

Potential of the Parasitoid *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) on the Control of *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae)

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Abstract

Helicoverpa armigera (Hubner, 1805) (Lepidoptera: Noctuidae) is an insect pest that attacks several cultures and has been a cause of concern in Brazil. The objective of this study was to evaluate parasitism in pupae and caterpillars of *H. armigera* by *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae), in laboratory, as well as checking if *T. howardi* parasitoid has the ability to locate and infect *H. armigera*. In the laboratory, pupae of *H. armigera* and *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) were exposed to 15 *T. howardi* females for 24 hours. Third, fourth and fifth instars of *H. armigera* were exposed to three *T. howardi* females for 24 hours. In the field experiment, cages were installed. Ten caterpillars of the fifth instar of *H. armigera* were released in each cage and waited for the necessary period for them to soak in the soil. After this period, 150 *T. howardi* females were released in each of four cages and in one cage there was no release of parasitoids. In the laboratory, the percentage of parasitized and emergent pupae was 100% in both hosts. The life cycle of *T. howardi* in *H. armigera* pupae was 15 days, and for *D. saccharalis* it was 16 days. The progeny was significantly larger for the host *H. armigera* (689.00) compared to *D. saccharalis* (358.27). The sex ratio was 0.91 and 0.87 for *H. armigera* and *D. saccharalis*, respectively. The longevity of males (25.60 days) and females (26.95 days) in the treatment with *H. armigera* differed from the treatment with *D. saccharalis*, which was 21.70 days for males and 22.95 days for females. For the experiment with *H. armigera* caterpillars, it was found that only two caterpillars from the fifth instar were parasitized with emergence of parasitoids in the pupal phase, with a progeny of 81 individuals; egg-adult cycle was 19.5 days; sex ratio of 0.88; male longevity was 19.6 days and for females 20.3 days. Despite the high rate of parasitism of *T. howardi* in *H. armigera* pupae in the laboratory, the same did not occur for caterpillars. In the field evaluation, of the total of 50 caterpillars released in the cages, 34 pupae were collected. Of these, 31 pupae had emergence of the *H. armigera* moth. No parasitism by *T. howardi* was found in *H. armigera* pupae. Further studies should be conducted to evaluate *T. howardi* parasitism in *H. armigera* pupae under field conditions.

Keywords: biological control, parasitoids, pest insects, soybean crop

1. Introduction

As Brazil has reached records of soybean *Glycine max* (L.) Merrill production with each harvest, it is the second largest soybean producer in the world, with great prospects of being the largest producer (Lima et al., 2019). However, the high production of this grain can be affected by the occurrence of several species of pest insects constantly present in the crop. Among the main species, *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) is of great concern, mainly because the insect in the caterpillar phase feeds preferentially on the plant's reproductive structures, such as inflorescences and pods, causing deformation, rot or fall of the reproductive structure (Ávila et al., 2013). Since 1999, *H. armigera* was considered a quarantine pest A1 in Brazil. In 2013, its occurrence was officially reported in high infestations in the country, in different states and

causing damage in many crops (Czepak et al., 2013; Specht et al., 2013; Tay et al., 2013). Integrated Pest Management (IPM) aims to integrate several tactics to optimize insect pest control in a sustainable and economically compatible manner (Prokopy & Kogan, 2003). Among the control tactics available, biological control is an important tool in the management of *H. armigera*, mainly because it can be associated with other types of control (Fathipour & Sedaratian, 2013). After detection of the species in Brazil in 2013, the use of *Helicoverpa armigera nucleopolyhedrovirus* in crops was approved (HearNPV) as well as the use of the egg parasitoid *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae) as the main promising biological control agents for the control of *H. armigera*. In Australia, 45 species of hymenoptera have been reported parasitizing eggs, caterpillars and pupae of *Helicoverpa* spp. (Johns & Whitehouse, 2004). In Iran, 36 parasitoids have been reported to be associated with immature forms of *H. armigera*, with natural biological control levels ranging from 5 to 80%, depending on the crop and the stage of development in which the pest is found (Fathipour & Sedaratian, 2013). In Brazil, the natural occurrence in *H. armigera* caterpillars of *Archytas marmoratus* (Townsend, 1915) (Diptera: Tachinidae) (Guerra et al., 2014) and *Campoletis sonorensis* (Cameron, 1886) (Hymenoptera: Ichneumonidae), *A. incertus* (Macquart, 1851) (Diptera: Tachinidae) parasitoids has been recorded, associated with *H. armigera*, in which the larval parasitism rate was higher than 41% in areas of structured cotton refuge, without the use of chemical insecticides (Luz et al., 2018).

Among the parasitoids found in agroecosystems, *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) is a pupal parasitoid, of Asian origin, widely distributed in northern Australia and northern China and in Western Pakistan (La Salle & Polaszek, 2007), being introduced from the Philippines to South Africa for biological control of the stem wireworm *Chilo partellus* (Swinhoe, 1885) (Lepidoptera: Pyralidae) (Kfir et al., 1993). In Brazil, there are reports of parasitism by *T. howardi* in lepidoptera pupae (Pereira et al., 2015) in different cultures, such as corn (*Zea mays*) and sorghum (*Sorghum bicolor*) (Cruz et al., 2011), sugarcane (*Saccharum officinarum*) (Vargas et al., 2011) and kale (*Brassica oleracea*) (Silva-Torres et al., 2010). And in laboratory conditions, in *H. armigera* pupae, there were good results of parasitism (Oliveira et al., 2016). However, in the agroecosystems, for *H. armigera* pupa in the soil (Ali & Choudhury, 2009), it is necessary to develop studies to clarify whether *T. howardi* has the capacity to locate and parasitize the pupae found in the soil. Therefore, the objective of the study was to evaluate the potential use of *T. howardi* in the control of *H. armigera*, in pupae and caterpillars, in the laboratory, as well as to verify if the parasitoid *T. howardi* has the capacity to locate and parasitize *H. armigera* pupae located in the soil, under field conditions, in soybean culture.

2. Material and methods

Three bioassays were performed using *H. armigera* pupae and caterpillars and *D. saccharalis* pupae from the breeding of the Entomology Laboratory of Embrapa Agropecuária Oeste (22°14'S; 54°49'W), bred initially on a modified artificial diet of Greene et al. (1976), and modified diet of Hensley and Hammond (1968), respectively. The *T. howardi* parasitoids used in the experiments were the result of breeding in a natural host *D. saccharalis* (Vargas et al., 2011). The experiments carried out in the laboratory were conducted at the Entomology Laboratory of Embrapa Agropecuária Oeste under controlled conditions of 25±2 °C, 60±10% relative humidity and 14 hour photophase. The field experiment was conducted in the experimental area of Fundação MS (22°08'S; 52°44'W, 425 m of altitude) located in the municipality of Maracaju, MS.

2.1 Bioassay 1: Compared Parasitism of *T. howardi* in Pupae of *H. armigera* and *D. saccharalis*

Helicoverpa armigera pupae (n = 30) with a mass between 0.328 and 0.418g and *D. saccharalis* pupae (n=30) (natural host) with a mass between 0.187 and 0.228g, both 24 hours after pupation were individualized in flat-bottomed glass tubes (8.5 × 2.5 cm) (height × diameter) and exposed to parasitism by 15 *T. howardi* females up to 24 hours old. The number of parasitoids per pupa was based on studies carried out by Vargas et al. (2013) and by Oliveira et al. (2016), who in the laboratory obtained 100% parasitism in pupae of *H. armigera* when exposed to 15 *T. howardi* females per pupa, providing an increase in the progeny without losing essential biological characteristics in a laboratory breeding. After 24 hours of parasitism, the parasitoids were removed from the tubes and the pupae of *H. armigera* and *D. saccharalis* remained under observation until the emergence of the new parasitoids. The glass tubes containing the pupae were sealed with cotton wool and kept in an air-conditioned room, with a temperature of 25±2 °C, relative humidity (RH) of 60±10% and a 14-hour photophase, until the potential emergence of the parasitoids. Percentage of parasitized pupae and emergent pupae, life cycle duration (egg-adult), progeny (number of parasitoids per pupa), sex ratio (number of females/number of progeny) and longevity of emerged parasitoids were assessed. To evaluate the parasitoids' longevity, 20 females and 10 males of *T. howardi* from each treatment were randomly selected, which were individualized in test tubes containing a drop of honey, where they remained until their death. The sex of *T. howardi* adults was determined according to the morphological characteristics of the antenna according to La Salle and Polaszek

(2007). The experimental design used was completely randomized, with two treatments (hosts) and 30 repetitions. The use of the natural host, *D. saccharalis*, was thought to be the normal pattern of development of the parasitoid. The data obtained were submitted to homogeneity and normality analysis, taking into account the conditions. Subsequently, the t test at 5% significance was performed.

2.2 Bioassay 2: *T. howardi* Parasitism in *H. armigera* Caterpillars and Their Effects on Host Mortality

Thirty individuals from third, fourth and fifth instars of *H. armigera* were individualized in flat-bottomed glass tubes (8.5 × 2.5 cm) (height × diameter) and exposed to parasitism for 24 hours by three copulated *T. howardi* females with up to 24 hours of age, with 30 caterpillars for each instar, and a control treatment, with caterpillars without exposure to the parasitoid. After the period of exposure to parasitism, the parasitoids were removed from the tubes and an artificial diet was provided for feeding the caterpillars. The glass tubes containing the caterpillars were sealed with cotton wool and kept in an air-conditioned room, with a temperature of 25±2 °C, relative humidity (RH) of 60±10% and a 14 hour photophase, until the potential emergence of the parasitoids. Percentage of parasitized caterpillars and with emergence of parasitoids, the duration of the life cycle (egg-adult), the progeny (number of parasitoids per pupa), the sex ratio (number of females/number of the progeny) and the longevity of emergent parasitoids from parasitized caterpillars were assessed. The effect of parasitism on caterpillar mortality was also evaluated. The experimental design used in a factorial scheme three (caterpillar instar) × 2 (without and with parasitoid) with five replications. Each repetition consisted of a group of six caterpillars. The data obtained were submitted to homogeneity and normality analysis, verifying the need for data transformation. To do this, Taylor's Power Law was followed, reaching the root transformation of $\sqrt{x} + 0.5$. After the transformation, the analysis of variance was performed, and the treatment averages compared by the Tukey test at 5% significance.

2.3 Bioassay 3: *T. howardi* Parasitism in *H. armigera* Pupae, Under Field Conditions, in Soybean Culture

In an area of approximately 15 hectares sown with soybean cultivar BMX Power RR in no-tillage system, cages made of PVC tube (1.00 × 1.00 m—width × height) covered with 'voile' fabric, were arranged at random, each covering approximately 12 soybean plants. The bases of the cages, close to the ground, were covered with soil, in order to prevent infestation by undesirable insects, as well as to prevent the escape of insects released inside. During the experiment the soybean plants were between stages R4 and R5.5 (Fehr & Caviness, 1977). On the upper leaves of the plants, 10 caterpillars of the fifth instar of *H. armigera* were released in each cage, and after a seven-day period, necessary for the caterpillars to pupate in the soil, 150 females of the parasitoid were released in each cage. The number of parasitoids per pupa was based on studies carried out by Vargas et al. (2013) and by Oliveira et al. (2016), as described above. After 24h of the release, pupae were collected from the soil and placed in Petri dishes and kept in the laboratory until the potential emergence of parasitoids. The experiment was conducted in randomized blocks with five replications and cages. In four cages, parasitoids were released and one was left without parasitoids being considered as the control treatment.

3. Results and Discussion

3.1 Compared Parasitism of *T. howardi* in Pupae of *H. armigera* and *D. saccharalis*

The percentage of pupae with parasitism and adult emergence was 100% for both hosts evaluated. The life cycle (egg-adult) of *T. howardi* in *H. armigera* pupae was 15 days, and for the host *D. saccharalis* it was 16 days. The progeny was significantly larger for the host *H. armigera* compared to *D. saccharalis* (Table 1).

Table 1. Biological aspects of *Tetrastichus howardi* in pupae of *Helicoverpa armigera* and *Diatraea saccharalis*. Temperature of 25±2 °C, relative humidity (RH) of 60±10% and 14 hour photophase

| Biological parameters | Host | | T test | CV (%) |
|-----------------------------|--------------------|-----------------------|----------|--------|
| | <i>H. armigera</i> | <i>D. saccharalis</i> | | |
| Parasitism (%) | 100 | 100 | - | - |
| Emergence (%) | 100 | 100 | - | - |
| Egg-adult cycle (days) | 15.00±0.00 | 16.00±0.00 | - | - |
| Progeny | 689.00±15.98 a | 358.27±14.54 b | 238.34** | 15.85 |
| Sex ratio | 0.91±0.00 a | 0.87±0.00 b | 14.39** | 3.88 |
| Longevity of ♂ ¹ | 25.60±0.30 a | 21.70±0.30 b | 106.11** | 3.58 |
| Longevity of ♀ ² | 26.95±0.18 a | 22.95±0.25 b | 152.00** | 4.11 |

Note. Means±standard error do not differ statistically by t test at 5% probability.

** Significant at 1% probability; ¹♂: Males; ²♀: Females.

The parasitism and emergence results obtained indicate that *H. armigera* pupae are good hosts for the breeding of the *T. howardi* species. According to Favero et al. (2013), a high reproductive performance of the parasitoid demonstrates its viability in using the host. In addition, the occurrence of parasitism is fundamental to the biological control applied in IPM and high rates of emergence contribute to the multiplication of parasitoids in mass breeding laboratories, as well as to high-volume releases in order to preserve these species in the field (Dias et al., 2008; Oliveira et al., 2016).

The life cycle duration of the parasitoids was statistically similar between the species studied (Table 1), being shorter than that reported by Costa et al. (2014), who evaluated biological aspects of *T. howardi* in *D. saccharalis* pupae, with a life cycle (egg-adult) duration of 20.26 days, and by Barbosa et al. (2015) when using *Erinnyis ello* (Linnaeus, 1758) (Lepidoptera: Sphingidae) as a host (20.12 days), and the study was conducted under the same temperature conditions, demonstrating that *H. armigera* does not affect important biological characteristics of the parasitoid, being a suitable host for its multiplication. The duration of the life cycle interferes in the speed of breeding the parasitoid in laboratory; therefore, in the field releases, with a shorter duration of the life cycle favoring a greater number of generations of the parasitoid in less time.

The sex ratio found in this study for *H. armigera* (0.91) (Table 1) was similar to that reported by Costa et al. (2014) in the host *D. saccharalis*, which was 0.93 females; when the parasitoid *T. howardi* was raised in *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae) pupae, it had a proportion of 0.94 females in the progeny (Oliveira, 2013) and when the host used was *E. ello*, the sex ratio was 0.96 (Barbosa et al., 2015). Sex ratio values close to one indicate the existence in the progeny of a greater number of females, being important for releases in the field, thus increasing the number of individuals produced in the next generation (Pereira et al., 2009). The sex ratio favors the retention of parasitoids in the crops and the proportion of 60% of females in the releases is enough for an efficient control (Campos-Farinha et al., 2010; Vacari et al., 2012). Other parasitoids of the Eulophidae family are characterized by the low number of males in the offspring, including *Trichospilus diatraeae* Cherian & Margabandhu, 1942 (Hymenoptera: Eulophidae) and *Palmistichus elaeisis* Delvare & Lasalle, 1993 (Hymenoptera: Eulophidae) (Chichera et al., 2012; Pastori et al., 2012; Favero et al., 2013).

The longevity of males and females in the treatment with *H. armigera* differed from the treatment with *D. saccharalis* (Table 1). In the host *E. ello*, the longevity of males and females was 15 and 12 days, respectively (Barbosa et al., 2015). This suggests that *H. armigera* is an alternative quality host, promoting greater longevity of the parasitoid *T. howardi*. It is noteworthy that the longevity of the parasitoid can result in greater pupal parasitism in potential releases of this biological control agent in the field. The increase in longevity is considered beneficial in the breeding of biological control agents, as it guarantees survival during operational procedures and favors greater stay of natural enemies in cultures (Sorensen et al., 2012; Vargas, 2013).

3.2 *Tetrastichus Howardi Howardi* Parasitism in *H. armigera* Caterpillars and Their Effects on Host Mortality

Only two caterpillars of *H. armigera* of the fifth instar were parasitized (6.67%), with emergence of parasitoids (Table 2).

Table 2. Evaluation of parasitism in *Helicoverpa armigera* caterpillars from different instars and effect on the mortality rates of host caterpillars and pupae. Temperature of 25±2 °C, relative humidity (RH) of 60±10% and 14-hour photophase

| Caterpillar Instar ^(A) | Parasitism ^(%) | | | Caterpillar Mortality (%) | | | Pupae Mortality (%) | | |
|-----------------------------------|---------------------------|------|------|--|----------|---------|----------------------------------|----------|---------|
| | Parasitoid ^(B) | | Mean | Parasitoid ^(B) | | Mean | Parasitoid ^(B) | | Mean |
| | Without | With | | Without | With | | Without | With | |
| 3 ^o | 0;00 | 0.00 | 0.00 | 6.68 aA | 13.36 aA | 10.02 a | 0.00 aB | 34.00 bA | 17.00 b |
| 4 ^o | 0.00 | 0.00 | 0.00 | 0.00 bA | 3.34 bA | 1.67 b | 3.34 aB | 38.04 bA | 20.69 b |
| 5 ^o | 0.00 | 6.67 | 3.33 | 0.00 bA | 0.00 bA | 0.00 b | 3.34 aB | 60.02 aA | 31.68 a |
| Mean | 0.00 | 2.35 | | 2.23 B | 5.57 A | | 2.22 B | 44.02 A | |
| - | | | | Test F ^(A) = 10.69** | | | Test F ^(A) = 2.88* | | |
| - | | | | Test F ^(B) = 3.10* | | | Test F ^(B) = 138.66** | | |
| - | | | | Test F ^(A*B) = 1.03 ^{ns} | | | Test F ^(A*B) = 3.82* | | |
| - | | | | CV (%) = 70.84% | | | CV (%) = 32.42% | | |

Note. Averages followed by the same lowercase letter in the column and uppercase in the row do not differ statistically from each other by the Tukey test at 5% probability. ^{ns} Not significant; * significant at 5% probability; ** significant at 1% probability. The data were transformed into $\sqrt{x + 0.5}$.

Regarding the parasitism of the two fifth instar caterpillars, the emergence of the parasitoids occurred in the pupal phase, presenting a progeny of 81 individuals; egg-adult cycle was 19.5 days; sex ratio of 0.88; male longevity was 19.6 days and for females 20.3 days. The rate of caterpillar parasitism and emergence of parasitoids found in this study was higher than that reported by Pereira et al. (2015), who verified the parasitism of *T. howardi* in different instars of the caterpillar *D. saccharalis*, which were exposed to seven females of the parasitoid for 96 hours of parasitism, where he identified 2% (n = 50) of parasitism only in caterpillars of fifth instar, of which, only 14% had emergence of parasitoids. Emergence of parasitoids occurred in the pupa phase of the host, as reported in this study for *H. armigera*. Vargas et al. (2013) reported parasitism by *T. howardi* in *D. saccharalis* caterpillars from second to fifth instar, and one parasitoid female was sufficient to cause the host's mortality, and the percentage of parasitism was influenced by the instars of the caterpillars, and was greater when combined the density of four *T. howardi* females. The low rates of parasitism of *H. armigera* caterpillars by *T. howardi* females suggest that the larval stage of *H. armigera* may have a stronger immune response against this parasitoid, similarly to what was reported for *D. saccharalis* caterpillars parasitized by *T. howardi* (Pereira et al., 2015). Cellular defenses, encapsulation and melanization of endoparasitoid eggs (Pennacchio & Strand, 2006; Andrade et al., 2010) may be some of the mechanisms involved in this phenomenon. The fact that *H. armigera* caterpillars present a slightly leathery coat may be one of the hypotheses for the low parasitism index, in addition to the caterpillar presenting a very aggressive defensive behavior. The caterpillar, different from the pupal phase, has movements and is able to defend itself from the parasitoid attack (Cónsoli & Vinson, 2009; Greeney et al., 2012).

Regarding the effects of parasitoid contact with caterpillars, a higher mortality rate was observed in third instar caterpillars (Table 2), possibly due to their smaller size, being more affected by substances injected during oviposition. Caterpillars that died as a result of parasitism acquired a dark color, and a flabby and then rigid integument, as fifth instar caterpillars were often in an intermediate stage between caterpillar and pupa, which was also observed by Vargas et al. (2013) in *D. saccharalis* caterpillars that were parasitized by *T. howardi* at different instars. Changes in the color and consistency of the body of *H. armigera* caterpillars parasitized by *T. howardi* may be related to the changes promoted by the fluids that are injected during oviposition. These substances cause physiological disturbances that help suppress the host's immune system, causing their death (Danneels et al., 2010; Colinet et al., 2013).

For the *H. armigera* caterpillars that passed to the pupal phase, differences were verified in relation to the control, with higher mortality rates for the pupae that in the previous phase (caterpillars) had been subjected to contact with the parasitoid (Table 2). Pupae from the fifth instar caterpillars exposed to parasitism had a higher mortality rate (60.02%) compared to the third (34.00%) and fourth instar (38.04%) (Table 2). The pupae that did not originate moths, were hollow, malformed and some were in an intermediate stage between caterpillar and pupa, showing potential stress suffered during the caterpillar phase. The *T. howardi* female, in some cases, does not cause the immediate death of the *D. saccharalis* caterpillars, this was evidenced when caterpillars of different instars died after reaching the pupal stage, which may also have occurred with the *H. armigera* caterpillars. It is suggested that the higher mortality of caterpillars and pupae that were exposed to parasitism can be explained by the ability of some parasitoids to inject toxins during oviposition, in order to overcome the immune response by encapsulation of the host (Schmid-Hempel, 2005; Bueno, 2015), which may also have caused the death of the host.

3.3 *Tetrastichus Howardi* Parasitism in *H. armigera* Pupae, Under Field Conditions, in Soybean Crop

Of the total of 50 caterpillars released inside the cages, 34 pupae were collected. Of these, three pupae died, and 31 pupae emerged from the *H. armigera* moth. Thus, *T. howardi* parasitism was not found in *H. armigera* pupae (Table 3).

Table 3. Average percentage of pupae collected from *Helicoverpa armigera*, pupae parasitized by *Tetrastichus howardi* and percentage of emergence of *H. armigera* moths, in the field, in soybean culture

| Parasitoid | Total caterpillars ⁽ⁿ⁾ | Pupae Collected ^(%) | Pupae Parasitized ^(%) | Adult Emergence ^(%) |
|------------|-----------------------------------|--------------------------------|----------------------------------|--------------------------------|
| Without | 10 | 70.00 | - | 100.00 |
| With | 40 | 67.50 | 0 | 96.00 |

The search for pupae in the soil was conducted up to 10 cm deep, as, according to the literature, it is in this range that pupae, in general, are located (Fragoso, 2014). The pupae that were recovered were between three and five

centimeters deep (Figure 1). This fact corroborates the results found by Martins et al. (2013), in which pupae of *H. armigera* were collected in cotton crop (n = 78), in high infestation of the pest, being located at an average depth of 3.1 cm.

In the search for pupae, initially the straw that was on the ground was carefully removed, as a hypothesis would be that the caterpillar could pupate under the straw, which was not verified, and after that, searched for possible holes that could indicate the pupae location. Only one hole was located where a pupa was collected (Figure 1a and 1b).

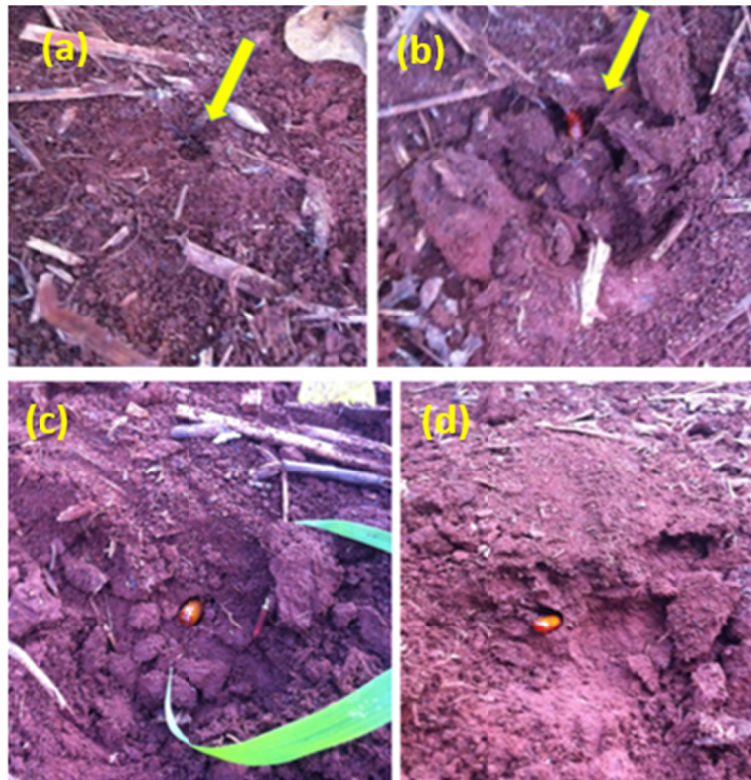


Figure 1. Location of *Helicoverpa armigera* pupae on the ground (a, b, c, d). Orifice indicating pupal location (a, b). (Photos: Juliana Simonato)

No other holes were observed in the soil indicating the location of pupae, as they were found as the excavation was carried out. A fact that may have interfered was the occurrence of heavy rain (30 mm) (22°08'S and 52°44'W, 425 m of altitude) two days before the release of the parasitoids, which may have obstructed the entrance to the pupae access gallery, preventing the parasitoids from reaching them. Perhaps the fact that there was no gallery that would allow the parasitoid to find the pupa made it difficult to locate them and, consequently, parasitism. Barbosa et al. (2018) evaluated parasitism by *T. howardi* in pupae of *D. saccharalis* in sugar cane under field conditions, where they found a parasitism rate of 46.4% when using 102 parasitoid females per pupa. Upon penetrating the sugar cane, the caterpillar *D. saccharalis* leaves a gallery, where the parasitoid manages to enter and perform the parasitism. However, as the *H. armigera* pupae are located in the soil, they may obstruct the entrance to the gallery, which prevents the parasitoid from locating the pupae and performing parasitism.

Based on the results obtained, it can be said that *T. howardi* successfully parasitized *H. armigera* pupae in the laboratory. However, *H. armigera* parasitism in caterpillars was not efficient, but it cannot be ruled out. With all, it was observed that caterpillars that were exposed to parasitism had a higher mortality rate, mainly in the pupa phase, suggesting that the effect of parasitism may have negatively affected this phase. Taking this fact into account, and that the parasitoid had a longevity of around 25 days, in field conditions it would be able to act in the different phases it encountered, parasitizing caterpillars of different instars, causing mortality even in the caterpillar phase and in the pupa phase of the host, being a contribution to the biological control of this pest. Studies focused on evaluating parasitism in *H. armigera* caterpillars, testing various densities of parasitoids, as well as the longer time of exposure to parasitism, may be conducted, in order to verify if the parasitoid is

efficient in the caterpillar phase. The efficiency of parasitism in pupae supports future evaluations for mass breeding of this parasitoid using pupae from this host. Although *H. armigera* parasitism by *T. howardi* did not occur under the conditions of this study, in the field, in soybean culture, it is important to carry out new experiments to validate this information.

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