one cabbage plant at the phenological stage III, and one fourth instar *P. xylostella* larva each. Wooden stakes were placed at each end of the vase and voile used to prevent larvae to escaping.

The treatments were composed of different densities of *T. howardi* females per *P. xylostella* larva on a cabbage plant. Mated and fed 24-hour-old *T. howardi* females were released into the vases at the densities of 1:1, 3:1, 6:1, 9:1, 12:1, 15:1 and 18:1 parasitoid females per *P. xylostella* larva. The densities of larvae per plant and of parasitoids per larva were determined through laboratory pre-tests. After 96 hours of parasitism, *P. xylostella* pupae and larvae were collected using a brush. Larvae were individualized in Petri dishes with cabbage leaves and the pupae in glass tubes (15 cm) covered with cotton and placed in a B.O.D. incubator with controlled temperature, humidity, and photoperiod of 25°C ± 2 ºC, 70 ± 10% and 12 hours, respectively.

2.4. Data analysis

The experiment was carried out in a completely randomized design (CRD) with seven treatments and a control (no *T. howardi* females). Fifty replications were used with a cabbage plant and one fourth instar *P. xylostella* larvae in each one.

Percentage of parasitism (%P) [(*P. xylostella* larvae with parasitoid emergence + larvae without *P. xylostella* adult emergence)/(total number of larvae) × 100]; total progeny (parasitoids emerged per parasitized host); emergence (% E) [(*P. xylostella* larvae with parasitoid emergence)/(number of parasitized larvae) × 100]; progeny per female (progeny of females as a function of the number of females per evaluated density); life cycle duration (development period of immature *T. howardi* from the day of parasitism to the emergence of its adults); and sex ratio (number of emerged females/number of total emerged adults) were the biological characteristics evaluated per treatment.

Data on the biological characteristics were submitted to analysis of variance (ANOVA) at 5% probability. Values significant were submitted to regression analysis and adjusted according to the model using all significant coefficients, based on the determination coefficient (R2), on the significance of the regression coefficients by the F test ($p \leq 0.05$) and on the adaptation to the biological phenomenon studied. The overall average of the data was performed for non-significant data.

3. Results

Tetrastichus howardi females found and parasitized fourth instar larvae of *P. xylostella* under greenhouse conditions with values of 50%, 62%, 72%, 84%, 78%, 72% and 72% with the densities of 1:1, 3:1, 6:1, 9:1, 12:1, 15:1 and 18:1 parasitoid females per *P. xylostella* larva, respectively, but it emerged only from this host pupae (Figure 1).

The characteristics of the parasitized larvae and pupae at all *T. howardi* female densities were body with losses of bright green coloration, integument dry and rigid and mummified appearance with brown coloration. The color of the *P. xylostella* pupae was caramel-brown with some immature *T. howardi* in their interior, usually, encapsulated and melanized, or with malformed individuals of this parasitoid.

The percentages of *T. howardi* emerged from *P. xylostella* pupae were similar with the different densities of this parasitoid females with values from 25 to 45.56%.

The duration of the life cycle (egg to adult) of *T. howardi* varied from 20.67 to 22.75 days with similar values with all the densities of this parasitoid females.

The progeny of *T. howardi* on *P. xylostella* varied with the density of this parasitoid with average values of 2.33 to 11.50 offspring per pupa at the densities of 18:1 and 9:1 respectively (Figure 2). The progeny of *T. howardi* per pupa of *P. xylostella* in greenhouse varied from six to 20 parasitoids.

The progeny of each *T. howardi* female per *P. xylostella* pupae declined as the density of this parasitoid increased with values from 3.00 and 0.16 for the densities of 1:1 and 18:1 parasitoid: host, respectively (Figure 3).

The sex ratio of *T. howardi* was inversely correlated with its female densities, with a higher value at 1:1 (Figure 4).

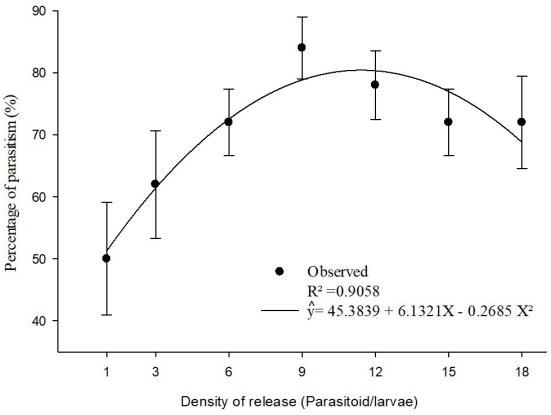

Figure 1. Parasitism percentage of fourth instar *Plutella xylostella* (Lepidoptera: Plutellidae) caterpillars by *Tetrastichus howardi* (Hymenoptera: Eulophidae) females at different densities in semi-field conditions.

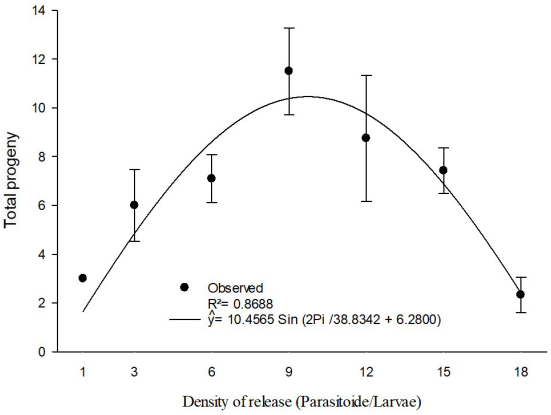

Figure 2. Total progeny of *Tetrastichus howardi* (Hymenoptera: Eulophidae) per pupa of *Plutella xylostella* (Lepidoptera: Plutellidae) with different densities of females of this parasitoid in semi-field conditions.

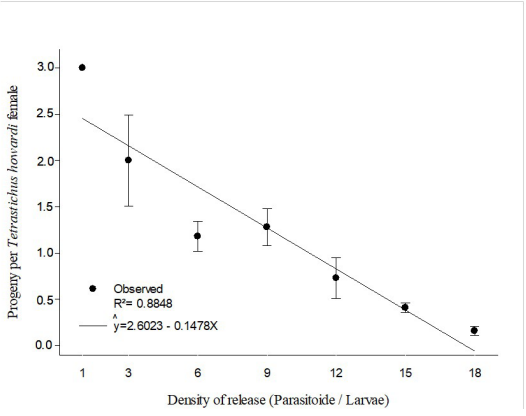

Figure 3. Progeny per *Tetrastichus howardi* (Hymenoptera: Eulophidae) female with a density of one, three, six, nine, 12, 15 or 18 females of this parasitoid per *Plutella xylostella* (Lepidoptera: Plutellidae) pupae.

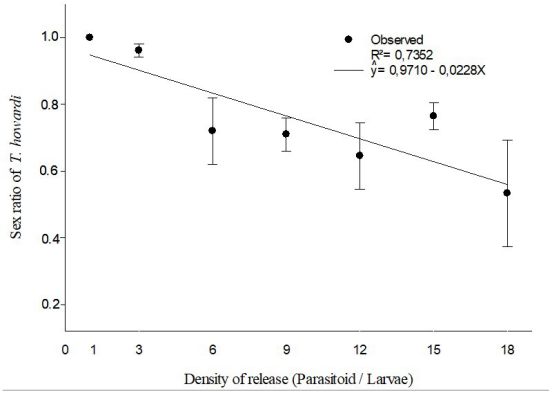

Figure 4. Sex ratio (number of females/(number of females + number of males)) of *Tetrastichus howardi* (Hymenoptera: Eulophidae) with different numbers of females per pupa of *Plutella xylostella*.

4. Discussion

The percentage of *P. xylostella* larvae parasitized at all densities of *T. howardi* females demonstrates the suitability of this host for the development of this parasitoid (Karindah et al., 2005). The success of *T. howardi* females parasitizing *P. xylostella* confirms that they overcome the immune defenses of this host as reported for this natural enemy parasitizing *D. saccharallis* larvae and emerging from pupae of this host (Rodrigues et al., 2021). The parasitism of 50% of the *P. xylostella* fourth instar larvae by *T. howardi* in greenhouse at density de 1:1 (*T. howardi*/ *P. xylostella*) is lower than the approximately 80% by one female of this natural enemy per fourth instar *P. xylostella* larva in the laboratory (Tiago, 2018). Difference in the parasitism rate may be due to controlled and confined environment while host location in greenhouse and field conditions is based mainly in chemical signals from plants, besides visual and physical stimuli (shape, texture, or host movement) (Kruidhof et al. 2019).

The increasing in the percentage of parasitism up to a density, and a decrease after it is similar to that of *T. howardi* and other Eulophidae such as *Palmistichus elaeisis* Delvare & LaSalle and *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) without a linear increase in the parasitism percentage as the number of female parasitoid increased (Pastori et al., 2012; Favero et al., 2015; Barbosa et al., 2019). The decline after the optimal parasitoid density could be due to parasitoid females regulating the number of eggs laid after identifying parasitized hosts (Godfray, 1994). The reduction in parasitism can characterize mutual interference when several females forage in the same location with greater interaction between them during host searching (Saini and Sharma, 2018).

The success of females of this parasitoid finding and parasitizing *P. xylostella* larvae under greenhouse conditions may be due to the process of injecting polydnavirus (PDV) and venom during oviposition, and teratocytes released into the host's hemolymph after the egg hatching as reported for the parasitoid *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae). Other parasitoids such as *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) release only venom during parasitism, which helps in this process, without affecting the host development (Bai et al., 2011). *Tetrastichus howardi*, being a parasitoid of the same family as *O. sololowskii*, may also only release venom during parasitism, which does not prevent the *P. xylostella* larva from becoming a pupa, and then completing its development into this last host stage.

Morphological characteristics of the pupae are altered and used for identification of parasitismo (Bae and Kim, 2004).The caramel-brown of parasitized pupae is related to the parasitoid invasion into the host's hemocele with its ovopositor and to the eggs inducing the release of substances generating immune responses and changes in the physiology and color of the host (Meng et al., 2016). The melanization and encapsulation of some parasitoids are due to the host immune defense, while the parasitoid injection, during parasitism, of poisons and other substances can reduce or prevent this process allowing its egg development. The finding of melanized and encapsulated parasitoids indicate that the substances released by the parasitoid did not, in some cases, overcome the host immune response (Meng et al., 2018).

The similar percentages of *T. howardi* emerged from *P. xylostella* pupae with all densities of this female parasitoid shows how much the parasitoid can adapt and that the nutrients in the host were sufficient for the development and multiplication of this natural enemy in greenhouse conditions (Harvey et al., 2013; Vargas et al., 2013; Barbosa et al., 2019).

The similar duration of the life cycle (egg to adult) of *T. howardi* with all its densities per *P. xylostella* pupae indicates that the food resource available to the immature parasitoid did not affect this parameter as found for this natural enemy with *D. saccharallis* pupa in the laboratory (Favero et al., 2015). The duration of the life cycle of this parasitoid in *P. xylostella* was similar to that with *D. saccharallis*, the original host of this parasitoid. This is important because the short cycle reduces the risk of early mortality of the parasitoid by antagonists, since the parasitoid is defenceless inside the host (Rodrigues et al., 2021).

The lower progeny of *T. howardi* per *P. xylostella* pupae at 18:1 parasitoid: host indicates an inadequate density of females per host decreasing oviposition or the survival inside the host pupae (Khatri et al., 2021). The progeny of *T. howardi* per pupa of *P. xylostella* in greenhouse from six to 20 parasitoids was lower than that for this parasitoid in the laboratory, four to 28 *T. howardi* per *P. xylostella* pupae (Karindah et al., 2005). The pupae of this host is smaller, 0.6 cm long and 4.83 mg in weight (Chagas Filho et al., 2010; Machekano et al. 2017) with a lower number of parasitoid produced per host pupa. Other host pupae such as those of *Erinnyis ello* (Linnaeus, 1758) (Lepidoptera: Sphingidae) measure between 4 to 6 cm long and with 3,602 mg produced 466 parasitoids each one (Barbosa et al., 2015). The offspring of this parasitoid is related to host size and the food availability (Pyñero et al., 2016).

The highest progeny per female at 1:1 and 3:1 densities of *T. howardi* and a decrease with higher parasitoid ratio per host female suggests that some female parasitoid decrease the number of eggs laid on the host (Vargas et al.,

2013). Another possibility is that the host supports a maximum number of immatures with high mortality over it (Pereira et al., 2015).

The inverse correlation between the parasitoid sex ratio with the number of its females per host pupae agrees with that reported for *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) with the second female of this parasitoid laying fewer eggs than the first, with the sex ratio tending to higher number of males (Campos-Farinha et al., 2000). This suggests that the higher sex ratio with the lower densities 1:1 and 3:1 is due to the lower number of parasitoid larvae inside the host with more food available favoring females. The higher sex ratio with fewer females is important for parasitoids in the field and its release at high sex ratio increases their efficiency (Vacari et al., 2012; Scheiner and Martin, 2020).

This is the first work evaluating the parasitism of *P. xylostella* larvae by *T. howardi* females at different densities per host in greenhouse conditions. *Tetrastichus howardi*, at all densities tested, parasitized *P. xylostella* larvae, but with higher parasitism rate with nine *T. howardi* females per *P. xylostella* larvae.

The use of *T. howardi* increases the perspectives for biological control programs of *P. xylostella* in vegetable growing agroecosystems in Brazil. Brassicaceae species are cultivated in small properties, which can be positive for the foraging of *T. howardi* females.

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