Reproduction of Tetrastichus howardi (Hymenoptera: Eulophidae) in Diatraea saccharalis (Lepidoptera: Crambidae) Pupae at Different Temperatures

Authors: Favero, Kellen, Pereira, Fabricio Fagundes, Torres, Jorge Braz, Oliveira, Harley Nonato De, Kassab, Samir Oliveira, et al.

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Reproduction of *Tetrastichus howardi* (Hymenoptera: Eulophidae) in *Diatraea saccharalis* (Lepidoptera: Crambidae) pupae at different temperatures

Kellen Favero[1], Fabricio Fagundes Pereira[1], Jorge Braz Torres[2], Harley Nonato De Oliveira[3], Samir Oliveira Kassab[1], and José Cola Zanuncio[4]*

Abstract

Temperature is a major abiotic factor affecting insects. The aim of this study was to evaluate the development of *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) on *Diatraea saccharalis* F., sensu Guenée (Lepidoptera: Crambidae) pupae at 6 constant temperatures (16, 19, 22, 25, 28, and 31 °C). This parasitoid developed at all temperatures with the shortest development time and lowest survival at 31 °C. *Tetrastichus howardi* females oviposited immediately after making contact with the host pupae. Parasitoids that were kept at 25 °C had the greatest fecundity, and those kept at 16 °C had the greatest longevity. The greatest net reproductive rate (R_0) occurred at 25 °C, and the intrinsic rates of increase (r_m) at 25, 28, and 31 °C were similar but significantly greater than at the lower temperatures. The generation time (T) of *T. howardi* was significantly the longest at 16 °C, which resulted in the slowest development and greatest female longevity. The fecundity of *Tetrastichus howardi* was greater in the 19 to 28 °C temperature range than at 16 °C and at 31 °C. These results are important for the multiplication of *T. howardi* in the laboratory, and for understanding its potential for the biological control of *D. saccharalis*.

Key Words: biological control; mass rearing; parasitism; sugarcane borer

Resumo

A temperatura é o principal fator abiótico que afeta insetos. O objetivo deste estudo é avaliar o desenvolvimento de *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) em pupas de *Diatraea saccharalis* F., sensu Guenée (Lepidoptera: Crambidae) em seis temperaturas constantes (16, 19, 22, 25, 28 e 31 °C) . Esse parasitóide se desenvolve em todas as temperaturas, com menor período de desenvolvimento e menor sobrevivência à 31 °C. Fêmeas de *T. howardi* podem ovipositar imediatamente após o contato com o hospedeiro, com maior fecundidade a 25 °C e sobrevivência a 16 °C. As tabelas de vida e de fertilidade mostraram uma taxa líquida de reprodução superior (R_0) a 25 °C e taxa intrínseca semelhante de aumento (r_m) com as temperaturas de 25, 28 e 31 °C. A duração de uma geração (T) de *T. howardi* foi maior a 16 °C, o que resultou no desenvolvimento mais lento e maior longevidade das fêmeas, nesta temperatura. *Tetrastichus howardi* tem maior desempenho, maior fecundidade e longevidade entre 19 a 28 °C. Estes resultados são importantes para a multiplicação de *T. howardi* em laboratório e para entender o seu potencial para o controle biológico de *D. saccharalis*.

Palavras Chave: controle biológico; criação massal; parasitismo; broca da cana-de-açúcar

Temperature is the main limiting factor affecting growth and survival of insects (Yu et al. 2013). Temperature affects development (Mawela et al. 2013), longevity (Rodrigues et al. 2013), fertility (Golizadeh et al. 2009), host location (Hance et al. 2007), and oviposition (Duale 2005) of parasitoids. The influence of temperature on the development and reproduction of insects is important in understanding parasitoid population dynamics (Golizadeh et al. 2009), efficiency in biological control programs (Roy et al. 2002), mass rearing (Yu et al. 2013), and the use of natural enemies in integrated pest management (Malekmohammadi et al. 2012).

The relationship between temperature and population growth of insects has been described using several methods (Yu et al. 2013). Life tables provide a complete description of the biological parameters of a population under certain environmental conditions, such as weather and nutritional resources (Golizadeh et al. 2009).

The intrinsic rate of the natural increase (r_m), one of the main results of life table analysis, indicates the possible success of a natural enemy (Coats 1976). A biological control agent is effective when its intrinsic rate of increase (r_m) is similar to that of the pest (Hondo et al. 2006). By determining the relationship of r_m to temperature, it is possible to identify the most favorable temperature for population growth, based on effects on the development, reproduction, and survival of insects (Castillo et al. 2006).

The sugarcane borer, *Diatraea saccharalis* F., sensu Guenée (Lepidoptera: Crambidae) is a major pest of sugarcane and of maize and sorghum (Cruz et al. 2011). The development of its larvae inside the

[1]Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados, 79804-970, Dourados, Mato Grosso do Sul, Brazil
[2]Depto de Agronomia/Entomologia, Universidade Federal Rural de Pernambuco, 52171900, Recife, Pernambuco, Brazil
[3]Embrapa Agropecuária Oeste, Caixa Postal 449, CEP 79804-970 Dourados, MS, Brazil
[4]Depto de Biologia Animal, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brazil
*Corresponding author; E-mail: samirkassab@gmail.com

plant stems hinders chemical control (Vacari et al. 2012) and provides a strong rationale for the development of biological control as an alternative to control this pest (Rodrigues et al. 2013).

The larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) can provide efficient control of *D. saccharalis* (Silva et al. 2012). This parasitoid has the ability to find its hosts within the sugarcane stem and to adapt to the various geographic areas where this crop is produced (Vacari et al. 2012).

Tetrastichus howardi (Olliff) (Hymenoptera: Eulophidae) can parasitize lepidopteran pests (Baitha et al. 2004; La Salle & Polaszek 2007; Prasad et al. 2007; Silva-Torres et al. 2010), including *D. saccharalis* in sugarcane (Vargas et al. 2011) and maize (Cruz et al. 2011). Furthermore, *T. howardi* was recorded from larvae and pupae of *D. saccharalis* (Vargas et al. 2011), and it can locate and parasitize this borer inside the galleries formed within sugarcane stems (Kfir et al. 1993). This shows that *T. howardi* could be included in the biological control of *D. saccharalis* as a supplement to the parasitism by *C. flavipes*.

A biological control program with parasitoids depends on knowledge of their biological characteristics under different temperature conditions (Rodrigues et al. 2013). Thus, the aim of this study was to evaluate the fertility life table parameters of *T. howardi* parasitizing *D. saccharalis* pupae at different constant temperatures.

Materials and Methods

The experiments were performed at the Laboratory of Entomology and Biological Control of the "Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)" of the "Centro de Pesquisa Agropecuária do Oeste (CPAO)" in Dourados, Mato Grosso do Sul State, Brazil.

MULTIPLICATION OF *DIATRAEA SACCHARALIS*

Eggs of *D. saccharalis* were obtained from the rearing stock of the Laboratory of Entomology and Biological Control of CPAO. Eggs were dipped in a 1% copper sulfate solution for disinfection and placed in 9 cm diameter Petri dishes lined with moistened filter paper. The eggs were kept in a climate chamber at 25 ± 1 °C and a 12:12 h L:D photoperiod until larvae hatched. Newly hatched larvae were fed with an artificial diet composed of soybean meal, wheat germ, vitamins, minerals, and preservatives (Hensley & Hammond 1968) until they reached the 4th instar inside glass vials (13 cm high and 8.5 cm diameter). Each newly molted 4th instar was individualized in a glass tube (8.5 cm height × 2.5 cm diameter) with artificial diet (Parra 2007) until they reached the pupal stage. The pupae were removed from the diet, sexed, and transferred to polyvinyl chloride (PVC) cages (10 cm diameter × 22 cm height). Each cage contained 24 adults (12 females and 12 males). These cages were covered with voile fastened with an elastic band, and lined internally with paper sheets as an oviposition substrate. The emerged adults were fed an aqueous 10% honey solution in plastic cylinders (4 cm height × 5 cm diameter) supplied to the insects by capillarity through a cotton swab inserted into the receptacle (Parra 2007).

MULTIPLICATION OF *TETRASTICHUS HOWARDI*

Rearing of *T. howardi* began with adults from a stock colony maintained at the Laboratory of Entomology and Biological Control (LECOBIOL) of the "Universidade Federal da Grande Dourados (UFGD)" in Dourados, Mato Grosso do Sul State, Brazil. Recently emerged (24-h-old) *Tetrastichus howardi* females were individualized and given access toto a 24-h-old *D. saccharalis* pupa in a glass tube (8.5 cm height × 2.5 cm diameter) closed with a cotton plug. Each parasitoid female was fed pure honey droplets inside the glass tube. Rearing was conducted in a room maintained at 25 ± 2 °C, 70 ± 10% RH, and a 12:12 h L:D photoperiod (Vargas et al. 2011).

EXPERIMENTAL DESIGN

Development and Reproduction of *T. howardi* at Various Temperatures

The fecundity and longevity of *T. howardi* were determined at 6 constant temperatures (16, 19, 22, 25, 28, and 31 °C). Thirty 24-h-old *T. howardi* females were isolated in glass tubes and fed with honey droplets at each temperature. One 24-h-old *D. saccharalis* pupa was offered daily to each parasitoid female until her death. Parasitized pupae were held in glass tubes at the same temperature until emergence of offspring.

The percent parasitism of the *D. saccharalis* pupae was recorded. The duration of the life cycle (egg to adult), sex ratio (number of females / number of adults), pre-oviposition and oviposition periods, fecundity, and longevity of *T. howardi* females were recorded. Parasitoids were sexed based on morphological characters (La Salle & Polaszek 2007). The natural mortality rate of *D. saccharalis* pupae at each of the temperatures was recorded in order to correct the mortality caused by the parasitoid by the formula of Abbott (1925). For this purpose, 20 non-parasitized *D. saccharalis* pupae were isolated in glass tubes per temperature, and the numbers of *D. saccharalis* adults that emerged were determined.

The results were subjected to regression analysis with the oviposition period, fecundity, and longevity as dependent variables (y) and the temperature as the independent variable (x). The equation for each variable was selected when all parameters were significant ($P < 0.05$) and based on greater coefficients of determination (R) as representative of the biological results.

Determination of the Life Table Parameters of Fertility

Data on the duration and viability of the immature stage (parasitism and adult emergence), sex ratio of the offspring, and daily production of offspring from parasitized pupae per each temperature were used to calculate the fertility life table parameters of *T. howardi* females (Birch 1948). Parameter means for the generation time (T, days), net reproductive rate (R_0), and intrinsic rate of increase (r_m) at each temperature were calculated (Maia et al. 2000) with the ProcLife test in SAS (SAS Institute 2002).

Results

DEVELOPMENT AND REPRODUCTION OF *T. HOWARDI* AT VARIOUS TEMPERATURES

The parasitoid *T. howardi* developed at all the temperatures tested. The duration of the life cycle (egg to adult) of this parasitoid in *D. saccharalis* pupae was inversely related to the temperature with values ranging from 15.0 ± 0.09 d at 31 °C to 53.2 ± 0.88 d at 16 °C. The smallest and greatest survival rates were 34% at 16 °C and 89% at 31 °C, respectively. The sex ratio of *T. howardi* in *D. saccharalis* pupae was 0.96 ± 0.01 (96% females) without differences ($P > 0.05$) between temperatures.

The fecundity of *T. howardi* was greatest in *D. saccharalis* pupae reared at 25 °C (165.4 ± 4.77) and smallest at 31 °C (77.2 ± 7.08) ($F = 19.32$; $P = 0.01$; $r^2 = 0.96$; Fig. 1C). The longevity of parasitoid females decreased with increasing temperature ($F = 53.69$; $P = 0.001$; $r^2 = 0.96$;

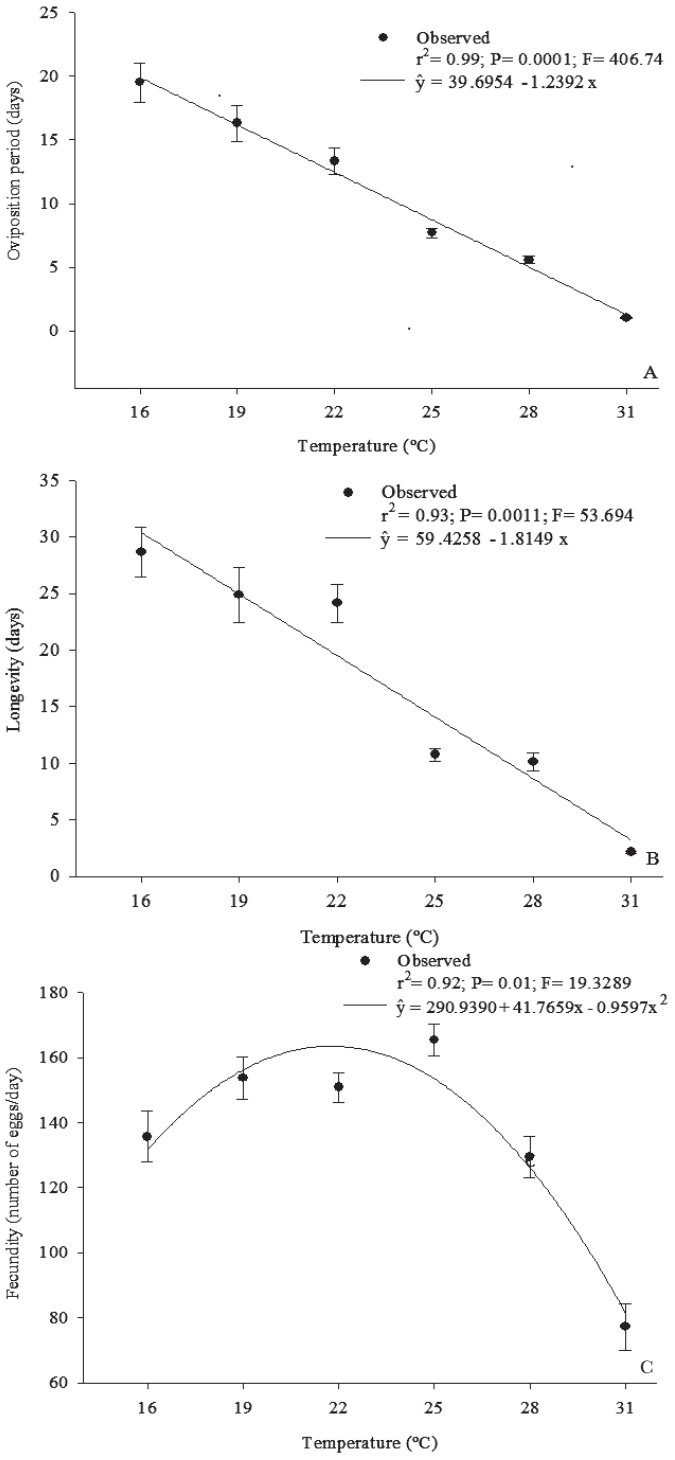

Fig. 1. Oviposition period (A), longevity (B) (days), and fecundity (number of eggs per day) (C) of *Tetrastichus howardi* on *Diatraea saccharalis* pupae at 6 constant temperatures and a 12:12 h L:D photoperiod.

Fig. 1B). The maximum longevity of *T. howardi* was 50 d at 16 °C and the minimum was 2 d at 31 °C. The longevity of this parasitoid was 21 d at 25 °C.

Tetrastichus howardi females began to oviposit on the same day they had contact with the host pupae. Parasitoid progeny production was greater when parasitism occurred during the 1st day of exposure to host pupae at all temperatures. About 90% of the offspring was produced when parasitism occurred during the first 5 d at 25, 28, and 31 °C and during the first 10 d at the lower temperatures (16, 19, and 22 °C). However, parasitism was recorded up to 13 and 42 d of adult parasitoid age at 25 and 16 °C, respectively. The oviposition period of *T. howardi* decreased with increasing temperature (F = 406.74; P < 0.0001; r^2 = 0.99; Fig. 1A).

FERTILITY LIFE TABLE PARAMETERS

The average generation time (T) of *T. howardi* developing in *D. saccharalis* pupae was inversely correlated with temperature and ranged from 12.9 d at 31 °C to 47.4 d at 16 °C (Table 1). The number of progeny produced per *T. howardi* female, characterized by the net reproductive rate (R_0), ranged from 46.0 to 107.3 females at 16 and 25 °C, respectively. The smallest reproductive rates were observed at 16 and 31 °C (P > 0.05) (Table 1). The intrinsic rate of increase (r_m) was positively correlated with the temperature up to 25 °C (Table 1), where it reached a peak (P > 0.05) (Table 1).

Discussion

Tetrastichus howardi developed and reproduced in *D. saccharalis* pupae at temperatures ranging from 16 to 31 °C, which shows its potential of adapting to a variable environment. This is important because the sugarcane borer occurs throughout the year at various temperatures in Brazil (Pinto et al. 2009). In addition, *T. howardi* produced 165.4 descendants per *D. saccharalis* pupae at 25 °C,which is a greater number of progeny per host pupa than the number it produced on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) pupae, i.e., 101.4 (Kfir et al. 1993). This difference in progeny produced per pupa may be due to differences in the host species acceptability by this parasitoid (Malekmohammadi et al. 2012), the immune response (Andrade et al. 2010), or the adaptation to the host and nutritional quality (Altoé et al. 2012; Favero et al. 2013a,b). Also, this difference in production of progeny is sometimes due to the host biomass (Pastori et al. 2013), but biomass of the *D. saccharalis* pupae is very similar to that of *H. armigera* pupae.

The longer longevity of *T. howardi* females at the lowest temperature and greatest number of descendants at the intermediate temperatures are important findings because fecundity is related to the female's longevity, especially for species with offspring throughout the reproductive period. This fact was demonstrated by the high parasitism rates of *T. howardi* in the first days of life. The presence of a particular host species (Kfir et al. 1993), the mating activity (Karindah et al. 2005), and the density of hosts also affect the parasitoid longevity.

Table 1. Fertility life table parameters of *Tetrastichus howardi* in *Diatraea saccharalis* pupae at various constant temperatures and a 12:12 h L:D photoperiod. R_0 is the net reproductive rate, r_m is the intrinsic rate of increase, and T is the mean generation time.

Temperature (°C)	R_0 (♀/♀)	r_m (♀/♀ × day)[a]	T (days)
16	46.0 ± 2.24 e	0.08 ± 0.00 d	47.4 ± 0.37 a
19	107.2 ± 4.18 b	0.15 ± 0.00 c	30.4 ± 0.17 b
22	86.7 ± 2.72 c	0.21 ± 0.00 b	20.5 ± 0.11 c
25	118.7 ± 3.09 a	0.30 ± 0.00 a	15.6 ± 0.05 d
28	77.0 ± 3.87 d	0.31 ± 0.00 a	13.7 ± 0.06 e
31	57.5 ± 6.44 e	0.31 ± 0.00 a	12.9 ± 0.05 f

Means (± 95% confidence interval) followed by the same letter per column do not differ by t-test at 5% probability. Errors estimated by the Jackknife method.
[a]rate of population increase per unit time.

The similar longevities of *T. howardi* at 25 °C and 28 °C indicate that this population was adapted to these temperatures. The population of *T. howardi* used in our experiments originated from Dourados, Mato Grosso do Sul State, Brazil (Vargas et al. 2011), which has an average annual temperature close to these temperatures. However, laboratory conditions might underestimate the effects of temperature variations on different stages of parasitoids compared with field conditions (Haghani et al. 2007; Krugner et al. 2007). The reduced longevity of this parasitoid at a constant temperature of 31 °C may affect its performance in the field. High temperatures negatively affect the early stages of parasitoids, and their development can be impaired or completely be prevented outside the ideal temperature range (Krugner et al. 2007). On the other hand, at Dourados, Mato Grosso do Sul State, temperatures at or above 31 °C may occur on summer days but only for short periods, and average daily temperatures do not reach 31 °C (Empresa Brasileira de Pesquisa 2014). Moreover, the prolonged longevity of *T. howardi* at lower temperatures is important for mass rearing because lower temperatures can allow retention of this parasitoid in its immature stages for longer periods to adjust its release into the field according to pest population denisty (Pomari et al. 2012).

Oviposition by *T. howardi* females began on the same day that they made contact with *D. saccharalis* pupae and continued up to 42 d, which is similar to oviposition observed using *H. armigera* pupae as host (Moore & Kfir 1995). The oviposition of *T. howardi* on the 1st day and for up to 19 d was observed in *Plutella xylostella* L. (Lepidoptera: Plutellidae) pupae (Karindah et al. 2005). The age when the parasitoids are the most productive must be determined in order to release them into the field at the best age (Silva-Torres et al. 2009). In addition, parasitoids should be replaced in mass rearing after oviposition has peaked, which is around 5 d for *T. howardi* at 25 °C.

The production of about 90% of the progeny of *T. howardi* females by the 5th day of life at 25, 28, and 31 °C and by 10 d at 16, 19, and 22 °C in *D. saccharalis* pupae confirms that this parasitoid performs similarly on the latter host as it did at 25 °C in *H. armigera* pupae (Cruz et al. 2011), in which 97% of the progeny were produced in the first 10 d after emergence (Kfir et al. 1993). This characteristic is important for biological control programs in the field, where the parastoid is exposed to other biotic and abiotic mortality factors and rapid oviposition enhances sucess (Bueno et al. 2012).

The shorter oviposition period of *T. howardi* females with increasing temperature occurred because this parasitoid's eggs mature using nutrients acquired during larval development (Kapranas & Luck 2008). Part of these nutrients is used for early egg maturation, and the remainder is conserved for future egg production (Jervis et al. 2008). Egg maturation is temperature dependent with longer maturation periods at lower temperatures, due to reduced parasitoid metabolism (Kapranas & Luck 2008). However, insects at lower temperatures may compensate for the lower number of eggs laid by ovipositing during a longer period of time (Legaspi & Legaspi 2005) as we observed in this study.

Tetrastichus howardi should be multiplied at 25 °C, but other temperatures, except temperatures below 16 °C and above 31 °C, can be used to delay or accelerate the development of progeny. At 25 °C, this parasitoid can increase its population at a maximum rate of 118 times every generation (Table 1). Lower R_0 values at 16 and 31 °C showed that adverse temperatures affect the development of parasitoids, as reported by Duale (2005), Castillo et al. (2006), and Pastori et al. (2013). Temperature extremes can reduce reproduction of *T. howardi* because of the lower number of eggs laid per host pupa and the lower survival and longer development of their immatures (Duale 2005).

Our finding that the intrinsic rate of increase (r_m) was near its maximum at 25 °C reinforces our conclusion that 25 °C is the most favorable temperature for *T. howardi* to parasitize the pupae of *D. saccharalis*. On the other hand, this variable should be considered along with the others, because a short generation period at the higher temperatures could overestimate their value in relation to r_m. This was demonstrated by the similarity of the r_m values at 25, 28, and 31 °C (Table 1). The estimation of the population growth based on r_m showed that each *T. howardi* female produced 165.36, 129.36, and 77.17 descendants at 25, 28, and 31 °C, respectively (Table 1).

The inverse relation between the generation time (*T*) of *T. howardi* with increasing temperatures was similar to that observed in *H. armigera* pupae (Kfir et al. 1993). The shorter generation time and longevity and the smaller progeny production of *T. howardi* at 31 °C indicate that high temperatures could reduce the foraging efficiency of parasitoids and their reproduction success (Denis et al. 2011). Besides, in some species, higher temperatures may increase the probability of a host defense against immature parasitoids or reduce the efficiency of the parasitoid polydnavirus injected by the female during oviposition (Hance et al. 2007).

The fertility life table and biological aspects show that *T. howardi* is a promising natural enemy of the sugarcane borer. *Tetrastichus howardi* parasitized and developed in *D. saccharalis* pupae at temperatures ranging from 16 to 31 °C, but with greater biological performance between 19 and 28 °C. This indicates that *T. howardi* has high thermal plasticity, which facilitates its use in regions with climatic variability. *Tetrastichus howardi* can be multiplied in the laboratory, and the number of parasitoids produced at a given constant temperature can be estimated.

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