



***Trichospilus diatraeae* (Hymenoptera: Eulophidae): development and reproduction in Lepidoptera palm oil pests**

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(With 2 figures)

Abstract

Brazil is a leading palm oil producer, but the defoliating caterpillars *Opsiphanes invirae* Hübner *Brassolis sophorae* L. (Lepidoptera: Nymphalidae) can reduce the productivity of this crop. The aim of this study was to evaluate the development and reproduction of the parasitoid *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) in pupae of these oil palm defoliators. Ten *O. invirae* or *B. sophorae* pupae with up to two days old were exposed each to 30 *T. diatraeae* females for 48 hours. Parasitism and emergence of the progeny of *T. diatraeae* were similar in pupae of both Lepidoptera defoliators. The life cycle of this parasitoid was shorter in *O. invirae* (21.50 ± 0.42 days) than with those of *B. sophorae* (27.60 ± 1.80 days). The number of the progeny (669.00 ± 89.62) and dead immature (217.13 ± 58.18) of *T. diatraeae* were higher in *B. sophorae* pupae than in those of *O. invirae* with 447.83 ± 51.52 and 13.50 ± 5.23 , respectively. The sex ratio and female and male longevity of *T. diatraeae* emerged from these hosts were similar. The reproductive traits, especially the number of individuals (offspring) of *T. diatraeae* were better with *B. sophorae* pupae than with those of *O. invirae*.

Keywords: biological control, caterpillars defoliating, *Elaeis guineensis*, parasitoid.

***Trichospilus diatraeae* (Hymenoptera: Eulophidae): desenvolvimento e reprodução em lepidópteros-praga da palma de óleo**

Resumo

O Brasil é um dos principais produtores de óleo de palma, porém as lagartas desfolhadoras *Opsiphanes invirae* Hübner e *Brassolis sophorae* L. (Lepidoptera: Nymphalidae) podem comprometer a produtividade dessa cultura. O objetivo deste trabalho foi avaliar o desenvolvimento e aspectos reprodutivos do parasitoide *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) em pupas desses desfolhadores da palma de óleo. Dez pupas de *O. invirae* ou de *B. sophorae* com até dois dias de idade, foram expostas, cada uma, ao parasitismo por 30 fêmeas de *T. diatraeae*, por 48 horas. O parasitismo e a emergência da progênie de *T. diatraeae* foram semelhantes em pupas de ambas as espécies de lepidópteros desfolhadores da palma de óleo. O ciclo de vida desse parasitoide foi mais curto em pupas de *O. invirae* ($21,50 \pm 0,42$ dias) que com as de *B. sophorae* ($27,60 \pm 1,80$ dias). O número de progênie ($669,00 \pm 89,62$) e de imaturos mortos ($217,13 \pm 58,18$) de *T. diatraeae* foram maiores em pupas de *B. sophorae* que naquelas de *O. invirae* com $447,83 \pm 51,52$ e $13,50 \pm 5,23$, respectivamente. A razão sexual e a longevidade de fêmeas e machos de *T. diatraeae* emergidos desses hospedeiros foram semelhantes. As características reprodutivas, especialmente, o número de indivíduos (progênie) de *T. diatraeae* foram melhores com pupas de *B. sophorae* que com as de *O. invirae*.

Palavras-chave: controle biológico, lagartas desfolhadoras, *Elaeis guineensis*, parasitoide.

1. Introduction

The oil palm (*Elaeis guineensis* Jacq.) is of African origin and the main agro-industrial activity in humid areas in the Brazilian Amazon, Colombia, Ecuador, Malaysia, Indonesia and several African countries (Hansen et al., 2015). This culture is a source of vegetable oil for food production and Bio fuels with high profitability, employment generation fixing the man in the field and reduced environmental impacts (Thawaro and Te-chato, 2010; Abdalla et al., 2008). *Elaeis guineensis* is well adapted to the Amazon conditions with this region being that with the largest area in the world to expanding activity (Chia et al., 2009).

The Pará state is the largest palm oil producer in Brazil with an average productivity of six tonnes of oil/ha/year (Chia et al., 2009), but Lepidoptera defoliators as *Opsiphantes invirae* Hübner and *Brassolis sophorae* L. (Lepidoptera: Nymphalidae) can compromise this crop productivity (Ribeiro et al., 2010). The damage of Lepidoptera pests is increased due to control measures deficiency without biological or synthetic chemical insecticides to be used against these pests (Ribeiro et al., 2010; Parra et al., 2009; Pereira et al., 2009a).

The natural biological control agents in palm tree crop in Brazil includes Chalcididae (Hymenoptera), Tachinidae and Sarcophagidae (Diptera) parasitizing *Brassolis astyra* Godart, *B. sophorae*, *O. invirae* and *Opsiphantes* sp. (Lepidoptera: Nymphalidae) larvae and pupae in the coconut (*Cocos nucifera*) (Marciano et al., 2007, 2009) and oil palm (*E. guineensis*) (Tinôco et al., 2012) culture. This shows the importance of manipulating natural enemies to increase the biological control (Dobbs and Potter, 2016; Pereira et al., 2015; Smith et al., 2015).

Parasitic Hymenoptera can control insect pests, but the biology of these natural enemies in natural or alternative hosts must be understood (Boyd Júnior and Held, 2016). This is necessary because the host type can affect the oviposition rate, longevity, sex ratio, body size and fecundity (Silva-Torres et al., 2009) besides life history of natural enemies (Martins et al., 2016; Valente et al., 2016).

The pupal parasitoid *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) of Asian origin is gregarious, with polyphagous habit but with high prevalence in lepidopteran with its record in 1942 in the sugarcane borers, *Diatraea venosata* Walker (Lepidoptera: Pyralidae) (Bennett et al., 1987). This parasitoid has been studied for the biological control of pests in cucurbitaceous, eucalyptus, corn, pasture, soybean and sugar cane (Oliveira et al., 2016; Silva et al., 2015; Zaché et al., 2010).

This objective was to study the biology of the parasitoid *T. diatraeae* in the lepidopteran oil palm *B. sophorae* and *O. invirae*.

2. Material and Methods

This work was performed at the Agropalma Plant Complex in the municipality of Tailândia, Pará State, Amazon region of Brazil, $48^{\circ}50'30.57''$ (West longitude)

and $02^{\circ}34'37.51''$ (South latitude) in a room at 25 ± 2 °C, relative humidity of $70 \pm 10\%$ and photophase of 12 hours.

2.1. Rearing the alternative host

Rearing *Tenebrio molitor* L. (Coleoptera: Curculionidae) started with larvae obtained from the Entomology Laboratory of Embrapa Eastern Amazon, Belém, Pará State. This host was kept in plastic trays (39.3 x 59.5 x 7.0 cm) in a room (25 ± 2 °C, $70 \pm 10\%$ RH and 12 hours photophase) with paper sheets covering the substrate of wheat bran where these insect laid eggs. Newly hatched *T. molitor* were fed wheat bran (97%), yeast (3%) and chayote slices as liquid supply until the adult emergence (Zanuncio et al., 2000).

2.2. Rearing the parasitoid

Adults of *T. diatraeae* were kept in glass tubes (14.5 x 2.0 cm) plugged with cotton and with droplets of honey. The alternative host pupae *T. molitor*, up to 24 hours old, were exposed to parasitism for 48 hours at a temperature of 25 ± 2 °C, relative humidity of $70 \pm 10\%$ and photophase of 12 hours followed by removal of the parasitoids (Pereira et al., 2009b).

2.3. Collecting pest species

Immature *O. invirae* and *B. sophorae* were collected in the field and transported to the laboratory of Agropalma, where they were placed in wooden cages (50 x 50 x 70 cm) and fed *ad libitum* with *E. guineensis* leaves until pupation.

2.4. Bioassay

Ten *O. invirae* and *B. sophorae* pupae weighing $1,500 \pm 90$ mg and $2,300 \pm 200$ mg, respectively, and up to 48 hours at the pupa stage were used. These pupae were individualized in glass tubes (14.5 x 2.0 cm) and exposed to parasitism by 30 *T. diatraeae* females, which provide high fertility and parasitoid individuals with better morphological conditions (Ribeiro et al., 2010). These females were removed from the tubes after 48 hours of exposure to the hosts. The pupae and the female parasitoid density were determined in preliminary tests. These pupae were kept in a room at 25 ± 2 °C, $70 \pm 10\%$ RH and 12 h photophase until emergence of adult parasitoids or moths.

The duration of the life cycle (egg to adult), parasitism percentage, percentage and number of individuals emerged, sex ratio (number of females/total number of individuals), number of immature and parasitoid pupae that did not complete its development (host pupae were opened at forty days after parasitism and number of immature and dead parasitoid pupae counted) and the offspring longevity (males and females) were evaluated. The sex of *T. diatraeae* adults was determined by the morphological characteristics of the antenna and abdomen of this parasitoid (Paron, 1999).

2.5. Statistical analysis

The experiment was conducted in a randomized design with two treatments represented by the hosts (*O. invirae* and *B. sophorae*) parasitized by *T. diatraeae* with 10 replications,

each with one host pupae. The percentage of parasitism and emergence of the progeny were subjected to nonparametric analysis of variance and when significant, compared with the Wilcoxon test at 5% significance level. The data of cycle length, parasitoid number, immature dead, sex ratio and longevity of males and females *T. diatraeae* per host were tested using the F 5% significance level (SAS Institute, 1997)

3. Results

Parasitism ($P=0.32$) and emergency ($P=0.59$) of *T. diatraeae* were similar in pupae of the two lepidopteran oil palm defoliator, with parasitism rates of 100% and 90% and the emergence of 60% and 50% from *B. sophorae* and *O. invirae* pupae, respectively (Figure 1).

The duration of egg-adult of *T. diatraeae* was shorter in *O. invirae* (21.50 ± 0.42 days) pupae than in those *B. sophorae* (27.60 ± 1.80 days) ($P=0.005$) (Table 1, Figure 2). Pupae of *B. sophorae* ($2,300 \pm 200$ mg) were heavier than those of *O. invirae* ($1,500 \pm 90$ mg).

The numbers of adults emerged ($P=0.04$) and immature mortality ($P=0.01$) of *T. diatraeae* were higher in *B. sophorae* pupae (689.00 ± 89.62 and 217.13 ± 58.18) than in those

of *O. invirae* (447.83 ± 51.52 and 13.50 ± 5.23) (Table 1). The sex ratio ($P=0.29$) and the longevity ($P=0.34$) of males ($P=0.29$) *T. diatraeae* emerged from *B. sophorae* and *O. invirae* pupae were similar (Table 1).

4. Discussion

The similar parasitism of *B. sophorae* and *O. invirae* pupae by *T. diatraeae* confirms the generalist habit of this parasitoid, as reported with *Anticarsia gemmatalis* Hübner, *Heliothis virescens* (Fabricius), *Spodoptera frugiperda* (Smith) (Noctuidae) and *Diatraea saccharalis* (Fabricius) (Pyralidae) (Paron and Berti-Filho, 2000), *Thyrintea arnobia* (Stoll) (Geometridae) (Pereira et al., 2008) *Tenebrio molitor* L. (Tenebrionidae) (Favero, 2009) and *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Oliveira et al., 2016) pupae. Moreover, the similar percentage of emergence from *O. invirae* and *B. sophorae* compared to *T. diatraeae* confirms the quality and/or nutritional values of these host to the parasitoid, because these factors can affect the onset and development of natural enemies (Brodeur and Boivin, 2004; Zanuncio et al., 2008).

The shortest duration from egg to adult *T. diatraeae* in *O. invirae* pupae than in those of *B. sophorae* can be attributed to the shorter pupa stage of the host, because the faster metabolism of the host can reduce the parasitoids

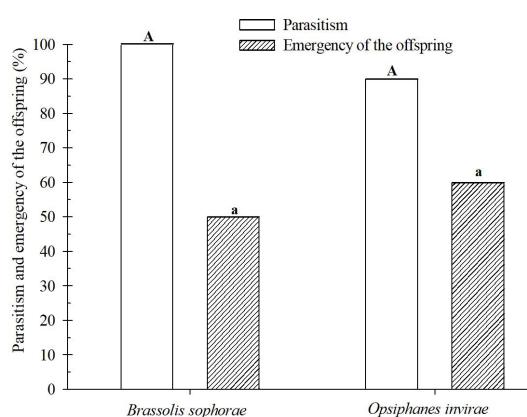


Figure 1. Parasitism and emergence (%) of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) in pupae of the *Opsiphanes invirae* *Brassolis sophorae* (Lepidoptera: Nymphalidae). Means followed by the same uppercase or lowercase letter do not differ by the nonparametric of Wilcoxon test ($p<0.05$).

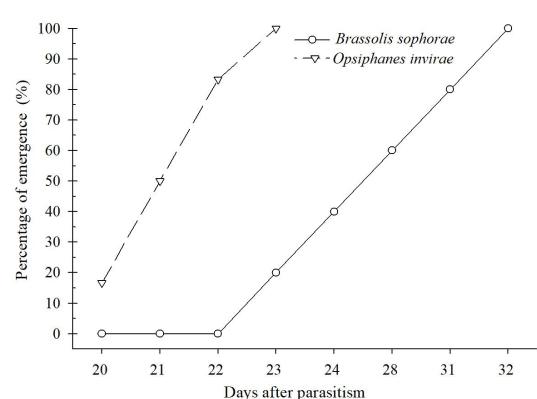


Figure 2. Accumulated percentage of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) individuals emerged from *Opsiphanes invirae* and *Brassolis sophorae* (Lepidoptera: Nymphalidae) pupae.

Table 1. Cycle length (days), progeny, number of immature, sex ratio, longevity (days) for males and females (mean \pm standard error) of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) in pupae of Lepidoptera palm oil defoliators.

Biological characteristics	<i>Opsiphanes invirae</i>	N	<i>Brassolis sophorae</i>	N
Egg - adult (days)	21.50 ± 0.42 b	6	27.60 ± 1.80 a	5
Progeny	447.8 ± 51.5 b	6	669.0 ± 89.6 a	5
Number of immature dead	13.5 ± 5.23 b	6	217.1 ± 58.2 a	8
Sex ratio	0.95 ± 0.01 a	6	0.97 ± 0.01 a	5
Longevity of females (days)	9.95 ± 1.25 a	20	12.0 ± 1.76 a	20
Longevity of males (days)	9.0 ± 1.35 a	10	7.3 ± 0.70 a	10

Means followed by the same letter per line do not differ by the F test ($p<0.05$).

cycle. The solitary endoparasitoid *Meteorus gyrtator* (Thunberg) (Hymenoptera: Braconidae) had shorter duration of the larval stage with *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) than with four other Noctuidae species, due to increased host speed development (*C. chalcites*) (Smethurst et al., 2004). Moreover, competition among immature *T. diatraeae* in the smaller *O. invirae* pupae and consequently the lower food resources available compared to larger *B. sophorae* pupae may also resulted in the development duration in that host (Aruna and Manjunath, 2010). The longest duration of *T. diatraeae* development in *B. sophorae* pupae can reduce the chances of host survival by it increases the period vulnerable to predators, pathogens or the host response (Benrey and Denno, 1997).

The larger *T. diatraeae* progeny in *B. sophorae* pupae that in those of *O. invirae* demonstrates a better quality of the first host for this parasitoid reproduction, what was helped by the greater pupae (Fidgen et al., 2000; Brodeur and Boivin 2004, Silva-Torres et al., 2009). Parasitoid females usually oviposit more eggs on larger hosts known as a principle of sex allocation (Jones, 1982), by having more food for immature stages (Uçkan et al., 2004). However, the immune defense of larger host can affect the development and survival of immature parasitoids (Strand and Pech, 1995; Andrade et al., 2010). The resource explored by immature *T. diatraeae* was similar in each host with 3.43 mg of fresh weight in *B. sophorae* [mean pupae weight ($2,300 \pm 200$ mg)/average number of progeny emerged (669.0 ± 89.6)] and 3.35 mg wet weight in pupae of *O. invirae* [weight ($1,500 \pm 90$ mg)/average number of progeny emerged (447.8 ± 51.5)] to produce each parasitoid adult. This confirms the hypothesis that host nutritional quality regulates the size of the population of *T. diatraeae* (Zaviezo and Mills, 2000; Bell et al., 2005). However, this differs from that observed for *Hyssopus pallidus* Askew (Hymenoptera, Eulophidae), where each milligram of wet weight of *Cydia molesta* Busck (lower host) produced 0.82 ± 3.8 and the same parasitoid of *C. pomonella* L. (Lepidoptera: Tortricidae) (bigger host) was able to produce only 0.27 ± 0.18 this parasitoid, showing nutritional quality between these different hosts (Häckermann et al., 2007).

The high number of immature dead in dissected *B. sophorae* pupae may be due to host immune defenses (Andrade et al., 2010) and/or the excessive egg numbers laid by *T. diatraeae* per host pupae (Jones, 1982). Competition among parasitoid larvae in the host can cause mortality and reduce the number of adults of these parasitoids emerged (Chong and Oetting, 2007).

A similarly high sex ratio of *T. diatraeae* in the lepidopteran *B. sophorae* and *O. invirae* pupae agrees with that reported for the parasitoid *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) in *Bombyx mori* L. (Lepidoptera: Bombycidae) pupae (Pereira et al., 2009a). This may be an Eulophidae (Pereira et al., 2010) and Ichneumonidae (Matos Neto et al., 2004) characteristic, facilitating these parasitoids to increase their populations in the laboratory and field (Matos Neto et al., 2005; Amalin et al., 2005). Furthermore, it indicates a high potential reproductive

capacity of *T. diatraeae* for integrated management of Lepidoptera oil palm defoliators.

A similar longevity of *T. diatraeae* males and females emerged from *B. sophorae* or *O. invirae* pupae indicates an adequate nutritional quality of these hosts, which can affect adult parasitoids (males and females) during its immature stage (Brodeur and Boivin, 2004). The parasitoid *T. diatraeae* has the potential for the biological control of Lepidoptera oil palm defoliators, especially, *B. sophorae*.

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