

Do parasitoid density and host age affect the parasitism of *Palmistichus elaeisis* (Hymenoptera: Eulophidae)?

*Densidade de parasitoides e idade do hospedeiro afetam o parasitismo de *Palmistichus elaeisis* (Hymenoptera: Eulophidae)?*

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ABSTRACT: The incidence of lepidopteran defoliants is one of the environmental factors that regulate the productivity of cultivated forests. The parasitoid *Palmistichus elaeisis* (Hymenoptera: Eulophidae) has significant importance for its efficiency in the parasitism of pupae of these Lepidoptera. The objective of this study was to evaluate the development and reproduction of *P. elaeisis* in different densities of pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) at different ages. Pupae of 24, 48, 72 and 96 hours were exposed at densities of 1:1, 4:1, 10:1, 19:1, 31:1 and 46:1 parasitoids/host, respectively. The parasitoids remained in contact with the pupae for 72 hours in 500 mL plastic pots, conditioned in an air-conditioned room, with temperature of $25 \pm 2^\circ\text{C}$, relative humidity of $70 \pm 10\%$ and photoperiod of 12 hours. It was concluded that the density of 10:1 presented great results of parasitism, and further increase of density was not needed. Pupae of 24 and 48 hours had a higher percentage of emergence. Biological variables were affected neither by parasitoid densities nor by host age.

KEYWORDS: mass rearing; biological control; natural enemy; parasitism.

RESUMO: A incidência de lepidópteros desfolhadores é um dos fatores ambientais que regulam a produtividade das florestas plantadas. O endoparasitoide *Palmistichus elaeisis* (Hymenoptera: Eulophidae) destaca-se pela eficiência no parasitismo e pode ser promissor no controle de Lepidoptera pragas em diversas culturas com importância econômica. Assim, avaliaram-se o desenvolvimento e a reprodução de *P. elaeisis* em pupas de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) com idades distintas submetidas a diferentes densidades do parasitoide. Pupas de 24, 48, 72 e 96 horas foram expostas às densidades de 1:1, 4:1, 10:1, 19:1, 31:1 e 46:1 parasitoides/hospedeiro. Os parasitoides permaneceram em contato com as pupas por 72 horas em potes plásticos de 500 mL, acondicionados em sala climatizada com temperatura $25 \pm 2^\circ\text{C}$, umidade relativa de $70 \pm 10\%$ e fotoperíodo de 12 horas. A densidade 10:1 apresentou resultados adequados de parasitismo. Pupas de 24 e 48 horas de idade apresentaram maior porcentagem de emergência. O ciclo de vida, o número de indivíduos emergidos, a razão sexual, a longevidade das fêmeas e o comprimento da cápsula cefálica e da tibia de *P. elaeisis* não foram afetados pelas densidades do parasitoide nem pela idade do hospedeiro.

PALAVRAS-CHAVE: criação massal; controle biológico; inimigo natural; parasitismo.

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INTRODUCTION

The incidence of pest insects is one of the environmental factors that regulate the productivity of cultivated forests (ZANUNCIO et al., 2009). The extension of planting, tree height and behavior of some insects reduce the efficiency of pesticide application (BITTENCOURT et al., 2004; ZANUNCIO et al., 2010). Thus, alternative methods to chemical control favor the conservation and sustainable use of natural resources, and among them, the use of natural enemies presents adequate characteristics to integrate pest management (BARBOSA et al., 2008).

The endoparasitoid *Palmistichus elaeisis* (DELVARE; LASALLE, 1993) (Hymenoptera: Eulophidae) is widely known for its efficiency in the parasitism of lepidopteran and coleopteran pupae (PEREIRA et al., 2008; ALVARENGA SOARES et al., 2009). This parasitoid can reproduce in alternative hosts such as *Bombyx mori* (Linnaeus, 1758) (Lepidoptera: Bombycidae), *Anticarsia gemmatalis* (Hubner, 1818), *Alabama argillacea* (Hubner, 1823) (Lepidoptera: Noctuidae) and *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) (PEREIRA et al., 2008; 2009; ZANUNCIO et al., 2008). This makes *P. elaeisis* an efficient alternative for multiplication and field release aiming the control of forests defoliator insects.

P. elaeisis also reproduces in pupae of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) (BITTENCOURT; BERTI FILHO, 1999), and caterpillars of this species can be reared in artificial diet. It makes this lepidopteran an ideal alternative host for the breeding of *P. elaeisis* in laboratory, since it is not necessary to use a natural diet for its feeding (SANTOS-CIVIDANES et al., 1996; OLIVEIRA et al., 2004).

The parasitoid density and age of the hosts pupae can affect parasitism capacity and reflect directly on parasitoid quality (THOMAZINI; BERTI FILHO, 2001; MATOS NETO et al., 2004), changing characteristics such as body size (BITTENCOURT; BERTI FILHO, 1999), longevity (SILVA-TORRES; MATTHEWS, 2003) and cycle length (BITTENCOURT et al., 2004). This shows the demand to improve the mass creation of parasitoids, aiming to know, especially, the ideal density and age in relation to the host to maximize their production (ZAKI et al., 1994; SAGARRA et al., 2000).

The objective of this study was to evaluate the development and reproduction of *P. elaeisis* in pupae of *S. frugiperda* at different densities and parasitoid/host ages.

MATERIALS AND METHODS

The study has been conducted in the Biological Control Laboratory (*Laboratório de Controle Biológico* — LCB) of the Universidade Federal dos Vales do Jequitinhonha e Mucuri

(UFVJM), in Diamantina, Minas Gerais, Brazil, in an air conditioned room, with temperature of $25 \pm 2^\circ\text{C}$, relative humidity of $70 \pm 10\%$ and photoperiod of 12 hours.

The parasitoid *P. elaeisis* was obtained from the LCB stock, where it was kept in 500-mL plastic pots with newly formed *T. molitor* pupae as an alternative host and honey droplets for adult feeding.

The *S. frugiperda* lepidopteran was grown in 100-mL plastic pots (caterpillars) and cylindrical polyvinyl chloride (PVC) cages with diameter of 20 cm and height of 50 cm (adults), in an air-conditioned room under the above-described conditions. The caterpillars were fed with artificial diet (PANTOJA et al., 1987) and adults with a solution containing water, corn glucose, sugar and ascorbic acid (OLIVEIRA et al., 1990).

The experimental design was completely randomized, in a 6×4 factorial scheme, with six densities — 1:1, 4:1, 10:1, 19:1, 31:1, 46:1 parasitoid/host —, four different ages of the host (24, 48, 72 and 96 hours) and ten replicates. Two hundred and forty pupae of *S. frugiperda* were weighed (260.32 ± 11.42 mg), individually conditioned in 250-mL plastic pots and exposed to the parasitism of *P. elaeisis* females at different densities. The endoparasitoid had no previous experience of oviposition and remained with the host for 72 hours, being fed with a drop of honey.

The percentage of parasitism was observed by discounting the natural mortality of the host (ABBOTT, 1925). Additionally, the percentage of emergence, duration of the life cycle (egg-adult), number of emerged individuals and sex ratio (No ♀ / No ♂ + No ♀) were observed.

Longevity was assessed using one female specimen from each replicate. They were conditioned in 14×2.2 cm test tubes, capped with cotton and fed with a drop of honey. After death, the insects were submitted to analysis of the morphometric variables. The size of the cephalic capsule at the median height of the eyes and the posterior tibial length were measured with an Optika OPTIKAM B5 camera coupled to a stereomicroscope with Optika Vision Lite 2.1 software.

The data were submitted to analysis of variance (ANOVA), and, when significant, the means were compared by the Tukey test ($p \leq 0.05$) or the Kruskal-Wallis test ($p \leq 0.05$).

RESULTS

The percentage of parasitism of *P. elaeisis* in *S. frugiperda* ($p = 0.79$; $F = 0.3446$; $gl = 3$) was not influenced by the pupae ages of this host (Fig. 1A). However, parasitism was higher in the proportions of 10:1, 19:1, 31:1 and 46:1 ($p < 0.05$, $F = 10,123$, $gl = 5$) when compared to 1:1 (Fig. 1B).

The adult emergence percentage of *P. elaeisis* was affected by *S. frugiperda* pupae age ($p < 0.01$; $F = 5.3211$; $gl = 3$) (Fig. 2A). However, it was not affected by the density of this parasitoid ($p = 0.5142$; $F = 0.38$; $gl = 5$) (Fig. 2B).

The life cycle of *P. elaeisis* (egg-adult) was affected neither by parasitoid densities ($p = 0.5236$; $F = 0.8662$; $gl = 5$) nor by host ages ($p = 0.05$; $gl = 3$) (Table 1).

The number of emerged individuals has not presented significant differences in relation nor to the densities ($p = 0.11251$; $F = 2.0313$; $gl = 5$) neither to the ages ($p = 0.1466$; $F = 1.9901$; $gl = 3$), ranging from 58.53 ± 34.56 to 196.7 ± 38.34 (Table 1).

Differences have not been observed in sex ratio regarding densities ($p = 0.3931$; $F = 1.1061$; $gl = 5$), neither in ages ($p = 0.52$; $F = 3.0934$; $gl = 3$), from 0.82 ± 0.07 to 9.4 ± 0.04 (Table 1).

Regarding longevity, differences have also not been observed due to tested *P. elaeisis* parasitism densities ($p = 0.5901$; $F = 0.7610$; $gl = 5$) nor *S. frugiperda* host ages ($p = 0.9628$; $F = 0.093$; $gl = 3$) (Table 1).

Differences in tibia length ($p = 0.171$; $F = 1.7788$; $gl = 5$) ($p = 0.4442$; $F = 0.9328$; $gl = 3$) and cephalic capsule ($p = 0.2724$; $F = 1.4041$; $gl = 3$) were not found in relation to the treatments (Table 1).

DISCUSSION

The pupae age of *S. frugiperda* have not reduced the rate of *P. elaeisis* parasitism. Thus, hosts pupae of varying ages can be efficiently parasitized by *P. elaeisis*. This is probably due to the ability to suppress immune response by immunomodulatory substances from their ovary placed in the host during oviposition (ANDRADE et al., 2010). The parasitoid *Brachymeria lasus* (Walker, 1841) (Hymenoptera: Chalcididae) did not discriminate pupae by age either, and it could be an adaptive and advantageous behavior of these insects in a condition of low density of the host (HUSNI; HONDA, 2001).

The greatest increase of parasitism of *P. elaeisis* observed indicates that the best density of *P. elaeisis* in pupae of *S. frugiperda* is 10:1 or above. Lower number of parasitoids within the host can better exploit the nutritional resources of the pupae (CHONG; OETTING, 2006). However, hosts may present defense mechanisms to parasitoids such as encapsulation of eggs by hemocytes (defense cells) (STRAND, 2008), and the increase in the density of parasitism may reduce pupal defense

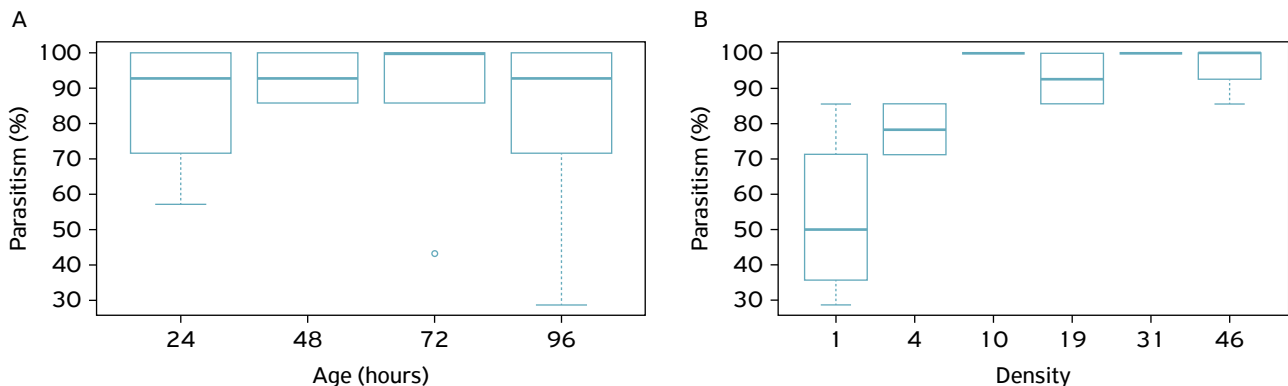


Figure 1. Parasitism (%) of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) at (A) different host ages and (B) different parasitoid densities ($25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ relative humidity and 12 hours photoperiod).

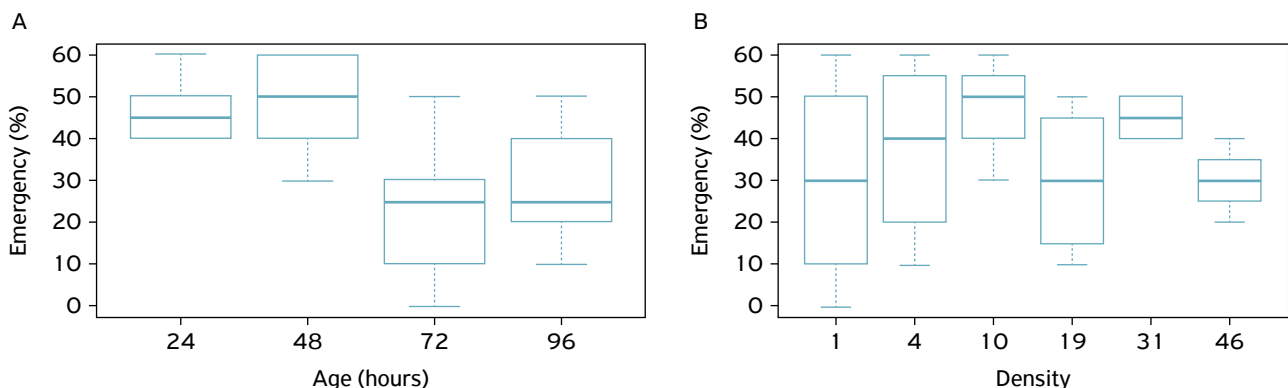


Figure 2. Emergence (%) of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) at (A) different host ages and (B) different parasitoid densities ($25 \pm 2^\circ\text{C}$, 60 to 80% $70 \pm 10\%$ relative humidity and 12 hours photoperiod).

due to substances released by female specimens during oviposition (UÇKAN et al., 2004; LI et al., 2007). Pupae of *A. gemmatalis* presented reduction in the number of hemocytes circulating in the hemolymph with the increase of the parasitoid density (ANDRADE et al., 2010).

The higher emergence rate of the *P. elaeisis* progeny from pupae with 24 and 48 hours shows that these ages are more adequate for reared of the parasitoids. However, PEREIRA et al. (2009) found higher emergence rate at *B. mori* pupae ages at 48 to 72 hours. The emergence of parasitoids is related to the combination of acceptability and survival of the offspring in the host (ABE, 2009). According to the literature, the observed difference may be related to the nutritional quality of the hosts, because the availability of nutrients decreases in the early stages of development, and due to physiological and morphological changes that may influence host adequacy by parasitoids (WANG; LIU, 2002). The percentage of emergence of *P. elaeisis* was not affected by its density, what suggests that *S. frugiperda* was a suitable host for maintenance of this parasitoid, because no effects of superparasitism or competition for space and food were observed, even in higher parasitism densities.

The development duration of *P. elaeisis* with different parasitoid densities and host age of *S. frugiperda* from 21.63 ± 1.07 to 24.75 ± 0.02 days was similar to that observed in *A. gemmatalis* pupae exposed to different densities of parasitoids, with 20 to 22 days (PASTORI et al., 2012).

The similarity between the number of individuals emerged from *P. elaeisis* at different densities of parasitism and host age indicates food adequacy even at higher densities and with advancement of the host age and low defense capacity against the parasitoid. In previous studies with alternative hosts, 70.07 ± 2.50 individuals of *P. elaeisis* were found per pupa of *T. molitor* (ZANUNCIO et al., 2008) and 111.60 ± 2.19 per pupae of *Diatraea saccharalis* (CHICHERA et al., 2012). These results suggest that pupae of *S. frugiperda* have potential to be used as alternative hosts for *P. elaeisis* production.

The sex ratio of *P. elaeisis* similar to parasitoid densities and host ages infers that the egg laying rate per female of *P. elaeisis* was adequate to the host without superparasitism (HUSNI; HONDA, 2001; CHONG; OETTING, 2006). Similar values of sexual ratio for *P. elaeisis* (0.89) were observed in pupae of *S. frugiperda* (BITTENCOURT; BERTI FILHO, 1999). The values found were considered high and important for the system of mass rearing and release of parasitoids in the field (AMALIN et al., 2005; VREYSEN; ROBINSON, 2011). In addition, higher values of sex ratio favor the retention of parasitoids in the field, and the high proportion of females in the releases is an important factor for an efficient biological control (VACARI et al., 2012).

Host age and density of parasitism do not affect longevity, showing adequacy in the development of the parasitoid in the host. It is expected that greater longevity favors the efficiency of biological control, being one of the important

Table 1. Biological variables (mean \pm standard deviation) of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) in pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) with different parasitism and host age densities at $25 \pm 2^\circ\text{C}$, 60 to 80% 70 \pm 10% relative humidity and 12 hours photoperiod.

Evaluated parameters	Density (number of parasitoids)						Age of the host (hours)			
	1	4	10	19	31	46	24	48	72	96
Life cycle (egg-adult) ¹	$24.75 \pm 4.02a$	$22.46 \pm 1.47a$	$22.22 \pm 0.8a$	$23.58 \pm 3.28a$	$22.09 \pm 1.07a$	$21.96 \pm 1.11a$	$24.12 \pm 2.69a^2$	$21.63 \pm 1.07a^2$	$22.72 \pm 2.96a^2$	$22.56 \pm 0.98a^2$
Number of progeny emerging ¹	$58.53 \pm 34.56a$	$113.26 \pm 52.85a$	$196.7 \pm 38.34a$	$178.71 \pm 108.44a$	$174.68 \pm 49.79a$	$136.1 \pm 27.85a$	$129.75 \pm 83.36a$	$191.76 \pm 60.41a$	$105.89 \pm 51.21a$	$130.87 \pm 44.07a$
Sex ratio ¹	$0.86 \pm 0.12a$	$0.93 \pm 0.07a$	$0.9 \pm 0.05a$	$0.89 \pm 0.06a$	$0.88 \pm 0.09a$	$0.82 \pm 0.06a$	$0.82 \pm 0.07a$	$0.88 \pm 0.06a$	$0.94 \pm 0.04a$	$0.9 \pm 0.09a$
Female longevity (day) ¹	$13.39 \pm 4.61a$	$12.8 \pm 3.82a$	$14.78 \pm 3.17a$	$13.01 \pm 2.94a$	$10.41 \pm 2.05a$	$14.27 \pm 4.17a$	$13.3 \pm 3.95a$	$13.24 \pm 2.76a$	$12.35 \pm 4.76a$	$13.38 \pm 2.93a$
Length of the cephalic capsule (mm) ¹	$0.54 \pm 0.01a$	$0.53 \pm 0.02a$	$0.52 \pm 0.04a$	$0.45 \pm 0.08a$	$0.47 \pm 0.05a$	$0.45 \pm 0.04a$	$0.53 \pm 0.05a$	$0.5 \pm 0.04a$	$0.46 \pm 0.04a$	$0.49 \pm 0.07a$
Length of posterior tibia (mm) ¹	$0.55 \pm 0.08a$	$0.56 \pm 0.03a$	$0.53 \pm 0.05a$	$0.53 \pm 0.05a$	$0.48 \pm 0.04a$	$0.48 \pm 0.04a$	$0.52 \pm 0.06a$	$0.55 \pm 0.04a$	$0.49 \pm 0.06a$	$0.53 \pm 0.05a$

¹Means followed by the same letter in the line do not differ by Tukey test ($p < 0.05$); ²means followed by the same letter in the row did not differ among the Kruskal-Wallis test ($p < 0.05$).

requirements for the quality control in mass rearing (VAN LENTEREN, 2000).

The similarity in tibia length at different densities of parasitoids and host ages is probably due to the similar competition of the larvae by nutrients and absence of superparasitism in this host. In the reared of natural enemies, it is expected to obtain individuals with greater body size, because they have positive correlation with quality indicators such as longevity, copula preference, fecundity, reproductive longevity, progeny emergence and sex ratio, which may indicate parasitoid efficiency (PASTORI et al., 2012).

The 10:1 density of individuals of *P. elaeisis* displayed adequate parasitism results, and further increase density in the mass rearing has not been required. Pupae of *S. frugiperda* with

24 and 48 hours of age had higher percentage of emergence of parasitoids. The biological variables of *P. elaeisis* were affected neither by the parasitoid densities nor by the age of the host.

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