BIOLOGICAL CHARACTERISTICS OF *TRICHOSPILUS DIATRAEAE* (HYMENOPTERA: EULOPHIDAE) ARE INFLUENCED BY THE NUMBER OF FEMALES EXPOSED PER PUPA OF *TENEBRIO MOLITOR* (COLEOPTERA: TENEBRIONIDAE)

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Abstract

Different numbers of parasitoid females confined with a host can variously affect the number, sex ratio and other characteristics of the parasitoid's progeny. The objective of this study was to elucidate the effects of various ratios of *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) females to pupae of one its hosts, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), primarily on the number of this parasitoid's progeny and their sex ratio. Both the parasitoid and the host used in this study were taken from cultures reared in the laboratory by standard methods. In order to minimize the effects of variations in host weight, 24 h-old *T. molitor* pupae weighing between 0.110 and 0.140 g were held as a single individuals in glass tubes (2.5 cm Ø × 14 cm L) with 48 h-old *T. diatraeae* females for 72 h to allow parasitization. After this period, female wasps were removed and the host pupa were transferred, one per glass tube, to a climate-controlled room at 25 ± 2 °C, 70 ± 10% RH and 12:12 h L:D. The experimental design was completely randomized with six treatments (parasitoid-host ratios) and 12 replicates per treatment. The ratios of *T. diatraeae* females per host used were: 1:1, 7:1, 14:1, 21:1, 28:1 and 32:1. The percentage parasitism of *T. diatraeae* on *T. molitor* pupae was 33.33, 82.00 and 83.33% at ratios of 1:1, 7:1 and 14:1, respectively, and 100% at all other ratios. The emergence of *T. diatraeae* adults from parasitized pupae was 75% at a parasitoid-host ratio of 1:1 and 100% at ratios of 21:1, 28:1 and 32:1. The duration of the parasitoid's life cycle ranged from 21.00 ± 2.22 to 24.00 ± 2.00 days at parasitoid-host ratios of 32:1 and 1:1, respectively. The number of *T. diatraeae* progeny per *T. molitor* pupa was highest at a ratio of 21:1 (246.50 ± 50.18). The proportion of *T. diatraeae* females in the offspring decreased as the parasitoid-host ratio increased, varying between 0.82 ± 0.06 and 0.97 ± 0.01. A parasitoid-host ratio of 21:1 *T. diatraeae* females per *T. molitor* pupa is considered the most adequate and appropriate for mass-rearing of this parasitoid. *Tenebrio molitor* appears to be a suitable alternate host for efficient mass-rearing of *T. diatraeae* for biological control of lepidopteran pests. At the parasitoid-host ratio of 21:1, each *T. molitor* pupa supported the production of 246.5 parsitoids of which 88% were females, i.e., 216.9 females and 29.6 males; each *T. diatraeae* female produced 9.55 ± 0.48 female progeny, and the developmental time from egg to adult was 20.4 days.

Key Words: alternate host, biological control, parasitism, pupal parasitoid, mass-rearing

Resumen

La variación en el número de hembras de parasitoides confinados con un hospedero puede afectar el desarrollo, la reproducción y la relación de sexos de la progenie del parasitoide. El objetivo de este trabajo fue estudiar el efecto de la variación del numero de hembras del parasitoide *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) por pupa del hospedero *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), primordialmente en numero de la progenie y su relación de sexos. Los parasitoides y hospederos utilizados en este estudio provinieron de colonias de laboratorio mantenidas con metodologías estandarizadas. Para minimizar la variación en el peso del hospedero se utilizaron pupas de *T. molitor* de 24 h de edad pesando entre 0.110 y 0.140 g, las cuales fueron mantenidas individualmente en tubos de vidrio (2.5 cm Ø × 14 cm L) con hembras de *T. diatraeae* de 48 h de edad por 72 h. Después de este periodo de parasitismo las avispas fueron removidas y las pupas del hospedero transferidas a nuevos tubos de vidrio y mantenidos en condiciones controladas de 25 ± 2 °C, 70 ± 10% HR y 12:12 h L:O. El diseño experimental fue completamente aleatorizado con seis tratamientos (numero de parasitoides/ hospedero) y 12 replicas por tratamiento. Los números de hembras de *T. diatraeae* por pupa de *T. molitor* utilizados fueron: 1:1, 7:1, 14:1, 21:1, 28:1 y 32:1 (parasitoides/hospedero). El porcentaje de parasitismo de *T. diatraeae* en pupas de *T. molitor* fué de 33.33, 82.00 y 83.33% en las relaciones de 1:1, 7:1 y 14:1 respectivamente, y de 100% en las otras relaciones de parasitoides /hospederos. La emergencia de los individuos de *T. diatraeae* fue de 75% en la densidad de 1:1 (parasitoides/hospedero) y 100% en las densidades de 21:1, 28:1 y 32:1. La duración del ciclo de vida del parasitoide varió de 21.00 ± 2.22 a 24.00 ± 2.00 días en las relaciones de 32:1 y 1:1, respectivamente. El número de descendientes de *T. diatraeae* por pupa de *T. molitor* fue mayor en la relación de 21:1 (246.50 ± 50.18). La proporción de hembras *T. diatraeae* en la progenie disminuyó con el aumento de la densidad del parasitoide, variando entre 0.82 ± 0.06 y 0.97 ± 0.01. La relación de 21:1 hembras de *T. diatraeae* por pupa de *T. molitor* fué considerada la más adecuada para la cría masiva de este parasitoide. *Tenebrio molitor* parece ser un hospedero alterno adecuado para la cría masiva de *T. diatraeae* para el control biológico de insectos lepidópteros plaga. En la relación parasitoide-hospedero de 21:1, cada pupa de *T. molitor* soporto la producción de 246.5 parasitoides de los cuales 88% fueron hembras, i.e., 216.9 hembras y 29.6 machos; cada hembra de *T. diatraeae* produjo 9.55 ± 0.48 hembras en su progenie, y el tiempo de desarrollo de huevo a adulto fue 20.4 días.

Palabras Clave: control biológico, hospedero alterno, parasitismo, parasitoide pupal, cría masiva

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Parasitoids are among the most common natural enemies of the class Insecta. The families Aphelinidae, Braconidae, Encyrtidae, Ichneumonidae, Pteromalidae, Trichogrammatidae and Eulophidae in the order Hymenoptera are the most commonly used parasitoids for biological control of insects (Van Driesche & Bellows 1996). *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) is a pupal parasitoid with potential for use in biological control of numerous lepidopteran pests. It has been reported to parasitize insects in the Arctiidae (Zaché et al. 2012), Crambidae (Paron & Berti-Filho 2000; Chichera et al. 2012; Rodrigues et al. 2013), Geometridae (Pereira et al. 2008; Zaché et al. 2010), Nymphalidae (Bouček 1976), Noctuidae (Andrade et al. 2010), Pyralidae (Bennett et al. 1987; Kazmi & Chauhan 2003; Melo et al. 2011) and Riodinidae (Zaché et al. 2011).

Mass-rearing is an important process of biological control programs. The nutritional quality, size, age, mechanical resistance and the immunological response of the host should be considered when selecting alternative hosts for mass-rearing of parasitoids (Pastori et al. 2008; Pereira et al. 2009; Pereira et al. 2010). *Trichospilus diatraeae* displays adequate biological characteristics when reared in pupa of the following alternate hosts: *Anticarsia gemmatalis* (Hübner), *Heliothis virescens* (Fabricius), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) (Paron & Berti-Filho 2000). *Trichospilus diatraeae* is a gregarious parasitoid, which makes it necessary to define the optimal ratio of females per host pupa to increase the production of descendants of quality similar to that of wild *T. diatraeae*. The ratio of female parasitoids per host affects the capacity of parasitism (Sagarra 2000a), production of progeny (Chong & Oetting 2006), the sex ratio of the offspring (Choi et al. 2001), the cycle duration, and the longevity of the adults (Silva-Torres & Matthews 2003). The assessment of these biological characteristics is important to increase the rearing efficiency in biological control programs.

Tenebrio molitor L. (Coleoptera: Tenebrionidae) can easily be mass-reared at low cost, and used as an alternate host for the parasitoid *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) (Zanuncio et al. 2008). Preliminary tests have shown that *T. diatraeae* can parasitize *T. molitor* pupae (Favero 2009). The objective of this study was to optimize the rearing of *T. diatraeae* in the laboratory by using different ratios of *T. diatraeae* females per *T. molitor* host pupa.

Materials and Methods

The experiments were conducted at the "Laboratório de Entomologia/Controle Biológico (LECOBIOL)" of the "Faculdade de Ciências Agrárias (FCA)" of the "Universidade Federal da Grande Dourados (UFGD)" in Dourados, Mato Grosso do Sul, Brasil.

Rearing of *Tenebrio molitor*

The pupae used in this study were reared in the LECOBIOL laboratory, where larvae of *T. molitor* were maintained in plastic trays (39.3 cm W × 59.5 cm L × 7.0 cm H), and fed wheat bran (97%), yeast (3%) and pieces of chayote (*Sechium edule*) according to the methodology of Zamperline & Zanuncio (1992).

Rearing of *Trichospilus diatraeae*

Trichospilus diatraeae adults were reared at the LECOBIOL laboratory in glass tubes (2.5 cm Ø × 8.5 cm L) sealed with cotton swabs. The insects were fed with droplets of honey. Twenty-four to 48 h-old *D. saccharalis* pupa were exposed to *T. diatraeae* females for 72 h. Parasitized pupa were separated and maintained individually at 25 ± 1 °C, 70 ± 10% RH and 14:10 h L:D until adult emergence (Pereira et al. 2008; Chichera et al. 2012).

Experimental Design

In order to minimize the effects of host weight variation, 24 h-old *T. molitor* pupae weighing between 0.110 and 0.140 g were held as single individuals in glass tubes (2.5 cm Ø × 14 cm L) with 48 h-old *T. diatraeae* females for 72 h to allow parasitization. After this period, female wasps were removed and the host pupa transferred to glass tubes in a climate-controlled room at 25 ± 2 °C, 70 ± 10% RH and 12:12 h L:D. The experimental design was completely randomized with 6 treatments (parasitoid-host ratios). The ratios of *T. diatraeae* females per host used were: 1:1, 7:1, 14:1, 21:1, 28:1 and 32:1, with 12 replicates per treatment.

The mean duration of the life cycle (egg to adult), the adult emergence rate (%), percent parasitism, number of offspring, sex ratio (SR = number of females/ number of females + number of males), and numbers of immature *T. diatraeae* that did not complete development were registered. The sex of adult parasitoids was determined by assessing the morphological characteristics of their antennae and abdomen (LaSalle 1994).

The natural mortality of the host was calculated (Abbott 1925) in the same environmental conditions as the experiment. *T. diatraeae* emergence and parasitism were analyzed using a generalized linear model with a binomial distribution ($P \leq 0.05$) with the R Statistical System (Ihaka & Gentleman 1996). This analysis was carried out using the original non-parametric data; however, the data are presented in percentage values to facilitate visualization.

The other parameters were subject to analysis of variance and if significant at 5% probability, regression analysis was conducted. Equations were selected using the linear, quadratic and cubic models, based on the coefficient of determination (R^2), significance of the regression coefficients (ßi) and regression by the F-test (up to 5% probability).

Results

Percent parasitism and adult emergence of *T. diatraeae* from *T. molitor* pupa were influenced by the number of parasitoids females per host. Percent of parasitism was 33.33% and 83.33% at ratios of 1:1 and 14:1, respectively, and 100% for all other ratios ($\chi^2 = 38.651$; $P = 0.001$) (Fig. 1 and Table 1). Adult emergence was 75% at a parasitoid-host ratio of 1:1, 83.33% at 14:1, and 100% for the ratios 21:1, 28:1 and 32:1, respectively ($\chi^2 = 42.548$; $P = 0.001$) (Fig. 1).

Developmental time from egg to adult for *T. diatraeae* in *T. molitor* pupa was 21.00 ± 2.22 days at a parasitoid-host ratio of 32:1, and 24.00 ± 2.00 days at ratio 1:1 ($F = 3.72$; $P = 0.03$; $R^2_{\text{Treat}} = 0.53$) (Fig. 2 and Table 1). The number of progeny per *T. molitor* pupa ($F = 10.39$; $P = 0.0001$; $R^2_{\text{Treat}} = 0.67$) increased with parasitoid-host ratio up to 21:1 (246.50 ± 50.18) (Fig. 3 and Table 1). The number of females produced per *T. diatraeae* female ($F = 56.01$; $P = 0.0001$; $R^2_{\text{Treat}} = 0.73$) was inversely proportional to the parasitoid-host ratio, and ranged between 4.53 ± 1.64 and 66.66 ± 9.22 females at ratios of 28:1 and 1:1, respectively (Fig. 4).

The number of dead immature *T. diatraeae* found inside *T. molitor* pupa was not influenced by parasitoid-host ratio ($P \leq 0.05$), with average values of 4.56 ± 2.87, 3.78 ± 3.38, 3.33 ± 2.73, 4.00

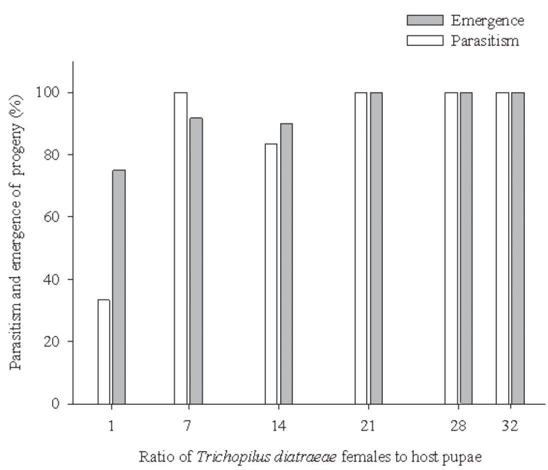

Fig. 1. Percentage of parasitism and emergence of adult *Trichospilus diatraeae* (Hymenoptera: Eulophidae) at 6 parasitoid-host ratios per *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupa (12:12 h L:D at 25 ± 2 °C and 70 ± 10% RH).

TABLE 1. BIOLOGICAL CHARACTERISTICS OF *TRICHOSPILUS DIATRAEAE* AT PARASITOID/HOST RATIOS OF 1:7, 1:14, 1:21, 1:28 AND 1:32 PARASITOID FEMALES PER *TENEBRIO MOLITOR* PUPA AT 25 ± 2 °C AND 70 ± 10% RH WITH A 12:12 H L:D PHOTOPERIOD.

Biological Characteristics (means)						
Parasitoid-host ratio	Parasitism (%)	Parasitoid Emergence (%)	Developmental time (days)	Progeny	Females produced per female	Sex ratio
1-1	33.3	75	24	68.3	62.2	0.97
7-1	100	91.6	21.27	128.7	16.66	0.9
14-1	83.3	90	20.88	171.44	12.22	0.86
21-1	100	100	20.41	246.5	9.6	0.88
28-1	100	100	21.83	151.41	4.5	0.85
32-1	100	100	21	177	4.7	0.85

± 3.38, 3.67 ± 1.0, and 4.64 ± 4.08 for parasitoid-host ratios 1:1, 7:1, 14:1, 21:1, 28:1 and 32:1, respectively. The sex ratio of the offspring, estimated as the proportion of females produced in *T. molitor* pupae (F = 8.47; P = 0.0006; R^2_{Treat} = 0.95) was highest (0.97 ± 0.01) at a 1:1 parasitoid-host ratio.

DISCUSSION

The biological characteristics (parasitism, adult emergence and progeny) of *T. diatraeae* per *T. molitor* pupa indicated that, for mass-rearing of this natural enemy, the optimal number of females parasitoids per host should be 21. Paron & Berti Filho (2000) demonstrated that exposure to one or many *T. diatraeae* females per pupa of *D. saccharalis*, *A. gemmatalis*, *H. virescens* and *S. frugiperda* did not affect emergence and parasitism rates. However, the biological characteristics of the descendants may be affected by competition among the parasitoids after oviposition (Chong & Oetting 2007).

The progeny of *T. diatraeae* were affected by the number of females per pupa of *T. molitor*. Low ratios of female parasitoids per host resulted in less oviposition per host. The ratio of parasitoid females per host pupa can affect fecundity and reduce the efficiency of mass-rearing systems, mainly due to an increase of competition by the immature stages within the same host pupa (Sagarra et al. 2000a). A similar effect was reported for *P. elaeisis* reared in *Bombyx mori* Linnaeus (Lepidoptera: Bombycidae) pupae (Pereira et al. 2010).

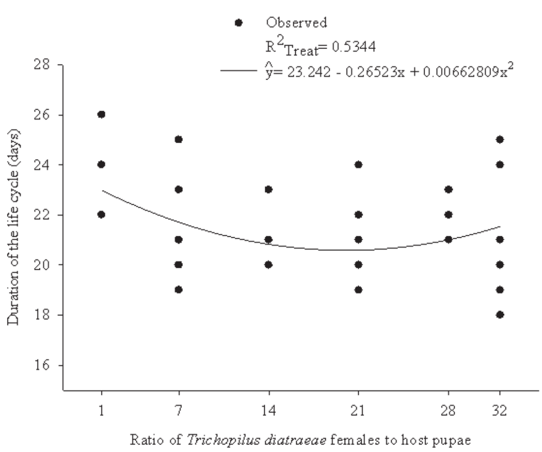

Fig. 2. Duration of the period egg to adult for *Trichospilus diatraeae* (Hymenoptera: Eulophidae) at 6 parasitoid-host ratios per *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupa (12:12 h L:D at 25 ± 2 °C and 70 ± 10% RH) (F = 3.7206; P = 0.0304; R^2_{Treat} = 0.5344).

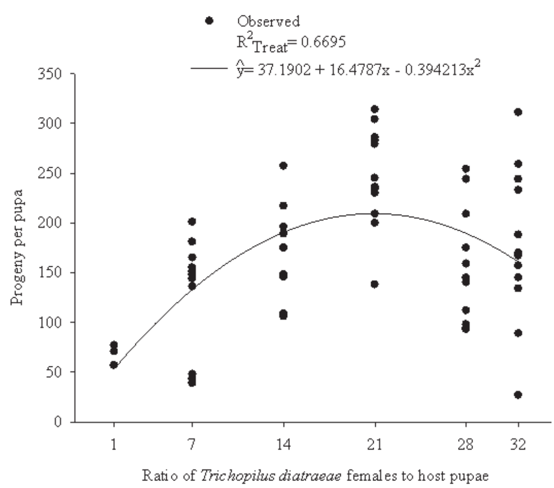

Fig. 3. Number of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) progeny at 6 parasitoid-host ratios per *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupa (12:12 h L:D at 25 ± 2 °C and 70 ± 10% RH) (F = 10.3936; P = 0.0001; R^2_{Treat} = 0.6695).

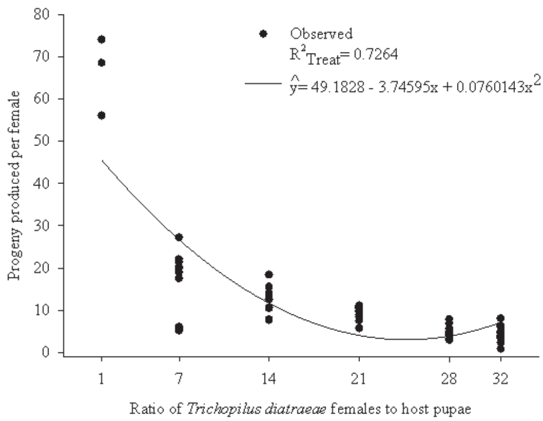

Fig. 4. Number of progeny per female *Trichospilus diatraeae* (Hymenoptera: Eulophidae) at 6 parasitoid-host ratios per *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupa (12:12 h L:D at 25 ± 2 °C and 70 ± 10% RH) (F = 56.0123; P = 0.0001; R^2_{Treat} = 0.7264).

The number of *T. diatraeae* descendants per host decreased at the highest ratios of female parasitoids per host. This suggests that *T. diatraeae* females have a strong tendency to avoid superparasitism (Sagarra et al. 2000a,b; Zanuncio et al. 2008; Pereira et al. 2009; Soares et al. 2009). Another factor that may explain the decreased numbers of parasitoid offspring at the highest ratios is that the host has a given carrying capacity above which food becomes a limiting factor for parasitoid development (Pereira et al. 2010).

The low rates of parasitism and adult emergence at parasitoid-host ratio 1:1 suggest that *T. molitor* pupa may have defense mechanisms against *T. diatraeae*. The host can have cellular defenses and reactions involving encapsulation and melanization of endoparasitoid eggs (Pennacchio & Strand 2006). Superparasitism could increase the survival of parasitoid progeny by overwhelming the cellular immune response of the host (Andrade et al. 2010). Parasitism by *P. elaeisis* on *B. mori* pupa showed similar trends to those in this study. Emergence of *P. elaeisis* descendants per *B. mori* pupa was observed only at a ratio of 45:1. The authors suggested that a given parasitoid-host ratio would neutralize the defense mechanisms of *B. mori* (Pereira et al. 2010).

The developmental period for *T. diatraeae*, from egg to adult emergence decreased at the highest ratios. However, the number of *T. diatraeae* per host did not affect the number of dead immatures inside *T. molitor* pupae. Reduced developmental period may be caused by competition between immature *T. diatraeae*, with physical combat or physiological suppression, which may reduce egg to adult development time of this parasitoid (Brodeur & Boivin 2004). Silva-Torres & Matthews (2003) suggested that high numbers of *Melittobia digitata* Dahms (Hymenoptera: Eulophidae) per pupa of *Neobellieria bullata* Parker (Diptera: Sarcophagidae) reduced the developmental time (egg-adult) of this parasitoid.

The number of females produced per female was inversely proportional to the number of descendants obtained, where low ratios produced the largest numbers of females. Generally, superparasitism decreases the number of female progeny (Soares et al. 2009). Andrade et al. (2012) reported that the offspring sex ratio is determined by the number of females that oviposit simultaneously; as this number increased, the number of fertilized eggs decreased. By contrast, in other cases the highest ratios produced the highest proportion of females (Silva-Torres & Matthews 2003; Chong & Oetting 2006).

The use of parasitoids for biological control programs depends on their development and reproduction in a preferred or suitable alternate host, which must present adequate nutritional conditions and low production cost. *Trichospilus diatraeae* can develop in *T. molitor* pupa and the information obtained in this study may facilitate efficient mass-rearing of this natural enemy for biological control of lepidopteran pests.

Conclusion

Tenebrio molitor pupa are adequate for rearing *T. diatraeae*. The ratio of 21 *T. diatraeae* females per *T. molitor* pupa produced the most parasitoid offspring and reduced variation in the biological characteristics of this natural enemy (Table 1). At this parasitoid-host ratio, each *T. molitor* pupa supported the production of 246.5 parsitoids of which 88% were females, i.e., 216.9 females and 29.6 males; each *T. diatraeae* female produced 9.55 ± 0.48 female progeny, and the developmental time from egg to adult was 20.4 days.

Acknowledgments

We thank the Brazilian institutions “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio ao Desenvolvimento do Ensino, and Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT)” and “Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)”. We thank undergraduate student Gabriela Piñeyro for translating the abstract to Spanish. Science Editing Experts corrected the English of this manuscript.

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