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THERMAL REQUIREMENTS AND GENERATION ESTIMATES OF *TRICHOSPILUS DIATRAEAE* (HYMENOPTERA: EULOPHIDAE) IN SUGARCANE PRODUCING REGIONS OF BRAZIL

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Abstract

Knowledge of the thermal requirements of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) is important if it is to be used successfully in to control *Diatraea saccharalis* (Lepidoptera: Crambidae) in sugarcane plantations. In the current study, the development of *T. diatraeae* was investigated in the pupae of *D. saccharalis* incubated at different temperatures. Seven *T. diatraeae* females were placed with host pupae for 24 h in tubes within chambers at 16, 19, 22, 25, 28 and 31 °C with 70 ± 10% RH and 14:10 h L:D. The life cycle duration of *T. diatraeae* decreased as the temperature increased, although no development was recorded at 31 °C. The number of *T. diatraeae* progeny per female ranged from 264.8 ± 40.7 (at 16 °C) to 385.1 ± 36.3 (at 25 °C), but no significant difference were recorded among temperature treatments ($P > 0.05$). The base temperature (T_b) and thermal constant (K) of *T. diatraeae* were 9.37 °C and 257.60 degree-days, respectively. The estimated average numbers of generations per year of *T. diatraeae* in pupae of *D. saccharalis* were 18.5, 19.93 and 17.73 for Dourados, Ivinhema and Ponta Porã municipalities of Mato Grosso do Sul State, Brasil, respectively.

Key Words: temperature requirements, biological control, *Diatraea saccharalis*, parasitoid, sugarcane production, *Trichospilus diatraeae*

Resumen

El conocimiento de las exigencias térmicas es importante para el uso de *Trichospilus diatraeae* (Hymenoptera: Eulophidae) en el control de *Diatraea saccharalis* (Lepidoptera: Crambidae) en plantaciones de caña de azúcar. Se estudio el desarrollo de *T. diatraeae* en pupas de *D. saccharalis* incubadas a diferentes temperaturas. Pupas de *D. saccharalis* fueron expuestas a siete hembras de *T. diatraeae* durante 24 h en tubos y luego colocadas en cámaras climatizadas a temperaturas de 16, 19, 22, 25, 28 y 31 °C con 70 ± 10% de humedad relativa y foto-periodo de 14 h. La duración del ciclo de vida de *T. diatraeae* disminuyó con el aumento de la temperatura. Este parasitoide no completó su desarrollo a 31 °C. La progenie de *T. diatraeae* varió de 264.75 ± 40.69 (16 °C) a 385.09 ± 36.28 (25 °C) individuos por hembra, sin presentarse diferencias significativas entre los tratamientos ($P > 0,05$). La temperatura base (T_b) y la constante térmica (K) de *T. diatraeae* fueron de 9.37 °C y 257.60 grados-día, respectivamente. El número medio estimado de generaciones por año de *T. diatraeae* en pupas de *D. saccharalis* fue de 18.5, 19.93 y 17.73 para los municipios brasileños de Dourados, Ivinhema y Ponta Porã, Mato Grosso do Sul, Brasil, respectivamente.

Palabras Clave: requerimientos de temperatura, control biológico, *Diatraea saccharalis*, parasitoide, producción de caña de azúcar, *Trichospilus diatraeae*

The pupal parasitoid *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) has been reported from species of various lepidopteran families, including Arctiidae (Zaché et al. 2012), Geometridae (Pereira et al. 2008; Zaché et al. 2010; Pastori et al. 2012), Nymphalidae (Bouček 1976), Noctuidae (Paron & Berti-Filho 2000), Oecophoridae (Oliveira et al. 2005), Pieridae (Kazmi & Chauhan 2003), Pyralidae (Bennett et al. 1987; Melo et al. 2011) and Riodin-

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idae (Zaché et al. 2011). This natural enemy can also parasitize and develop in pupae of *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) (Paron & Berti-Filho 2000), indicating its potential for use in the biological control of this insect borer of sugar cane.

Diatraea saccharalis is the most important pest of sugarcane in Brazil (Dinardo-Miranda et al. 2012). Feeding injury to the stem results in direct damage, where as indirect losses occur because of microorganism growth that results in decrease of sugar and alcohol production (Pinto et al. 2009). Chemical insecticides have low efficiency against *D. saccharalis* because its larvae develop in galleries within the sugarcane stem. This increases the importance of using parasitoids for biological control of this pest. In Brazil, the larval endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) is the most efficient means of controlling *D. saccharalis* (Silva et al. 2012), but the egg parasitoid *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae) also shows promise in controlling pest populations (Parra & Zucchi 1997).

Diatraea saccharalis is attacked by *T. diatraeae* (La Salle 1994). However, little is known about this parasitoid, including its temperature range. The relation between environmental temperature and insect development can be used to determine the best conditions to rear insects that will be released in the field and to predict the number of generations of the pest and its natural enemies (Nava et al. 2007; Wanderley et al. 2007; Pereira et al. 2011).Temperatures above or below an optimal value can affect insect development (Rodrigues et al. 2004). Determining the optimum temperature can allow production of a large number of individuals with adequate survival, and with high reproductive and parasitism rates, both in laboratory and in the field (Medeiros et al. 2004; Pereira et al. 2004; Pratissoli et al. 2006; Zago et al. 2006; Pasini et al. 2010). The ability of natural enemies to survive at different temperatures can also increase their impact in biological control programs (Pratissoli et al. 2005; Iranipour et al. 2010).

The objective of the current study was to determine the thermal requirements of *T. diatraeae* in *D. saccharalis* pupae in the laboratory and to estimate the number of generations of this parasitoid in three municipalities that are major sugarcane producers in Mato Grosso do Sul State, Brasil.

Materials and Methods

The experiment was conducted at the Laboratory of Entomology/Biological Control (LECOBIOL) at the Faculdade de Ciências Agrárias (FCA) of the Universidade Federal da Grande Dourados (UFGD) in Dourados, Mato Grosso do Sul, Brasil.

Rearing of *Diatraea saccharalis*

Eggs of *D. saccharalis* were obtained from the company Biosoluções. Newly hatched first-instar larvae were placed in screened vials with an artificial diet until pupa stage was reached. Pupae were then collected, sexed and groups of 50 pupae (20 males and 30 females) were placed in PVC cages (10 cm diam × 22 cm ht). Each cage was wrapped with moistened bond paper and contained a Petri dish lined with filter paper as an oviposition substrate. Each PVC cage was closed with bond paper and rubber bands. Emerging adults were fed with a 10% water and honey solution supplied to the insects through a cotton wick inserted into plastic containers (3 cm diam × 4 cm ht), modified according to Parra (2001).

Rearing of *Trichospilus diatraeae*

Adults of *T. diatraeae* were obtained from a rearing laboratory at LECOBIOL and kept in glass tubes (2.5 cm diam × 8.5 cm ht) sealed with cotton wool. The insects were fed with droplets of honey. Twenty-four-to 48-h-old *D. saccharalis* pupae were exposed to the parasitoid for a period of 72 h. Parasitized pupae were isolated and maintained at 25 ± 1 °C, 70 ± 10% RH and 14:10 h L:D until adult emergence (Pereira et al. 2008).

Experimental Design

Single 24-h-old *D. saccharalis* pupae each weighing 0.202 g were placed in glass tubes (2.5 cm diam × 14 cm ht) and exposed to seven 24- or 48-h-old *T. diatraeae* females for 24h. The parasitoids were then removed and the host pupae transferred to climatic chambers at 16, 19, 22, 25, 28 or 31 °C with 70 ± 10% RH and 14:10 h L:D.

The duration of the preimaginal development, the number and percent of emerged adults, and the number and sex ratio of the offspring [SR= number of females/(number of females + number of males] were evaluated. In addition, 15 *T. diatraeae* females were selected per treatment to evaluate their longevity. Each female was placed individually in a glass tubes (2.5 cm diam × 8.5 cm ht) with a droplet of honey. Each tube was closed with cotton and the longevity of the parasitoid recorded. The sex of each adult parasitoid was determined based on morphological characteristics of the antenna and the abdomen (Delvare & Lasalle 1993).

The experimental design was completely randomized with 6 treatments (temperatures) and 12 replications. Percentages of *T. diatraeae* emergence were analyzed through a generalized linear model with binomial distribution ($P \leq 0.05$) with the R Statistical System (Ihaka & Gentleman 1996). Analysis of variance was conducted on the other variables and, if significant at 5% prob-

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ability, to regression analysis. The equation was selected using the linear and cubic models, based on the determination coefficient (R^2), significance of the regression coefficients ($ß_i$) and regression by the F-test (up to 5% probability).

The base temperature (T_b) and thermal constant (K) were calculated according to the hyperbole method (Haddad et al. 1999) using the Mobae program based on the duration of the cycle (egg-adult) of *T. diatraeae* at each temperature tested. The number of annual generations of this parasitoid was estimated for the municipalities of Dourados, Ivinhema, and Ponta Porã, Mato Grosso do Sul State, Brasil, with the equation: NG= {T (T_m-T_b)/K}, where K= thermal constant, T_m= average temperature for each location studied, T_b = base temperature and T = number of days per month. This was based on the normal thermal conditions of sugar cane production. Climatic data (annual temperature and relative humidity averages between 1999 and 2009) at these locations were provided by the National Institute of Meteorology (INMET).

Results

The duration of the egg to adult cycle of *T. diatraeae* in *D. saccharalis* pupae decreased as the temperature increased between 16 and 28 °C (R^2 = 0.79; *F* = 174.0; *P* = 0.0001; df_{error} = 47) (Fig. 1A). The parasitoid did not complete its development at 31 °C, therefore, its biological characteristics were not evaluated at this temperature.

Temperatures between 16 and 28 °C variously affected the emergence of *T. diatraeae* from *D. saccharalis* pupae. The percentage of *D. saccharalis* pupae from which the parasitoid emerged ranged from 100 to 0%, with lower values at 22, 28 and 31 °C, and higher values at 16, 19 and 25 °C (Fig. 1B). The average number of *T. diatraeae* progeny varied from 264.8 ± 40.7 (16 °C) to 385.1 ± 36.3 (25 °C), with no significant difference in values (*P* > 0.05) at these temperatures.

The longevity of *T. diatraeae* females was highest at 16 °C (34.4 ± 1.5 d) and lowest at 28 °C (1.5 ± 0.19 d) (R^2 = 0.883; *F* = 178.37; *P* = 0.0001; df_{error} = 74) (Fig. 2A). The sex ratio of *T. diatraeae* offspring varied from 0.80 to 0.90 at 16-28 °C (*P* > 0.05). The thermal requirements for the immature stage of this parasitoid in *D. saccharalis* pupae was based on the model Y = (1/D) = -0.036393 + 0.003882x (R^2 = 95.64). The values of the base temperature (T_b) and thermal constant (K) were 9.37 °C and 257.60 degree-days (DD), respectively (Fig. 2B).

The average numbers of *T. diatraeae* generations per year in *D. saccharalis* pupae can be estimated based on the mean temperatures from 1999 to 2009 for Dourados municipality, Mato Grosso do Sul, Brasil. Climate conditions of Dourados municipality might allow 18.5 generations of this parasitoid per year, whereas this number is 5.4 for *D. saccharalis* (Fig. 3A).The numbers of generations per year of *T. diatraeae* were 19.93 and 17.3 for Ivinhema and Ponta Porã, Mato Grosso do Sul, Brasil, respectively (Figs. 3B and C).

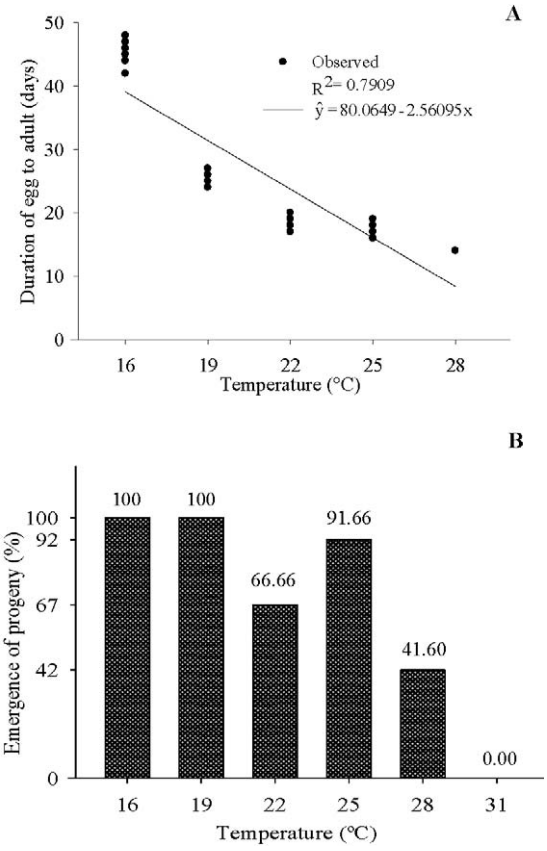


Fig. 1. Duration of egg to adult (days) (*F*= 174.0391; *P* = 0.0001) of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) (A) and percentage of *Diatraea saccharalis* (Lepidoptera: Crambidae) pupae (B) from which of *T. diatraeae* emerged at different temperatures and at 70 ± 10% RH and 14:10 h L:D.

Discussion

Trichospilus diatraeae developed in *D. saccharalis* pupae under temperature conditions of 16-28 °C. However, at 31 °C, 91.7% of host pupae contained dead pre-pupae of the parasitoid, which indicates that the upper thermal limit for this stage of this parasitoid is above 28 °C and below 31 °C. The population of *T. diatraeae* used in our experiments was from Viçosa, Minas Gerais State, Brasil, which has an average annual temperature of 19.4 °C. This population may not be adapted to high temperatures which might have contributed to the fact that the parasitoid was unable to complete its life cycle at 31 °C (Pereira et

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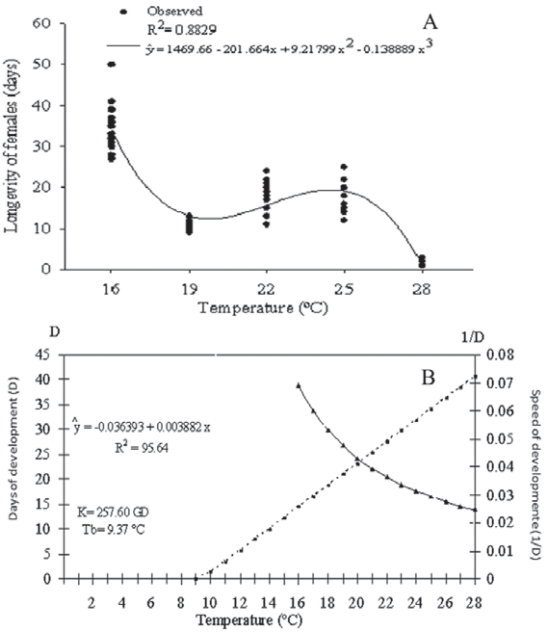


Fig. 2. Longevity (F = 178.3684; P = 0.0001) in days (A) and development speed (B) of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) females that emerged from *Diatraea saccharalis* (Lepidoptera: Crambidae) pupae at different temperatures and at 70 ± 10% RH and 14:10 h L:D.

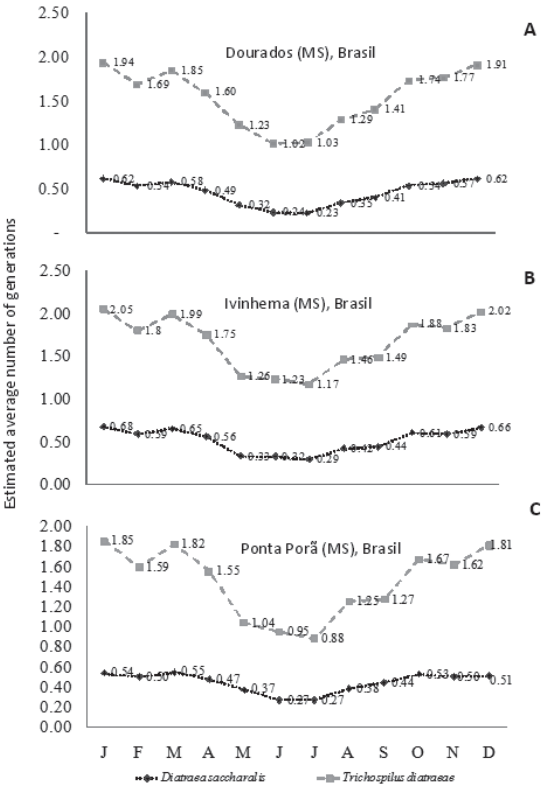


Fig. 3. Estimated average number of generations per month of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) in *Diatraea saccharalis* (Lepidoptera: Crambidae) in sugar cane producing municipalities in Mato Grosso do Sul State, Brasil (A, B and C).

al. 2008). High temperatures negatively affect the early stages of parasitoids and their development can be lower or totally unviable outside the ideal temperature range (Krugner et al. 2007). However, laboratory conditions might under estimate the effects of temperature variations on different stages of parasitoids compared with field conditions (Haghani et al. 2007; Krugner et al. 2007). Therefore, *T. diatraeae* might complete its development in the field even at temperatures above 30 °C as long as these do not coincide with crucial development stages of this parasitoid.

The mean longevity of *T. diatraeae* was 18 days at 22-25 °C, but there was no emergence of females of this parasitoid at 31 °C from *D. saccharalis*, although they were able to survive for 34 days at this temperature. The life cycle of the parasitoid can increase at 16 °C in the laboratory to decrease the number of generations during lower demand for parasitoid or absence of the host. This could be used to synchronize parasitoid emergence with the presence of its host at the right stage in the field to improve the efficiency of the mass rearing and release of this natural enemy in biological control programs (Sagarra et al. 2000; Krugner et al. 2007; Bueno et al. 2008). Thermal requirements allow calculation of the time needed to complete the development of both a pest and its natural enemies (Zanuncio et al. 2004; Pereira et al. 2011). The thermal requirements of *T. diatraeae* in the laboratory were similar to those of *D. saccharalis* (T_b = 8.0 °C and K = 371.88 DD) (Paron & Berti-Filho 2000). However, this parasitoid showed a shorter development cycle compared with *D. saccharalis* and, therefore, a higher number of generations. This might help reduce or stabilize the population density of *D. saccharalis* at a low level in the field (Pastori et al. 2008; Pereira et al. 2011).

The development of *T. diatraeae* in *D. saccharalis* pupae at temperatures between 16 and 28 °C indicates that releases of parasitoid adults to control *D. saccharalis* must be conducted during the morning or late afternoon. In addition, temperatures should be below 31 °C because this parasitoid population might not provide adequate levels of control at high temperatures. Parasitism alone might not be sufficient to adequately control *D. saccharalis* in the field. However, the overlapping of *T. diatraeae* generations may reduce *D. saccharalis* infestations in the field. The data obtained by this current study might facilitate

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the biological control of *D. saccharalis* with *T. diatraeae* in sugarcane producing regions, especially in Mato Grosso do Sul State, Brasil.

Conclusion

Trichospilus diatraeae develops in the laboratory between 16 and 28 °C in *D. saccharalis* pupae. The estimated number of generations of *T. diatraeae* per yr is higher than that of *D. saccharalis*, which might contribute to the successful biological control of this pest using this parasitoid species.

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