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Reproduction of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) in the pupae of *Diaphania hyalinata* (Lepidoptera: Crambidae) of various ages

Isabel Moreira Da Silva^{1,*}, Teresinha Vinha Zanuncio², Fabricio Fagundes Pereira³, Carlos Frederico Wilcken⁴, José Eduardo Serrão⁵, and José Cola Zanuncio²

Abstract

Trichospilus diatraeae Cherian & Margabandhu (Hymenoptera: Eulophidae) is a gregarious parasitoid of a wide variety of lepidopterans including melonworm, *Diaphania hyalinata* L. (Lepidoptera: Crambidae), pupae in the field. The latter is a key pest of Cucurbitaceae; it primarily damages their leaves and secondarily their fruit, and it reduces the productivity of the plants. The aim of this study was to determine the effect of the age of *D. hyalinata* pupae on the development of *T. diatraeae*. *Diaphania hyalinata* pupae aged 24, 48, 72, 96, 120, and 144 h were individualized and exposed to eight 48-h-old *T. diatraeae* females for 24 h. The emergence and parasitism rates, sex ratios, and longevities of *T. diatraeae* males and females were found to be similar with *D. hyalinata* pupae of different ages. The number of progeny, width of the head capsule, and duration of the life cycle (egg to adult) of *T. diatraeae* decreased with the increasing age of *D. hyalinata* pupae. Importantly, the number of parasitoid progeny per host pupa and size of the parasitoid adults were larger when 24-h-old host pupae were parasitized than when 48- to 144-h-old pupae were parasitized. *Trichospilus diatraeae* was very effective in parasitizing *D. hyalinata* pupae of ages ranging from 24 to 144 h.

Key Words: host pupa age; oviposition; host quality

Resumo

Trichospilus diatraeae Cherian e Margabandhu (Hymenoptera: Eulophidae) é um parasitoide gregário de uma ampla variedade de Lepidoptera e parasitou pupas de *Diaphania hyalinata* L. (Lepidoptera: Crambidae) em campo. Este inseto é praga chave e causa desfolha e redução da produtividade de Cucurbitaceae. O objetivo deste estudo foi avaliar o efeito da idade de pupas de *D. hyalinata* no desenvolvimento de *T. diatraeae*. Pupas de *D. hyalinata* com idades de 24, 48, 72, 96, 120 e 144 h foram individualmente expostas a oito fêmeas de *T. diatraeae* com 48 h de idade, por 24 h. A taxa de emergência e parasitismo, razão sexual e longevidade de machos e fêmeas de *T. diatraeae* foram semelhante para pupas de diferentes idades de *D. hyalinata*. A progênie, largura da cápsula cefálica e duração do ciclo de vida (ovo a adulto) de *T. diatraeae* diminuiu com o aumento da idade de pupas de *D. hyalinata*. *Trichospilus diatraeae* foi eficaz no parasitismo de pupas de *D. hyalinata* com diferentes idades.

Palavras Chave: idade da pupa; oviposição; qualidade do hospedeiro

Cucurbitaceae (Cucurbitales) such as *Citrullus lanatus* (Thunb.) Matsumara & Nakai, *Cucurbita maxima* Duchesne, *Cucurbita moschata* Duchesne, and *Cucurbita pepo* L. are rich in substances that help preventing diseases (Achu et al. 2005). Pests can reduce the productivity of plants of this family throughout the vegetative and reproductive stages (Gonring et al. 2003; Dhillon et al. 2005; Santana et al. 2012). *Diaphania* species are widely distributed in America, and they feed on Cucurbitaceae leaves, twigs, and shoots (Surís et al. 1997; Gonring et al. 2003; Melo et al. 2011).

Parasitoids are important in the context of their diversity and the parasitism levels they inflict on the host populations (Olivera & Bordat 1996; Pikart et al. 2011; Tavares et al. 2012). Eulophidae (Hymenoptera), the largest family of Chalcidoidea with 4,472 species in 297 genera, occur in temperate and tropical areas and are ectoparasitoids (Eulophinae and Euderinae) or endoparasitoids (Entedoninae and Tet-

rastichinae) of insects of different orders (Pereira et al. 2008; Zanuncio et al. 2008; Talebi et al. 2011; Zaché et al. 2011). The use of parasitoids in biological control programs may depend on the availability of a host species with suitable characteristics for the development and maturation of these natural enemies (Lemos et al. 2003; Pastori et al. 2012; Tavares et al. 2012). The generalist habit of *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) renders this parasitoid as a promising agent for biological control (Ribeiro et al. 2013; Rodrigues et al. 2013). *Trichospilus diatraeae* parasitized *Diaphania hyalinata* L. (Lepidoptera: Crambidae) pupae in the field at a level that showed a considerable potential for its use in the biological control of this pest (Melo et al. 2011). *Trichogramma* species (Hymenoptera: Trichogrammatidae) and Tachinidae species have been reported to parasitize *D. hyalinata* eggs and larvae, respectively (Gonring et al. 2003; Polanczyk et al. 2011).

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The quality of the host used to mass rear parasitoids for biological control can affect the reproduction and parasitism capacity of these natural enemies (Tran & Takasu 2000; Pereira et al. 2009). Koinobiont parasitoid species parasitize young or early-stage hosts in which they can proceed through their development, and they usually do not kill the host until the parasitoid reaches its final developmental stage (Kant et al. 2012). In contrast, idiobiont parasitoid species, such as *T. diatraeae*, usually parasitize an immobile stage of the host, i.e., egg or pupa—but some also parasitize larvae—and prevent its further development. The age and quality of the host pupa at the time of parasitism can affect the quality of the progeny of the idiobiont (Imandeh 2006).

Parasitoids depend on the host as a single resource for their offspring, and the host age determines its nutritional quality, which can affect the progeny of the parasitoids (Husni & Honda 2001; Imandeh 2006; Caron et al. 2010). The host age can affect sex ratio, progeny size (Hansan et al. 2009; Pereira et al. 2009), life cycle (Husni & Honda 2001), and longevity (Imandeh 2006) of parasitoids. Furthermore, the hormonal and cellular host defenses against parasitism depend on the host's developmental stage (Hegazi & Khafagi 2008). *Trichospilus diatraeae* has been reported to parasitize *D. hyalinata*, wherefore the aim of this study was to evaluate the parasitism and development of this parasitoid in this host's pupae of different ages.

Materials and Methods

The study was conducted at the Laboratory of Biological Control of Insects (LCB) of the Institute of Biotechnology Applied to Agriculture (BIOAGRO) of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil.

REARING

Diaphania hyalinata larvae were kept in plastic pots (3 L) each of which had a spout, and sealed with organza. The larvae were fed daily with chayote (*Sechium edule* [Jacq.] Sw.; Cucurbitales: Cucurbitaceae) leaves from the 1st instar to pre-pupa stage. Then, they were placed in plastic containers lined with paper towels for pupation. For adult emergence, the pupae were transferred to closed wooden cages with screened sides. Adult Lepidoptera received a cotton wad soaked with a nutrient solution composed of honey and water (1:1) at the bottom of the cage on a Petri dish. Pumpkin leaves were placed inside the cages as oviposition site, and egg masses were collected and placed in plastic pots.

Trichospilus diatraeae adults were kept in glass tubes (14.0 × 2.2 cm) containing drops of honey as food and plugged with cotton. Pupae of the alternative host *Anticarsia gemmatilis* Hübner (Lepidoptera: Noctuidae) were obtained from the rearing facility of the LCB/Dep. de Biologia Animal (DBA)/UFV, and these pupae were exposed individually to parasitism by 10 *T. diatraeae* females for 24 h for rearing of this parasitoid (Pereira et al. 2009).

EVALUATION OF THE EXPERIMENT

Twelve *D. hyalinata* pupae (100–120 mg) per age (24, 48, 72, 96, 120, and 144 h) were individualized and exposed for 24 h to eight 72-h-old *T. diatraeae* females. These pupae were isolated in glass tubes (2.2 × 14.0 cm) capped with cotton in a room at 25 ± 0.2 °C, 70 ± 10.8% RH, and a 12:12 h L:D photoperiod until the emergence of either parasitoid adults or host adults (Pereira et al. 2009).

We evaluated the duration of the life cycle (egg to adult), the percentage of parasitism and emergence, the number of parasitoids emerged, sex ratio (SR = the number of females/number of adults),

width of the head capsule, and longevity of *T. diatraeae* males and females. Sex was determined by morphological characteristics of the antenna and abdomen of these parasitoids (Paron 1999), and the size of the head capsule and body was measured with an ocular micrometer attached to a stereomicroscope. The longevity and the size of the head capsule of *T. diatraeae* were evaluated in 20 females and 15 males.

The completely randomized design experiments were conducted with 12 *D. hyalinata* pupae per treatment. Data were subjected to analysis of variance (ANOVA) at the 5% level, and when significant, they were subjected to regression analysis. Parasitism and emergence rates of *T. diatraeae* from *D. hyalinata* pupae were subjected to ANOVA and the non-parametric Kruskal–Wallis test (SAS Institute 1997).

Results

The age of *D. hyalinata* pupae when first parasitized did not affect percentage of parasitism and percentage of emergence of *T. diatraeae*, because the rate of parasitism was 100% in all treatments (Fig. 1), and the rate of emergence from 72-, 96-, and 120-h-old pupae was 83.33% and that from 24-, 48-, and 144-h-old pupae was 91.67% (Fig. 1). The number of *T. diatraeae* offspring per pupa declined with increasing age of the *D. hyalinata* pupae, and the largest number of progeny was obtained from 24-h-old pupae ($F = 138.036$, $P = 0.0003$, $R^2 = 0.9718$) (Fig. 2A). The duration of the lifecycle of *T. diatraeae* (egg to adult) peaked in 48- to 72-h-old pupae and then decreased with the increasing age of the *D. hyalinata* pupae ($F = 49.9171$, $P = 0.0197$, $R^2 = 0.9520$) (Fig. 2B). The widths of the head capsule of females ($F = 49.9171$, $P = 0.0197$, $R^2 = 0.8241$) and males ($F = 13.9139$, $P = 0.0203$, $R^2 = 0.7767$) *T. diatraeae* decreased with the increasing age of the *D. hyalinata* pupae (Figs. 2C and 2D, respectively), but the longevities of males and females of this parasitoid did not vary significantly with the age of the host pupa (Table 1). The sex ratios (proportions of females) of *T. diatraeae* adult progeny that emerged from *D. hyalinata* pupae that had been parasitized at ages ranging from 24 h to 144 h were similar and varied between 0.91 and 0.94 (Table 1).

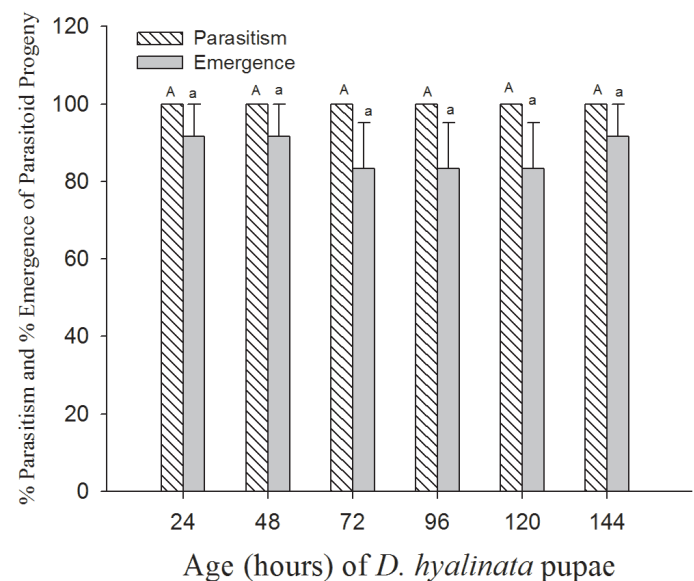


Fig. 1. Percentage of parasitism of *Diaphania hyalinata* pupae and percentage of emergence of *Trichospilus diatraeae* progeny from *D. hyalinata* hosts parasitized as 24-, 48-, 72-, 96-, 120-, and 144-h-old pupae. Bars with the same uppercase or lowercase letter do not differ by the non-parametric Kruskal–Wallis test ($P > 0.05$).

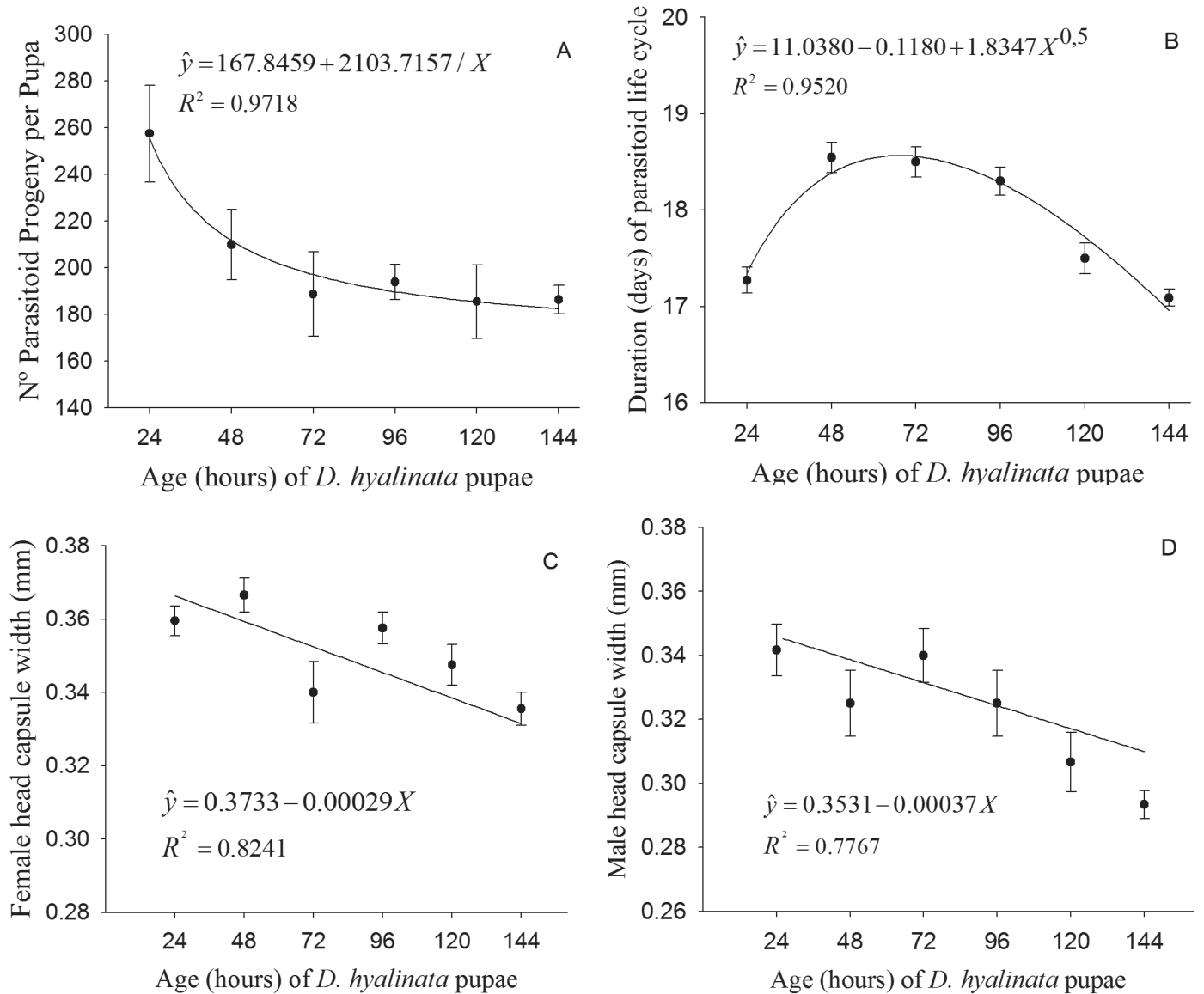


Fig. 2. *Trichospilus diatraeae* progeny produced in *Diaphania hyalinata* pupae; (A) number of progeny; (B) duration of the *T. diatraeae* life cycle; (C) width of the *T. diatraeae* female head capsule; (D) width of the *T. diatraeae* male head capsule (mean \pm SE). Measurements were made on *T. diatraeae* that emerged from *D. hyalinata* hosts parasitized as 24-, 48-, 72-, 96-, 120-, and 144-h-old pupae.

Discussion

Because the age of *D. hyalinata* pupae when first parasitized did not affect percentage of parasitism and percentage of emergence of *T. diatraeae*, young pupae (24 h) can be used to reduce the rearing costs. The independence of these important life parameters from the age of host pupae should help the parasitoid to maintain its populations in the field.

The percentages of parasitism and emergence of *T. diatraeae* from *D. hyalinata* pupae of different ages differ from those observed for *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae) from *Plutella xylostella* L. (Lepidoptera: Plutellidae) pupae (Wang & Liu 2002). The parasitism capacity of *D. collaris* decreased with increasing age of its host due to selection and preference for younger pupae that present better nutrition quality for the development of its progeny (Wang & Liu 2002; Pizzol et al. 2012). In comparison, despite the possible decline in the nutritional quality of *D. hyalinata*, *T. diatraeae* showed

greater flexibility during oviposition. The age of the host can reduce its suitability due to the hardening of its puparium, which hinders the penetration of the ovipositor, but female parasitoids can change the oviposition place on the pupa (King 2011).

The reduction of the number of progeny of *T. diatraeae* that emerged from older *D. hyalinata* pupae may be due to decreased food quality resulting from the conversion of hemolymph into adult tissues (Minot & Leonard 1976; Pereira et al. 2009). The presence of dead *T. diatraeae* individuals inside 144-h-old *D. hyalinata* pupae showed that this parasitoid had oviposited in this host at this age. The diminished production of offspring per host pupa in 144-h-old pupae indicated that pupae of advanced age were less suitable for the development of immature parasitoids, probably due to morphological and physiological changes, which can influence the acceptability and suitability of hosts by parasitoids (Wang & Liu 2002), as was reported for the reduction in number of progeny of *Nesolynx thymus* (Girault) and *Palmistichus elaeis* Delware & LaSalle (Hymenoptera: Eulophidae) with the increasing age of pupae of *Exorista sorbillans* (Wiedemann) (Diptera:

Table 1. Mean \pm SE longevity of females and males in days and sex ratio of *Trichospilus diatraeae* that emerged from *Diaphania hyalinata* hosts parasitized as 24-, 48-, 72-, 96-, 120-, and 144-h-old pupae.

Host age (h)	Longevity (d)				Sex ratio (no. of females / no. of males) ^{ns}	
	Females ^{ns}	V. I. (n) ^a	Males ^{ns}	V. I. (n) ^a	Mean	V. I. (n) ^a
24	11.20 \pm 0.83	8–18 (20)	9.73 \pm 0.26	6–13 (15)	0.94 \pm 0.01	0.90–0.96 (12)
48	10.30 \pm 0.73	6–16 (20)	10.47 \pm 0.50	6–15 (15)	0.94 \pm 0.01	0.92–0.98 (12)
72	10.25 \pm 0.64	8–15 (20)	10.07 \pm 0.41	4–12 (15)	0.92 \pm 0.01	0.88–0.96 (12)
96	11.00 \pm 0.53	7–13 (20)	11.67 \pm 0.52	7–15 (15)	0.92 \pm 0.01	0.89–0.96 (12)
120	10.65 \pm 0.80	8–16 (20)	10.33 \pm 0.70	8–17 (15)	0.93 \pm 0.01	0.89–0.98 (12)
144	10.50 \pm 0.61	7–13 (20)	10.80 \pm 0.82	3–15 (15)	0.94 \pm 0.01	0.91–0.97 (12)

^{ns} Not significant by the *F*-test at 5% probability.

^a V. I., variation interval; *n*, number of replications.

Tachinidae) and *Bombyx mori* L. (Lepidoptera: Bombycidae), respectively (Hansan et al. 2009; Pereira et al. 2009). *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) eggs aged more than 4 d were not parasitized by *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), suggesting reduced suitability of *S. cerealella* eggs with their increasing age (Fatima et al. 2009).

Gregarious endoparasitoids depend on host nutrients for their embryonic development (Haeckermann et al. 2007). Females of *T. diatraeae* may have laid similar numbers of eggs, regardless of the host pupa age, but a smaller number of immatures may have completed the life cycle in older hosts likely due the diminished food quality (increasing competition) (Wylie 1963; Husni & Honda 2001). Alternatively, the parasitoid females may have laid fewer eggs in older hosts with poor physiological suitability for the development of their larvae (Pizzol et al. 2012). The latter occurs when female parasitoids have oviposition strategies to maximize reproductive fitness, including survival of their immature progeny (Hegazi & Khafagi 2008). However, the use of older pupae by parasitoid species may be advantageous for their survival when host density is sparse (Husni & Honda 2001).

The shorter time of development of *T. diatraeae* with the increasing age of the *D. hyalinata* pupae differs from that of *Brachymeria lasus* (Walker) (Hymenoptera: Chalcididae) and *Melittobia acasta* (Walker) (Hymenoptera: Eulophidae), which had life cycle durations independent of the increasing age of the host (Husni & Honda 2001; Imandeh 2006). However, when *Trichogramma cacaeciae* Marchal (Hymenoptera: Trichogrammatidae) females parasitized older *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae) eggs, their progeny had a shorter development period than that developing in younger host eggs (Pizzol et al. 2012). These reports show that parasitoids differ in their capacities to utilize resources from hosts of different ages (Tran & Takasu 2000).

The similar sex ratios of *T. diatraeae* from *D. hyalinata* pupae of diverse ages suggests that the older host did not affect the parasitoid progeny sex ratio and that high numbers of fertilized eggs were laid regardless of this parameter (Husni & Honda 2001). By contrast, the parasitoid *N. thymus* had lower proportions of females with older (Hansan et al. 2009) or super-parasitized hosts (Nieuwenhove & Ovruski 2011). This shows the ability in some species of female parasitoids to regulate the offspring sex ratio in response to the host and environmental conditions (Chow & Mackauer 1996; Caron et al. 2010; Nieuwenhove & Ovruski 2011). The similar survival of *T. diatraeae* offspring with differing ages of *D. hyalinata* pupae again showed that the parasitoid could adapt to this condition with the development of its immatures, as observed for *Gronotoma micromorpha* (Perkins) (Hymenoptera: Figitidae) (Abe 2009).

The smallest width of the head capsule of *T. diatraeae* individuals emerging from the oldest *D. hyalinata* pupae indicated that the

advanced development of this host, especially with 144-h-old pupae, reduced the food available to the parasitoid offspring (also effecting a shorter life cycle) and thus resulted in smaller individuals of this natural enemy. Similarly, the parasitoid *B. lasus* had smaller individuals in older hosts, which may indicate inadequate or reduced food availability (Husni & Honda 2001).

In conclusion, *D. hyalinata* pupae of various ages were parasitized by *T. diatraeae*, but the number of parasitoid progeny per host pupa and the size of the parasitoid adults were larger when 24-h-old host pupae were parasitized than when 48- to 144-h-old pupae were parasitized.

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