Anais da Academia Brasileira de Ciências (2010) 82(2): 323-331
(Annals of the Brazilian Academy of Sciences)
ISSN 0001-3765
www.scielo.br/aabc

The density of females of *Palmistichus elaeisis* Delvare and LaSalle (Hymenoptera: Eulophidae) affects their reproductive performance on pupae of *Bombyx mori* L. (Lepidoptera: Bombycidae)

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Manuscript received on March 6, 2009; accepted for publication on July 30, 2009

ABSTRACT

Palmistichus elaeisis Delvare and LaSalle (Hymenoptera: Eulophidae) is a gregarious and polyphagous parasitoid mainly of Lepidoptera pupae. The objective of this paper was to study the development of parasitoid on *Bombyx mori* L. (Lepidoptera: Bombycidae) pupae exposed to one, nine, 18, 27, 36, 45 or 54 female *P. elaeisis*, respectively. The females of the parasitoid remained in contact with pupae for 24 hours in glass tubes (14.0 $\times$ 2.2 cm), packed in a climatic chamber regulated at 25 $\pm$ 2°C, 70 $\pm$ 10% relative humidity and photo phase of 12 hours. With the exception of density 1:1 (72.72%), in other densities parasitism was 100%. Adults of *P. elaeisis* did not emerge from pupae at densities of 1:1 and 9:1, but 100.0% of parasitoid emergence was observed at the density of 45:1 and 54.54% at 54:1. The duration of the life cycle of this parasitoid ranged from 20 to 28 days. *P. elaeisis* produced 49 to 589 descendants per pupa of *B. mori*. The sex ratio of *P. elaeisis* ranged from 0.93 $\pm$ 0.01 to 0.97 $\pm$ 0.01 without differences with 18, 27, 36, 45 and 54 females/host. This parasitoid should be reared with the density of 45 females per pupa of *B. mori*.

Key words: alternative host, biological control, mass rearing, parasitism rate, parasitoids.

INTRODUCTION

The family Eulophidae comprises 297 genera and 4.472 species worldwide. They are end or ectoparasitoids; idiobionts or koinobionts; solitary or gregarious; primary or secondary parasitoids; specialists or generalists (Noyes 2003, Pereira et al. 2008b). Many eulophid species are studied and used with success in biological control programs (Gauthier et al. 2000, Pereira et al. 2008a).

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Palmistichus elaeisis Delvare and LaSalle (Hymenoptera: Eulophidae) is a gregarious endoparasitoid mainly of Lepidoptera pupae. This species was recorded in Brazil in *Eupseudosoma involuta* (Sepp) (Lepidoptera: Noctuidae) and *Euselasia eucerus* Hewitson (Lepidoptera: Riodinidae) (Delvare and LaSalle 1993), *Sabulodes* sp. (Lepidoptera: Geometridae) (Bittencourt and Berti Filho 1999) and *Thyrinteina arnobia* (Stoll) and *Thyrinteina leucoceraea* Rindge (Lepidoptera: Geometridae) in eucalyptus plants in the Minas Gerais State, Brazil (Pereira et al. 2008b), being one important natural enemy due to their attack rate (Zanuncio et al. 2009).

The mass rearing represents an important stage of control programs (Parra et al. 2002, Pastori et al. 2008, Pereira et al. 2009), and the nutritional quality, size, age, mechanical resistance and capacity of immunological response of parasitoids should be considered to select alternative hosts (Godfray 1994). *P. elaeisis* showed a good reproductive performance (adequate parasitism, emergence, progeny per pupa, and size of the body, besides longevity of males and females and sex ratio) when reared on pupae of the alternative host *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae). In addition, the low cost to rear this alternative host indicates that it could be easily used to mass rear the parasitoid *P. elaeisis* for biological control programs (Zanuncio et al. 2008).

The density of parasitoids per host affects the parasitoid offspring (Thomazini and Berti Filho 2000, Matos Neto et al. 2004), the sex ratio (Choi et al. 2001), the parasitism capacity (Sampaio et al. 2001), the duration of the life cycle, the size of the body, and the longevity of the imagines (Silva-Torres and Matthews 2003). To minimize the cost and labor and to maximize the production of parasitoids in mass rearing programs, it is, therefore, necessary to study the density of the parasitoids in relation to the host (Sagarra et al. 2000a).

Palmistichus elaeisis are reared on pupae of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae), *Anticarsia gemmatalis* Hubner, *Heliothis virescens* (Fabricius), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) and *T. arnobia* (Bittencourt and Berti Filho 1999, 2004, Pereira et al. 2008b), but the number of females of *P. elaeisis* per pupae is not known. This is important because *P. elaeisis* is a gregarious parasitoid, which makes necessary to define the ideal density of its females per pupa of the host to increase the production of descendants with similar quality to native ones.

Bombyx mori L. (Lepidoptera: Bombycidae) has been reared with low cost, and its pupa has high nutritional quality (Greiss et al. 2003, Wang-Dun et al. 2004). For this reason, it can be an adequate host for pupa endoparasitoids. The objective of this paper was to study the development of *P. elaeisis* with different numbers of females of this parasitoid per pupae of *B. mori*.

MATERIALS AND METHODS

The experiment was developed in the Laboratory of Biological Control of the Department of Animal Biology of the "Universidade Federal de Viçosa" (UFV) at Viçosa, Minas Gerais State, Brazil, as:

REARING *B. mori*

The Sericiculture Laboratory of the Department of Animal Biology (UFV) provided first instar caterpillars of *B. mori* that were placed in plastic trays (39.3 × 59.5 × 7.0 cm) with *Morus alba* L leaves, which were changed daily. The pupae obtained of *B. mori* were transferred to plastic trays (28.3 × 36.0 × 7.0 cm) and placed in an acclimatized chamber at 25 ± 2°C, 70 ± 10% relative humidity (RH) and a photo phase of 12 hours.

REARING THE PARASITOID

Adults of *P. elaeisis* were kept in glass tubes (14.0 × 2.2 cm) with honey droplets as food. The tubes were closed with a cotton plug. Forty-eight to 72 hours old pupae of *B. mori* were removed from their cocoons and exposed to the parasitoid females during 24 hours in the acclimatized chamber.

EFFECT OF THE PARASITOID DENSITY ON THE BIOLOGY OF *P. elaeisis*

Pupae of *B. mori* (weight 1.06 ± 0.01g) and females of *P. elaeisis*, both with 72 hours old, at the densities of 1:1, 9:1, 18:1, 27:1, 36:1, 45:1 and 54:1 (parasitoid: host), were reared in an acclimatized chamber at 25 ± 2°C, 70 ± 10% relative humidity (RH) and a photo phase of 12 hours. Each pupa was exposed to the parasitism by females of *P. elaeisis* into glass tubes (14.0 cm × 2.2 cm) for 24 hours under the conditions user. These females were removed from the tubes at the end of this period.

The parameters evaluated were: the duration of the life cycle (egg-adult), the percentage of parasitism [without the natural mortality of the host (Abbott 1925)], the percentage of emergence of the progeny, the number and size of parasitoids that emerged per pupa, the number of immature that did not complete their development, the sex ratio (which was calculated by the equation SR = number of females/number of males and females). The sex of *P. elaeisis* was determined by morphologic characteristics in the antenna and the gaster as described by Delvare and LaSalle (1993).

The treatments were represented by the densities of one, nine, 18, 27, 36, 45 and 54 *P. elaeisis* females per pupa of *B. mori* in 12 replications in an entirely casualized design. The data of the duration of the life cycle, the number of parasitoids that emerged per pupa and the percentage of dead immature were submitted to a variance analysis. The regression analysis was done with the computer program SigmaPlot 8.0 when the data presented differences at 5% probability. The percentages of parasitism and emergency of *P. elaeisis* were submitted to the analysis of general linear models (GLM) with binomial distribution ($P \leq 0.05$) with the computer program R Statistical System (Ihaka and Gentleman 1996). This analysis was carried through with the original data that are not-parametric, but these had been expressed in percentage to facilitate the visualization. The data of the sex ratio and those of the size of the body of females and males of *P. elaeisis* were submitted to a variance analysis and to the test of Scott-Knott with the computer program SAEG 9.0 when they were different at 5% probability.

RESULTS

The density of females of *P. elaeisis* affected their percentage of parasitism and the number of specimens of this parasitoid that emerged per pupa of *B. mori*. A total of 72.72% of pupae of this host were parasitized at the density of 1:1, while this number was 100.00% for other densities (P = 0.0312) (Fig. 1). No specimens of *P. elaeisis* emerged from pupae of *B. mori* at the densities of 1:1 and 9:1, but those of 45:1 and 54:1 had 100.00% and 54.54% of pupae with parasitoid emergence, respectively (P = 0.0055) (Fig. 1).

The duration of the life cycle (egg-adult) (P = 0.0001; $df_{error} = 30$) decreased as the density of *P. elaeisis* increased with values from 20 to 28 days (Fig. 2). The density of this parasitoid per pupa of *B. mori* (F = 4.7025; P = 0.0170; $df_{error} = 31$) affected the progeny that was produced. The number of offspring per pupa of *B. mori* ranged from 49 to 589, but no offspring was produced at the densities 1:1 and 9:1 (Fig. 3). The progeny per female of *P. elaeisis* per pupa of *B. mori* was similar, with 18, 27, 36, 45 e 54 females of this parasitoid per pupae of this host (F = 1.2789; P = 0.2674; $df_{erro} = 30$) (Fig. 4).

The density of females of *P. elaeisis* affected the percentage of dead immature of this parasitoid (F = 8.8088; P = 0.0006; $df_{error} = 49$) (Fig. 5) with lower numbers (6.83 ± 1.72) at the density of 45:1. The sex ratio of *P. elaeisis* ranged from 0.93 ± 0.01 to 0.97 ± 0.01, with similar values at all densities (F = 2.12; P = 0.06962) (Table I).

The length of the body of adult *P. elaeisis* and the width of the head capsule differed among the densities of females of this parasitoid. The length of the body of females and males of *P. elaeisis* ranged from 1.67 ± 0.04 to 1.89 ± 0.02 mm (F = 3.707; P = 0.00964) and from 1.34 ± 0.01 to 1.54 mm ± 0.03 (F = 5.449; P = 0.00091), respectively (Table I). The width of the head capsule of females and males of *P. elaeisis* ranged from 0.47 ± 0.01 to 0.50 ± 0.01 mm (F = 3.355; P = 0.01577), and from 0.38 ± 0.01 to 0.44 ± 0.01 mm (F = 4.186; P = 0.00497), with the densities of 18 and 54 females of this parasitoid per pupae of *B. mori* (Table I).

DISCUSSION

The percentage of parasitism and emergence of *P. elaeisis* per pupa of *B. mori* showed that the optimal number of females of this parasitoid per pupae of this host should be 45 for its mass rearing. The progeny of *P. elaeisis* can be affected by the number of females of *P. elaeisis* per pupae of *B. mori*. This indicates that inadequate numbers of females of this parasitoid can reduce the number of eggs laid per host and the amount of toxins injected in the pupa by the female wasps. The toxins are necessary to reduce the immunological response from the pupa, a defense mechanism used by the pupa to encapsulate eggs and/or larvae of the parasitoid (Schmid-Hempel 2005). So, eggs of *P. elaeisis* may be encapsuled and dead by pupal hemocyts when the female density of this parasitoid per host is low to suppress the immune response. Nevertheless, high doses of toxins injected by many female *P. elaeisis* per pupa may kill the host before the complete development of the immature parasitoid occurs (Strand and Pech 1995).

The reproductive parameters of *P. elaeisis* differed from those of *Trichospilus diatraeae* Cherian and Margabandhu (Hymenoptera: Eulophidae) and from pupae of *D. saccharalis*, *A. gemmatalis*, *H. virescens* and *S. frugiperda* exposed to one or several females of this parasitoid. The number of offsprings of *T. diatraeae* per host almost duplicated, and 100.00% of parasitism was

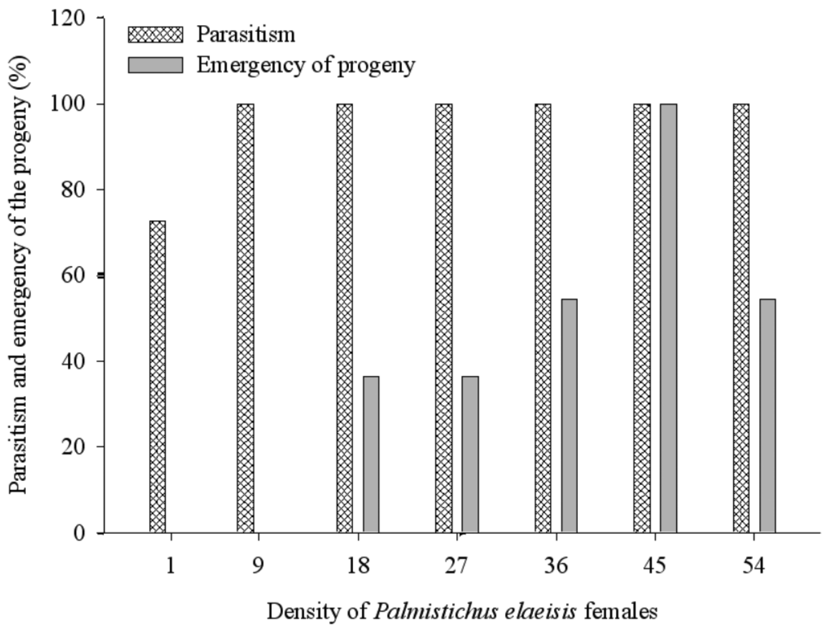

Fig. 1 – Percentage of parasitised pupae and emergence of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) with one, nine, 18, 27, 36, 45 or 54 females per pupa of *Bombyx mori* (Lepidoptera: Bombycidae) at 25 ± 2°C, 70 ± 10% relative humidity and 12 hours photo phase. (No adults of this parasitoid emerged with the densities 1:1 and 9:1).

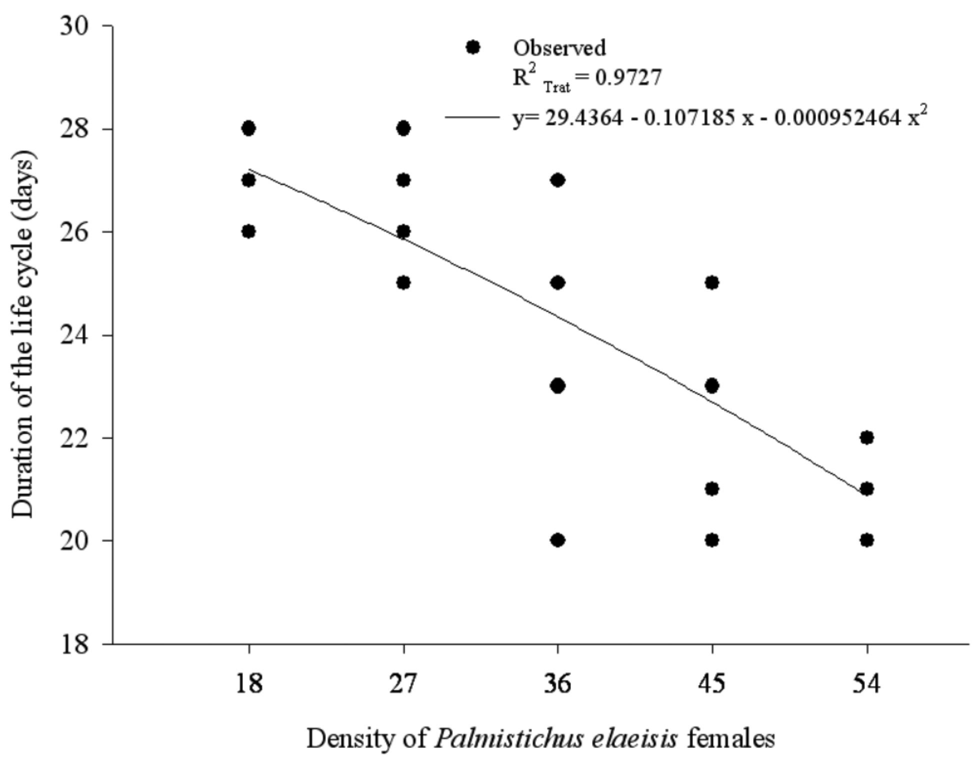

Fig. 2 – Duration of the life cycle of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) with one, nine, 18, 27, 36, 45 or 54 females per pupa of *Bombyx mori* (Lepidoptera: Bombycidae) at 25 ± 2°C, 70 ± 10% relative humidity and 12 hours photo phase.

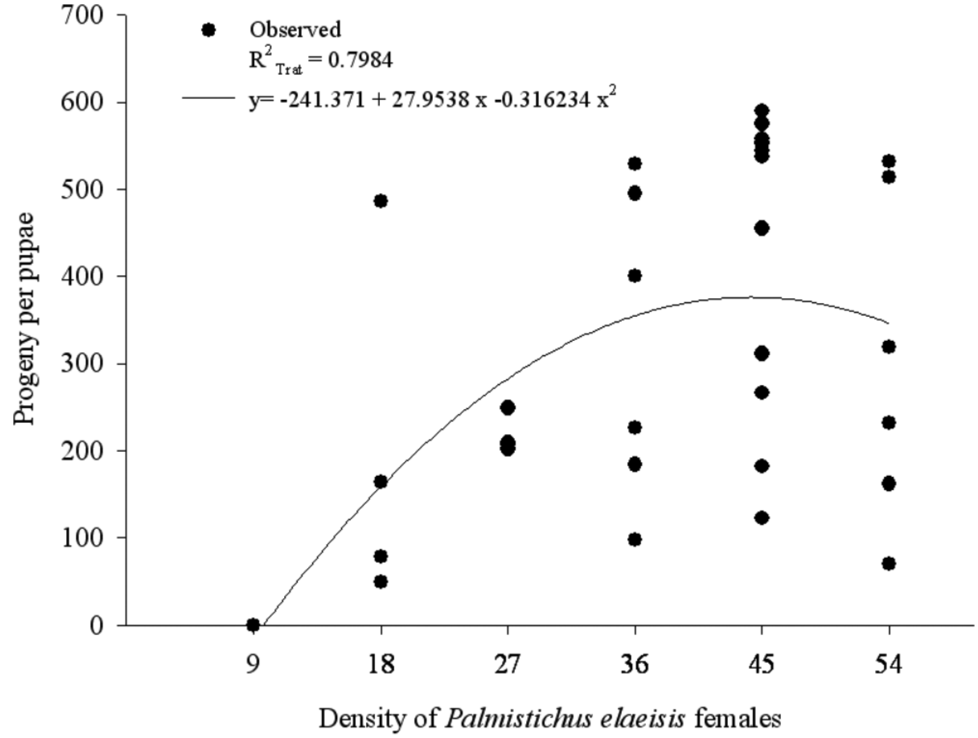

Fig. 3 – Progeny of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) with one, nine, 18, 27, 36, 45 or 54 females of this parasitoid per pupa of *Bombyx mori* (Lepidoptera: Bombycidae) at 25 ± 2°C, 70 ± 10% relative humidity and 12 hours photo phase.

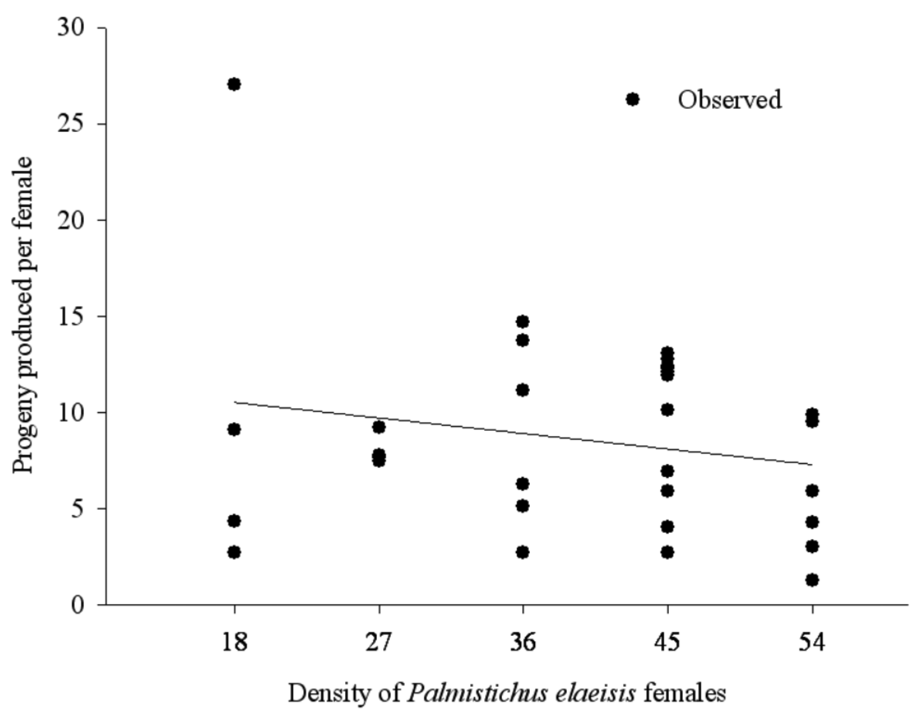

Fig. 4 – Progeny produced per female of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) with one, nine, 18, 27, 36, 45 or 54 females of this parasitoid per pupa of *Bombyx mori* (Lepidoptera: Bombycidae) at 25 ± 2°C, 70 ± 10% relative humidity and 12 hours photo phase.

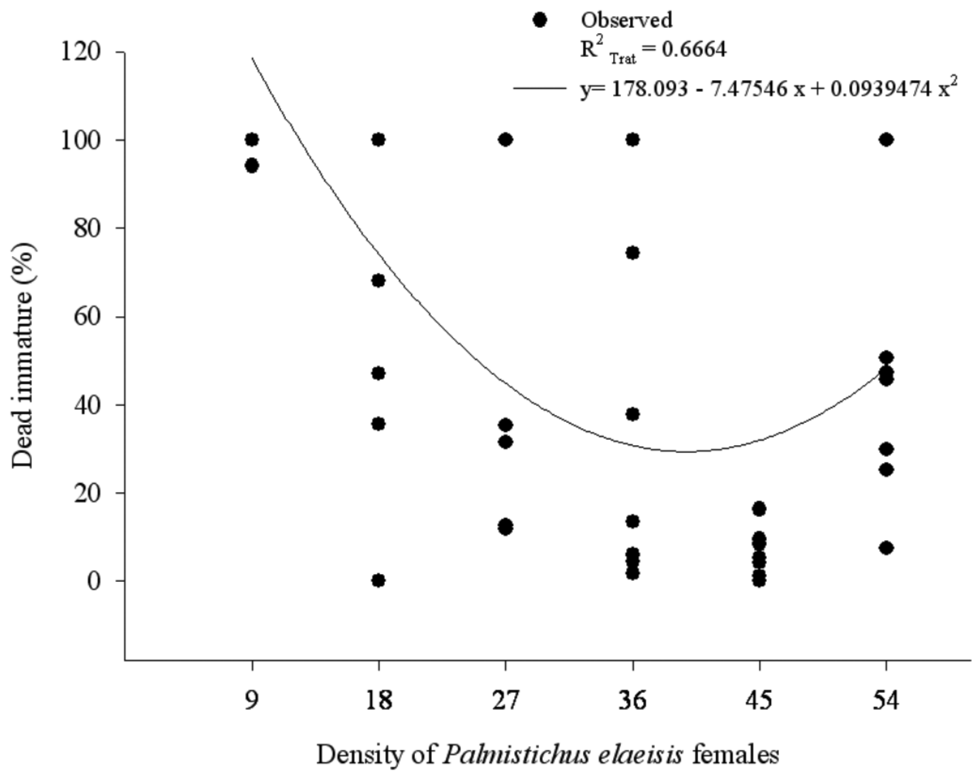

Fig. 5 – Percentage of dead immature of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) with one, nine, 18, 27, 36, 45 and 54 females of this parasitoid per pupa of *Bombyx mori* (Lepidoptera: Bombycidae) at 25 ± 2°C, 70 ± 10% relative humidity and 12 hours photo phase.

TABLE I

Means (± SE) of the sex ratio, body length and width of the head capsule of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) with one, nine, 18, 27, 36, 45 or 54 females of this parasitoid per pupa of *Bombyx mori* (Lepidoptera: Bombycidae) at 25 ± 2°C, 70 ± 10% relative humidity and 12 hours photo phase.

Parasitoid: host density	N	Sex ratio	Body length (mm)		Head capsule (mm)	
			female	male	female	male
1:1	—	—	—	—	—	—
9:1	—	—	—	—	—	—
18:1	12	0.95 ± 0.01a	1.81 ± 0.05a	1.54 ± 0.03a	0.48 ± 0.02b	0.44 ± 0.01a
27:1	12	0.95 ± 0.01a	1.83 ± 0.03a	1.49 ± 0.05a	0.49 ± 0.01b	0.41 ± 0.02a
36:1	12	0.93 ± 0.02a	1.83 ± 0.06a	1.49 ± 0.03a	0.49 ± 0.01b	0.42 ± 0.01a
45:1	12	0.93 ± 0.01a	1.89 ± 0.02a	1.46 ± 0.03a	0.50 ± 0.01a	0.44 ± 0.01a
54:1	12	0.97 ± 0.01a	1.67 ± 0.04b	1.34 ± 0.01b	0.47 ± 0.01b	0.38 ± 0.01b

N = number of parasitoids measured. / Means followed by the small letter in the same column do not differ among densities by the Scott-Knott test ($P \leq 0.05$). / — Without emergence of *Palmistichus elaeisis*.

obtained with several females of this parasitoid per pupa (Paron and Berti Filho 2000). On the other hand, the percentage of parasitism and the emergence of *Aphidius colemani* Viereck (Hymenoptera: Aphididae) were similar with different densities of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). This suggests that *M. persicae* does not present any nutritional or physiological barriers for the development of *A. colemani* (Sampaio et al. 2001). *B. mori* might have an efficient defense mechanism against *P. elaeisis* because the emergence of this parasitoid was observed only from 18:1 and higher densities of the parasitoid per pupae of this host.

The absence of offsprings of *P. elaeisis* at the density 1:1 can be due to the encapsulation of its eggs by the immune defense of the pupa of *B. mori*. This mechanism and the melanization constitute the main defense of insects against strange materials in their bodies (Schmid-Hempel 2005). On the other hand, parasitoids can avoid the encapsulation by injecting particles that are similar to virus, polidnaviroses or poisons into the host to interfere with its immunological recognition (Strand and Pech 1995, Schmidt et al. 2001, Nakamatsu and Tanaka 2003), as well as the depletion of the immune system that has high energy cost, is finite and can be neutralized by toxins from parasitoids (Schmid-Hempel 2005). These hypotheses may explain the high number of *P. elaeisis* per pupa of *B. mori* at the in density of 54:1, suggesting that this amount of female parasitoids is sufficient to neutralize the immune response of this host. The decrease on parasitoid progeny with the density 54:1 parasitoid:host suggests the occurrence of superparasitism.

The shorter life cycle duration of *P. elaeisis* in the densities 36:1; 45:1 and 54:1, indicates that the competition for nutrients reduced the development of these immature parasitoids. This agrees with reports for different densities of *Melittobia digitata* Dahms (Hymenoptera: Eulophidae) per pupa of *Neobellieria bullata* Parker (Diptera: Sarcophagidae) (Silva-Torres and Matthews 2003). The duration of the immature stages of *P. elaeisis* with *A. gemmatalis*, *D. saccharalis*, *S. frugiperda*, *T. arnobia* and *H. virescens* was 18.9, 19.5, 19.7, 20.2 and 22.0 days, respectively. These results demonstrate that the host species also affects the development period of this parasitoid (Bittencourt and Berti Filho 2004).

The similar number of progeny per female of *P. elaeisis* in the densities 18:1, 27:1, 36:1, 45:1 and 54:1, parasitoid:host shows that this natural enemy control the number of eggs per ovipositon and recognizes non parasitized, parasitized or super-parasitized hosts. However, the competition, size and age of the parasitoid, besides the number of eggs encapsulated by the host, can also affect the number of offsprings (Godfray 1994, Sagarra et al. 2000a, b).

The sex ratio of *P. elaeisis* was similar considering the host *T. molitor* (Zanuncio et al. 2008) and other eulophid parasitoids such as *Melittobia clavicornis* Cameron (Gonzáles et al. 2004), *Melittobia australica* Girault and *M. digitata* (Silva-Torres and Matthews 2003), suggesting that a high sex ratio is characteristic to Eulophidae.

The smaller size of emerging adult *P. elaeisis* at the density 54:1 indicates the occurrence of competion for nutrients during the larva stage of this parasitoid. This is important because the size of the body is positively correlated with the longevity, fecundity, reproductive period, emergence of the progeny and sex ratio, which are indicators of quality and efficiency of the parasitoids. Large females and males of *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae) lived longer (35.4 ± 10.0; 29.16 ± 6.5 days) than small ones (27.9 ± 9.6; 18.4 ± 5.7 days), respectively, but females of *A. kamali* to mate with males large rather or small, no preference. However, the fecundity of *A. kamali* was positively correlated with the size of the females, with values of 37 ± 21 eggs for small females, and 96 ± 43 for large females. The parasitism capacity, daily oviposition rate and the number of offsprings per female of *A. kamali* showed higher values for large specimens. This suggests the necessity of using larger specimens and to monitor mass rearing facilities in order to maximize the production of parasitoids (Sagarra et al. 2001).

Palmistichus elaeisis parasitized *B. mori* pupae at the density of 1:1, 9:1, 18:1, 27:1, 36:1, 45:1 and 54:1 (parasitoid: host), but densities below or above 45 parasitoids per pupa are inadequate for producing low number of descendants, besides favoring the auto-immune reply of *B. mori* pupae. This parasitoid should be reared with the density of 45 females per pupa of *B. mori* in laboratory and economic studies (Monteiro et al. 2006), and in field (Pratissoli et al. 2005a, b) it must be conducted to be applied in biological control programs.

ACKNOWLEDGMENTS

To Dr. Christer Hansson, Lund University, Sweden and Dr. Marcelo Teixeira Tavares, Universidade Federal do Espírito Santo for the identification of the parasitoid. To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

RESUMO

Palmistichus elaeisis Delvare e LaSalle (Hymenoptera: Eulophidae) é um parasitóide polífago, que inviabiliza, principalmente, pupas de lepidópteros. O objetivo desse trabalho foi estudar o desenvolvimento do parasitóide em pupas de *Bombyx mori* L. (Lepidoptera: Bombycidae) expostas a uma, nove, 18, 27, 36, 45 ou 54 fêmeas de *P. elaeisis*, respectivamente. As fêmeas do parasitóide permaneceram em contato com as pupas por 24 horas em tubos de vidro (14,0 × 2,2 cm), acondicionadas em câmara climatizada regulada a 25 ± 2°C, 70 ± 10% de UR e fotofase de 12 horas. Com exceção da densidade 1:1 (72,72%), nas demais densidades o parasitismo foi 100%. Adultos de *P. elaeisis* não emergiram nas densidades de 1:1 e 9:1, mas observou-se 100% de emergência do parasitóide na densidade 45:1 e 54,54% em 54:1. A duração do ciclo de vida desse parasitóide variou de 20 a 28 dias. *P. elaeisis* produziu de 49 a 589 descendentes por pupa de *B. mori*. A razão sexual de *P. elaeisis* variou de 0,93 ± 0,01 a 0,97 ± 0,01 sem diferenças nas densidades de 18, 27, 36, 45 e 54 fêmeas/hospedeiro. Esse parasitóide deve ser criado na densidade de 45 fêmeas por pupa de *B. mori*.

Palavras-chave: hospedeiro alternativo, controle biológico, criação massal, taxas de parasitismo, parasitóides.

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