Parasitism and Development of *Tetrastichus howardi* (Hymenoptera: Eulophidae) in Immature *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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**Abstract**

*Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) is an endoparasitoid mainly of Lepidoptera. Its potential as a biological control agent has been investigated for several agricultural and forestry species in different countries. The objective of our study was to evaluate the biological characteristics of *T. howardi* in larvae, prepupae and pupae of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), under laboratory conditions. The first bioassay was performed with *S. frugiperda* larvae in the fifth and sixth instar and prepupal phases, which were exposed to the parasitism of 7 female *T. howardi* for 24 h. The second bioassay was conducted with *S. frugiperda* pupae, with parasitism allowed for 24 h, at six parasitoid of *T. howardi* densities, for one host pupa of *S. frugiperda* (1, 7, 14, 21, 28 or 35 parasitoids: 1 host). The parasitism of *T. howardi* in *S. frugiperda* larvae was confirmed by larval mortality and the emergence of parasitoid descendants. Female *T. howardi* parasitized and emerged from *S. frugiperda* larvae of fifth and sixth instars, prepupae, and pupae under laboratory conditions, which is the first record of such in the literature.

**Keywords:** biological control, endoparasitoid, parasitoid-host interaction, fall armyworm

1. **Introduction**

The fall armyworm *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) causes significant damage to corn due to the defoliation caused by the larvae during the entire developmental cycle of this cereal (Arthur, Arthur, & Machi, 2016; Fernandes, Abreu, Faria, Gobbi, & Afonso-Rosa, 2016; Fernandes, Abreu, Christ, & Rosa, 2019).

The larval stage of *S. frugiperda* has six developmental instars (Silva et al., 2012). Initially, the larvae cause damage known as 'scrapped leaves'. As the larva develops, it damages the corn husk, causing significant losses in the crop (Rubin, 2009).

At the end of its larval development, the larva heads to the ground, where it builds a cocoon, inside which it passes to the prepupae and pupal stage. The pupae are reddish-brown and 13 to 16 mm in size (Rubin, 2009). The pupal stage varies from 10 to 12 days during the hottest times of the year (Santiago, Pádua, Silva, Carvalho, & Maia, 2008). This species is considered a polyphagous pest, consuming soybeans, cotton, rice, wheat, oats, and especially corn (Silva et al., 2017).

The manner that corn leaves develop shelter and protect *S. frugiperda* larvae makes their control difficult (Sparks, 1979; Prowell, Mcmichael, & Silvain, 2004). In more developed plants, the caterpillars penetrate the stem, where...
they make galleries, this makes their control difficult, which when chemical, must be carried out before the caterpillar penetrates the stem, this behavior serves as protection for the caterpillar, considering that the chemical insecticide does not can reach the inside of the (Valicente, 2015). Therefore, the biological control of S. frugiperda can be achieved through parasitoid insects of the genus Trichogramma, which have the potential to parasitize moth eggs, providing a population balance of this lepidopteran in the crop (Figueiredo, Cruz, Silva, & Foster, 2015). Tetrastichus howardi (Olliff, 1893) (Hymenoptera: Eulophidae) is another parasitoid for potential biological control and has been recorded in several hosts, mainly Lepidoptera (Rodrigues, Pereira, & Barbosa, 2021). In corn, T. howardi was recorded parasitizing pupae of Diatraea saccharalis (Fabricius, 1794) (Crambidae: Lepidoptera) (Cruz, Redoan, Silva, Figueiredo, & Enteadodias, 2011).

The potential of T. howardi as a biological control agent has been investigated for several species of agricultural interest in different countries (Moore & Kfir, 1995; Felix, Gonzalez, Montes de Oca, Ravelo, & Baitha, 2005; La Salle & Polaszek, 2007; Barbosa et al., 2019; Rodrigues et al., 2021). T. howardi can develop in different stages of host development, such as larva, prepupa, pupa, and even adult D. saccharalis (Pereira et al., 2015). The parasitism by T. howardi of larvae and pupae of Oxydia vesulia (Cramer, 1779) (Lepidoptera: Geometridae) was also recorded under laboratory conditions, confirming its excellent potential for biological control (Favoreto et al., 2021).

The parasitism, emergence, progeny, and longevity of female and male T. howardi can be influenced by different parasitoid densities and parasitism periods (Costa et al., 2014; Pastori et al., 2012a, Barbosa, Couri, & Coelho, 2008). Thus, studies are necessary to verify how the natural enemy will respond functionally and numerically to increased host density (Faria, Torres, & Farias, 2000; Polanczyk et al., 2011).

The host’s developmental stage, larval instar, and parasitoid density are biotic factors that can influence parasitism and parasitoid development. Therefore, the objective of this study was to evaluate the biological characteristics of T. howardi in larva, prepupa, and pupa of S. frugiperda, with different parasitoid densities.

2. Material and Methods

2.1 Laboratory Rearing of T. howardi and S. frugiperda

The experiments were developed from stock rearings of the parasitoid T. howardi and the host S. frugiperda, kept in an acclimatized room with a temperature of 25±2 °C, 70±10% relative humidity (RH), and a 12 h photophase. The voucher specimens are deposited in the Entomological Collection of UFES, Department of Biological Sciences, Federal University of Espírito Santo (Vargas, Pereira, Tavares, & Pastori, 2011). Accession numbers: 150792 to 150816 to 157187, data available online at Splink. The parasitoids were identified by Dr. Marcelo Teixeira Tavares.

Spodoptera frugiperda larvae were reared on an artificial diet, based on beans and wheat germ, in individualized disposable containers, until the formation of pupae. Adults were kept in PVC cages and fed a solution containing water and 10% honey (Parra, 2001).

Adult T. howardi were kept in glass tubes (100 × 10 mm) covered with cotton and containing a droplet of honey as food for the insects. One S. frugiperda pupae at 24 to 48 h old were exposed to parasitism by seven female T. howardi for 24 h at 25±2 °C, RH of 70±10% and 14 h photophase, in a climatized chamber (Vargas et al., 2011).

2.2 Experimental Development

Preliminary tests were conducted with larvae from the second, third, fourth, fifth, and sixth instars, at densities 1 and 7, where parasitism was observed in all instars and densities evaluated. As the emergence of adult T. howardi only occurred when seven female T. howardi were used per fifth and sixth instar larva, this density was selected to conduct the experiment.

2.2.1 Parasitism by T. howardi of Fifth and Sixth Instar Larvae and Prepupae of S. frugiperda

Caterpillars of fifth and sixth instars as well as the prepupal S. frugiperda were individualized (1 host) in Petri dishes and exposed to parasitism by 7 female T. howardi that were 24 h old (Costa et al., 2014). Parasitism was allowed for 24 hours. The plates were sterilized, and the larvae were fed daily, for minimal interference in the parasitism process and development of the T. howardi progeny. The material was maintained in an acclimatized room at 25±1 °C, 14 h photophase, and 70±10% RH.

2.2.2 Parasitism of S. frugiperda Pupae by Different Densities of T. howardi

Spodoptera frugiperda pupae weighing 0.140±0.003 g and 24 h old were individually exposed to parasitism by T. howardi (24 hours old), at 6 different densities in glass test tubes 100 mm in length by 10 mm in diameter (Costa et al., 2014). The densities tested were 1, 7, 14, 21, 28, and 35 parasitoids to one S. frugiperda (Smith, 1797)
pupa. Parasitism was allowed for 24 h and the pupae were kept in an acclimatized room at 25±2 °C, 70±10% RH, and a 14 h photophase until the emergence of adult parasitoids. The natural mortality of the host was calculated using the Abbott’s (1925) formula.

2.3 Experimental Evaluation

The biological characteristics evaluated for the two experiments were: percentage of parasitism and emergence of parasitoids; life cycle duration (egg-adult); progeny (number of individuals per host); progeny per female (number of individuals per parasitoid); longevity of adults (with food), and sex ratio (number of females/total number of individuals).

2.4 Statistical Analysis

The experimental design used was completely randomized (DIC). In the first experiment, three treatments (larval phases) and five repetitions were performed, with each repetition containing ten individuals. For the second experiment, six treatments were prepared (T. howardi density). Each treatment consisted of five replicates, each containing a group of 10 pupae, totaling 50 experimental units per treatment. Data on life cycle duration, percentage of parasitism and emergence of parasitoids, number of individuals per host and per female parasitoid, sex ratio, and longevity were submitted to analysis of variance and, when significant, to regression with 5% of probability (Sigmaplot 12.0 Software). The choice of the most suitable equation was based on the Determination Coefficient (R²), the significance of the Regression Coefficients (ᵦᵢ), and the Regression by the F Test (at 5% probability).

3. Results

3.1 Parasitism of S. frugiperda Fifth and Sixth Instar Larva, Prepupae and Pupae by T. howardi

Adult female T. howardi were able to parasitize, develop, and emerge from S. frugiperda fifth and sixth instar larvae as well as prepupae.

The highest parasitism and emergence of T. howardi were observed in sixth instar larvae, at 28.00±6.23% and 76.39±10.91%, respectively (Table 1). The parasitized S. frugiperda larvae had a swollen, dry, and rigid appearance, with brown and black coloration. The parasitism and emergence from prepupal S. frugiperda were greater than 90% (Table 1).

T. howardi had the shortest life cycle (egg-adult) in the prepupal phase of S. frugiperda, with 20.10±0.22 days (Table 1). The progeny of T. howardi increased as the instars of S. frugiperda increased.

The greatest number of T. howardi descendants were obtained in the prepupal phase of S. frugiperda, with 363.27±44.61 offspring. The progeny per female T. howardi was also higher in prepupal S. frugiperda, with 47.61±6.09 individuals per female (Table 1).

The sex ratio of T. howardi that emerged from the fifth and sixth instar and prepupal larvae was similar, with an overall mean of 0.90±0.02 (Table 1).

There was no difference in the longevity of adult female T. howardi emerged from the fifth and sixth instars and prepupal S. frugiperda, with an overall mean of 20.40±0.33 days. The longevity of males was lower when adults emerged from prepupal S. frugiperda, with 18.70±0.40 days (Table 1).
Table 1. Means (±standard error) of the biological characteristics of *Tetrastichus howardi* (Hymenoptera: Eulophidae) emerged from fifth and sixth instar larvae and prepupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) with seven female *T. howardi*, with 24 h of parasitism at 25±2 °C, 70±10% RH, and 14 h photophase

| Biological characteristics | 5° Instar | 6° Instar | Prepupa | P | C.V. |
|---------------------------|-----------|-----------|---------|---|-----|
| Parasitism (%)            | 10.00±4.47 b (n = 10) | 28.00±6.23 b (n = 10) | 90.00±5.24 a (n = 10) | 0.0001 | 40.46 |
| Emergency (%)              | 37.50±15.14 b (n = 4) | 76.39±10.91 ab (n = 9) | 92.50±5.24 a (n = 10) | 0.00001 | 38.82 |
| Life cycle length (days)   | 31.00±0.45 a (n = 2) | 22.44±0.97 b (n = 8) | 20.10±0.22 b (n = 10) | 0.0003 | 9.31 |
| Progeny                   | 86.50±36.45 b (n = 2) | 225.18±16.23 ab (n = 8) | 363.27±44.61 a (n = 10) | 0.47 | 41.55 |
| Progeny/female            | 11.64±4.95 b (n = 2) | 4.25±1.38 b (n = 8) | 47.61±6.09 a (n = 10) | 0.003 | 58.17 |
| Sex ratio                 | 0.87±0.03 a (n = 2) | 0.92±0.03 a (n = 8) | 0.91±0.01 a (n = 10) | 0.025 | 7.71 |
| Longevity of females (days)| 20.40±0.20 a (n = 20) | 20.95±0.19 a (n = 20) | 19.85±0.62 a (n = 20) | 0.0004 | 8.57 |
| Longevity of males (days) | 19.90±0.38 ab (n = 10) | 20.00±0.26 a (n = 10) | 18.70±0.40 b (n = 10) | 0.065 | 5.66 |

Note. Means followed by the same letter in the column do not differ from each other by Tukey’s Test at 5% probability. N = number of repetitions.

The parasitism of *T. howardi* on *S. frugiperda* occurred in all larval instars of this host. After parasitism, *S. frugiperda* larvae had a swollen, dry, and rigid appearance with brown and black coloration. Seven fifth or sixth instar *S. frugiperda* larvae that tried to transition to the prepupal phase died, remaining in an intermediate phase between the two.

The percentage of parasitism, emergence, life cycle duration (egg-adult), sex ratio, and longevity of *T. howardi* females in *S. frugiperda* pupae were similar in the different densities evaluated (Table 2).

The mean percentage of *T. howardi* parasitism and emergence in *S. frugiperda* pupae was 99% in the evaluated densities of female parasitoids. The duration of the life cycle (egg-adult) of *T. howardi* in pupae of *S. frugiperda* was similar, with an overall mean of 16.30±0.10 days.

The sex ratio of *T. howardi*, with a general mean of 0.85±0.02, did not differ when the number of females increased. The longevity of the parasitoid also exhibited no statistical difference between the densities tested, with means of 22.10±0.64 to 23.60±0.80.

Table 2. Means (±standard error) of biological characteristics of *Tetrastichus howardi* (Hymenoptera: Eulophidae) in pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) at densities 1:1, 7:1, 14:1, 21:1, 28:1, and 35:1 (parasitoid: host), at 25±2 °C, 70±10% RH, and 14 h photophase

| Biological characteristics | 1:1 | 7:1 | 14:1 | 21:1 | 28:1 | 35:1 |
|----------------------------|-----|-----|------|------|------|------|
| Parasitism (%)             | 94.00±0.04 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | n.s. |
| Emergency (%)              | 96.00±0.02 | 98.00±2.00 | 100.00±2.00 | 98.00±2.00 | 94.00±3.00 | 100.00±0.00 | n.s. |
| Life cycle length (days)   | 16.52±0.24 | 16.00±0.00 | 16.25±0.27 | 16.60±0.40 | 16.40±0.27 | 16.00±0.00 | n.s. |
| Sex ratio**                | 0.78±0.04 | 0.88±0.02 | 0.88±0.00 | 0.92±0.00 | 0.85±0.01 | 0.81±0.01 | n.s. |
| Longevity of females (days)**| 22.1±0.64 | 22.2±0.46 | 23.60±0.80 | 22.90±0.50 | 23.10±0.67 | 22.60±0.63 | n.s. |

Note. n.s. = Not significant (p > 0.05). * Number of individuals, ** Number of days and *** Number of females/(Number of males + Number of females).

The density of the progeny produced in the *S. frugiperda* pupae was positively influenced by the density of *T. howardi*, with means ranging from 99.1±6.09 to 513.82±18.66 of offspring per pupa at densities 1:1 and 28:1, respectively (Figure 1).
The progeny per female *T. howardi* in *S. frugiperda* pupae decreased as the density of this parasitoid increased, decreasing from 99.10±6.09 to 11.14±0.98 offspring, which was inversely proportional to the increase in parasitoid density (Figure 2).
4. Discussion

*Tetrastichus howardi* parasitized, developed, and emerged in all tested phases of *S. frugiperda*, allowing its use for the biological control of this insect. Similar results were observed in the parasitism capacity of *T. howardi* in different stages of *Helicoverpa armigera*, (Hübner, 1809) (Lepidoptera: Noctuidae), in which 2 fifth instar larvae were parasitized with emergence of parasitoids in the pupal phase, with a progeny of 81 individuals; the egg-adult cycle was 19.5 days; the sex ratio was 0.88; the longevity of males was 19.6 days and females 20.3 days, demonstrating significant parasitism in the larval and pupal stages of *H. armigera* (Simonato, Oliveira, J. F. Gigolli, M. M. K. Gigolli, & Silva, 2020).

Our study is the first to record, under laboratory conditions, the parasitism of *T. howardi* in the larvae and prepupae of *S. frugiperda*. This information is very relevant because this insect is easy to raise on an artificial diet and can be used as an alternative host for large-scale production (Parra, 2001).

In relation to parasitism by *T. howardi* in the fifth and sixth instar and prepupal larvae of *S. frugiperda*, we can consider, parasitism generally increased with the more advanced development of the host *S. frugiperda*, with values of 10.00±4.47; 28.00±6.23; and 90.00±5.24, respectively. The emergence of adult parasitoids was 37.50±15.14 for the fifth instar, 76.39±10.91 for the sixth instar, and 92.50±5.24 for the prepupal phase. This increase may be associated with the nutritional quality of the host larvae, which increases as it grows and develops (Cônsoli & Vinson, 2009). This increase may be associated with the nutritional quality of the host larvae, which increases as it grows and develops (Cônsoli & Vinson, 2009). These results demonstrate that caterpillars, pre-pupae and pupae of *S. frugiperda* fit perfectly as hosts for the multiplication of *T. howardi* and, as the host develops and grows, the more the parasitoids multiply better.

Another important factor is that larvae in more advanced phases can be more aggressive, demonstrating that larger hosts can defend themselves better than smaller ones, which are in early phases of development (Kouamé & Mackauer, 1991). The fifth instar larvae presented a defensive behavior that reduced parasitism by *T. howardi*, through body movements and regurgitation. A similar situation was observed for third, fourth, and fifth instar larvae of *H. armigera* that exhibited aggressive movements towards *Eriborus argenteopilosus* (Cameron) (Hymenoptera: Ichneumonidae), which interrupted the oviposition of the parasitoid female or moved her away (M. E. Pascua & L. T. Pascua, 2004).

In the sixth instar, the larva prepares to change to the prepupal phase; thus, it tends to remain immobile. After the last larval instar, the larva transforms into the prepupa, at which point it completely stops feeding and prepares to pupate. Initially, the prepupa remains shrunken, which facilitates parasitism, as occurred for *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae), a soybean caterpillar (Fernandes, 2018).

When parasitizing fifth and sixth instar larvae, *T. howardi* emerged both from the larvae and pupae of *S. frugiperda*. The stage change of these larvae may have been influenced by parasitism or the cessation of feeding. The cessation of feeding promotes reduced metabolism; with this, the secretions of brain cells start to stimulate the prothoracic gland at levels sufficient to promote molt (Costa, Ide, & Simonka, 2006).

After parasitism, *S. frugiperda* larvae have a swollen, dry, and rigid appearance with brown and black coloration, because parasitoid females of the Eulophidae family inject venom during oviposition and reduce the number of circulating hemocytes in the host. This provides a favorable environment for the development of its immature phase, probably due to immunosuppression of the defense system, to prevent the parasitoid larvae from dying due to encapsulation, asphyxia, or activation of substances antagonistic to their development (Uckan, Sinan, Savasci, & Ergin, 2004; Nappi & Christensen, 2005; Carton, Poirié, & Nappi, 2008; Andrade et al., 2010). The same aspects were observed in the parasitism of *A. gemmatalis* larvae by *T. howardi* in laboratory and semi-field conditions (Fernandes, 2018).

The parasitism and emergence of *T. howardi* in prepupal *S. frugiperda* above 90%, demonstrates a great advantage for this parasitoid and its excellent potential to be used as an alternative host for rearing in biofactories, to increase the speed of the entire process. Similar results were found for *T. howardi* parasitism of in prepupal *A. gemmatalis* (Fernandes, 2018). This corroborates with several studies, where the use of younger or newly formed hosts facilitates excellent development of *T. howardi*, as females of this parasitoid prefer newly formed pupae and prepupal phases as described in the results of this and other studies (Moore & Kfir, 1995; Prasad, Aruna, Kumar, & Kariappa, 2007; Cruz et al., 2011; Barbosa et al., 2015).

Seven *S. frugiperda* larvae in the fifth or sixth instar that tried to make the transition to the prepupal phase died, remaining in an intermediate phase between the two, in which the host does not have a sufficient immune response, such as to the encapsulation of eggs, to complete its development cycle. Parasitism can cause changes...
in juvenile hormones, ecdysteroids, and neuropeptides in the host, preventing some larvae from evolving into the next stage (Strand & Peck, 1995).

The duration of the life cycle (egg-adult) of *T. howardi* emerged from *S. frugiperda* exposed to seven female parasitoids was statistically different depending on the phase. The life cycle was longer in the fifth instar (31.00±0.45) and shorter in the sixth instar (22.44±0.97) and prepupa (20.10±0.22). This may be because late instar larvae provide more nutrients to the parasitoids than younger larvae, resulting in rapid growth and development (M. E. Pascua & L. T. Pascua, 2004). The shorter life cycle of *T. howardi* is satisfactory for commercialization because it facilitates faster rearing of adults, meeting the requirements of biofactories.

The highest total progeny and progeny per female of *T. howardi* occurred in the prepupal phase of *S. frugiperda*, confirming its potential as an alternative host for mass rearing. *T. howardi* is a parasitoid of pupae and, thus, preferred the prepupal phase, which is the closest to their natural host stage. A similar preference was observed for the phases of *D. saccharalis* parasitized by *T. howardi* (Rodrigues et al., 2021). *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae) also had greater progeny in the later instars of *Anastrephaphraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) (Van Nieuwenhove & Ovruski, 2011).

In the larval stage of *S. frugiperda*, changes occur in the structure of various tissues and their metabolism, which interfere with the availability of nutrients in the larvae’s hemolymph (Cônsoli & Vinson, 2009), directly interfering with parasitism.

The sex ratio of *T. howardi* was similar in the fifth and sixth instars and prepupae of *S. frugiperda* larva, demonstrating a high capacity of this parasitoid to develop at any stage, if necessary, with an excellent result with a mean greater than 90%. This parameter is very relevant for biological control, since parasitoid females are the agents responsible for parasitism and successful pest control, providing an increase in mass rearing and its performance in the field (Rodrigues et al., 2021; Costa et al., 2014; Kumar, Baitha, & Bareliya, 2016).

The longevity of *T. howardi* females was similar in the three phases tested, with a general average of 20 days. The behavior of this species guarantees its reproductive success because the female is responsible for the parasitism and continuation of the species. This survival will guarantee the location of the host in the habitat, evaluating and choosing those that present the best quality for development of offspring, directly interfering with the performance of the progeny (Royer, Fournet, Brunel, & Boivin, 1999; Pereira, Zanuncio, Pastori, Pedrosa, & Oliveira, 2010a; Pereira et al., 2010b). Even if descendant adults do not emergence, the parasitism alone is enough to control and prevent the continuation of the arthropod-pest cycle.

The emergence of *T. howardi* males is essential for copulation with females to occur in the first hours after emergence (González, Oca, & Ravelo, 2003). The longevity of *T. howardi* males was lower in the prepupal phase of *S. frugiperda*, confirming that this phase is a good alternative for breeding this species. In the absence of the pupa, the prepupa will have as promising results as the pupal stage.

In the field all, stages of *S. frugiperda* are found; thus, parasitism occurring in all larval phases is extremely important to control the fall armyworm in corn.

In relation to parasitism of *S. frugiperda* pupae by *T. howardi* with different densities, we can consider that, the percentages of parasitism and emergence of *T. howardi* from *S. frugiperda* pupae were not influenced by the density of female parasitoids. This demonstrates the host’s suitability for the development of the parasitoids, which must satisfy their nutritional requirements and prevent the host’s immune system from eliminating them (Strand & Pech, 1995; Cônsoli & Vinson, 2009).

The increase in the density of *T. howardi* per *S. frugiperda* pupa did not affect the duration of the parasitoid cycle. Similar results were obtained for the parasitism of *T. howardi*, at various densities, on *D. saccharalis* (Vargas, 2013). Generally, the development period is shortened at high densities of parasitoids. When *T. howardi* were reared in pupae of *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae), the increased number of females per pupa of the coleopteran reduced the life cycle of this parasitoid, which was 20.19±0.36 days at 1:1 density and 18.88±0.55 days at 32:1 density (Oliveira, 2013). This was also evidenced in the reproduction of *Trichosphus diatraeae* Cherian & Margabandhu, 1942 (Hymenoptera: Eulophidae) in *T. molitor* pupae (Favero et al., 2013). *Palmistichus elaeisis* Delvare & La Salle, 1993 (Hymenoptera: Eulophidae) in pupae of *Bombyx mori* Linnaeus, 1758 (Lepidoptera: Bombycidae) obtained between 49 and 589 descendants at densities of 1:1, 9:1, 18:1, 27:1, 36:1, 45:1, and 54:1 (Pereira et al., 2010). The differences between these results may be related to the capacity to support the host (Pereira et al., 2010), competition, as well as the age and size of the parasitoid (Godfray, 1994).
The sex ratio of *T. howardi* per pupa of *S. frugiperda* resulted in 0.85±0.02 females. This result was similar to the sex ratio of *T. howardi* when reared in the pupa of *M. morio*, with a proportion of 94% females in the progeny (Oliveira, 2013). The low number of males in the descendant population is also characteristic of other parasitoids in the Eulophidae family, including *T. diatraeae* and *P. elaeisis* (Chichera et al., 2012; Pastori et al., 2012a; Favero et al., 2013).

The best densities for total progeny per pupa were 21 and 28, with approximately 500 individuals in each density. The progeny produced by the *S. frugiperda* pupa was positively influenced by the density of *T. howardi* (Figure 1). Many parasitoids in the Eulophidae family can parasitize hosts of different sizes, developmental stages, families, and orders. Because the host species affects the development of parasitoids, different results for their biological parameters have been observed (Baitha, Jala, Rabindra, Venkatesan, & Rao, 2004, La Salle & Polaszek, 2007; Prasad et al., 2007; Silva-Torres, Pontes, Torres, & Barros, 2010; Pastori et al., 2012a, 2012b).

The density of parasitoid females is reported to influence the production of total *P. elaeisis* progeny per *T. arnobia* pupa, with a minimum of 30 and a maximum of 724 offspring of the parasitoid produced at densities of 1 and 15 per host pupa, respectively (Barbosa, Zanuncio, Pereira, Kassab, & Rossoni, 2016). Similarly, 10 *T. diatraeae* females per pupa of *A. gemmatalis* are indicated for mass rearing of this parasitoid, with the best density producing an average of 300 individuals per pupa (Oliveira et al., 2018).

The total progeny of *T. howardi* was also positively influenced by the density of females per prepupa of *A. gemmatalis*, where the total progeny ranged from 47.27±4.43 (1:1) to 389.40±4.03 descendants (25:1). For parasitism in the larval phase, the total progeny ranged from 73.11±8.89 to 271.88±38.44 of *T. howardi* descendants produced at densities of 1 and 10 parasitoids per host, respectively (Fernandes, 2018).

The progeny produced by each female *T. howardi*, at density 1, had a satisfactory result, producing almost 100 offspring. The progeny of *T. howardi* produced by females in *S. frugiperda* pupae was inversely proportional to the density of female parasitoids (Figure 2). Hence, one female *T. howardi* is considered sufficient for the parasitism, development, and emergence of this parasitoid. We propose the possibility of optimizing breeding in the laboratory, to obtain a greater number of parasitoids per host using the proportion of only one female for each host pupa, adding results and reducing maintenance costs. Similar data were found in the progeny of *T. howardi* per female in *D. saccharalis*, which presented an overall mean of 106.92±6.04, when a female *T. howardi* was exposed to different densities of *D. saccharalis* pupae (Vargas, 2013).

Up to a density of 7, *T. howardi* presented good progeny, with quality for its rearing in the laboratory, aimed at a possible use in applied biological control. At densities 14, 21, 28, and 35, each female of *T. howardi* produced less progeny. With gregarious parasitoids, when the number of females per host is increased, the average number of female offspring decreases simultaneously (Rabinovich, Jorda, Bernstein, & 2000), because female progeny exhibits greater conspecific competition within a single host (Dorn & Beckage, 2007). This was clearly evidenced in this research with *T. howardi* reared in *S. frugiperda* pupae, as the number of females tended to reduce as a function of density.

Due to the high rate of parasitism and emergence in pupae (94%) by a female *T. howardi*, the high biological potential of these parasitoid females is evidenced, proving that immature *T. howardi* efficiently exploited the nutritional resources of the host. Parasitoid females have developed strategies throughout their co-evolution process with arthropod-pests to overcome immune defenses and make the most of their nutritional resources, using, for example, substances from the ovary to block host’s defenses, kill or paralyze the host, or protect the eggs against the host’s immune system (Kaeslin, Pfister-Wilhelm, Molina, & Lanzerein, 2005). These strategies indicate the direct relationship of the parasitoid in the suppression of the host’s cellular defense, reflected in the effective development of immature forms of the progeny (Andrade et al., 2010).

The longevity of *T. howardi* females ranging from 22.10±0.64 to 23.60±0.80 was similar at different densities. In mass rearing of parasitoids, survivability is one of the requirements for quality control (Carneiro, Fernandes, & Cruz, 2009). In addition, greater longevity is a favorable characteristic for the species, as it gives parasitoid females longer time to search for hosts in the field under conditions of host scarcity (Foerster & Avanci, 1999). *Spodoptera frugiperda* in its pupal stage has great potential as an alternative host for *T. howardi* rearing, because in addition to being easy to handle, it allows parasitism and emergence rates of 100%, with 250 parasitoids per pupa, which is important for biological control laboratories aiming at field releases.

Consideration should be given to the possibility of using *T. howardi* in combination with other parasitoid species of lepidopteran pest eggs, such as *Trichogramma spp.* or *Ooencyrtus submetallicus* Howard, 1897 (Hymenoptera: Encyrtidae) (Faca et al., 2021), to control *S. frugiperda*, which is a proposal for future research. Additionally, the
population dynamics of the parasitoid together with the behavior of the Lepidoptera pest in the field should also
be taken into account in future studies, given that the pupae of this Lepidoptera are usually located in the first
centimeters of the soil and are not located by the parasitoid in natural conditions. The number of female
parasitoids that emerged per host, as well as their duration in days, are important factors to be verified in the
quality control of laboratory rears and mass releases, as they interfere with biological aspects of the parasitoid,
such as the rate of parasitism, viability, progeny, and sex ratio (Carneiro et al., 2009).

From the results obtained and the discussion raised, this work provides a basis and opens the possibility for
future studies with other species of lepidopterans, with possible implementations of new protocols for rearing
and field releases of this parasitoid species, in a synchronized way, to balance pest populations in the field.

5 Conclusions

*Tetrastichus howardi* parasitized, developed, and emerged in all immature stages of *S. frugiperda*, which is the
first record in the literature.

Overall, the total progeny produced by the *S. frugiperda* pupa increased as the density of *T. howardi* increased.

The progeny produced by each female *T. howardi* in the *S. frugiperda* pupae decreased as the parasitoid density
increased. One female was sufficient for parasitism.

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