Parasitism of Lepidopteran Defoliators of Urban Plants by Palmistichus elaeisis (Hymenoptera: Eulophidae)

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Parasitism of lepidopteran defoliators of urban plants by *Palmistichus elaeisis* (Hymenoptera: Eulophidae)

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Abstract

Heraclides anchisiades capys (Lepidoptera: Papilionidae), *Citioica anthonilis* (Lepidoptera: Saturniidae), and *Methona themisto* (Lepidoptera: Nymphalidae) are tree and shrub pests commonly found in urban areas. The parasitism capacity of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) was evaluated on pupae of these lepidopteran pests as well as on 2 commonly used alternative hosts, *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) and *Tenebrio molitor* (Coleoptera: Tenebrionidae). The number of parasitoids produced per mg of host pupal biomass was significantly greater on *H. anchisiades capys* and *T. molitor* compared with the other 3 lepidopteran species. The mean number of parasitoids emerging per host pupa was also significantly more on *H. anchisiades capys* compared with *C. anthonilis*, *M. themisto*, *A. gemmatalis*, or *T. molitor*. All host species tested in this study show the potential to be used in mass rearing *P. elaeisis* in the laboratory. However, the lower parasitism and emergence of *P. elaeisis* on *H. anchisiades capys* pupae suggests that this lepidopteran pest may not be a suitable host for this parasitoid in the field.

Key Words: alternative host; mass rearing; parasitism rate; *Tenebrio molitor*

Resumen

Heraclides anchisiades capys (Lepidoptera: Papilionidae), *Citioica anthonilis* (Lepidoptera: Saturniidae) y *Methona themisto* (Lepidoptera: Nymphalidae) son plagas de árboles y arbustos, comúnmente, encontrados en áreas urbanas. La capacidad de parasitismo de *Palmistichus elaeisis* (Hymenoptera: Eulophidae) fue evaluada en pupas de estos lepidópteros plaga, así como en dos hospederos alternativos comúnmente usados, *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) y *Tenebrio molitor* (Coleoptera: Tenebrionidae). El número de parasitoides producido por miligramo de biomasa de pupa del hospedero fue significantemente mejor en *H. anchisiades capys* y *T. molitor* comparado con otras tres especies de lepidópteras. El número promedio de parasitoides emergidos por pupa de hospedero fue significantemente mayor en *H. anchisiades capys* comparado con *C. anthonilis*, *M. themisto*, *A. gemmatalis y T. molitor*. Todas las especies de hospederos probados en este estudio, mostraron potencial para la cría en masa de *P. elaeisis* en laboratorio. Sin embargo, el bajo parasitismo y emergencia de *P. elaeisis* en las pupas de *H. anchisiade scapys*, sugiere que este lepidóptero plaga no sea un hospedero adecuado para ese parasitoide en el campo.

Palabras Clave: cría en masa; hospedero alternativo; tasa de parasitismo; *Tenebrio molitor*

Thagona tibialis Walker (Lepidoptera: Erebidae) (= *Stilpnotia tibialis* Walker) is considered a public health problem because its larval hairs and scales from adult moths cause allergies in humans (Tavares et al. 2014). This pest has been reported from Argentina to Costa Rica as well as Brazil including Belo Horizonte and Viçosa, Minas Gerais State; Morro Reuter, Rio Grande do Sul State; and Brasília, Federal District (Tavares et al. 2014), where it is a serious defoliator of *Terminalia catappa* L. (Combretaceae). Larvae have been reported to infest urban areas, such as gardens, parks, parking lots, and street lines where *T. catappa* trees occur (Tavares et al. 2014). Adults of the gregarious pupa endoparasitoid, *Palmistichus elaeisis* Delvare & La Salle (Hymenoptera: Eulophidae) have been observed to emerge from *T. tibialis* pupae collected on a *T. catappa* tree in Viçosa (Tavares et al. 2012, 2013a). This biological control agent parasitizes pupae of several coleopteran and lepidopteran species, including *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Worldwide, *T. molitor* often is used as an alternative host to mass rear this parasitoid (Zanuncio et al. 2008). Biological control programs using generalist parasitoids are a pest management option for urban areas where people and animals preclude the use of many traditional chemical control options (Tavares et al. 2011, 2013b; Zanuncio et al. 2013). We believe that generalist parasitoids, such as *P. elaeisis*, may provide a useful tool for screening a variety of suitable

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alternative hosts that could augment the high number of mass reared individuals required for biological control programs for agriculture and forestry pests. We report here on a study to evaluate the parasitism capacity of *P. elaeisis* on pupae of foliage pests of shrubs and trees commonly found in urban areas for possible use in biological control programs aimed at controlling *T. tibialis*. In addition, the suitability of 2 widely used alternative hosts of the parasitoid, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) and *T. molitor*, also was evaluated under laboratory conditions.

Materials and Methods

INSECTS

Thagona tibialis pupae were collected during May 2010 in an urban area in Viçosa (20.7500°S, 42.8500°W; 650 masl). The location is characterized by subperennial tropical forest-type vegetation of the Atlantic Forest biome. Fifty *T. tibialis* pupae, unknown age, were collected using thick-tip tweezers (150 mm H × 0.4 mm W) from 10 *T. catappa* trees spaced 5 m apart. These pupae were placed in 500 mL plastic pots lined with cotton wool. These pots were brought to the Laboratory of Biological Insect Control at the Universidade Federal de Viçosa in Viçosa, Minas Gerais State, Brazil, where they were kept at 25 ± 1 °C, 70 ± 10% RH, and 12:12 h (L:D) photoperiod. *Terminalia catappa* was identified by morphology of its reproductive organs (Ivani et al. 2008). Samples of this plant were deposited in the herbarium of the Departamento de Biologia Vegetal at the Universidade Federal de Viçosa.

Thagona tibialis pupae were individually placed into glass test tubes (14 cm L × 2.2 cm diam) lined with cotton wool. Four male and 4 female *T. tibialis* emerged from these pupae, then were mounted and identified as an atypical form of this species, lacking dots on the forewings. In addition to the adults, pupae, egg masses, and larvae also were mounted and deposited in the Regional Museum of Entomology at the Universidade Federal de Viçosa. Adult specimens also were deposited in the Instituto Uiraçu in Camacan, Bahia state, Brazil.

Fifty-four *P. elaeisis* individuals emerged from a *T. tibialis* pupa and 10 individuals were used to identify this species using the taxonomic keys of Delvare & La Salle (1993). Another 10 individuals were deposited in the Department of Biology at Lund University in Sölvegatan, Lund, Sweden, and in the Regional Museum of Entomology at the Universidade Federal de Viçosa. The final 34 individuals were used to start a colony of *P. elaeisis* in the laboratory using methods described by Zanuncio et al. (2008).

Host caterpillars were captured on shrubs and trees of 80 species from 21 plant families grown on the Universidade Federal de Viçosa campus adjacent to *T. catappa* trees (up to 2 km away) when most *T. tibialis* were in the pupal stage. Around 100 final-instar larvae were collected from each location using a fine-tip brush; these were *Citioica anthonilis* (Herrich-Schaeffer) (Lepidoptera: Saturniidae) on *Piptadenia gonoacantha* (Martin) Macbride (Fabaceae); *Heraclides anchisiades capys* (Hübner) (Lepidoptera: Papilionidae) on *Citrus* sp. (Rutaceae), and *Methona themisto* (Hübner) (Lepidoptera: Nymphalidae) on *Brunfelsia uniflora* (Pohl) D. Don (Solanaceae) and *Inga edulis* Martius (Fabaceae). Caterpillars were placed in 500 mL plastic pots lined with cotton wool and transported to the laboratory at the Universidade Federal de Viçosa where they were subsequently transferred to screened rearing cages (30 cm W × 30 cm H × 30 cm L). The petiole of branches from their plant hosts were inserted into a 50 mL plastic vial with water in screen cages until pupation at 25 ± 1 °C, 70 ± 10% RH, and 12:12 h (L:D) photoperiod. Frass and plant debris were removed from cages and fresh foliage provided ad libitum daily.

Fifty 24-h-old pupae (mean ± SE) of *A. gemmatalis* (279.90 ± 7.30 mg), *C. anthonilis* (748.80 ± 18.08 mg), *H. anchisiades capys* (429.12 ± 11.98 mg), *M. themisto* (427.92 ± 12.64 mg), and *T. molitor* (93.70 ± 1.13 mg) each were placed in a glass test tube (14 cm L × 2.2 cm diam) with four 72-h-old *P. elaeisis* females previously mated at 48 h from the laboratory colony at the Universidade Federal de Viçosa. Honey was provided ad libitum as food for parasitoids and tubes were sealed with a cotton swab. All 250 pupae were subjected to parasitism at the same time. First generation (F_1) parasitoids obtained from field-collected *T. tibialis* pupae were used in all bioassays. Pupae were exposed to parasitoids for 24 h (Zanuncio et al. 2008) and remained in tubes until emergence of parasitoids or adult hosts.

Anticarsia gemmatalis pupae were obtained from a larval laboratory colony reared on an artificial diet (Ferreira et al. 2008) for approximately 5 yr. *Tenebrio molitor* pupae were obtained from larvae grown on an alternative artificial diet (Tavares et al. 2017) from an established laboratory colony in culture for approximately 10 yr. Insect hosts (*C. anthonilis*, *H. anchisiades capys*, and *M. themisto*) (Leite et al. 2010; Barbosa & Costa 2013; Miranda et al. 2015) and plant species (*B. uniflora*, *Citrus* sp., *I. edulis*, and *P. gonoacantha*) (Filipowicz et al. 2012) were identified by comparing them with taxonomic keys and descriptions. Each treatment (host species) consisted of 50 replicate pupae. Mean numbers of pupae parasitized were compared between treatments using a completely randomized study design.

PARASITOID PRODUCTION

The parasitoid variables evaluated in this study were developmental time from egg to adult (d), parasitism and parasitoid emergence (%), proportion of females (number of females ÷ number of insects), body length, head capsule width (mm), male and female longevity (d), and mean number of parasitoids emerged per pupa. Bioassay results were expressed as mg of host pupal biomass. Parasitism of host pupae by *P. elaeisis* was evaluated according to three outcomes: successfully parasitized, unsuccessfully parasitized, and not parasitized. Successfully parasitized pupae were caramel in color and produced *P. elaeisis* adults. Unsuccessfully parasitized pupae were black, hollow, shrunken, and eventually died. Non-parasitized pupae produced adult moths or beetles depending on host species (Zanuncio et al. 2008). *Palmistichus elaeisis* males and females were identified by analyzing morphological characteristics of their antennae and abdomen (Delvare & La Salle 1993). Measurements of body length and head capsule width of *P. elaeisis* adults were obtained with a Model WF ocular micrometer (10×) attached to a stereomicroscope Luxeo 4D (Labo America, Inc., Fremont, California, USA).

STATISTICS

The data were distributed normally and the variances were homogenous (PROC UNIVARIATE; GPLOT PROC), so data transformation was not necessary. Data were analyzed with one-way analysis of variance and means compared using the Tukey multiple comparison test ($\alpha = 0.05$) (Tukey 1949) except those of parasitism and emergence rates, which were analyzed by general linear models (GLM) of binomial distributions (PROC GLM) ($\alpha = 0.05$). This analysis was performed with the original data expressed as percentages. All statistical analyses used SAS software, version 9.2 (SAS Institute 2008).

Results

Significantly fewer *H. anchisiades capys* pupae (40.5 ± 2.2%) were parasitized as compared to *A. gemmatalis*, *C. anthonilis*, *M. themisto*, and *T. molitor* pupae (100%) (χ^2 = 4.880; *P* = 0.0026). Parasitoid emergence was highest from *C. anthonilis*, *M. themisto* (100%) and *T. molitor* (92.3 ± 4.7%) pupae; intermediate from *A. gemmatalis* (72.1 ± 2.9%) pupae, and lowest from *H. anchisiades capys* (40.4 ± 1.6%) pupae (χ^2 = 7.240; *P* = 0.0013). The parasitoid developmental period from egg to adult was longer in *T. molitor* pupae (23.42 ± 0.18 d) than in *A. gemmatalis* (21.60 ± 0.16 d), *H. anchisiades capys* (21.15 ± 0.15 d), *M. themisto* (20.42 ± 0.15 d), or *C. anthonilis* pupae (19.34 ± 0.14 d) (χ^2 = 5.010; *P* = 0.0019) (Table 1). The longevity of parasitoid females was greater when reared in *C. anthonilis* pupae (26.41 ± 0.82 d) than with those in *M. themisto* (23.91 ± 1.04 d), *H. anchisiades capys* (23.72 ± 1.15 d), *A. gemmatalis* (23.15 ± 2.32 d), or *T. molitor* pupae (22.65 ± 1.13 d) (*F* = 5.31; df = 4; *P* = 0.0017) (Table 1). The longevity of males was greatest when reared in *H. anchisiades capys* pupae (30.10 ± 2.42 d), intermediate in *C. anthonilis* (28.89 ± 3.04 d), *T. molitor* (28.30 ± 2.38 d), and *M. themisto* (27.53 ± 2.01 d), and lowest in *A. gemmatalis* (24.30 ± 4.17 d) (*F* = 4.29; df = 4; *P* = 0.0044) (Table 1).

The head capsule of males was widest when reared in *T. molitor* pupae (0.45 ± 0.01 mm), intermediate in *C. anthonilis* (0.43 ± 0.01 mm), and narrowest in *M. themisto* (0.40 ± 0.01 mm), *H. anchisiades capys* (0.40 ± 0.02 mm), and *A. gemmatalis* (0.39 ± 0.01 mm) (*F* = 3.87; df = 4; *P* = 0.0087) (Table 2). The head capsule of females was widest when reared in *T. molitor* pupae (0.58 ± 0.01 mm), intermediate in *C. anthonilis* (0.55 ± 0.02 mm), *M. themisto* (0.54 ± 0.01 mm), and *H. anchisiades capys* (0.52 ± 0.02 mm), and narrowest in *A. gemmatalis* (0.50 ± 0.01 mm) (*F* = 4.91; df = 4; *P* = 0.0025) (Table 2).

The body length of *P. elaeisis* males was longer when reared in *M. themisto* (1.34 ± 0.02 mm), *T. molitor* (1.34 ± 0.02 mm), *C. anthonilis* (1.34 ± 0.01 mm), and *A. gemmatalis* (1.32 ± 0.03 mm) pupae than in *H. anchisiades capys* (1.28 ± 0.04 mm) (*F* = 4.14; df = 4; *P* = 0.0052) (Table 2). The body length of females was longest when reared in *T. molitor* (2.00 ± 0.03 mm) and *C. anthonilis* (2.00 ± 0.01 mm) pupae, intermediate in *M. themisto* (1.94 ± 0.02 mm), and shortest in *H. anchisiades capys* (1.92 ± 0.04 mm) and *A. gemmatalis* (1.88 ± 0.03 mm) (*F* = 4.94; df = 4; *P* = 0.0081) (Table 2).

The mean number of *P. elaeisis* individuals produced per mg of host pupal biomass was higher from *H. anchisiades capys* (0.7529 ± 0.0317) and *T. molitor* (0.7364 ± 0.0619) than with those of *A. gemmatalis* (0.3859 ± 0.0233), *C. anthonilis* (0.3824 ± 0.0160), and *M. themisto* (0.3232 ± 0.0071) (*F* = 4.81; df = 4; *P* = 0.0021) (Table 3). The mean numbers of parasitoids per host pupa (*F* = 3.85; df = 4; *P* = 0.0002) and total number of parasitoids produced per host species (*F* = 3.85; df = 4; *P* = 0.0002) was highest in *H. anchisiades capys* (323 ± 38 and 6,460 ± 760), intermediate in *C. anthonilis* (286 ± 29 and 5,720 ± 580), *M. themisto* (138 ± 9 and 2,760 ± 180), and *A. gemmatalis* (108 ± 17 and 2,160 ± 340), and lowest in *T. molitor* (69 ± 7 and 1,380 ± 140), respectively (Table 4). The proportion of females was similar for pupae of *A. gemmatalis* (0.96 ± 0.01), *H. anchisiades capys* (0.95 ± 0.02), *M. themisto* (0.95 ± 0.02), *C. anthonilis* (0.95 ± 0.01), and *T. molitor* (0.94 ± 0.01) (*F* = 3.01; df = 4; *P* = 0.06) (Table 4).

Discussion

In our study, life expectancy of *P. elaeisis* females reared in *A. gemmatalis* pupae was similar to that reported by Pereira et al. (2010, 2013) for laboratory-reared colonies of this species. Indeed, we found that the longevity of *P. elaeisis* adults exceeded 10 d, which has been considered sufficient for oviposition on lepidopteran hosts (Chichera et al. 2012). The head capsule width of both *P. elaeisis* sexes from *A. gemmatalis* pupae was similar to that of colonized individuals reared for 6 generations on artifical diet (Pereira et al. 2010). Wide head capsules of male and female *P. elaeisis* indicate greater robustness with higher parasitism and offspring production capacity (Camilo et al. 2015).

The body lengths of *P. elaeisis* males and females from *A. gemmatalis* pupae were similar when compared with the same colonized species at the sixth generation (1.34 ± 0.02 mm and 1.88 ± 0.03 mm) (Pereira et al. 2010). Generally, larger *P. elaeisis* females have greater reproductive potential and longevity during food shortages (Chichera et al. 2012). The number of *P. elaeisis* individuals produced per *A. gemmatalis* pupa was similar to that for colonized parasitoids of this species reared during the first (110.20 ± 19.37) (Pereira et al. 2010) and sixth generation (493.27 ± 1.04). This suggests that *P. elaeisis* is capable of adaptation to laboratory conditions (Pereira et al. 2010). In our study, the proportion of females of *P. elaeisis* that emerged from *A. gemmatalis* pupae was similar to that for this parasitoid in similar host pupae stored for 12 d at 12 °C (0.94 to 0.95) (Pereira et al. 2013).

We believe that the mass rearing of *P. elaeisis* for use in biological control programs for urban ornamental defoliators depends on suitable alternative hosts. *Citioica anthonilis*, *H. anchisiades capys*, or *M. themisto* pupae may not be suitable because of limited seasonal availability. Moreover, we found that the low parasitism and emergence rates of *P. elaeisis* on *H. anchisiades capys* pupae suggest this host is not suitable for this parasitoid in the field. This leads to *A. gemmatalis* and *T. molitor* pupae being preferable for mass rearing of the parasitoid. *Palmistichus elaeisis* is considered a generalist endoparasitoid of lepidopteran pupae that could facilitate its use in pest management programs. We believe that mass production and augmentive release

Table 1. Developmental time from egg to adult, and male and female *Palmistichus elaeisis* longevity (mean ± SE) on *Anticarsia gemmatalis*, *Citioica anthonilis*, *Heraclides anchisiades capys*, *Methona themisto*, and *Tenebrio molitor* pupae reared at 25 ± 1 °C, 70 ± 10% RH, and 12:12 h (L:D) photoperiod.

Species	Egg to adult (d)	Male longevity (d)	Female longevity (d)
A. gemmatalis	21.60 ± 0.16 B	24.30 ± 4.17 B	23.15 ± 2.32 B
C. anthonilis	19.34 ± 0.14 B	28.89 ± 3.04 B	26.41 ± 0.82 B
H. anchisiades capys	21.15 ± 0.15 B	30.10 ± 2.42 A	23.72 ± 1.15 B
M. themisto	20.42 ± 0.15 B	27.53 ± 2.01 B	23.91 ± 1.04 A
T. molitor	23.42 ± 0.18 A	28.30 ± 2.38 C	22.65 ± 1.13 B
CV (%)	12.2	15.8	8.5
ANOVA	(*F* = 5.01; df = 4; *P* = 0.0019)	(*F*= 5.31; df = 4; *P* = 0.0017)	(*F* = 4.29; df = 4; *P* = 0.0044)

CV = Variation coefficient. Means within the same column followed by the same letter do not differ (*P* < 0.05) by Tukey's multiple comparison test.

Table 2. Head capsule widths and body lengths (mean ± SE) of male and female *Palmistichus elaeisis* from *Anticarsia gemmatalis*, *Citioica anthonilis*, *Heraclides anchisiades capys*, *Methona themisto*, and *Tenebrio molitor* pupae reared at 25 ± 1 °C, 70 ± 10% RH, and 12:12 h (L:D) photoperiod.

Species	Male head capsule (mm)	Female head capsule (mm)	Male body length (mm)	Female body length (mm)
A. gemmatalis	0.39 ± 0.01 C	0.50 ± 0.01 C	1.32 ± 0.03 A	1.88 ± 0.03 C
C. anthonilis	0.43 ± 0.01 B	0.55 ± 0.02 B	1.34 ± 0.01 A	2.00 ± 0.01 A
H. anchisiades capys	0.40 ± 0.02 C	0.52 ± 0.02 B	1.28 ± 0.04 B	1.92 ± 0.04 C
M. themisto	0.40 ± 0.01 C	0.54 ± 0.01 B	1.34 ± 0.02 A	1.94 ± 0.02 B
T. molitor	0.45 ± 0.01 A	0.58 ± 0.01 A	1.34 ± 0.02 A	2.00 ± 0.03 A
CV (%)	5.5	9.6	8.3	10.7
ANOVA	(*F* = 3.87; df = 4; *P* = 0.0087)	(*F* = 4.91; df = 4; *P* = 0.0025)	(*F* = 4.14; df = 4; *P* = 0.0052)	(*F* = 4.94; df = 4; *P* = 0.0081)

CV = Variation coefficient. Means within a column followed by the same letter do not differ ($P < 0.05$) by Tukey's multiple comparison test.

Table 3. *Palmistichus elaeisis* (mean ± SE) individuals produced per mg of *Anticarsia gemmatalis*, *Citioica anthonilis*, *Heraclides anchisiades capys*, *Methona themisto*, and *Tenebrio molitor* pupa biomass and range at 25 ± 1 °C, 70 ± 10% RH, and 12:12 h (L:D) photoperiod.

Species	Individuals per mg	Range
A. gemmatalis	0.3859 ± 0.0233 B	[0.3626 – 0.4092]
C. anthonilis	0.3824 ± 0.0160 B	[0.3664 – 0.3990]
H. anchisiades capys	0.7529 ± 0.0317 A	[0.7212 – 0.7846]
M. themisto	0.3232 ± 0.0071 B	[0.3161 – 0.3303]
T. molitor	0.7364 ± 0.0619 A	[0.6745 – 0.7983]
CV (%)	9.85	
ANOVA	(*F* = 4.81; df = 4; *P* = 0.0021)	

CV = Variation coefficient. Means within a column followed by the same letter do not differ ($P < 0.05$) by Tukey's multiple comparison test.

Table 4. Individuals emerging per pupa and total number produced per host species (mean ± SE), and the proportion *Palmistichus elaeisis* that were females when reared on *Anticarsia gemmatalis*, *Citioica anthonilis*, *Heraclides anchisiades capys*, *Methona themisto*, and *Tenebrio molitor* pupae at 25 ± 1 °C, 70 ± 10% RH, and 12:12 h (L:D) photoperiod.

Species	Individuals per pupa	Total production	Proportion
A. gemmatalis	108 ± 17 C	2,160 ± 340 C	0.96 ± 0.01 A
C. anthonilis	286 ± 29 B	5,720 ± 580 B	0.95 ± 0.01 A
H. anchisiades capys	323 ± 38 A	6,460 ± 760 A	0.95 ± 0.02 A
M. themisto	138 ± 9 C	2,760 ± 180 C	0.95 ± 0.02 A
T. molitor	69 ± 7 D	1,380 ± 140 D	0.94 ± 0.01 A
CV (%)	39.4	39.4	4.5
ANOVA	(*F* = 3.85; df = 4; *P* = 0.0002)	(*F* = 3.85; df = 4; *P* = 0.0002)	(*F* = 3.01; df = 4; *P* = 0.06)

CV = Variation coefficient. Proportion of females = number of females ÷ number of insects. Means in each column followed by the same letter do not differ ($P < 0.05$) by Tukey's multiple comparison test.

of *P. elaeisis* represents a plausible avenue for biological control of defoliating caterpillars in urban trees and shrubs especially when several different lepidopteran pest species are present.

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