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Original Article

Tetrastichus howardi (Hymenoptera: Eulophidae): first report of parasitism in *Oxydia vesulia* (Lepidoptera: Geometridae)

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Abstract

The adaptation of native lepidopteran species to eucalyptus plantations reduces the productivity of this crop in Brazil. *Oxydia vesulia* Cramer (Lepidoptera: Geometridae) is a secondary pest, frequently reported in eucalyptus plantations with population outbreaks and economic damages. Methods of biological control of this pest may include the use of the exotic pupae endoparasitoid *Tetrastichus howardi* Olliff (Hymenoptera: Eulophidae), reported as efficient to controlling lepidopteran pests. The parasitism of *O. vesulia* caterpillars and pupae by *T. howardi* was evaluated under controlled conditions (25 ± 1 ºC, 60 ± 20% humidity and 12:12 h L:D). Each *O. vesulia* caterpillar or pupae was individually placed in a flat-bottom tube with 10 and 15 females of *T. howardi* for 48h, respectively. The parasitoids were removed after that period, the caterpillars were fed and the pupae were maintained until emergence of the parasitoid or formation of pupae and emergence of adults of this pest. The fourth-instar caterpillars of *O. vesulia*, after the parasitism period, were kept in pots with *Eucalyptus urophylla* leaves, changed daily until the end of the experiment. A total of 40% of the caterpillars died before the pre-pupae stage, 40% reached the pupae stage and died due to inadequate adult formation and 20% generated moths, but none adult parasitoid emerged from the caterpillars. All pupae of *O. vesulia* were parasitized and showed emergence of parasitoids. The parasitism of *O. vesulia* caterpillars and pupae by T. *howardi* shows the potential of this natural enemy for the integrated management of this defoliator pest in eucalyptus plantations.

Keywords: biological control, caterpillar and pupae parasitoid, *Eucalyptus*.

Tetrastichus howardi (Hymenoptera: Eulophidae): primeiro relato de parasitismo em *Oxydia vesulia* (Lepidoptera: Geometridae)

Resumo

A adaptação de espécies nativas de lepidópteros a plantios de eucalipto reduz a produtividade dessa cultura no Brasil. *Oxydia vesulia* Cramer (Lepidoptera: Geometridae) é praga-secundária, frequentemente, relatada em plantios de eucalipto com surtos populacionais e danos econômicos. Métodos de controle biológico, dessa praga, podem incluir o uso do endoparasitoide exótico de pupas *Tetrastichus howardi* Olliff (Hymenoptera: Eulophidae) relatado como eficiente para o controle de lepidópteros-pragas. O parasitismo de lagartas e pupas de *O. vesulia* por *T. howardi* foi avaliado em condições controladas (25 ± 1º C, 60 ± 20% umidade e 12h fotoperíodo). Cada lagarta ou pupa de *O. vesulia* foi colocada individualmente em tubos de fundo chato com 10 e 15 fêmeas de *T. howardi* por 48h, respectivamente e, após esse período, os parasitoides foram removidos, as lagartas alimentadas e as pupas mantidas até a emergência do parasitoide ou formação de pupas ou de adultos dessa praga. As lagartas de quarto ínstar de *O. vesulia*, após o período de parasitismo, foram mantidas em potes com folhas de *Eucalyptus urophylla* trocadas, diariamente, até o fim do experimento. Um total de 40% das lagartas de *O. vesulia* morreu antes da fase de pré-pupa, 40% atingiram o estágio de pupa e morreram devido à má formação ou problemas e 20% originaram mariposas, porém nenhum parasitoide

adulto emergiu das lagartas. Todas as pupas de *O. vesulia* foram parasitadas e tiveram emergência de parasitoides. O parasitismo de lagartas e pupas de *O. vesulia* por *T. howardi* mostra o potencial desse inimigo natural para o manejo integrado desse desfolhador em cultivos de eucalipto.

Palavras-chave: controle biológico, parasitoide de pupas e de lagartas, *Eucalyptus*.

1. Introduction

Oxydia vesulia Cramer (Lepidoptera: Geometridae) is a native moth observed in population outbreaks, damaging eucalyptus plantations in Brazil (Campos and Cure, 1993; Santos et al., 2002; Oliveira et al., 2003; Zanuncio et al., 2018). This insect has six or seven instars lasting around 27 days for males and 31 for females, and the leaf consumption by its caterpillars increase considerably from the fourth instar (Zanuncio et al., 1993; Espindola and Gonçalves, 2000). *Oxydia vesulia* female moths live an average of 12 days and can lay 1,970 eggs in up to nine postures (Espíndola and Gonçalves, 2000). This specie is considered a secondary pest and its populations tend to increase with the period of cultivation of eucalyptus in the same area (Zanuncio et al., 2018).

Biological control, including bioinsecticides based on the entomopathogenic bacterium *Bacillus thuringiensis* Berliner and releases of parasitoids and predators, should be preferred in the management of lepidopteran pests in eucalyptus plantations (Zanuncio et al., 1992; Barbosa et al., 2016).

Tetrastichus howardi Olliff (Hymenoptera: Eulophidae) is an exotic pupae endoparasitoid with primary or hyperparasitoid behavior associated with lepidopteran pests and have been used to effectively control many lepidopteran species in important crops (Baitha et al., 2004; La Salle; Polaszek, 2007). The first report of this parasitoid in Brazil was in *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) pupae obtained from corn plants (Cruz et al., 2011) and *D. saccharalis* pupae from sugar cane (Vargas et al., 2011). This natural enemy has parasitized caterpillars and pupae of *D. saccharalis* (Pereira et al., 2015) and species of the families Crambidae, Noctuidae, Plutellidae and Sphyngidae (Moore and Kfir, 1995; Cruz et al., 2011; Vargas et al., 2011; Barbosa et al., 2015), but it has not been reported for the Geometridae family, which contains some of major eucalyptus primary pests (Zanuncio et al., 1994).

The objective was to evaluate *T. howardi* parasitism on *O. vesulia* caterpillars and pupae.

2. Material and Methods

The experiments were conducted at the Laboratory of Biological Control of Forest Pests (LCBPF) of the São Paulo State University (UNESP), School of Agriculture, in Botucatu, São Paulo state, Brazil. The caterpillars of *O. vesulia* were collected on field during an outbreak in Minas Gerais State in 2018 and transported to São Paulo State University where they were identified. The rearing of *O. vesulia* caterpillars were kept in cages in a room with a controlled environment (25 ± 1 ºC, 60 ± 20% humidity and 12:12 h L:D), with *Eucalyptus urophylla* leaves changed daily, up to the pupae stage of this insect. The procedure for rearing *T. howardi* were to keep them in sealed glass tubes, fed with pure honey and multiplied in *D. saccharalis* pupae up to 48 hours old. The parasitized pupae were transferred to 2-liter plastic pots after a period of exposure of 72 hours, which were maintained under a controlled-environment room (25 ± 1 ºC, 60 ± 20% humidity and 12:12 h L:D).

Ten fourth-instar caterpillars of *O. vesulia* were maintained per 1-liter plastic pot, receiving daily *Eucalyptus urophylla* leaves. The parasitism was allowed for 48 hours with 100 individuals of *T. howardi* (24 h old) per plastic pot in the proportion of 10 parasitoid females/*O. vesulia* caterpillar. Pupae of *O. vesulia* (weight between 482-773 mg) at 48 h of age were individualized in flat-bottom tubes (8.5 cm long × 2.5 cm in diameter) with 15 *T. howardi* females (48 h old and fed with honey drops) sealed with "voil" fabric. After this period, the parasitoids were removed and the *O. vesulia* caterpillars and pupae remained under the same controlled conditions. The caterpillar keep eating *E. urophylla* leaves and compared with control caterpillars to observe their behavior. The emergence of the progeny, length cycle (egg-adult), number of parasitoids emerged per *O. vesulia* caterpillar or pupae (progeny) and the sex ratio of *T. howardi* were evaluated.

3. Results

Tetrastichus howardi parasitized and killed 40% of the *O. vesulia* caterpillars, but none adult parasitoid emerged from them. It parasitized 100% of the *O. vesulia* pupae, emerged from and killed 100% of the pupae of this Lepidoptera (Figure 1). It is life cycle (egg-adult) was 16.4 ± 0.25 days. The progeny of *T. howardi* was 321 ± 28.7 parasitoids/pupa of *O. vesulia* with a sex ratio of 0.807 ± 0.022.

The color, body consistency and behavior varied, and the consumption of eucalypt leaves was lower for the *O. vesulia* caterpillars which were parasitized by *T. howardi* females, while the control caterpillars kept feeding until the pupae stage. A total of 40% of caterpillars died before the pre-pupa stage, 40% reached the pupa stage and died due to inadequate adult formation and 20% generated moths (Figure 2).

The emergence of *T. howardi* from 100% of the *O. vesulia* pupae demonstrates that this host is adequate for the development of this parasitoid.

The results report for the first time in controlled conditions the capacity of *T. howardi* to parasitize pupae of *O. vesulia*.

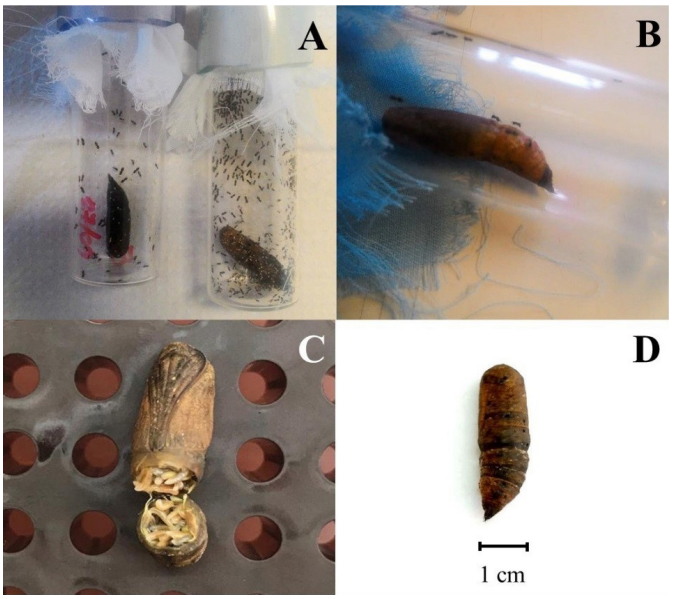

Figure 1. Adults of *Tetrastichus howardi* (Hymenoptera: Eulophidae) emerged from the *Oxydia vesulia* (Lepidoptera: Geometridae) pupae (A), *Tetrastichus howardi* females parasitizing *Oxydia vesulia* pupae (B), larvae of *Tetrastichus howardi* in development (C) and *Oxydia vesulia* pupae after the parasitoid emergence (D).

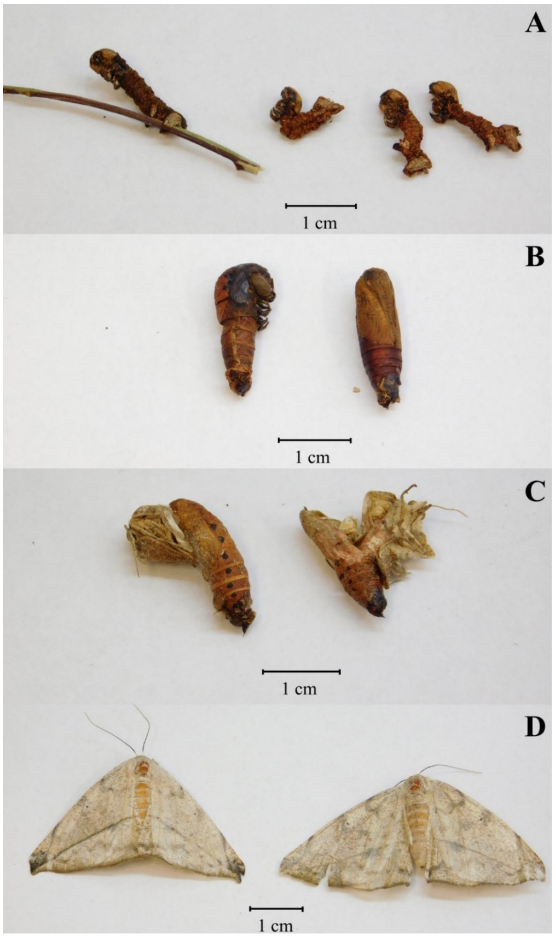

Figure 2. *Oxydia vesulia* (Lepidoptera: Geometridae) caterpillars that did not reach the pupae stage (A), malformation of pupae and adults (B and C) and adults that survived the *Tetrastichus howardi* (Hymenoptera: Eulophidae) parasitism (D).

4. Discussion

A parasitism rate of 40% by *T. howardi* in *O. vesulia* larvae and the 2% of parasitism by *T. howardi* in *D. saccharalis* larvae suggest that this life stage may poses a stronger immune response against this parasitoid (Pereira et al., 2015).

Changes in the color and consistency of the *O. vesulia* caterpillar body and the reduction of consumption of *E. urophylla* leaves after parasitism by *T. howardi* might be due to fluids injected during oviposition by this parasitoid, suppressing the host immune system (Asgari and Rivers 2011; Colinet et al. 2013) and causing its death. *Oxydia vesulia* adults, originated from caterpillars of this insect submitted to *T. howardi*, indicate that they may have been favored by frequent feeding, increasing the immune response against parasitism.

The emergence of parasitoids from all *O. vesulia* pupae represents the first report of this activity on laboratory, and thus includes another host species for *T. howardi*, suppressing the cellular defense of this Lepidoptera and consuming its nutritional resources for the development of its progeny, as reported for *Palmistichus elaeisis* Delvare and LaSalle and *T. diatraeae* (Hymenoptera: Eulophidae) in the host *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) (Andrade et al., 2010).

The egg-adult cycle of *T. howardi* in pupae of *O. vesulia* was shorter when compared to *D. saccharalis*, around 20 days less (Pereira et al., 2015), which may be associated with nutritional availability, size, immune response or pupae stage period of the host (Favero et al., 2013, Ribeiro et al., 2019).

The progeny of *T. howardi* in *O. vesulia* pupae was lower than that of this parasitoid in *Erinnyis ello* Linnaeus (Lepidoptera: Sphyngidae) pupae. However, *O. vesulia* is an adequate host for *T. howardi*, because the pupae of this host, with medium weight of 522.9 mg, generated 321 ± 28.7 parasitoids (one parasitoid offspring produced per 1.63 mg of host pupa), while the mean weight of *E. ello* pupae was 3602 mg with a progeny of 466 parasitoids/pupa (Barbosa et al., 2015) with parasitoid offspring consuming 7.73 mg weight of host pupae. Therefore *O. vesulia* pupae produced 4.5 times more parasitoid offspring per host tissue weight compared to *E. ello*, demonstrating that the progeny of this parasitoid varies with the host species and biomass (Favero et al., 2013). This is similar to that reported for *Trichospilus diatraeae* Cherian and Margabandhu (Hymenoptera: Eulophidae) and *T. howardi* on pupae of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), with 373 mg generating 669.3 *T. howardi* parasitoids and 816.11 *T. diatraeae* parasitoids, each consuming 0.56 mg and 0.46 mg of the host per individual produced, respectively. *Tetrastichus howardi* offspring consumed around 1.2 times more host mass than *T. diatraeae*, with a difference of 146.8 parasitoids produced from the host *H. armigera*. This variation in the number of individuals per host pupae for these endoparasitoids is probably due to their size, with *T. howardi* larger than *T. diatraeae* (Oliveira et al., 2016). The parasitoid *T. diatraeae* produced 868 parasitoids per pupae of the host *Spodoptera cosmioides* Walker (Lepidoptera: Noctuidae) (Zaché et al., 2012). The sex ratio of *T. howardi*, with a predominance of females, may increase the efficiency of this parasitoid in the biological control, since they are responsible for parasitism and host control (Cañete and Foerster 2003; Zacarin et al., 2004).

The species *O. vesulia* has potential to be used as an alternative host to mass rearing *T. howardi*, with a production of 321 ± 28.7 parasitoids/host pupa. The parasitism of *O. vesulia* caterpillars and pupae by *T. howardi* shows the potential of this natural enemy for the integrated management of this defoliator pest in eucalyptus plantations.

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