Parasitism and emergence of *Tetrastichus howardi* (Hymenoptera: Eulophidae) on *Diatraea saccharalis* (Lepidoptera: Crambidae) larvae, pupae and adults

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Diatraea saccharalis (Fabricius) (Lepidoptera: Crambidae), a major pest of sugarcane in the Americas (White & Wilson 2008; Dinardo-Miranda et al. 2012; Svedese et al. 2013) is capable of causing loss in biomass, death of the apical meristem and reduction in sugar and alcohol production (Simões et al. 2012; Rossato et al. 2013).

Diatraea saccharalis caterpillars develop inside the sugarcane stalks, which diminishes the efficacy of the insecticides used to control them (Pinto et al. 2009); hence the interest in the use of biological control to suppress *D. saccharalis* infestations (Cruz et al. 2011; Rodrigues et al. 2013). Hymenopteran parasitoids are frequently used as natural enemies in numerous applied biological control programs targeting *D. saccharalis* (Zanuncio et al. 2008; Mafi & Ohbayashi 2010; Tavares et al. 2011, 2013).

The parasitoid *Tetrastichus howardi* Olliff (Hymenoptera: Eulophidae) has been recorded from the pupae of several lepidopteran families, including: Crambidae, Noctuidae and Plutellidae (Kfir et al. 1993; Moore & Kfir 1995; Kfir 1997; Baitha et al. 2004; Hayat & Shahi 2004; Prutz et al. 2004; Silva-Torres et al. 2010; Duong et al. 2011; Costa et al. 2014), and it has been used to effectively control several lepidopteran pests. *Tetrastichus howardi* was also found parasitizing *D. saccharalis* larvae (Vargas et al. 2011), which motivated this study on the parasitism of *T. howardi* on various life stages of this important sugarcane pest. The aim of this study was to evaluate the biological characteristics of *T. howardi* parasitizing *D. saccharalis* eggs, larvae, pupae and adults stages.

Experiments were performed in the Laboratory of Entomology/ Biological Control (LECOBIOL) of the "Faculdade de Ciências Agrárias" of the "Universidade Federal da Grande Dourados (UFGD)" in Dourados, Mato Grosso do Sul State, Brazil. Life stages of *D. saccharalis* (eggs, larvae, pupae and adults) were obtained from *D. saccharalis* rearing of LECOBIOL (Parra 2007). *Tetrastichus howardi* females were reared on *D. saccharalis* caterpillars (Vargas et al. 2011).

Each experimental parcel contained 10 of the following stages of *D. saccharalis*: egg masses, larvae, pupae or adults, which were individualized with 7 *T. howardi* females. Each experiment was replicated 5 times (50 *D. saccharalis* individuals in total were used per treatment).

The parasitism intervals were as follows: 24 h for *D. saccharalis* eggs and pupae and 96 h for fifth instar caterpillar larvae and adults. Following parasitism, the *T. howardi* females were removed, *D. saccharalis* stages were placed in glass tubes (13-cm height and 8.5-cm diam), and transferred to climatic chambers set at 25 ± 2 °C, 70 ± 10% RH and 14:10 h L:D.

Percent parasitism adjusting for natural host mortality (Abbott 1925), percentage of emergence, life cycle duration (from egg to adult), the number of parasitoids that emerged (progeny) from the different stages of *D. saccharalis,* and the sex ratio of *T. howardi* were evaluated. The sex of *T. howardi* was determined based on morphological characteristics (La Salle & Polaszek 2007) under a stereoscopic microscope.

Tetrastichus howardi did not parasitize *D. saccharalis* eggs. Parasitism by *T. howardi* was evident in fifth instar larvae (Figs. 1A, C and D), pupae (Fig. 1B, Figs. 2A, B and C) and adults of *D. saccharalis* (Fig. 2D) showing rates of parasitism of 2%, 56% and 68%, respectively. The emergence of *T. howardi* was 14%, 100% and 100% for fifth instar larvae, pupae, and adults of *D. saccharalis*, respectively.

The life cycle (egg-adult) durations of *T. howardi* in pupae, fifth instar larvae and adult stages of *D. saccharalis* were 20.00 ± 0.03, 27.00 ± 0.01 and, 33.00 ± 0.00 days, respectively. *Tetrastichus howardi* also showed a higher fecundity and sex ratio (females) with the *D. saccharalis* pupae at 70.44 ± 5.22 and 0.85 ± 0.41 individuals, respectively.

Tetrastichus howardi parasitized and developed within the larvae, pupae and adults of *D. saccharalis*. The greatest number of offspring and greatest sex ratio (female based) for this parasitoid was observed in the pupal stage of the sugarcane borer, which suggests that this host stage is the most suitable for parasitism and development by this natural enemy. The low parasitism rates of *D. saccharalis* larvae and adult stages by *T. howardi* females suggests that these life stages may possess a stronger immune response against this parasitoid, as reported for *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) parasitizing the sugarcane borer larvae (Mahmoud et al. 2012). Cellular defenses, encapsulation and melanization of the endoparasitoid eggs (Pennacchio & Strand 2006; Andrade et al. 2010) can be some of the mechanisms involved in this phenomenon.

Tetrastichus howardi parasitized the fifth instar *D. saccharalis* larvae and emerged in the pupal stage. Also *T. howardi* parasitized young *D. saccharalis* pupae and emerged from the adults. This is the first time that this pattern of behavior and development of *T. howardi* has been reported.

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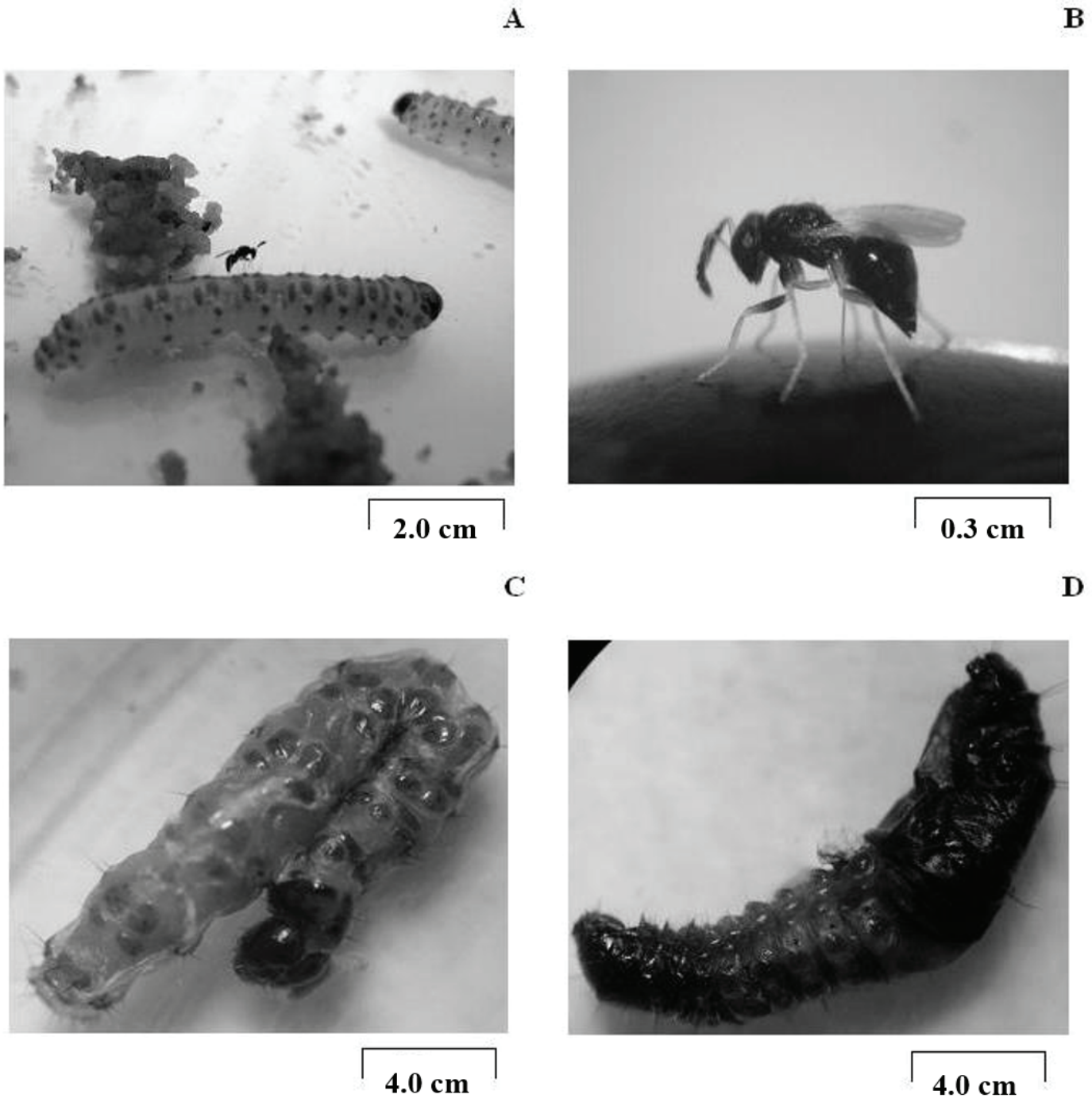

Fig 1. *Tetrastichus howardi* (Hymenoptera: Eulophidae) female parasitizing fifth instar caterpillar and pupa of *Diatraea saccharalis* (Lepidoptera: Crambidae) (A and B) and hosts after parasitism (C and D).

Parasitism of the different biological stages of *D. saccharalis* by *T. howardi* revealed the ability of this natural enemy to regulate the development of various sugarcane borer life stages, and this can be attributed the longer life span of the adult stage of *T. howardi* (Vargas et al. 2011) compared with that of the egg parasitoids *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) (Pereira-Barros et al. 2005; Oliveira et al. 2013) and the larval parasitoid *C. flavipes* (Simões et al. 2012). Thus, in sugarcane fields, this eulophid has more time than other studied parasitoid species to locate and parasitize its host.

Tetrastichus howardi displays high plasticity by parasitizing larvae, pupae and adults of *D. saccharalis*. However, further studies on the cell biology, physiology and ecology of this parasitoid are necessary to better understand the parasitoid-host relationships and to increase the chances of success of this important natural enemy in biological control programs.

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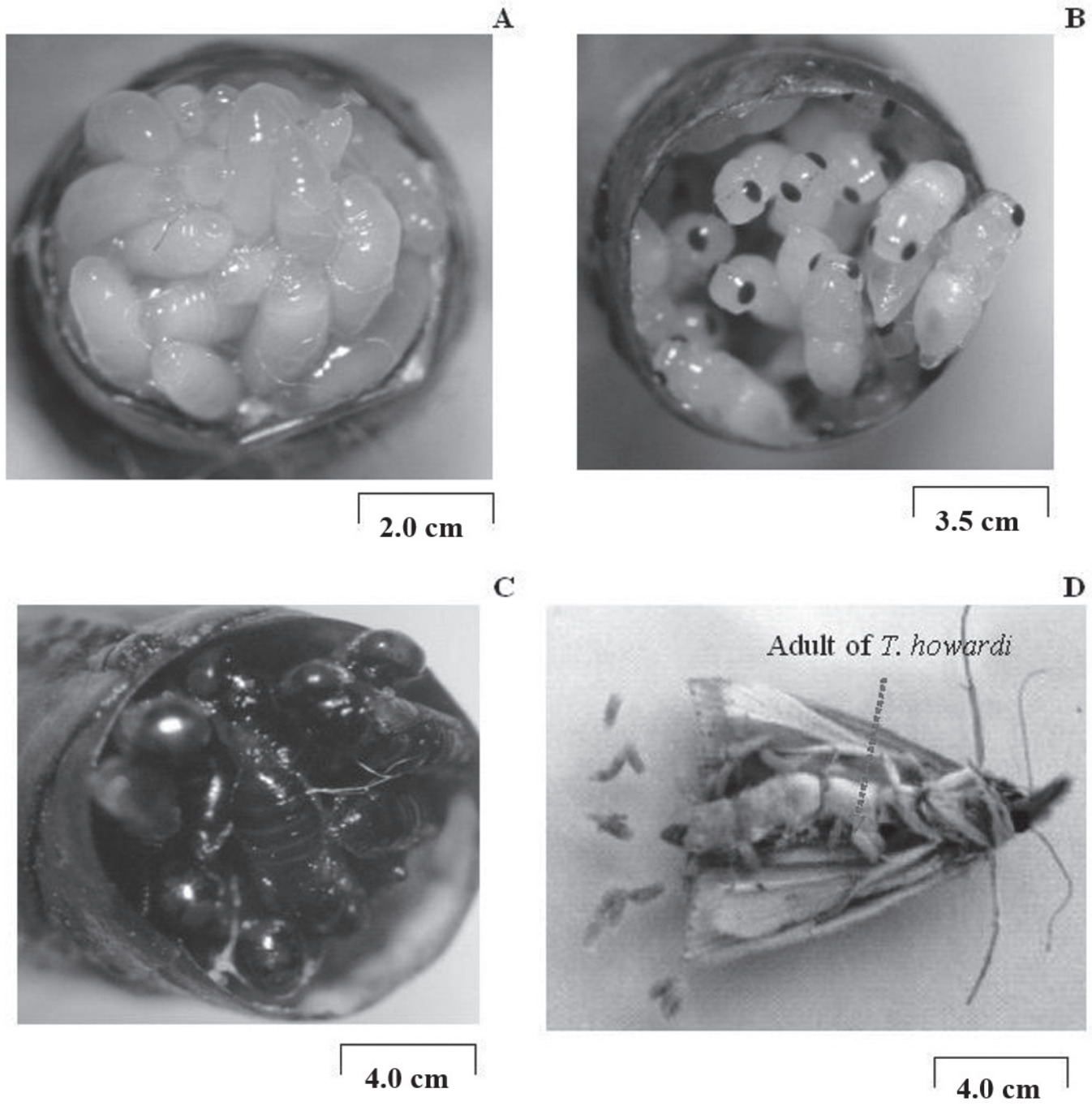

Fig. 2. Larvae, pupae and adults of *Tetrastichus howardi* (Hymenoptera: Eulophidae) in pupae of *Diatraea saccharalis*(Lepidoptera: Crambidae) (A, B, C); *D. saccharalis* adult parasitized by *T. howardi* (D).

Summary

Tetrastichus howardi Olliff (Hymenoptera: Eulophidae) parasitizes the larvae, pupae and adults of *Diatraea saccharalis*, and therefore seems to be a suitable candidate for the biological control of *D. saccharalis* in commercial sugarcane in Brazil and other industries where this stem borer is an important pest. The aim of our study was to analyze the biological characteristics of this natural enemy on sugarcane borer. The research was conducted in the Laboratory of Entomology/Biological Control (LECOBIOL) at the "Faculdade de Ciências Agrárias" of the "Universidade da Grande Dourados (UFGD)" in Dourados, Mato Grosso do Sul State, Brazil. Ten of each of the following life stages: 24-h old egg masses, fifth instar larvae, pupae and adults of *D. saccharalis* were isolated and exposed to parasitism by seven *T. howardi* parasitoids females. Parasitism rates by *T. howardi* of the adult, fifth instar larva and pupal stages of *D. saccharalis* were 2%, 56% to 68%, respectively. Emergence rates of 14%, 100% and 100% were recorded for adult, fifth instar larvae, and adults, respectively. The duration of each life cycle (egg to adult) of *T. howardi* on the pupae, fifth instar larvae and adults of *D. saccharalis* were 20 ± 0.03, 27.00 ± 0.01, 33 ± 0.00 days, respectively. Fecundity and the female-based sex ratio of *T. howardi* were

greatest in the pupae of *D. saccharalis*, at 70.44 ± 5.22 and 0.85 ± 0.41, respectively. Parasitism and the emergence of *T. howardi* from the fifth instar larvae, pupae and adults of *D. saccharalis* revealed the ability of this natural enemy to establish itself in culture, even in the absence of host pupae.

Key Words: biological control, larval and pupal parasitoid, sugarcane, sugarcane borer

Sumário

Tetrastichus howardi Olliff (Hymenoptera: Eulophidae) parasita larvas, pupas e adultos de *D. saccharalis* (Fabricius) (Lepidoptera: Crambidae), e, portanto, parece ser um candidato adequado para o controle biológico de *D. saccharalis* em plantios comerciais de cana-de-açúcar do Brasil e outras indústrias onde a broca do caule é uma importante praga. O objetivo do nosso estudo foi analisar as características biológicas desse inimigo natural na broca da cana-de-açúcar. A pesquisa foi realizada no Laboratório de Entomologia/Controle Biológico (LECOBIOL) na "Faculdade de Ciências Agrárias" da "Universidade da Grande Dourados (UFGD)", em Dourados, Mato Grosso do Sul, Brasil. Dez de cada uma das seguintes fases da vida: massas de ovos com 24 horas de idade, larvas de quinto instar, pupas e adultos de *D. saccharalis* foram isoladas e expostas ao parasitismo por sete fêmeas de *T. howardi*. A taxa de parasitismo por *T. howardi* em adulto, a larva de quinto estádio e pupa de *D. saccharalis* foram de 2 %, 56 % e 68 %, respectivamente. As taxas de emergência foram de 14 %, 100% e 100% para adultos, larvas de quinto ínstar e pupas de *D. saccharalis*, respectivamente. A duração de cada ciclo de vida (ovo a adulto) de *T. howardi* em pupa, as larvas quinto ínstar e adultos de *D. saccharalis* foram de 20 ± 0,03, 27,00 ± 0,01, 33 ± 0,00 dias, respectivamente. A progênie e razão sexual de *T. howardi* foram maiores em pupas de *D. saccharalis* com 70,44 ± 5,22 e 0,85 ± 0,41, respectivamente. O parasitismo e a emergência de *T. howardi* em larvas de quinto ínstar, pupas e adultos de *D. saccharalis* revelam a capacidade, desse inimigo natural, em se estabelecer na cultura, mesmo com ausência as pupas hospedeiras.

Palavras-Chave: broca, controle biológico, cana-de-açúcar, parasitoide larval e pupal

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