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

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(With 2 figures)

**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*.
adulto emergiu das lagartas. Todas as pupas de _O. vesulia_ foram parasitadas e tiveram emergência de parasitóides. 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 (Espindola and Gonçalves, 2000). This species 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_ pupa 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 Sphingidae (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_.

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**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 pose 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 (Asgar and Rivers 2011; Colinet et al. 2013) and causing its death. *Oxycia 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 *Erinnys ello* Linnaeus (Lepidoptera: Sphingidae) 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 *Trichosprotus 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 pupa 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) (Zachê 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; Zacarín 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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