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Reproductive biology of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) with alternative and natural hosts

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ABSTRACT. Mass rearing of parasitoids depends on choosing appropriate alternative hosts. The objective of this study was to select alternative hosts to rear the parasitoid *Palmistichus elaeisis* Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae). Pupae of the lepidopterans *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae), *Bombyx mori* Linnaeus, 1758 (Lepidoptera: Bombycidae) and *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) were exposed to parasitism by females of *P. elaeisis*. The duration of the life cycle of *P. elaeisis* was 21.60 ± 0.16 and 24.15 ± 0.65 days on pupae of *A. gemmatalis* and *B. mori*, respectively, with 100.0% parasitism of the pupae and 71.4 and 100.0% emergence of parasitoids from the first and second hosts, respectively. The offspring number of *P. elaeisis* was 511.00 ± 49.70 and 110.20 ± 19.37 individuals per pupa of *B. mori* and *A. gemmatalis*, respectively. The reproduction of *P. elaeisis* from pupae of *T. arnobia* after six generations was similar to the other hosts.

KEY WORDS. *Anticarsia gemmatalis*; *Bombyx mori*; parasitoids; *Thyrinteina arnobia*.

Parasitoids are important for biological control of insect pests in agro-ecosystems due to their diversity and to the high levels of population regulation of insects in different orders (Pennacchio & Strand 2006). Most of these natural enemies belong to several families of Hymenoptera, including Eulophidae, which are comprised of 297 genera and 4,472 species, thus far described in tropical and temperate areas exhibiting a wide range of biological traits. For example, some species are endo or ectoparasitoids, idiobionts or koinobionts. They may be solitary or gregarious, primary or hyperparasitoids, specialists or generalists and many of these species have been studied and used with success in biological control programs (Gauthier *et al.* 2000).

Palmistichus elaeisis Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae) is a polyphagous parasitoid with reports of parasitism on pupae of several lepidopteran hosts, including *Eupseudosoma involuta* (Sepp, 1852) (Lepidoptera: Arctiidae), *Euselasia eucerus* Hewitson, 1872 (Lepidoptera: Riodinidae), *Sabulodes* sp., *Thyrinteina arnobia* (Stoll, 1782), and *T. leucoceraea* Rindge, 1961 (Lepidoptera: Geometridae) (Delvare & LaSalle 1993, Bittencourt & Berti Filho 1999, Pereira *et al.* 2008b), which occur in eucalyptus plantations of Brazil. The generalist habit of *P. elaeisis* demonstrates that this natural enemy can be used to control Lepidoptera eucalyptus defoliators, primarily species of the genus *Thyrinteina* (Pereira *et al.* 2008b). However, rational evaluations, supported by ecological data, are necessary to prevent possible unpredictable negative impact of this natural enemy after its release, due to its generalist habit (Waage 2001).

The success of biological control with parasitoids species depends on basic studies of hosts and other environmental features which could affect their development (Pratissoli *et al.* 2005, Pastori *et al.* 2007, Pereira *et al.* 2009, 2010). Moreover, these natural enemies, reared on alternative hosts with lower production costs and without reduction on their efficiency when compared with their natural hosts, may have economic benefits (Pratissoli *et al.* 2005, Zanuncio *et al.* 2008).

The silkworm, *Bombyx mori* Linnaeus, 1758 (Lepidoptera: Bombycidae) can be reared with low cost and the pupae contain high levels of protein (Greiss *et al.* 2003, Wang-Duu *et al.* 2004). *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) has a short life cycle and can also be maintained on artificial diet in the laboratory (Greene *et al.* 1976). The parasitoid *P. elaeisis* also has been reared on pupae of *B. mori* in the laboratory (Bittencourt & Berti Filho 1999, 2004). Therefore, *B. mori* and *A. gemmatalis* are potential alternative hosts for *P. elaeisis* and, for this reason the objective of this study was to select the most appropriate host for rearing the parasitoid and to evaluate its reproductive success from these alternative hosts, as opposed to the natural host *T. arnobia*.

MATERIAL AND METHODS

Considering rearing the lepidopteran hosts of the parasitoid *P. elaeisis*: 1) *Dirphia moderata* Bouvier, 1929 (Lepidoptera: Saturniidae) was reared using a methodology adapted from Pereira *et al.* (2008a). The parasitoid *P. elaeisis* was reared for one generation with the host *D. moderata* to avoid its conditioning before rearing it on *B. mori* and *A. gemmatalis* pupae. 2) *Bombyx mori* caterpillars were reared in plastic trays (39.3 x 59.5 x 7.0 cm) with *Morus alba* Linnaeus, 1753 (Rosales: Moraceae) leaves supplied daily. The cocoons were transferred to plastic trays (28.3 x 36.0 x 7.0 cm) at 25 ± 2 ºC, 70 ± 10% relative humidity and photophase of 14 h. 3) Eggs of *A. gemmatalis* were maintained on moistened paper filter inside Petri dishes (10.0 x 2.5 cm). Newly-emerged caterpillars were transferred to plastic containers where they were provided with artificial diet (Greene *et al.* 1976) at 25 ± 1ºC, 70 ± 10% relative humidity and photophase of 14 h until pupation. 4) *Thyrinteina arnobia* caterpillars were placed in glass tubes (8.5 x 2.4 cm), covered with cotton and provided with artificial diet (Wilcken & Berti Filho 2006).

Adults of the parasitoid *P. elaeisis* were reared in glass tubes (14.0 x 2.2 cm) closed with a cotton wad and with honey droplets as food. Forty-eight to 72 h old pupae of *B. mori* were removed from the cocoons and exposed to parasitism by *P. elaeisis* females over the course of 24 h at 25 ± 2ºC, 70 ± 10% relative humidity and a photophase of 14 h. Forty-eight hour old pupae of *D. moderata* were exposed to *P. elaeisis* during 24 h. After emergence, adults of this parasitoid were divided into two groups and reared for three generations on alternative hosts *A. gemmatalis* or *B. mori* to remove preimaginal conditioning of parasitoids. Fifteen *A. gemmatalis* pupae (0.24 ± 0.004 g) and 15 24 h old *B. mori* pupae (1.2 ± 0.05 g) were placed individually in glass tubes (14.0 x 2.2 cm) and exposed to parasitism by six and 45 *P. elaeisis* females (Pereira *et al.* 2010), respectively, during 24 h at 25 ± 1ºC, 70 ± 10% relative humidity and photophase of 14 h. The ideal number of *P. elaeisis* for the different hosts was previously estimated based on the minimum number needed to overcome the host immunity (see Pereira *et al.* 2010). All *P. elaeisis* were removed from the tubes at the end of this period.

The reproductive performance of *P. elaeisis* on natural host *T. arnobia* was determined with adults reared for six generations on *A. gemmatalis* or *B. mori* pupae. Twenty-eight 48 h old pupae of *T. arnobia* were sexed, weighed (7 female = 0.60 ± 0.04 g and 7 male = 0.13 ± 0.01 g; 7 female = 0.69 ± 0.07 g and 7 male = 0.24 ± 0.02 g), and separately placed in glass tubes where they were exposed over 72 h to parasitism by *P. elaeisis* females reared for six generations on *A. gemmatalis* or *B. mori* pupae. Based on preliminary tests, we used six females of *P. elaeisis* per male pupa of *T. arnobia*, and 15 females per female pupa.

The duration of the life cycle (egg-adult); percent parasitism – discounting natural mortality of the host (Abbott 1925) –; percent emerging; number of parasitoid individuals emerged per pupa of each host; longevity of descendants and sex ratio were evaluated.

The experimental design was completely randomized using two treatments where *P. elaeisis* was reared with each alternative host (*A. gemmatalis* or *B. mori*) with 15 replications each with one host pupa. The same design was used, but each treatment had 14 replications represented by the biological aspects of *P. elaeisis* obtained from pupae of *T. arnobia* but on which they had been previously reared on *A. gemmatalis* or *B. mori*. The treatments were submitted to analysis of variance (ANOVA) followed by the F test using the software SAEG 8.0. Percent parasitism and emergence of *P. elaeisis* were analyzed with general linear models (GLM) of binomial distributions ($p \leq 0.05$) using the R Statistical System (Ihaka & Gentleman 1996).

RESULTS

The life cycle (egg to adult) of *P. elaeisis* was shorter when reared on *A. gemmatalis* than on *B. mori* (F = 11.369, p = 0.003). *Palmistichus elaeisis* parasitism reached 100% on *B. mori* and on *A. gemmatalis* pupae. The emergence of progeny was 100% on parasitized pupae of *B. mori* and 71.4% on *A. gemmatalis* (χ^2 = 5.900, p = 0.015) (Tab. I). More *P. elaeisis* emerged from *B. mori* pupae than from *A. gemmatalis* pupae (F = 45.502, p = 0.0001). However, a larger number of females was produced per female of *P. elaeisis* from *A. gemmatalis* pupae (F = 5.563, p = 0.028) (Tab. I).

The length of the body (head to the abdominal extremity) (F = 15.445, p = 0.0005) and the width of the head capsule (F = 35.196, p = 0.0001) of *P. elaeisis* females emerging from *B. mori* pupae increased when compared to those of *A. gemmatalis*. However, the length of the body (F = 2.135, p = 0.155) and the width of the head capsule (F = 3.903, p = 0.06) of males were similar on both hosts (Tab. I).

The longevity of *P. elaeisis* females and males emerging from *B. mori* and *A. gemmatalis* was similar (F = 2.147, p = 0.147) for females (F = 0.89, p = 0.0001) for males (Tab. I). The sex ratio was similar for offspring originating from *A. gemmatalis* and *B. mori* (Tab. I).

There were no differences in parasitism (χ^2 = 2.9392, p = 0.086), emergence of offspring (χ^2 = 1.3426, p = 0.247), life cycle length (egg to adult) (F = 2.402, p = 0.137), progeny per pupa (F = 1.842, p = 0.190), female (F = 2.267, p = 0.144) and male (F = 1.499, p = 0.232) body length, female (F = 3.099, p = 0.090) and male (F = 2.278, p = 0.143) head capsule width, female (F = 0.037, p > 0.05) and male (F = 0.007, p > 0.05) longevity and sex ratio (F = 2.442, p = 0.134) in *T. arnobia* parasitized by *P. elaeisis* after being previously reared on *A. gemmatalis* or *B. mori* (Tab. II). On the other hand, the number of offspring per female was higher in *T. arnobia* pupae (F = 5.009, p = 0.037) after the parasitoid was reared with *A. gemmatalis* pupae (Tab. II).

Table I. Reproductive characteristics (mean ± standard error) of *P. elaeisis* when reared on pupae of *B. mori* or *A. gemmatalis*.

Reproductive characteristics	*Bombyx mori* (Means ± SE)[1]	(n)	*Anticarsia gemmatalis* (Means ± SE)[1]	(n)
Life cycle duration (days)	**24.15 ± 0.65**	13	**21.60 ± 0.16**	10
Progeny per pupa	**511.00 ± 49.70**	13	**110.20 ± 19.37**	10
Females produced per female	**10.64 ± 1.02**	13	**17.68 ± 3.15**	10
Female body length (mm)	**1.85 ± 0.03**	15	**1.65 ± 0.04**	15
Male body length (mm)	1.38 ± 0.03	10	1.32 ± 0.03	10
Female head capsule width (mm)	**0.52 ± 0.01**	15	**0.46 ± 0.01**	15
Male head capsule width (mm)	0.41 ± 0.01	10	0.39 ± 0.01	10
Female longevity (days)	17.55 ± 1.84	40	14.63 ± 0.78	40
Male longevity (days)	16.40 ± 2.26	10	13.70 ± 1.50	10
Sex ratio (female/total)	0.94 ± 0.01	13	0.96 ± 0.01	10

[1] Values which differ between hosts (F-test at 5% probability) are shown in bold.

Table II. Reproductive characteristics (mean ± standard error) of *P. elaeisis* when reared on pupae of *T. arnobia* after six generations on pupae of *B. mori* or *A. gemmatalis*.

Reproductive characteristics	*Bombyx mori* (Means ± SE)[1]	n	*Anticarsia gemmatalis* (Means ± SE)[1]	n
Life cycle duration (days)	20.64 ± 0.28	11	21.00 ± 0.30	11
Progeny per pupa	319.45 ± 74.87	13	493.27 ± 1.04	10
Females produced per female	**24.64 ± 3.50**	11	**39.31 ± 4.67**	11
Female body length (mm)	1.95 ± 0.03	15	1.88 ± 0.03	15
Male body length (mm)	1.38 ± 0.02	15	1.34 ± 0.02	15
Female head capsule width (mm)	0.59 ± 0.02	15	0.55 ± 0.01	15
Male head capsule width (mm)	0.42 ± 0.00	15	0.41 ± 0.00	15
Female longevity (days)	23.75 ± 2.12	40	23.15 ± 2.32	40
Male longevity (days)	24.80 ± 4.10	10	24.30 ± 4.17	10
Sex ratio (female/total)	0.95 ± 0.01	11	0.93 ± 0.01	11

[1] Values which differ between hosts (F-test at 5% probability) are shown in bold.

DISCUSSION

The offspring of *P. elaeisis* showed a shorter development time on *A. gemmatalis* pupae, which may be due to differences of nutritional resources between hosts. Thus, *A. gemmatalis* may have a reduced availability of nutritional resources which stimulates the parasitoids to develop faster, but with a smaller size. On the other hand, parasitoids can adjust the number of its population according to the size of the natural host, thus avoiding sibling competition for a limited resource (Zaviezo & Mills 2000). Furthermore, the parasitoids had increased emergence from *B. mori* than from *A. gemmatalis*, indicating that host quality can vary with the host (Vinson & Iwantsch 1980, Jervis *et al*. 2008). These results demonstrate that the host species affect the development period of this parasitoid (Bittencourt & Berti Filho 2004).

The size of *P. elaeisis* adults from *B. mori* and *A. gemmatalis* pupae were adequate for quality control in their production and use due to similarities in the biological parameters of *P. elaesisis* from *T. arnobia*. This is important, because the body size in many parasitoids is positively correlated with important functional traits including mating, fecundity, reproductive longevity, emergence of progeny and sex ratio (Sagarra *et al*. 2001, Moreira *et al*. 2009).

The similar longevity of *P. elaeisis* females and males when reared on *B. mori* and *A. gemmatalis* pupae shows that this parasitoid was not affected by these hosts. This has also been reported for *Hyssopus pallidus* (Askew, 1964) (Hymenoptera: Eulophidae) on *Cydia molesta* (Busck, 1916) and *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae) pupae (Häckermann *et al*. 2007). However, it differs from results for *M. acasta* when developing on *C. erythrocephala* pupae of different ages (Imandeh

2006). This indicates that some parasitoids are not able to convert energy gained to increase their longevity (Hein & Dorn 2008).

The offspring sex ratio of *P. elaeisis* was similar when developing on *B. mori* and *A. gemmatalis* pupae. This was also reported for this parasitoid on *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) (Bittencourt & Berti Filho 1999) and previously refrigerated *B. mori* pupae (Pereira *et al.* 2009). This was also similar to *Melittobia clavicornis* (Cameron, 1908) (Hymenoptera: Eulophidae) developing on *Trypoxylon politum* Say, 1837 (Hymenoptera: Sphecidae), *Neobellieria bullata* (Parker, 1916) (Diptera: Sarcophagidae) and *Antrax* sp. (Diptera: Bombyliidae) pupae (Gonzáles *et al.* 2004). However, the sex ratio of Hymenoptera can vary with the stage, age or nutritional status of the host as well as the age of the parasitoid (Uçkan & Gülel 2002), besides the temperature (Pereira *et al.* 2004, Pratissoli *et al.* 2006, Pandey & Tripathi 2008, Pastori *et al.* 2008).

The reproductive success, the capacity to produce females, and the complete development characteristics of *B. mori* and *A. gemmatalis* pupae indicate that they are appropriate hosts for *P. elaeisis*. Moreover, the development and reproduction of *P. elaeisis* on pupae of the natural host *T. arnobia* were not reduced after six generations on *A. gemmatalis* and *B. mori* pupae, which demonstrates that this parasitoid can be easily reared with *A. gemmatalis* and *B. mori* for the biological control of *T. arnobia*.

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