e-ISSN 1983-4063 - *www.agro.ufg.br/pat* - Pesq. Agropec. Trop., Goiânia, v. 52, e72978, 2022

Research Article

Parasitism of *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) on *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae) pupae at different ages[1]

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ABSTRACT

Tetrastichus howardi (Olliff, 1893) (Hymenoptera: Eulophidae) is an adult parasitoid of lepidopteran pests, and the age of the host has a strong influence on its reproduction. This study aimed to evaluate the biological characteristics of *T. howardi* reared on different aged pupae of *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae). For that, pupae with 24, 48, 72, 96, 120, 144 or 168 h of age were exposed to parasitism. The experimental design was completely randomized, with seven treatments (pupal age) and ten replications, with each replication consisting of five pupae individualized with one female parasitoid at the respective ages. *Tetrastichus howardi* parasitized and emerged in pupae of all the evaluated ages. The duration of the cycle (egg-adult) in days, number of parasitoids that emerged per pupa (progeny), progeny per female, sex ratio of the parasitoid and longevity of adults were similar. This is the first record of *T. howardi* reproducing in *C. includens* pupae under laboratory conditions. The highest parasitism and development of *T. howardi* was obtained in 24- to 96-hour-old pupae of *C. includens*, indicating that this age range is more favorable to rear this parasitoid under laboratory conditions.

KEYWORDS: Biological control, pupal parasitoids, soybean looper, integrated pest management.

RESUMO

Parasitismo de *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) em pupas de *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae) de diferentes idades

Tetrastichus howardi (Olliff, 1893) (Hymenoptera: Eulophidae) é um parasitoide adulto de lepidópteros-praga, e a idade do hospedeiro exerce forte influência sobre sua reprodução. Objetivou-se avaliar as características biológicas de *T. howardi* criado em pupas de *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae) de diferentes idades. Para tanto, pupas com 24, 48, 72, 96, 120, 144 ou 168 horas de idade foram expostas ao parasitismo. O delineamento experimental foi inteiramente casualizado, com sete tratamentos (idade de pupa) e 10 repetições, sendo que cada repetição foi constituída por cinco pupas individualizadas com uma fêmea do parasitoide nas respectivas idades. *Tetrastichus howardi* parasitou e emergiu em pupas de todas as idades avaliadas. A duração do ciclo (ovo-adulto) em dias, número de parasitoides emergidos por pupa (progênie), progênie por fêmea, razão sexual do parasitoide e longevidade de adultos foram semelhantes. Este é o primeiro registro de *T. howardi* se reproduzindo em pupas de *C. includens* em condições de laboratório. O maior parasitismo e desenvolvimento de *T. howardi* foi obtido em pupas de *C. includens* com 24 a 96 horas, sendo essa faixa etária favorável para a criação desse parasitoide em condições de laboratório.

PALAVRAS-CHAVE: Controle biológico, parasitoides de pupas, lagarta-falsa-medideira, manejo integrado de pragas.

INTRODUCTION

Brazil is the biggest soybean [*Glycine max* (L.) Merrill] producer in the world (Campeão et al. 2020, Conab 2021), and the soybean looper (*Chrysodeixis includens*) (Walker, [1858]) (Lepidoptera: Noctuidae) has become one of the main pests of the crop in the country (Carvalho et al. 2012, Fidelis et al. 2019).

This polyphagous species can feed on different crops and defoliate soybean, feeding on the leaf blade until only the veins remain, what causes a lacey appearance of the leaves. This feeding reduces the leaf area, impairing the plant photosynthetic capacity and, consequently, directly affecting yield. These caterpillars can also attack pods that have already formed, increasing damage and causing

[1] Received: June 01, 2022. Accepted: Sep. 30, 2022. Published: Oct. 31, 2022. DOI: 10.1590/1983-40632022v5272978.
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great economic losses, especially if the attack occurs during the crop reproductive period (Sosa-Gómez et al. 2014, Specht et al. 2015, Tomquelski et al. 2015).

Chemical insecticides are the most used method to control *C. includens*; however, when applied incorrectly, they lead to the selection of resistant insect populations and suppression of beneficial insects (Korrat et al. 2019, Perini et al. 2019, Stacke et al. 2019). The use of natural enemies to regulate the population of *C. includens* is a successful and extremely important alternative, as these insects are fundamental in the implementation of control tactics within integrated pest management (Carvalho et al. 2013, Pereira et al. 2018).

Among the beneficial insects used in biological control, the *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) parasitoid has the ability to parasitize multiple insect pests. However, *T. howardi* prefers lepidopteran pupae (Barbosa et al. 2015, Piñeyro et al. 2016, Sanomia et al. 2020), what may be related to the parasitoid's immune response (Andrade et al. 2010, Meng et al. 2018). When the host is parasitized, it responds to the parasitism through the immune system, but parasitoids also have an efficient immune system, where they can inject toxins, which modify the host's immune responses and interrupt the production of humoral components (intermediary reactive oxygen, nitrogen and melanin species) (Moreau & Asgari 2015, Liu et al. 2018, Tang et al. 2019).

For a successful biological control using parasitoids, knowledge on their behavior, when reared under laboratory conditions, is very important (Costa et al. 2014, Oliveira et al. 2016, Rolim et al. 2020). Thus, the percentage of parasitism and emergence, cycle (egg-adult) duration, progeny, progeny per female, sex ratio and longevity of males and females are factors that may be easily affected by the host species, exposure period and host age (Barbosa et al. 2015, Ueno 2015, Piñeyro et al. 2016, Tiago et al. 2019, Rodrigues et al. 2021).

Thus, this study aimed to evaluate the parasitism, as well as the biological and reproductive development, of *T. howardi* in *C. includens* pupae at different ages, under laboratory conditions.

MATERIAL AND METHODS

The rearing of the insects and the experimental bioassays were carried out at the Universidade Federal da Grande Dourados (22º11'56.56''S; 54º56'1.396"W), in Dourados, Mato Grosso do Sul state, Brazil, in 2021, in air-conditioned rooms, at 25 ± 2 ºC, relative humidity of 70 ± 20 % and photoperiod of 14 h.

Initially, the *Chrysodeixis includens* were reared from eggs acquired from a commercial company. After the larvae hatched, they were kept in 500 mL plastic cups, with the bottom coated with an artificial diet (adapted from Greene et al. 1976) and sealed with perforated lids, until they reached the third larval instar. Then, they were individually transferred to new plastic cups with the bottom lined with artificial diet, where they remained until they became pupae.

The pupae were sexed using a stereomicroscope (Motic SMZ-168), and later grouped into 75 females and 75 males, distributed in 300 mL plastic pots with a paper towel-lined bottom and closed with voile tissue, remaining for six days in these containers. On the seventh day, the pupae were transferred to PVC tube cages (25 cm in diameter × 30 cm in height) with an interior lining of pink bond paper serving as an oviposition substrate after the emergence of the adults. The upper part of the cage was sealed with voile fabric held by elastic gums. The cages were placed on light green plastic trays (adapted from Barbosa et al. 2018). After the emergence of the adults, 50 mL plastic cups with a cotton swab soaked in an aqueous solution of honey were introduced into the PVC cages as food for the adults, and this process was used to obtain the adults and their oviposition.

The cages were placed on steel shelves under artificial lighting from two parallel fluorescent lamps (MAXXI® F 18W T8 Pink Gro-lux type and SYLVANIA® F 20W T10 5000K Luz do Dia Plus), which were connected to a digital timer for a photoperiod of 14 h of light, to stimulate copulation and oviposition (Adapted from Morando 2014).

Under artificial lighting, the oviposition of the moths started on the fourth day after the emergence of the adults, with a maximum peak reached on the seventh day. These were placed in 7,000 mL plastic pots with the upper part sealed by voile fabric. After the larvae hatched, they remained in the pots and were fed an artificial diet until they reached the third instar. Then, they were grouped into 100 individuals and transferred to new 7,000 mL plastic pots and fed an artificial diet until they turned into pupae, restarting the cycle (Adapted from Barbosa et al. 2018).

The production of *T. howardi* parasitoid started from an already established rearing. The proliferation of *T. howardi* began with pupae reared on *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae). These 24-h-old pupae were placed inside test tubes (2.5 cm in diameter × 8.5 cm in length), together with five female *T. howardi*. The upper part of the tubes was sealed with pieces of cotton.

A droplet of pure honey introduced into the inner wall of the tubes served as food for the female parasitoids. Parasitism was allowed for 24 h. After this period, the females were removed, leaving only the pupae, until the emergence of the new adult parasitoids, resuming the parasitoid rearing cycle.

To evaluate the influence of pupal age on the host, a 24-h-old female *T. howardi*, which had been fed pure honey, was initially introduced into a glass tube (150 mm long × 20 mm wide) containing one *C. includens* pupa with 24, 48, 72, 96, 120, 144 or 168 h of age, at a density of 1:1 (parasitoid and pupa), and these tubes were sealed with cotton. To determine the sexes of the parasitoids, the taxonomic identification of the parasitoid was based on the dimorphism of the antennae of *T. howardi*. The females' antenna has three dark funicular segments and scape without a sensory plate on the ventral margin. The males' antenna has four segments, with only the club being dark, and their scape has a sensory plate on the ventral margin (La Salle & Polaszek 2007) (Figure 1).

After 24 h of exposure, the *T. howardi* females were removed from the tubes with the aid of a fine bristle brush and discarded. The pupae were kept in the tubes until the emergence of the parasitoids (adapted from Barbosa et al. 2015).

The percentage of parasitism [(number of pupae with parasitoid emergence + pupae without adult parasitoid emergence)/(total number of pupae) × 100], emergence percentage [(number of pupae with adult parasitoid emergence)/(number of parasitized pupae) × 100], duration of the cycle (egg-adult) in days, number of emerged parasitoids per pupa (progeny), progeny per female, sex ratio (number of females/number of adults) of the parasitoids and longevity of off-spring males and females (adapted from Barbosa et al. 2015, Tiago et al. 2019) were evaluated.

The natural mortality of *C. includens* was determined by placing 50 pupae individually in 100 mL plastic pots and counting the number of emerged adults. Thus, the natural mortality of the host was defined by the Abbott (1925) formula: Mc (%) = (%Mo - %Mt)/(100 - %Mt) × 100, where Mc is the corrected mortality, Mo the observed mortality and Mt the control mortality.

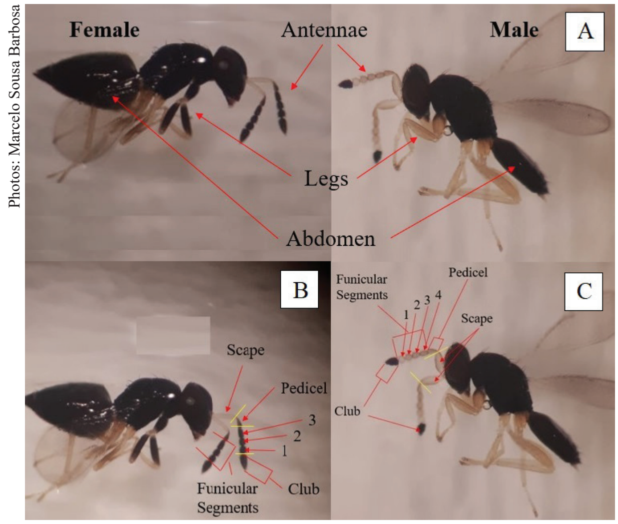

Figure 1. Sexual dimorphism of *Tetrastichus howardi* (A) and antennae detail of the female (B) and male (C).

The experimental design was completely randomized, with seven treatments (pupal ages) and ten replications, with each replication consisting of five individualized pupae at the respective age with female parasitoids.

The data were submitted to regression analysis at 5 % of probability. The equation that best fit the data was chosen based on the coefficient of determination (R^2), significance of the regression coefficients (βi) and regression, using the F test (up to 5 % of probability). The analyzes were processed using the SigmaPlot software (Systat Software Inc, San Jose, CA 95131, USA).

RESULTS AND DISCUSSION

The parasitism percentage of *C. includens* pupae by *T. howardi* females under laboratory conditions was similar at the pupal ages of 24 and 48 h, with 64.00 ± 4.99 and 62.00 ± 5.54 (%), respectively (Figure 2).

Results for the parasitism percentage by *T. howardi* females in pupae of *Erinnyis ello* (Linnaeus, 1758) (Lepidoptera: Sphingidae) obtained by Barbosa et al. (2015) reached 64 %. This result corroborates the experiment at the ages of 24 and 48 h, indicating that these ages are viable for *T. howardi* parasitism of *C. includens* pupae. This is the first record of *T. howardi* reproducing in *C. includens* pupae under laboratory conditions.

The pupae parasitism at 72 and 96 h were similar, with 70.00 ± 6.83 and 70.00 ± 7.45 (%). Treatments at 120 and 144 h exhibited a decreased parasitism percentage with 42.00 ± 8.67 and 40.00 ± 8.94 (%). After 168 h, a decrease in parasitism percentage was observed [34.00 ± 6.00 (%)], with the pupae at this age being less suitable for *T. howardi* parasitism (Figure 2).

Tetrastichus howard parasitized and developed in *C. includens* pupae that were 24 to 96 h old, but reduced at 120 h onward. *T. howardi* did not differentiate pupae from *C. includens* at these advanced ages (Figure 2).

The pupal stage development includes a process called histolysis, with physiological modification, deterioration and dissolution of organic tissues. Histogenesis occurs before this process, in which the development of different embryonic tissues occurs (Andrade et al. 2010, Costa et al. 2014), but does not interfere with the parasitism of *C. includens* pupae by *T. howardi*.

The emergence percentage of *T. howardi* adult females from *C. includens* pupae was similar for the treatments at 24 and 48 h old, with 97.50 ± 2.50 (%) at both ages. At the age of 120 h, a 100 % increase in the emergence rate of *T. howardi* was observed, in relation to the previous treatments. At 144 and 168 h, the emergence percentage decreased again, but remained above 85 %, with 86.67 ± 13.33 and 85.71 ± 9.91 (%), respectively (Figure 3).

Studies that evaluated the biological characteristics of *T. howardi* on *Tenebrio molitor* Linnaeus, 1758 (Linnaeus) (Coleoptera: Tenebrionidae) pupae confirmed the parasitism capacity of *T. howardi* in this host and the emergence percentage, which, at 25 ± 2 ºC, was 100 % for both the biological characteristics, making *T. molitor* a viable option for rearing the parasitoid under laboratory conditions (Tiago et al. 2019). The choice of host is very important for the production of the parasitoid under laboratory conditions, as it influences the percentage of parasitism and parasitoid emergence, in addition to minimizing the rearing costs and the period of field release of the parasitoid (Costa et al. 2014, Rolim et al. 2020).

Tetrastichus howardi parasitized and emerged from *C. includens* pupae in all treatments, demonstrating its potential for laboratory production with that host. The obtained emergence percentage indicates the biological capacity of this parasitoid to

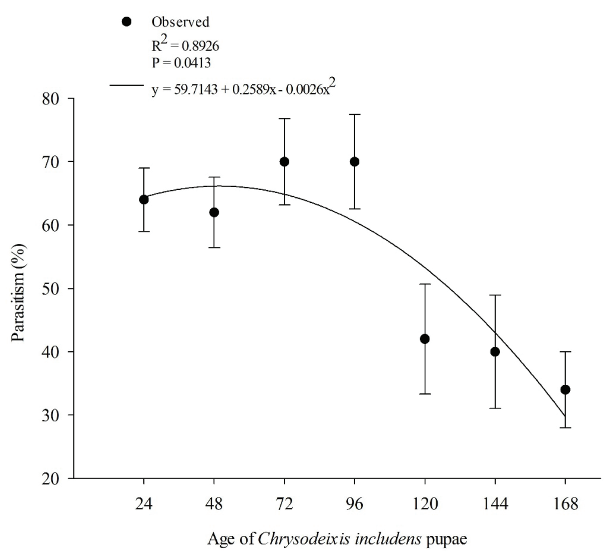

Figure 2. Parasitism of *Tetrastichus howardi* (Hymenoptera: Eulophidae) on *Chrysodeixis includens* (Lepidoptera: Noctuidae) pupae as a function of host age (hours) (25 ± 2 ºC; relative humidity of 70 ± 20 %; photoperiod of 14 h).

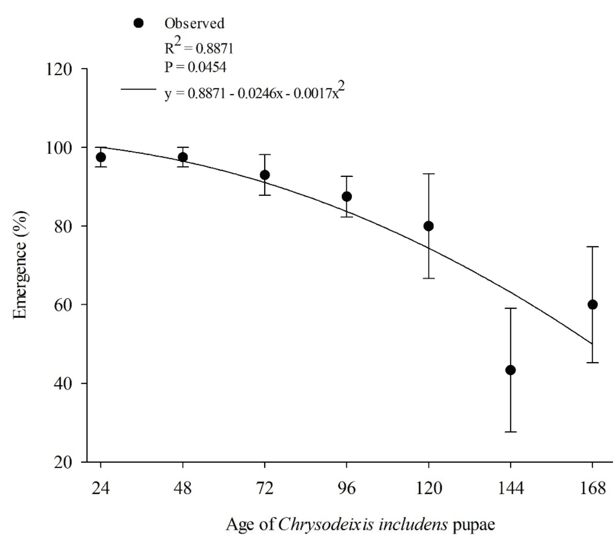

Figure 3. Emergence of *Tetrastichus howardi* (Hymenoptera: Eulophidae) on *Chrysodeixis includens* (Lepidoptera: Noctuidae) pupae as a function of host age (hours) (25 ± 2 ºC; relative humidity of 70 ± 20 %; photoperiod of 14 h).

Table 1. Biological characteristics (means ± standard error) of *Tetrastichus howardi* (Hymenoptera: Eulophidae) reared on *Chrysodeixis includens* (Lepidoptera: Noctuidae) pupae as a function of the parasitoid age (hours) (25 ± 2 ºC; relative humidity of 70 ± 20 %; photoperiod of 14 h).

Biological characteristics	Age (hours) of *Chrysodeixis includens* pupae							
	24	48	72	96	120	144	168	
Cycle duration (days)	18.02 ± 0.19	17.29 ± 0.07	17.58 ± 0.21	17.77 ± 0.21	19.29 ± 0.84	18.00 ± 0.49	19.24 ± 0.66	ns[1]
Progeny per pupa (%)	64.05 ± 3.28	79.62 ± 3.90	70.57 ± 4.54	72.94 ± 4.32	67.29 ± 14.99	73.87 ± 18.02	62.74 ± 7.04	ns
Sex ratio (%)	0.95 ± 0.01	0.92 ± 0.04	0.95 ± 0.00	0.95 ± 0.01	0.88 ± 0.06	0.87 ± 0.04	0.96 ± 0.01	ns
Progeny per female (%)	60.36 ± 3.10	72.88 ± 4.28	67.42 ± 4.37	68.67 ± 4.35	69.41 ± 14.86	69.33 ± 17.69	60.87 ± 8.33	ns
Female longevity (days)	23.15 ± 1.31	23.85 ± 1.03	21.95 ± 1.24	19.60 ± 1.20	24.10 ± 1.21	16.15 ± 1.25	16.80 ± 1.04	ns
Male longevity (days)	22.70 ± 2.88	33.80 ± 2.46	23.50 ± 1.92	25.20 ± 2.61	30.00 ± 2.35	22.30 ± 2.62	26.10 ± 2.03	ns

[1] ns: not significant ($p \geq 0.05$).

develop in the host, with the immature being able to exploit the nutritional resources available in the *C. includens* pupae.

Tetrastichus howardi is an endoparasitoid of pupae, and its reproductive success occurs in pupae that are less than 24 h (Costa et al. 2014, Tiago et al. 2019). The biological characteristics of *T. howardi* were favorable when reared on *Bombyx mori* Linnaeus, 1758 (Lepidoptera: Bombycidae) pupae (Piñeyro et al. 2016).

The age of the *C. includens* pupae did not affect the other biological characteristics of *T. howardi* (Table 1). The duration of the egg-to-adult cycle ranged from 17.29 ± 0.07 to 19.29 ± 0.84 days, the progeny per pupa from 62.74 ± 7.04 to 79.62 ± 3.90, the sex ratio from 0.88 ± 0.06 to 0.96 ± 0.01, the female progeny from 60.36 ± 3.10 to 72.88 ± 4.28, the female longevity from 16.15 ± 1.25 to 24.10 ± 1.21 days and the male longevity from 22.30 ± 2.62 to 33.80 ± 2.46 days (Table 1).

The life cycle duration (egg to adult) of *T. howardi* was similar in all evaluated ages, suggesting that the *C. includens* pupae are viable for parasitoid development. Studies with *T. howardi* showed that the life cycle duration of this parasitoid in *B. mori* pupae is greater than 17 days (Barbosa et al. 2015, Piñeyro et al. 2016). The life cycle duration (egg to adult) depends on several aspects, as well as on the host species (Zago et al. 2006).

Tetrastichus howardi females that mate after emergence have a greater chance of success in parasitism, with females responsible for parasitism and for progeny per pupa and per female (Amalin et al. 2005, Sanomia et al. 2020).

The *T. howardi* sex ratio was equivalent in all evaluated pupal ages. The ideal sex ratio index for mass rearing of the parasitoid is equal to or greater than 0.5 (Dias et al. 2008). Rates greater than 0.9 % were observed when *T. howardi* was reared on *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) pupae (Oliveira et al. 2016). The male parasitoids perform the function of fertilizing the females; they do not parasitize the insect pests and, therefore, do not help to reduce the pest action. Thus, the high sex ratio, i.e., the emergence of a greater number of females, is favorable for biological control programs (Navarro 1998).

The longevity of males and females of the parasitoid were similar at all ages (hours) of *C. includens* pupae, with males living longer. Longevity of more than 10 days is a sufficient period for female parasitoids to reproduce and find their hosts, when they are released in the field (Pratissoli et al. 2005).

The capacity for parasitism and development of *T. howardi* at different pupal ages is important. Pupae between 48 and 96 h of age provide a longer period of time to use the host to rear the parasitoid in the laboratory, as well as to be used in biological control programs for field release, which may help to reduce production costs (Costa et al. 2014).

The parasitism and the biological and reproductive development of *T. howardi* in different ages of the host (pupae) demonstrated the potential of this natural enemy for quality proliferation in *C. includens* pupae, providing a satisfactory rearing under laboratory conditions.

CONCLUSION

The highest parasitism, as well as biological and reproductive development, of *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) was obtained in *Chrysodeixis includens* (Walker,

[1858]) (Lepidoptera: Noctuidae) pupae between 24 and 96 h old, indicating that this age group is favorable for the rearing of this parasitoid under laboratory conditions.

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