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PALMISTICHUS ELAEISIS (HYMENOPTERA: EULOPHIDAE) PARASITIZING PUPAE OF *CITIOICA ANTHONILIS* (LEPIDOPTERA: SATURNIIDAE) COLLECTED ON *PIPTADENIA GONOACANTHA* (FABACEAE)

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ABSTRACT. The moth *Citioica anthonilis* (Herrich-Schaeffer, [1854]) (Lepidoptera: Saturniidae: Ceratocampinae) occurs in areas of preserved forests, where it is a significant defoliator of *Piptadenia gonoacantha* (Martius) Macbride (Fabaceae) trees. In this study, caterpillars of fourth instar *C. anthonilis* were collected from the ground after falling from a *P. gonoacantha* tree in a herbarium and were reared in the laboratory. Pupae of *C. anthonilis*, the velvetbean caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae), and the flour beetle *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae) were each parasitized by mated parasitoid females wasp *Palmistichus elaeisis* Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae). Data were collected relating to the levels of parasitism and emergence rates of *P. elaeisis* per host pupa, and the size of the host pupae. Our results show that the fecundity of *P. elaeisis* was highest in *C. anthonilis* hosts, probably because of the greater size of these pupae, which supported the development of an increased number of parasitoids. Therefore, *C. anthonilis* is a suitable host for rearing *P. elaeisis* in the laboratory, which could be a means of rearing parasitoids for the biological control of this defoliator of *P. gonoacantha* and other pests in Brazil.

Additional key words: biological control, forest insects, host, parasitism, pupal parasitoid

Citioica anthonilis (Herrich-Schaeffer, [1854]) (Lepidoptera: Saturniidae: Ceratocampinae) occurs in Central America, Guyana, Amazonia, the Andean region from Mexico to Bolivia and southern Brazil, in tropical forests with an annual rainfall of between 250 mm and 2500 mm, and is a bioindicator of habitat conservation (Regier et al. 2008, Stefanescu et al. 2009). *Citioica anthonilis* completes its larval development on *Robinia pseudoacacia* L. (Fabaceae) and *Salix caprae* L. (Salicaceae); its larvae are green in color with a black stripe on each side (Prestes et al. 2009). The length of the forewing *C. anthonilis* females collected in Iraí, Rio Grande do Sul State, Brazil (Atlantic Forest biome) was 36–43 mm, in contrast to that of the male, which was 26–31 mm long; the flight period of this insect is January in this region (Prestes et al. 2009).

The parasitoid wasp *Palmistichus elaeisis* Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae) is an important natural enemy of pests on palms (Arecaceae), eucalyptus (Myrtaceae), passion fruit (Passifloraceae) and *Terminalia catappa* L. (Combretaceae) (Gil-Santana & Tavares 2006, Pereira et al. 2011, Tavares et al. 2012a),

attacking the pupae of lepidopteran defoliators (Delvare & LaSalle 1993, Pereira et al. 2010a, Tavares et al. 2012b). Importantly, it can also be reared in the laboratory on alternative lepidopteran and coleopteran host pupae (Zanuncio et al. 2008, Pereira et al. 2009, 2010b), including those of species of the genus *Hylesia* Hübner, [1820] and *Dirphia moderata* Bouvier, 1919 (Lepidoptera: Saturniidae) (Pereira et al. 2008, Soares et al. 2009).

The objective of this study was to assess the suitability of alternatives hosts for rearing *P. elaeisis* in the laboratory. We used pupae of its natural host, *C. anthonilis*, and also of the velvetbean caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae), and the flour beetle *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae). We recorded the rates of parasitism, and emergence of *P. elaeisis* from pupae of these species, as well as the length and width of each host pupa.

Materials and Methods

On May 4, 2011, 25 fourth-instar caterpillars of *C. anthonilis* were collected from the ground after falling from a *Piptadenia gonoacantha* (Martius) Macbride (Fabaceae) tree in the herbarium of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil (20º45'S, 42º51'W, 651 m above sea level; Atlantic Forest biome) (Tavares et al. 2011a). The occurrence of *P. gonoacantha* is widespread throughout the Atlantic Forest biome of Brazil (Marques et al. 2009, Braga et al. 2011). The caterpillars were brought to the Laboratory of Biological Control of Insects (LCBI) from UFV and kept at 25 ± 1ºC, under a 12-h photoperiod and 70 ± 10% relative humidity (RH) in 1L plastic cups with a thin layer of sand in the bottom and *P. gonoacantha* branches until they pupated, which occurred on May 10 and 11, 2011.

In total, 20 newly formed *C. anthonilis* pupae were each placed in a test tube (14 cm length × 2.2 cm diameter) closed with a cotton swab and kept for 10 days with 40 mated *P. elaeisis* females of a first generation emerged from pupa of *Thagona tibialis* Walker, 1855 (Lepidoptera: Lymantriidae). This number of females was used based on results of a preliminary test using 10, 20, 30, 40 or 50 *P. elaeisis* females per *C. anthonilis* pupa. Pupae of *T. tibialis* had been collected from a *T. catappa* tree in the campus of UFV (Tavares et al. 2012a, 2012b). A drop of honey was placed inside the test tubes as food for the parasitoid females. Recordings were made of the rates of parasitism and emergence, as well as the number of parasitoids that emerged per *P. elaeisis* pupa. In addition, the width and length of each *C. anthonilis* pupa were recorded.

To study the parasitism by *P. elaeisis* of *A. gemmatalis* and of *T. molitor*, 20 newly formed pupae of each host species were each placed in a test tube (14 cm length × 2.2 cm diameter) for 48 hours with six mated *P. elaeisis* females, based on the methodology proposed for *A. gemmatalis* (Pereira et al. 2010c) and *T. molitor* (Zanuncio et al. 2008). The pupae were obtained from the mass rearing of these insects from the LCBI of the UFV (Pereira et al. 2010b, Zanuncio et al. 2011). The same data recordings were made as for *C. anthonilis* detailed above.

The parasitism of *C. anthonilis*, *A. gemmatalis* and *T. molitor* pupae by *P. elaeisis* was evaluated according to the following criteria: parasitized pupae turned caramel in color, whereas unviable pupae were black in color, lost weight, became hollow and then died and non-parasitized pupae had emergence of adult (Zanuncio et al. 2008, Pereira et al. 2010c). The design was entirely randomized with three treatments represented by *C. anthonilis*, *A. gemmatalis* and *T. molitor*. Each treatment had 20 replications. Data of the mean ± standard error of mean of parasitism and emergence rates were obtained. Data relating to the number of parasitoids of *P. elaeisis* that emerged per host pupa, and the width and length of the pupae were submitted to Analysis of Variance (ANOVA) and the means (± standard error of mean) compared between species by Tukey test at 5% probability, using the software Statistical Analysis Software (SAS/STAT 1989) (Supplier: UFV).

Adult females of *P. elaeisis* were photographed in the UFV. These pictures were sent to the Department of Biology of Lund University in Sölvegatan, Lund, Sweden for identification by Dr. Christer Hansson. Five pupae of *C. anthonilis* were kept in plastic cups until adult emergence. Four adult females were sent to the Department of Zoology of Federal University of Paraná in Curitiba, Paraná State, Brazil for identification by Dr. Olaf Hermann Hendrik Mielke. Second (Fig. 1), fourth (Fig. 2) and fifth instar (Fig. 3) caterpillars, and adult (Fig. 4) *C. anthonilis* were photographed by Leroy Simon and these pictures are available at http://www.silkmoths.bizland.com/phlsimon.htm.

Results

Pupae of *C. anthonilis* were longer (3.82 ± 0.28 cm) and wider (0.8 ± 0.04 cm) than those of *A. gemmatalis* (1.68 ± 0.19 cm and 0.67 ± 0.02 cm, respectively), which, in turn, were longer and wider than those of *T. molitor* (1.47 ± 0.12 cm and 0.59 ± 0.01 cm, respectively) ($F_{2,57}$; $P<0.05$ in both cases).

In total, *P. elaeisis* parasitized 100% of *C. anthonilis* pupae, 90% of *T. molitor* pupae and 70.0% of *A.*

FIG. 1. Caterpillar of second instar of *Citioica anthonilis* Herrich-Schäeffer, 1854 (Lepidoptera: Saturniidae).

FIG. 3. Caterpillar of fifth instar of *Citioica anthonilis* Herrich-Schäeffer, 1854 (Lepidoptera: Saturniidae).

gemmatalis pupae. The same values applied to the emergence of *P. elaeisis* from the pupae of each species (i.e. parasitoids emerged from each of the pupae that had been parasitized).

More *P. elaeisis* emerged per *C. anthonilis* pupa (286 ± 29 insects) than from *A. gemmatalis* pupae (108 ± 17 insects), totals that were both higher than from *T. molitor* pupae (69 ± 7 insects) ($F_{2,57}$; $P<0.05$).

DISCUSSION

This is the first report of the parasitism of *C. anthonilis* pupae by *P. elaeisis* in the laboratory. The parasitism of *C. anthonilis* by progeny of *P. elaeisis* collected from *T. tibialis* pupa sampled from *T. catappa* trees in Viçosa confirms the ability of a wild strain of *P. elaeisis* to parasitize *C. anthonilis* pupae in the laboratory. However, wild strains of parasitoids might require several generations in the laboratory to develop an adequate parasitic ability on alternative hosts, including *P. elaeisis* collected in the field in Viçosa on pupae of *A. gemmatalis*, *Bombyx mori* Linnaeus, 1758 (Lepidoptera: Bombycidae) and *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) (Pereira et al. 2010b, 2011).

Parasitism of *C. anthonilis* pupae by *P. elaeisis* suggests that this host is suitable for rearing this parasitoid in the laboratory. The occurrence of *C. anthonilis* in preserved areas of native forests in several

FIG. 2. Caterpillar of fourth instar of *Citioica anthonilis* Herrich-Schäeffer, 1854 (Lepidoptera: Saturniidae).

FIG. 4. Adult of *Citioica anthonilis* Herrich-Schäeffer, 1854 (Lepidoptera: Saturniidae).

States and in the Federal District of Brazil and the adaptation of *P. elaeisis* and other hymenopteran parasitoids in urban areas to *T. catappa* and to plantations of crop species from the Arecaceae, Myrtaceae and Passifloraceae suggests the need to maintain areas of original vegetation near agricultural and forest crops to increase natural biological control (Woodcock & Vanbergen 2008, Pickett et al. 2009), particularly by eulophid parasitoids on pupae of arctiid, geometrid, lymantriid, noctuid and saturniid defoliators (Murakami & Hirao 2010). This was shown by fewer outbreaks of lepidopterous pests on *Eucalyptus grandis* Hillex Maiden (Myrtaceae) plantations near areas of native forest due to the refuges for natural enemies (Zanuncio et al. 2001). Natural enemies might be more frequent in mixed environments, as plants of *Crotalaria juncea* L. (Fabaceae) grown near to *Zea mays* L. (Poaceae) act as a refuge for these organisms and so aid the natural biological control of herbivores of this important crop (Tavares et al. 2011b, 2012c, 2012d).

As in our investigation, more individuals of *P. elaeisis* have been recorded to emerge per *C. anthonilis* pupa than from *T. molitor* pupae in other studies [only 71 individuals (Zanuncio et al. 2008)] or from pupae of *Thyrinteina leucoceraea* Rindge, 1961 (Lepidoptera: Geometridae) [only 194 individuals (Pereira et al. 2008)]. This can be explained by the larger size in terms of length and width of *C. anthonilis* pupae compared with those of *T. molitor*, *T. leucoceraea* and *A. gemmatalis*. Pupae of *B. mori*, which are of a similar or larger size to those of *C. anthonilis*, produced 550 individuals of *P. elaeisis*, but rearing this host is difficult in the laboratory (Pereira et al. 2009).

Our study verified that a wild strain of *P. elaeisis* was more successful at parasitizing pupae of *C. anthonilis* than those of *A. gemmatalis* and *T. molitor* in the laboratory. This is the first report of parasitism by *P. elaeisis* of *C. anthonilis* pupae in the laboratory and provides background information for the laboratory rearing of *P. elaeisis* as a means of biological control of *C. anthonilis* and other pests.

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