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Parasitism of *Helicoverpa armigera* pupae (Lepidoptera: Noctuidae) by *Tetrastichus howardi* and *Trichospilus diatraeae* (Hymenoptera: Eulophidae)

Parasitismo de pupas de *Helicoverpa armigera* (Lepidoptera: Noctuidae) por *Tetrastichus howardi* e *Trichospilus diatraeae* (Hymenoptera: Eulophidae)

Harley Nonato de Oliveira[1*]; Juliana Simonato[2];
Daniele Fabiana Glaeser[3]; Fabricio Fagundes Pereira[4]

Abstract

Helicoverpa armigera is a pest that was recently detected in Brazil, which causes significant losses in various crops in different regions of the country. Biological control has been reported to offer a promising alternative in the management of this pest. Thus, this study evaluated the ability of the parasitoids *Tetrastichus howardi* and *Trichospilus diatraeae* to parasitize pupae of *H. armigera* in the laboratory. *H. armigera* pupae were individually exposed to 15 female *Trichospilus diatraeae* or 15 female *Tetrastichus howardi* for 24 h. The pupae remained under observation until the emergence of parasitoids. Parasitism by both parasitoids was observed in 100% of the pupae. Further, 100% of *Tetrastichus howardi* and 90% of *Trichospilus diatraeae* emerged. The life cycle of *Tetrastichus howardi* parasitizing pupae of *H. armigera* was 15 ± 0.0 days, while that of *Trichospilus diatraeae* was 15.90 ± 0.10 days. The numbers of offspring from *Tetrastichus howardi* and *Trichospilus diatraeae* were 669.3 ± 33.47 and 816.11 ± 89.22 per pupae, with a sex ratio of 0.91 ± 0.01 and 0.94 ± 0.01, respectively. *Tetrastichus howardi* and *Trichospilus diatraeae* were shown to successfully parasitize *H. armigera* pupae in laboratory conditions, suggesting that this host could be used for rearing both parasitoids. However, further detailed studies of the host-parasitoid relationship are needed to assess the feasibility of including these parasitoids in integrated management programs of *H. armigera.*
Key words: Biological control, insect pests, parasitoid

Resumo

Helicoverpa armigera é uma praga recentemente identificada no Brasil ocasionando perdas significativas em várias culturas em várias regiões do país. O controle biológico tem sido relatado como uma das alternativas promissoras para o manejo desta praga. O objetivo deste trabalho foi avaliar o parasitismo de pupas de *H. armigera* pelos parasitoides *Tetrastichus howardi* e *Trichospilus diatraeae*, em laboratório. Pupas de *H. armigera* foram individualizadas e expostas ao parasitismo por 15 fêmeas de *Trichospilus diatraeae* ou por 15 fêmeas de *Tetrastichus howardi*, durante 24 h. Após este período, as pupas permaneceram em observação até a emergência dos parasitoides. As porcentagens de parasitismo de *Tetrastichus howardi* e *Trichospilus diatraeae* foram de 100%. Em relação à emergência,

[1] Pesquisador, Dr., Embrapa Agropecuária Oeste, Dourados, MS, Brasil. E-mail: harley.oliveira@embrapa.br
[2] Discente do Curso de Doutorado do Programa de Pós-Graduação em Entomologia e Conservação da Biodiversidade, Universidade Federal da Grande Dourados, UFGD, Dourados, MS, Brasil. E-mail: ju_simonato@hotmail.com
[3] Bolsista PNPD/CNPq, Embrapa Agropecuária Oeste, Dourados, MS, Brasil. E-mail: daniglaeser@yahoo.com.br
[4] Prof. Dr., Faculdade de Ciências Biológicas e Ambientais, UFGD, Dourados, MS, Brasil. E-mail: fabriciofagundes@ufgd.edu.br
[*] Author for correspondence

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a porcentagem foi de 100% para *Tetrastichus howardi* e de 90% para *Trichospilus diatraeae.* O ciclo de vida de *Tetrastichus howardi* em pupas de *H. armigera* foi de 15,0 ± 0,0 dias, enquanto que *Trichospilus diatraeae* completou o desenvolvimento em 15,90 ± 0,10 dias. O número de indivíduos de *Tetrastichus howardi* e *Trichospilus diatraeae* emergidos por pupa foi de 669,30 ± 33,47 e 816,11 ± 89,22, e razão sexual de 0,91 ± 0,01 e 0,94 ± 0,01, respectivamente. Os parasitoides *Tetrastichus howardi* e *Trichospilus diatraeae* parasitam com sucesso pupas de *H. armigera* em condições de laboratório, demonstrando que esse pode ser um bom hospedeiro para criação de ambos os parasitoides. Contudo, estudos mais detalhados da relação parasitoide-hospedeiro são necessários para avaliar a viabilidade de inclusão desses parasitoides em programas de manejo integrados de *H. armigera.*
Palavras-chave: Controle biológico, inseto-praga, parasitoide

Helicoverpa armigera (Lepidoptera: Noctuidae) is distributed worldwide, occurring in countries of Asia, Africa, Europe, Oceania (GUO, 1997), and South America (CZEPAK et al., 2013). In Brazil, it was considered an A1 pest species until January 2013. It was detected in the states of Goiás, Bahia, and Mato Grosso in the first quarter of 2013, the first recorded cases in the Americas, and was primarily associated with damage of cotton and soybean crops (CZEPAK et al., 2013).

In the southern state of Paraná, a severe attack of this pest was observed during initial stages of soybean development in the state of Mato Grosso do Sul, and *H. armigera* caterpillars were found to cause cotton and soybean crop losses in areas neighboring Chapadão do Sul. In other regions of the state, several winter corn producers reported the loss of corn (Bt) due to *H. armigera* (ÁVILA et al., 2013).

Biological control is an important tool for the control of *H. armigera*. However, because this species is a newly detected pest in Brazil, there have not yet been any in-country evaluations of the effectiveness of biological control in eggs, larvae, and pupae of *H. armigera*. Information, therefore, is restricted to international literature (ÁVILA et al., 2013).

The use of *Helicoverpa armigera nucleopolyhedrovirus* (HearNPV) for the control of *H. armigera* on an emergency basis has been approved by the Ministry of Agriculture. In addition, the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) has been released in crops for the control of this pest (CHIAPPINI, 2014). Eulophidae parasitoids, natural enemies of many Lepidoptera, including species in the Noctuidae and Crambidae families, are also promising biological control agents for the control of *H. armigera* (ANDRADE et al., 2010; PEREIRA et al., 2008; VARGAS et al., 2011).

Tetrastichus howardi (Hymenoptera: Eulophidae) is an Asian parasitoid species that was introduced into South Africa for the purpose of biological control of the *Chilo partellus* (Lepidoptera: Pyralidae) (MELO et al., 2011). In Brazil, *Tetrastichus howardi* has been recorded as parasitizing the pupae of *Diatraea saccharalis* (Lepidoptera: Crambidae) in sugarcane (VARGAS et al., 2011) and in corn (CRUZ et al., 2011) and pupae of *Plutella xylostella* in cabbage (SILVA-TORRES et al., 2010). Moreover, studies have demonstrated the ability of this natural enemy to parasitize and develop in the caterpillar stage of *D. saccharalis* (VARGAS et al., 2011).

Another parasitoid that has been studied for the control of insect pests is the endoparasitoid *Trichospilus diatraeae* (Hymenoptera: Eulophidae), which shows a preference for parasitizing Lepidoptera pupae. In Brazil, this species was first reported in pupae of Arctiidae (PARON; BERTI-FILHO, 2000); it has since been reported in Geometridae (PEREIRA et al., 2008) and Pyralidae (MELO et al., 2011). So far, there are no reports to indicate whether *Trichospilus diatraeae* parasitizes *H. armigera* under natural conditions.

With the recent detection of *H. armigera* in Brazil, biological control methods for the management of this pest are an important consideration. The objective of this study was to evaluate *Tetrastichus howardi* and *Trichospilus diatraeae* parasitism of *H. armigera* pupae in the laboratory.

This study was conducted under controlled conditions by using environmental chambers at 25 ± 2 °C, 60 ± 10% relative humidity, and a 12-h photoperiod. For the bioassay, *H. armigera* pupae from the Embrapa Agropecuária Oeste Entomology Laboratory colony were fed artificial diet described by Greene et al. (1976) and adapted by Parra (2001). The parasitoids used in the experiment were maintained on the host *D. saccharalis*.

Pupae of *H. armigera* (0.328–0.418 g) were individually placed in glass tubes (8.5 x 2.5 cm) 24 h after pupation. Ten pupae were exposed to 15 mated 24 h-old *Trichospilus diatraeae* females, and another 10 pupae were offered to 15 mated 24 h-old *Tetrastichus howardi* females. The number of parasitoids per pupa was defined by density tests with parasitoids of the Eulophidae family (VARGAS et al., 2011). After 24 h of parasitism, the parasitoids were removed from the tubes and parasitized pupae remained under observation until the emergence of the parasitoids.

The experimental design was completely randomized, with 10 repetitions (pupae) for each parasitoid. We evaluated the percentage of parasitized pupae, rate of pupal emergence, duration of the life cycle (egg-adult), number of progeny (number of parasitoids per pupa), and sex ratio of progeny (number of females/progeny number) of *Trichospilus diatraeae* and *Tetrastichus howardi*.

The percentages of *H. armigera* pupa parasitized by both species of parasitoids, *Trichospilus diatraeae* and *Tetrastichus howardi*, were 100%. Further, 100% of *Tetrastichus howardi* and 90% of *Trichospilus diatraeae* emerged. The life cycle (egg-adult) of *Tetrastichus howardi* in *H. armigera* pupae was 15 ± 0.0 d, while that of *Trichospilus diatraeae* in *H. armigera* pupae was 15.9 ± 0.10 d.

The number of *Tetrastichus howardi* individuals that emerged per pupa was 669.3 ± 33.47, with 609.9 ± 33.67 females and 59.40 ± 5.26 males, and a sex ratio of 0.91 ± 0.01. Regarding *Trichospilus diatraeae*, the number of individuals that emerged was 816.11 ± 89.22, with 771.33 ± 86.56 females and 44.78 ± 5.03 males, and a sex ratio of 0.94 ± 0 01. *Trichospilus diatraeae* had a greater number of offspring per pupa than *Tetrastichus howardi*, probably because the size difference between the parasitoids, T*etrastichus howardi* is larger than *Trichospilus diatraeae.*

These results indicate that *H. armigera* pupae can serve as a host for both parasitoids, because, according to Favero et al. (2013), high reproductive performance of the parasitoid demonstrates viable host use. In addition, parasitism is central to applied biological control, a part of Integrated Pest Management, and the high emergence rates could facilitate the amplification of parasitoids in mass rearing laboratories and the preservation of these species in the field following inundative releases.

The data regarding the parasitoid *Tetrastichus howardi* obtained in this study, including the length of the life cycle and the sex ratio in pupae of *H. armigera*, were similar to results reported by Costa et al. (2014), a study that evaluated the biological aspects of *Tetrastichus howardi* in pupae of *D. saccharalis* (weight range, 0.190–0.220 g). In spite of this, the number of *Tetrastichus howardi* progeny from *H. armigera* pupae was higher (669.3 ± 33.47) than that observed in the host *D. saccharalis* (254.78 ± 4.60). It is important to note, however, that each *D. saccharalis* pupa was exposed to seven parasitoid females in the earlier study, while each *H. armigera* pupa was exposed to 15 parasitoid females in the present experiment. In a study carried out by Cruz et al. (2011), a single *Tetrastichus howardi* female was able to produce up to 66 offspring per pupa in *D. saccharalis*. Thus, the production of offspring can increase in proportion to the density of parasitoids per pupa and the size of female parasitoid hosts.

Calado et al. (2014) evaluated the biological characteristics of *Trichospilus diatraeae* in pupae of *D. saccharalis* (Lepidoptera: Crambidae) and *Bombyx mori* (Lepidoptera: Bombycidae) by using 21 parasitoid females per pupa, a number greater than was used in the present study. However, even though they were exposed to fewer parasitoids, *H. armigera* pupae yielded a higher rate of emergence (90%) than the hosts *D. saccharalis* (75%) and *B. mori* (66.6%). In addition, the number of parasitoid offspring was higher in *H. armigera* (816.11 ± 89.22) than in *D. saccharalis* (354.50 ± 41.88) and *B. mori* (469.11 ± 15.19), which demonstrates the suitability of *H. armigera* as a host for this parasitoid.

Both *Trichospilus diatraeae* and *Tetrastichus howardi* successfully parasitized *H. armigera* pupae in the laboratory. This information suggests the need to evaluate mass rearing of these parasitoids on *H. armigera* pupae, as well as the need to attempt releases of these parasitoids in the field for the control of the pest species. Thus, further studies should be conducted in order to verify the capacity and efficiency of *H. armigera* as a host for these parasitoids, since *H. armigera* pupae are typically located on the ground. The dynamics of species of the Noctuidae family of Lepidoptera on crops should also be taken into account in this evaluation.

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