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Scientific note

Reproduction of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) in pupae of two lepidopterans defoliators of eucalypt

Reproducción de *Trichospilus diatraeae* (Hymenoptera: Eulophidae) en pupas de dos lepidópteros defoliadores de eucalipto

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Abstract: Biological control of lepidopteran defoliators using parasitoids is a promising alternative. The objective of this work was to evaluate the reproduction of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) in pupae of the eucalypt defoliators *Thyrinteina arnobia* (Lepidoptera: Geometridae) and *Hylesia paulex* (Lepidoptera: Saturniidae). Host pupae were individualized in glass tubes (14 x 2.2 cm) with six parasitoid females for 24 h under controlled conditions [25 ± 2ºC; 70 ± 10% (RH) and; 14 h photo phase]. *T. diatraeae* parasitized 95.8 ± 2.85% pupae of *T. arnobia* and 79.2 ± 6.72% of *H. paulex*, with an emergence rate of 89.6 ± 5.03% and 69.8 ± 6.13%, respectively. However, *H. paulex* pupae yielded large parasitoid progenies. No difference in the parasitoid sex ratio, adult size and longevity were observed between both hosts. The successful parasitism and development of *T. diatraeae* in pupae of *T. arnobia* and *H. paulex* suggest that this parasitoid can be an alternative for the biological control of these defoliators in eucalyptus plantations.

Key words: Biological control. *Hylesia paulex*. Lepidoptera. Parasitoids. *Thyrinteina arnobia*.

Resumen: El control biológico con parasitoides de los lepidópteros defoliadores con parasitoides es una alternativa prometedora. El objetivo de este trabajo fue evaluar la reproducción de *Trichospilus diatraeae* (Hymenoptera: Eulophidae) en pupas de los defoliadores del eucalipto *Thyrinteina arnobia* (Lepidoptera: Geometridae) e *Hylesia paulex* (Lepidoptera: Saturniidae). Pupas del anfitrión fueron individualizadas en tubos de vidrio (14 x 2,2 cm) con seis hembras del parasitoide durante 24 h en condiciones controladas [25 ± 2°C, 70 ± 10% (HR) y foto-período de 14 horas]. *T. diatraeae* parasita 95,8 ± 2,85% de las pupas de *T. arnobia* y 79,2 ± 6,72% de *H. paulex*, con un índice de emergencia de 89,6 ± 5,03% y 69,8% ± 6,13, respectivamente. Sin embargo, las pupas de *H. paulex* produjeron grandes progenies del parasitoide. No se observaron diferencias en la proporción de sexos, tamaño de adulto y longevidad de los parasitoides en las progenies. El éxito del parasitismo y desarrollo de *T. diatraeae* en las pupas de *T. arnobia* y *H. paulex* sugieren que este parasitoide puede ser una alternativa para el control biológico de estos defoliadores en plantaciones de eucalipto.

Palabras clave: Control biológico. *Hylesia paulex*. Lepidoptera. Parasitoides. *Thyrinteina arnobia*.

Introduction

The increase in the area with eucalyptus plantation in Brazil facilitates the adaptation of native Lepidoptera that can cause economical damage to these forests. The abundance of resources and the low occurrence of natural enemies can explain the loss inflicted by these insects in reforestations (Withers 2001). *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) is the main defoliator of eucalypt during population outbreaks. However, species of the genus *Hylesia* Hübner, 1820 (Lepidoptera: Saturniidae) are receiving more attention due to the damage in several agricultural and forest systems as well as to the health problems caused by the induction of dermatits in humans (Specht *et al.* 2006).

Between Eulophidae (Hymenoptera: Chalcidoidea) some are gregarious parasitoids, mainly of Lepidoptera (Paron and Berti Filho 2000; Pereira *et al.* 2008a; Pereira *et al.* 2008b; Zaché *et al.* 2010). *Trichospilus diatraeae* Cherian and Margabandhu, 1942 (Hymenoptera: Eulophidae) is a polyphagous endoparasitoid (Paron and Berti Filho 2000) that has received special attention in Brazil as it has been investigated as a potential biological control agent of eucalyptus defoliator pests (Pereira *et al.* 2008a). However, the use of parasitoids in biological control programs depends on the knowledge of their production in natural (Pastori *et al.* 2007) or alternative hosts in the laboratory (Pereira *et al.* 2009) for later release in the field.

The extension of eucalyptus plantations and the necessarily high volume of spraying is a constraint to the chemical control of defoliators in forest systems. Therefore, alternative control methods, such as the use of biological control with natural enemies (Paron e Berti Filho 2000, Pereira *et al.* 2008a), is required because they are economically and ecologically preferable (Monteiro *et al.* 2006).

Thus, our objective was to assess the suitability of pupae of *T. arnobia* and *Hylesia paulex* Dognin, 1922 (Lepidoptera: Saturniidae) for the development of *T. diatraeae* knowing their primary potential to control these pest species.

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Materials and Methods

Rearing the lepidopterans hosts and the parasitoid: **1)** Eggs of *T. arnobia* were obtained from a laboratory colony reared on branches of *Eucalyptus cloeziana* plants into organza fabric bags (0.70 x 0.40 cm). Larvae were removed and new food was offered every three days. Pupae were collected, sexed, separated in pairs and located in ventilated plastic pots (500 mL) under controlled conditions (25 ± 2ºC; 70 ± 10% relative humidity (RH) and 12 h photophase for egg laying. **2)** Eggs of *H. paulex* were obtained from a laboratory colony reared in ventilated plastic pots (500 mL) whit leaves of *Eucalyptus cloeziana* as food, which were replaced daily. Adult pairs were obtained, fed a 10% honey solution and placed into screened cages (30 x 30 x 30 cm) containing branches of *E. cloeziana* for egg laying and food. **3)** Adults of *T. diatraeae* were maintained in glass tubes (14 x 2.2 cm) and fed honey droplets. The tubes were closed with a cotton plug. Twenty-four to 48 h-old pupae of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) were exposed to the parasitism for 24 h under the controlled conditions earlier described for parasitoid development and adult emergence.

Experimental work. *T. diatraeae* were reared for one generation in pupae of both of the hosts in order to eliminate a likely pre-imaginal conditioning by creating the alternative host, *A. gemmatalis*. Each pupae of host, *T. arnobia* (187.4 ± 10.14 mg) and *H. paulex* (468.7 ± 24.30 mg), with up to 24 hours, were individualized in glass tubes (14 x 2.2 cm) with six females of *T. diatraeae* for 24 hours under controlled conditions [25 ± 2ºC; 70 ± 10% relative humidity (RH) and 14 h photo phase]. Females of this parasitoid were removed from the tubes after 24 hours and the parasitized pupae returned to the acclimatized chamber at described conditions where they were maintained until the emergence of the parasitoids.

Parasitism rate, emergence rate, number and size of the parasitoids emerged per pupa, duration of the cycle (egg-adult), sex ratio (based on the characteristics of the antennas and calculated with the equation SR= number of females/number of adults) and longevity were recorded.

The experimental design was fully randomized with 16 replications each with a group of tree host pupae for determine of parasitism rate, emergence rate, number and size of the parasitoids emerged per pupa, duration of the cycle and sex ratio and 12 and 24 replications constituted by 12 males and 24 females, selected on the descendants of each treatment for longevity and head capsule width of males and females, respectively. The averages were compared by ANOVA test with the test F at 5% probability level.

Results

The parasitism (F = 1.1710; df = 30.0; P = 0.2878) of *T. arnobia* and *H. paulex* pupae by *T. diatraeae* was 95.8 ± 2.85% and 89.6 ± 5.03% with emergence (F = 1.0630; df = 30.0; P = 0.3107) of 79.2 ± 6.72% and 69.8 ± 6.13%, respectively, without differences (Table 1). The total of individuals of this parasitoid emerged was 2.4 times larger from pupae of *H. paulex* (341.8 ± 33.65) than from those of *T. arnobia* (141.4 ± 17.27) (F = 28.070; df = 30.0; P ≤ 0.00001), but the average number of parasitoids emerged per gram of the host was similar (F = 0.0152; df = 30.0; P ≤ 0.9028) (Table 1).

The duration of the life cycle (egg-adult) of *T. diatraeae* was longer with pupae of *T. arnobia* (F = 10.6450; df = 30.0; P ≤ 0.0027) (Table 1). The sex ratio of this progeny was 0.99 ± 0.00 from both hosts (F = 0.0000; df = 30.0; P = 0.6117) (Table 1).

The longevity of females (F = 1.7723; df = 46.0; P = 0.1896) and males (F = 3.3777; df = 22.0; P = 0.0796) of *T. diatraeae* from pupae of *T. arnobia* and the size of head capsule of its females (F = 2.2530; df = 46.0; P = 0.1401) and males (F = 0.4940; df = 22.0; P = 0.4896) were similar to those emerged from *H. paulex* pupae (Table 1).

Discussion

Parasitism and emergence of *T. diatraeae* from the natural hosts *T. arnobia* and *H. paulex* suggest that this natural enemy can be present in the field when populations of these hosts are even at low numbers (Pereira *et al.* 2008b). Parasitism rate near 90.0% was also found for *T. diatraeae* in *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae); *A.*

Table 1. Biological parameters (Mean ± SE) of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) of the hosts *Hylesia paulex* (Lepidoptera: Saturniidae) and *Thyrinteina arnobia* (Lepidoptera: Geometridae) pupae. 25 ± 2ºC, RH: 70 ± 10% and photophase: 14 hours.

Biological parameters	*Thyrinteina arnobia*[1]	*Hylesia paulex*[1]
Parasitism (%)	95.80 ± 2.85 a	89.60 ± 5.03 a
Emergence (%)	79.20 ± 6.72 a	69.80 ± 6.13 a
Total of parasitoids emerged (n)	141.40 ± 17.27 b	341.80 ± 33.65 a
Parasitoids emerged for weight (grams) of host (n)	0.78 ± 0.10 a	0.76 ± 0.09 a
Life cycle (egg-adult) (days)	21.00 ± 0.62 a	18.50 ± 0.45 b
Sex ratio	0.99 ± 0.00 a	0.99 ± 0.00 a
Longevity of females (days)	23.62 ± 1.38 a	20.29 ± 2.09 a
Longevity of males (days)	16.91 ± 1.97 a	12.58 ± 1.29 a
Head capsules (females) (mm)	0.71 ± 0.01 a	0.73 ± 0.01 a
Head capsules (males) (mm)	0.55 ± 0.02 a	0.57 ± 0.02 a

[1] Means followed by the same letter, per line, are similar by the test F at 5%.

gemmatalis; *Spodoptera frugiperda* (J. E. Smith, 1797) and *Heliothis virescens* (Fabricius, 1781) (Lepidoptera: Noctuidae) in the laboratory (Paron and Berti Filho 2000). This indicates that some parasitoid of the Eulophidae family presents capacity to adaption to different hosts (Pereira *et al.* 2008b). The largest number of individuals of *T. diatraeae* emerged from pupae of *H. paulex* shows that its adult can produce more numerous progeny with the same initial individuals, such as observations with pupae of *D. saccharalis*, *A. gemmatalis*, *S. frugiperda* and *H. virescens* (Paron e Berti Filho 2000). This occurs because females of this parasitoid can determine the size of its host (Zaviezo and Mills 2000) and, for this reason, females of *T. diatraeae* probably laid lower number of eggs on pupae of the host *T. arnobia*. On the other hand, the larger reproductive success with *H. paulex* than with *T. arnobia* can be due to the largest nutritional resource of the first host.

The shorter duration of the life cycle (egg-adult) and the largest number of individuals of *T. diatraeae* per pupa of *H. paulex* indicate possible competition between immature of this parasitoid for nutrients, which does not influence the size of head capsule, as found for *Melittobia digitata* Dahms, 1984 (Hymenoptera: Eulophidae) with pupae of *Neobellieria bullata* (Parker, 1916) (Diptera: Sarcophagidae) (Silva-Torres and Matthews 2003). Although, differences on nutrient conversion show that the size of the offspring increased with the size of the host, as observed for *Hyssopus pallidus* (Askew, 1964) (Hymenoptera: Eulophidae) with larvae of *Cydia molesta* (Busck, 1916) and *C. pomonella* (L., 1758) (Lepidoptera: Tortricidae) (Häckermann *et al.* 2007) and *Melittobia clavicornis* (Cameron, 1908) (Hymenoptera: Eulophidae) with pupae of *Trypoxylon politum* Say, 1837 (Hymenoptera: Sphecidae), *N. bullata* and *Anthrax* sp. (Diptera: Bombyliidae) (González *et al.* 2004). This demonstrates that host species can affect parasitoid development (Paron and Berti Filho 2000; Jervis *et al.* 2008, Pereira *et al.* 2008b).

The similar sex ratio of *T. diatraeae* between the hosts *T. arnobia* and *H. paulex* demonstrates that their pupae are appropriate for the reproduction of this parasitoid, due to the fact that female are responsible for the production of the progeny (Pastori *et al.* 2007). The number of females produced depends on the host as reported for *Cirrospilus coachellae* Gates, 2000 (Hymenoptera: Eulophidae) in *Marmara gulosa* Guillén, Davis and Heraty, 2001 (Lepidoptera: Gracillariidae) and it should be considered when choosing parasitoids for mass rearing (Guillén *et al.* 2007).

The similar longevity of individuals of *T. diatraeae* (males and females) from pupae of *T. arnobia* and *H. paulex* indicates that nutritional resources of the both host allowed to supply the nutrients required by the parasitoid for reproduction and the energy necessary to maintain these function during life time (Imandeh 2006).

The development of *T. diatraeae* from *H. paulex* increases the range of hosts of this parasitoid with a species of the family Saturniidae (Lepidoptera) because this parasitoid was reported in Lepidoptera species of, at least, five families (Crambidae, Noctuidae, Arctiidae, Nymphalidae and Geometridae) (Pereira *et al.* 2008a).

The reproductive success of *T. diatraeae* with pupae of *T. arnobia* and *H. paulex* supplies basic information of the interaction of this parasitoid with these eucalyptus pests and shows perspectives of using it in biological control of these defoliators in reforestations areas with this plant in Brazil.

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