Foraging Activity of Palmistichus elaeisis (Hymenoptera: Eulophidae) at Various Densities on Pupae of the Eucalyptus Defoliator Thyrinteina arnobia (Lepidoptera: Geometridae)

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Foraging activity of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) at various densities on pupae of the eucalyptus defoliator *Thyrinteina arnobia* (Lepidoptera: Geometridae)

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Abstract

Parasitoids are the main component of biological control of Lepidoptera defoliators in forested areas, and the densities of host insects can affect the efficiency of these natural enemies. The aim of this work was to study the biology and parasitism of *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) at various densities of females on individual pupae of *Thyrinteina arnobia* Stoll (Lepidoptera: Geometridae), the major Lepidoptera pest in eucalyptus (Myrtaceae) plantations in Brazil. *Thyrinteina arnobia* pupae were exposed to *P. elaeisis* females at various parasitoid-to-host ratios: (1:1, 3:1, 6:1, 9:1, 12:1, 15:1, 18:1, and 21:1), with 12 replications. The parasitism (81.8%) and emergence (100%) rates of *P. elaeisis* on *T. arnobia* pupae were highest with 15 ovipositing females per host. The life cycle (egg to adult) of *P. elaeisis* was shortest (19.8 ± 0.5 d) at a ratio of 21:1. The largest production of *P. elaeisis* per *T. arnobia* pupa was obtained at the ratios of 15:1 (298.4 ± 5.0) and 18:1 (287.4 ± 4.3). The female sex ratio of parasitoid offspring was highest (0.97 ± 0.01) with a parasitoid-to-host ratio of 12:1. The density of 15 *P. elaeisis* females per *T. arnobia* pupa was the most appropriate one for providing high parasitism (81.8%), emergence (100%), and progeny (298.4 ± 5.0) of this parasitoid.

Key Words: biological control; looper of eucalyptus; parasitism; pupal parasitoid

Resumo

Parasitoides são os principais componentes de controle biológico de lagartas desfolhadoras em áreas florestais e o número de fêmeas por hospedeiro pode afetar a eficiência desses inimigos naturais. O objetivo deste trabalho foi estudar a biologia e o parasitismo de *Palmistichus elaeisis* Delvare e LaSalle (Hymenoptera: Eulophidae) em diferentes densidades de fêmeas desse parasitoide por pupa de *Thyrinteina arnobia* Stoll (Lepidoptera: Geometridae), principal lepidóptero-praga em plantações de eucalipto no Brasil. Pupas de *T. arnobia* foram expostas ao parasitismo por fêmeas *P. elaeisis* nas seguinte proporções de parasitoides/hospedeiro: 1:1, 3:1, 6:1, 9:1, 12:1, 15:1, 18:1 e 21 :1, com 12 repetições. O parasitismo (81,8%) e emergência (100%) de *P. elaeisis* em pupas de *T. arnobia* foram maiores com 15:1 parasitoides/hospedeiro. O ciclo de vida (ovo-adulto) de *P. elaeisis* foi mais curto (19,8 ± 0,5 dias) em uma proporção de 21:1. A maior produção de *P. elaeisis* por pupa de *T. arnobi*a foram obtidas nas densidades de 15:1 (298,4 ± 5,0) e 18:1 (287,4 ± 4,3), respectivamente. A razão sexual do parasitoide foi maior (0,97 ± 0,01), com 12:1. A densidade de 15 *P. elaeisis* fêmeas por pupa de *T. arnobia* foi o mais adequado por apresentar maior parasitismo (81,8%), emergência (100%) e progênie (298,4 ± 5,0) do parasitoide.

Palavras Chave: controle biológico; lagarta do eucalipto; parasitismo; parasitoide de pupa

Parasitoids are important natural enemies for the equilibrium of agroecosystems because they have high species and hosts diversity (Oliveira et al. 2000; Pratissoli et al. 2005). *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) parasitizes and develops in Lepidoptera pupae of the families Arctiidae (Pereira et al. 2008), Bombycidae (Pereira et al. 2010), Crambidae (Bittencourt & Berti-Filho 2004; Chichera et al. 2012), Lymantriidae (Tavares et al. 2011; Zaché et al. 2012), Muscidae (Zaché et al. 2013), Noctuidae (Bittencourt & Berti-Filho 2004; Andrade et al. 2010; Pereira et al. 2013), Nymphalidae (Tavares et al. 2013a), Notodontidae (Zanuncio et al. 2015), Papilionidae (Tavares et al. 2013b), and Saturniidae (Pereira et al. 2008); it also develops in Coleoptera pupae of the family Tenebrionidae (Zanuncio et al. 2008). Furthermore, this parasitoid was recorded in pupae of *Thyrinteina arnobia* Stoll and *Thyrinteina leucoceraea* Rindge (Lepidoptera: Geometridae), which characterizes it as a potential biological control agent in *Eucalyptus* (Myrtaceae) plantations (Pereira et al. 2008, 2011).

Thyrinteina arnobia is the major lepidopteran pest in eucalypt plantations with outbreaks in several regions of Brazil (Oliveira et al. 2005) and causes significant yield losses (Bragança et al. 1998). This pest has been controlled with insecticides that have the potential to cause mortality of natural enemies and contaminate the environment (Oliveira et

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al. 2011). Hence, there is need to develop lower-impact control methods for this pest (Pereira et al. 2008).

Natural enemies can reduce outbreaks of defoliator Lepidoptera in eucalypt plantations (Zanuncio et al. 1993; Zanuncio et al. 1998; Guedes et al. 2000; Zanetti et al. 2003), and the use of *P. elaeisis* in biological control programs depends on the development of mass rearing methods for this natural enemy (Pereira et al. 2011; Menezes et al. 2012). The density of female parasitoids per host can affect parasitism (Sampaio et al. 2001), progeny sex ratio (Chong & Oetting 2006, 2007), life cycle length, and the longevity of the offspring (Pereira et al. 2010).

Palmistichus elaeisis is a gregarious parasitoid, and the optimal proportion of its females per host pupa can increase efficiency and production of this natural enemy. The aim of this work was to study the biology and parasitism of *P. elaeisis* in *T. arnobia* pupae with various densities of females of this parasitoid.

Materials and Methods

The experiment was conducted at the Laboratory of Biological Control of Insects of the Animal Biology Department/Instituto de Biotecnologia Aplicada á Agropecuária (BIOAGRO) of the Universidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais, Brazil.

REARING OF THE INSECTS

Rearing of the Host

Eggs of *T. arnobia* were obtained from the laboratory rearing stock of the Laboratory of Biological Control of Insects of the UFV. Larvae, soon after hatching, were placed in organza cloth bags (0.70 × 0.40 cm) containing eucalypt branches (from 6-mo-old plants of *Eucalyptus cloeziana* F. Muell.) and provided with fresh branches every 3 d, until they reached the pupal stage. Pupae were removed from these bags, sexed, separated in couples, and placed in plastic pots (500 mL) with a hole in the center of the plastic lid sealed with an organza mesh screen. Paper strips were fixed to the inside of the lids for oviposition, and pots were kept in a room at 25 ± 2 °C, 70 ± 10% RH, and a 14:10 h L:D photoperiod (adapted from Oliveira et al. 2010).

Rearing of the Parasitoid

Adults of *P. elaeisis* were kept in glass tubes (14 cm height × 2.5 cm diameter) sealed with cotton and containing a drop of honey to feed the parasitoids. *Bombyx mori* L. (Lepidoptera: Bombycidae) pupae (obtained from the company Fiação de Seda Bratac S/A, Brazil), 48 to 72 h old, were exposed to parasitism by *P. elaeisis* for 24 h at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod to maintain a colony of the parasitoid (Pereira et al. 2008).

EXPERIMENT

Thyrinteina arnobia pupae that were 24 to 48 h old were individually exposed to 72- to 96-h-old *P. elaeisis* females (Andrade et al. 2012) in glass tubes (14 cm height × 2.5 cm diameter) and placed in a chamber maintained at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod, at density treatments of 1, 3, 6, 9, 12, 15, 18, and 21 parasitoids per host. After 48 h, the *P. elaeisis* females were removed from the tubes. The duration of the life cycle (egg to adult), percentage of parasitism [(number of pupae of *T. arnobia* with emergence of parasitoid + pupae without adult emergence of *T. arnobia*) ÷ (total number of pupae) × 100] (discounting the natural mortality of the host) (Abbott 1925), emergence [(number of pupae of *T. arnobia* with adult emergence of parasitoids)÷(number of parasitized pupae) × 100], progeny (adults of *P. elaeisis* emerged per *T. arnobia* pupa), body length (mm), width of the head capsule (mm) (size measurements were performed with an ocular micrometer attached to a stereomicroscope), and sex ratio (number of females per number of adults) were obtained. The sex of adult parasitoids was determined by the morphological characteristics of their antennae and abdomen. Compared with males, females are usually larger in size, have a larger abdomen, and have no pigmentation between the thorax and abdomen (LaSalle 1994). The treatments were set up with 12 replications in a completely randomized design.

DATA ANALYSES

The data on duration of life cycle and the number of parasitoids emerged per *T. arnobia* pupa were subjected to analysis of variance and, when significant at 5% probability, to a regression analysis. The parasitism and emergence rates of *P. elaeisis* were subjected to a binomial distribution analysis ($P \leq 0.05$). The data of sex ratio, body length (mm), and width of the head capsule (mm) of *P. elaeisis* females and males were subjected to analysis of variance and, when significant at 5% probability, to the Scott–Knott test.

Results

The percentage of parasitism was influenced by the densities of *P. elaeisis* per *T. arnobia* pupa and ranged from 58.3 to 81.8% (Fig. 1). The percentage of parasitoid emergence was 28.6, 42.9, 62.5, 75.0, 87.5, 100, 89.9, and 50.0% at the densities of 1, 3, 6, 9, 12, 15, 18, and 21 parasitoids per host, respectively (Fig. 1).

The duration of the life cycle (egg to adult) decreased with increasing density of *P. elaeisis* females from 25 to 18 d ($\hat{y} = 24.518 - 1.0024x + 0.09066327x^2 - 0.00254969x^3$; $F = 14.2446$; $P = 0.0001$; $R^2 = 0.7102$) (Fig. 2A). The density of *P. elaeisis* females per *T. arnobia* pupa influenced the progeny of this parasitoid ($\hat{y} = 133.217 + 140.043 / (1 + \exp(-(x - 13.8951) / 0.0248825))$; $F = 4.1382$; $P = 0.0117$; $R^2 = 0.85$), with a minimum of 30 and a maximum of 724 *P. elaeisis* offspring produced at the densities of 1 and 15 parasitoids per host pupa, respectively (Fig.

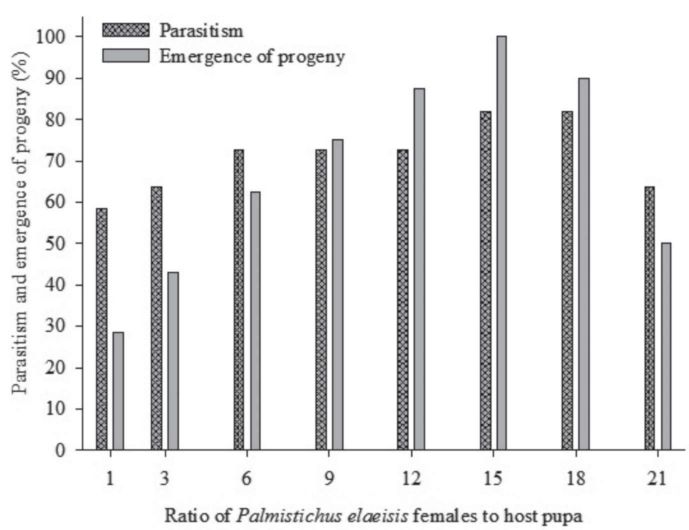

Fig. 1. Percentage of pupae parasitized and percentage of emergence of *Palmistichus elaeisis* with a density of 1, 3, 6, 9, 12, 15, 18, or 21 ovipositing females per *Thyrinteina arnobia* pupa at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod. Statistical significance: parasitism, $P = 0.3770$; emergence, $P = 0.034$.

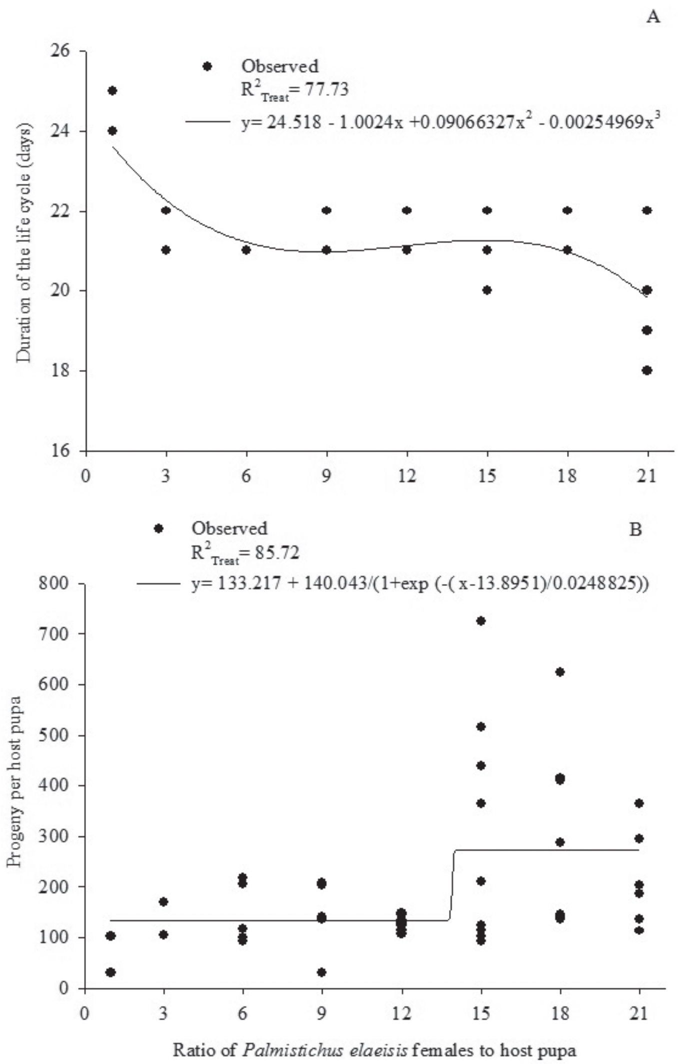

Fig. 2. (A) Duration of life cycle (egg to adult) and **(B)** numbers of *Palmistichus elaeisis* progeny with a density of 1, 3, 6, 9, 12, 15, 18, or 21 ovipositing females per *Thyrinteina arnobia* pupa at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod.

2B). The sex ratio of *P. elaeisis* emerged from *T. arnobia* pupae ranged from 0.46 ± 0.45 to 0.97 ± 0.01 (number of females per total number of adults) without differences between the densities of 3 to 21 parasitoids per host (*P* > 0.05) (Table 1).

The body length (mm) of emerged *P. elaeisis* females ranged from 2.02 ± 0.01 to 2.28 ± 0.02 and that of males from 1.71 ± 0.02 to 1.78 ± 0.01, with no differences between the different densities of this parasitoid (*P* > 0.05) (Table 1). The width of the head capsule (mm) of emerged *P. elaeisis* females did not differ between treatments (*P* > 0.05), whereas that of males ranged from 0.47 ± 0.01 to 0.51 ± 0.01, with differences between treatments (P ≤ 0.05).

Discussion

The density of *P. elaeisis* females per *T. arnobia* pupa affected the percentage of parasitism, percentage of emergence, and viability of the offspring of this parasitoid. *Thyrinteina arnobia* pupae may have effective defense mechanisms against *P. elaeisis* because the emergence of these parasitoids was low at the densities of 1 and 3 parasitoids per host. Another hypothesis is that the size of this host requires a larger number of parasitoids to neutralize its immune system (Smilanich et al. 2009; Pereira et al. 2010; Altoé et al. 2012; Hood et al. 2012).

The reproduction of *P. elaeisis* was different from that of the parasitoid *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) in pupae of *Anticarsia gemmatalis* Hübner, *Heliothis virescens* (F.), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) exposed to one or more females of this parasitoid (Paron & Berti-Filho 2000; Favero et al. 2013). The variability in reproductive success may be related to inadequate proportions of female parasitoids per host, which reduced oviposition and toxin injections necessary to reduce the immune response of the host (Andrade et al. 2010; Cusumano et al. 2010; Harvey et al. 2013). In contrast, the parasitism and emergence ratios of *P. elaeisis* from *B. mori* pupae were similar with different densities of this parasitoid, suggesting that *B. mori* did not present nutritional or physiological barriers for the development of *P. elaeisis* (Pereira et al. 2010).

The short life cycle (egg to adult) of *P. elaeisis* in *T. arnobia* pupae can be explained by competition among parasitoid larvae and by physical or physiological suppression by the host, as reported for this parasitoid in *B. mori* pupae (Pereira et al., 2010). This pattern has also been reported with different densities of *T. diatraeae* in pupae of *T. molitor* (Favero et al. 2013) and *D. saccharalis* (Rodrigues et al. 2013). Besides the parasitoid density, the host species can affect the development period of parasitoids. This effect can be attributed to differences in nutritional quality and size of pupae, because the host is the food source and shelter for immatures of these natural enemies and, if inappropriate, impedes their development (Pastori et al. 2012).

Table 1. Sex ratio (number of females / [number of females + males]), body size, and width of the head capsule of *Palmistichus elaeisis* (mean ± standard error) progeny emerged with a density of 1, 3, 6, 9, 12, 15, 18, or 21 ovipositing females per *Thyrinteina arnobia* pupa at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod.

				Body size (mm)		Head capsule (mm)	
Density	*n*	Sex ratio (females per progeny)	*n*	Female	Male	Female	Male
1	2	0.46 ± 0.45b	12	2.23 ± 0.02a	1.78 ± 0.01a	0.54 ± 0.01a	0.49 ± 0.01b
3	3	0.92 ± 0.05a	12	2.02 ± 0.05c	1.71 ± 0.02b	0.53 ± 0.01a	0.47 ± 0.01b
6	5	0.94 ± 0.01a	12	2.02 ± 0.01c	1.82 ± 0.02a	0.53 ± 0.01a	0.47 ± 0.01b
9	6	0.94 ± 0.02a	12	2.28 ± 0.02a	1.82 ± 0.02a	0.54 ± 0.01a	0.50 ± 0.01a
12	7	0.97 ± 0.01a	12	2.11 ± 0.04b	1.82 ± 0.01a	0.54 ± 0.01a	0.48 ± 0.01b
15	9	0.96 ± 0.01a	12	2.26 ± 0.05a	1.82 ± 0.01a	0.55 ± 0.01a	0.50 ± 0.01a
18	8	0.95 ± 0.01a	12	2.28 ± 0.02a	1.80 ± 0.01a	0.54 ± 0.01a	0.51 ± 0.01a
21	6	0.94 ± 0.01a	12	2.27 ± 0.03a	1.79 ± 0.02a	0.54 ± 0.01a	0.50 ± 0.01a

Means followed by the same lowercase letter per column do not differ by the Scott–Knott test at 5% probability.

The greatest number of *P. elaeisis* individuals produced per *T. arnobia* pupa at the densities of 15 and 18 parasitoids per host shows that 15 females of this natural enemy are sufficient to counteract defense mechanisms of this host. On the other hand, densities of 1, 3, 6, 9, and 21 females per *T. arnobia* pupa reduced the number of progeny of this parasitoid. This finding indicates that inadequate proportions of females per host result in reduced oviposition or increased competition and death of immature parasitoids (Pereira et al. 2010). The density of the parasitoid females per host pupa can reduce fertility and efficiency of mass rearing, primarily due to mortality of immatures within the pupae (Harvey et al. 2013) and competition between adults during oviposition (Pereira et al. 2013).

The high sex ratio (i.e., the predominance of females) of *P. elaeisis* offspring in *T. arnobia* pupae is important because parasitoid females are responsible for the parasitism and progeny production (Rodrigues et al. 2013) as reported for this parasitoid with *A. gemmatalis*, *B. mori*, and *D. saccharalis* pupae (Pereira et al. 2010, 2013; Chichera et al. 2012). Moreover, the sex ratio of *P. elaeisis* in *T. arnobia* pupae was higher than in *Sarsina violascens* (Herrich-Schaeffer) (Lepidoptera: Lymantriidae), likely due to differences in nutritional value, physiological barriers, and defense capacity of *S. violascens* pupae (Zaché et al. 2012). A decrease in sex ratio as a function of host species may reduce the efficacy of *P. elaeisis* parasitism, because parasitism is maximized when a large number of females is produced (Pereira et al. 2010).

The size range of *P. elaeisis* adults with different densities of parasitoid females can be explained by the limited resources of the host pupa as the number of larvae developing inside the host increases (Tian et al. 2008; Harvey et al. 2013). A reduced body size may reduce the efficiency of biological control, because body size is positively correlated with indicators of quality of parasitoids such as longevity, fecundity, progeny emerged, and sex ratio (Pereira et al. 2010).

The parasitism and development of *P. elaeisis*, independent of the density of its females, on *T. arnobia* pupae demonstrate that this natural enemy has potential to reduce outbreaks and possible damage caused by this insect defoliator in eucalyptus plantations. Successful rearing of this parasitoid will depend on the density of ovipositing females per host pupa.

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