Biological parameters and thermal requirements of *Trichogramma pretiosum* reared on *Helicoverpa armigera* eggs

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Abstract – The objective of this work was to determine the biological parameters and thermal requirements of *Trichogramma pretiosum* TM strain reared on *Helicoverpa armigera* eggs. A card containing 20 eggs of *H. armigera* was offered for each *T. pretiosum* female, at temperatures of 18, 20, 22, 25, 28, 30 and 32°C. All life stages of *T. pretiosum* were observed under the given temperature conditions. The optimum temperature for the parasitism of *T. pretiosum* was 25°C, and the highest longevity was found at the same temperature. The highest parasitoid emergence rates occurred at temperatures of 22, 25 and 28°C. The highest proportion of *T. pretiosum* individuals emerged per egg was observed at 18°C. Concerning the sex ratio, the highest proportion of females occurred at 28°C, and the highest percentage of parasitism viability was observed at this same temperature. The lowest thermal threshold for total preimaginal development and thermal requirements of *T. pretiosum* were 10.82°C and 134.55 degree-days, respectively. The estimated number of generations of *T. pretiosum* in the average temperatures of municipality of Bom Jesus, state of Piauí, Brazil is four per month.

Index terms: Trichogrammatidae, biological control, parasitoid, temperature.

Parâmetros biológicos e exigências térmicas de *Trichogramma pretiosum* sobre ovos de *Helicoverpa armigera*

Resumo – O objetivo deste trabalho foi determinar os parâmetros biológicos e as exigências térmicas de *Trichogramma pretiosum* linhagem TM sobre ovos de *Helicoverpa armigera*. Para cada fêmea, foi oferecida uma cartela contendo 20 ovos de *H. armigera*, às temperaturas de 18, 20, 22, 25, 28, 30 e 32°C. Todos os estádios de vida de *T. pretiosum* foram observados sob as dadas condições de temperatura. A temperatura ótima para o parasitismo de *T. pretiosum* foi de 25°C e a maior longevidade foi verificada à mesma temperatura. As maiores taxas de emergência do parasitoide foram às temperaturas de 22, 25 e 28°C. A maior proporção de indivíduos de *T. pretiosum* emergidos por ovo foi observada a 18°C. Quanto à razão sexual, a maior proporção de fêmeas ocorreu a 28°C, e o maior percentual de viabilidade do parasitismo foi observado à mesma temperatura. O limiar térmico inferior para o desenvolvimento pré-imaginal total e as exigências térmicas de *T. pretiosum* foram de 10,82°C e 134,55 grau-dias, respectivamente. O número estimado de gerações de *T. pretiosum* às temperaturas médias do Município de Bom Jesus, Piauí, foi de 4 por mês.

Termos para indexação: Trichogrammatidae, controle biológico, parasitoide, temperatura.

Introduction

Helicoverpa armigera (Hübner, 1805) (Lepidoptera: Noctuidae) was observed in several regions in Brazil, and caused significant losses to many crops. Among these, the soybean (*Glycine max* L.) crop stands out, and that pest damaged the vegetative and reproductive stage in soybean production (Sosa-Gómez et al., 2016). Due to the lack of studies on the integrated management of *H. armigera*, the main control method is represented by the use of chemical insecticides, which results in disadvantages because of the high use of toxic substances that are not very selective, showing slow environmental degradation and also potential to bioaccumulation and biomagnification. At the same time, these chemical pesticides can lead to serious consequences such as the target pest resistance, elimination of natural enemies, environmental contamination and risks to human health (Amichot et al., 2016).

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Thus, alternative control measures must be used in order to attempt to minimize the damage caused by this lepidopteran in agro-ecosystems, consequently reducing the use of agrochemicals. Among these management tactics, the biological pest control using the parasitoid *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) stands out, being considered a micro-hymenoptera egg parasitoid of several insects of economic relevance. This parasitoid includes several species that have been used as control agents by preventing the development of host species (Molnár et al., 2016).

However, the successful use of biological control with *Trichogramma* spp. depends on factors such as the knowledge of the species potential and/or strain to be employed, the host to be controlled, the thermal requirements and the influence of the prevailing weather conditions; thus, these factors may be decisive for the success of releases in the field (Marchioro et al., 2015; Coelho Jr. et al., 2016). The study of thermal requirements facilitates the understanding of the relationship between temperature and the biological control agent in addition to the prediction of the number of generations in the field (Reznik & Voinovich, 2015).

As explained above, the information on the determination of the optimum temperature under laboratory conditions in the study of *Trichogramma* sp. can provide the basis to obtain the desired number of parasitoids, with a forecast of survival, life cycle duration and females' reproductive capacity at known temperatures (Pereira et al., 2009). In addition to this, there are few studies that involve the use of biological control involving native species of the studied region, especially *T. pretiosum*, which can provide important information for the integrated management of the target pest in soybean crops.

The objective of this work was to determine the biological parameters and thermal requirements of *Trichogramma pretiosum* TM strain reared on *Helicoverpa armigera* eggs.

Materials and Methods

The experiment was carried out at the laboratory of plant science at the Universidade Federal do Piauí, Campus Professora Cinobelina Elvas, municipality of Bom Jesus, state of Piauí, Brazil.

The population of *H. armigera* used in this experiment came from the insect breeding laboratory of the Universidade Federal do Piauí, where they were reared on artificial diet adapted from Kasten Júnior et al. (1978) at temperature of 25±1°C, and relative humidity (RH) of 60±1%. Neonate larvae (0 to 24 hours old) were individualized and transferred to 100 mL plastic containers with lid containing artificial diet until they reached the pupal stage. After emergence, moths were transferred to 40×30 cm diameter polyvinyl chloride (PVC) cages, internally coated with white paper sheets serving as oviposition substrate. The moths were fed with a honey-based solution (10%, v/v) and kept under controlled conditions (25±5°C, 60±10% RH, 12 hours photophase). The eggs were collected and stored (25±5°C) in plastic pots of 100 mL until emergence of caterpillars. *H. armigera* eggs not used in the experiments were used for the maintenance of the colony in the laboratory.

Trichogramma pretiosum (Riley, 1879) TM strain was collected in traps, using *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) eggs as an alternative host in soybean commercial crop in the Cerrado biome, in the municipality of Baixa Grande do Ribeiro, state of Piauí (PI), Brazil (08º40'56.4"S, 45º05'39.2"W). This municipality has sub-humid tropical climate, with temperatures ranging from 26 to 36°C and average rainfall ranging from 700 mm to 1,200 mm between December and May (Aguiar & Gomes, 2004). The species *T. pretiosum* was identified by morphological characteristics according to Querino & Zucchi (2003).

Trichogramma pretiosum TM strain was reared and multiplied in *A. kuehniella* eggs, which received a diet based on whole wheat flour (97%) and yeast (3%), at 25±1°C, 60±1% RH, and 14 hours of photophase, according to the method proposed by Parra (1997).

The diet was placed in plastic trays containing precut corrugated cardboard strips that are intended to provide support and also to be a place for pupation. In each tray 0.35 g of *A. kuehniella* eggs were placed and covered by a plastic bag with an opening in voile fabric allowing internal ventilation. After that, the trays were kept in larval development room, where they remained until the beginning of adult emergence.

A. kuehniella adults were collected using an adapted vacuum cleaner for sucking the insects from inside the breeding boxes, and then the adults were transferred to plastic containers (2.5 L). At the end of the process, the eggs, which fell through a fine mesh into the plastic containers, were collected.

T. pretiosum TM strain was raised according to the methodology by Stein & Parra (1987). Once collected, the host's eggs were placed in blue cardboard (8.0 × 2.0 cm) fixed with gum arabic diluted in water (50%, m/v) and subjected to *T. pretiosum* parasitism.

Before installing the experiments, the *T. pretiosum* TM strain was kept for one generation on *H. armigera* eggs in order to eliminate a possible conditioning by the breeding of the alternative host (*A. kuehniella*).

Females of the *T. pretiosum* TM strain (0-24 hours of age) were individualized in glass tubes (12 mm diameter and 75 mm height). Twenty eggs of *H. armigera*, considered not feasible, were offered for each female, in each of the temperature treatments. A droplet of pure honey was offered to females as food. The parasitism was allowed for 24 hours inside climatic chambers set at constant temperatures of 18, 20, 22, 25, 28, 30 and 32°C (treatments) according to Bueno et al. (2012), where all these treatments were kept at 70±10% RH, and 14 hours of photophase.

The experimental design was completely randomized with seven treatments (different temperatures) and 20 repetitions per treatment, totaling 140 females of *T. pretiosum* (20 females per treatment) and 980 eggs of *H. armigera* (140 eggs per treatment).

The following biological parameters were observed at each temperature: cycle duration (egg-adult), conducted through daily observations, always at the same time, in a 24-hour interval; parasitism, by counting the number of darkened eggs; emergence percentage, carried out under a stereoscopic microscope, by counting the host eggs in which there was an orifice intended for the adults exit; number of emerged adults per egg, calculated by dividing the total number of adults by the total number of orifices observed in each treatment (Bueno et al., 2012); sex ratio (SR = number of females/number of females + number of males), determined according to Bowen & Stern (1966); percentage of viability, by counting the host eggs in which there was an orifice for adults exit; longevity, carried out by daily observations, always at the same time, in a 24-hour interval, for mortality accounting adapted from Bueno et al. (2012).

The results were analyzed for normality and homoscedasticity to verify the statistical assumptions. The data were submitted to the ANOVA analysis of variance and the averages were compared by Tukey test at 5% probability and they were analyzed using the statistical software R version 3.0.3 (R Core Team, 2014). In addition to this, a regression analysis was performed to better understand the relationship between temperatures and the results found, based on the study of Altoé et al. (2012), and the model chosen was based on the best fit of the determination coefficient (R^2) and 5% probability.

The calculations of the lowest thermal threshold for development (Tb) and thermal requirement (K) were obtained by the hyperbole method according to Haddad et al. (1999), through the MOBAE program (Statistical Models Applied to Entomology), based on the duration of the period (egg–adult) at temperatures studied for *T. pretiosum* TM strain on *H. armigera* eggs. The generation number (GN) of *T. pretiosum* TM reared on *H. armigera* for the municipality of Bom Jesus, state of Piauí, Brazil, covering the grain producing region of municipalities of Currais, Baixa Grande do Ribeiro, Uruçuí, and Palmeira, in state of Piauí, was estimated by the equation: GN = {D(Tm-Tb)/K}, in which: K, thermal requirement; Tm, average temperature for each studied location; Tb, lower thermal threshold; and D, time (days). Thus, the number of generations of the parasitoid was calculated for the period from October to May, corresponding to the soybean crop season, using monthly averages from 10 years obtained from meteorological stations at the locations.

Results and Discussion

The period duration of *Trichogramma pretiosum* TM strain development on eggs of *H. armigera* was significantly affected by the evaluated temperatures. The cycle duration values showed an inverse relationship based on the measured temperature, providing an increase in the development speed as the temperature increased. For the temperatures of 18 and 20°C, 14.8 days on average were required for the complete development of the parasitoid, while at temperatures of 30 and 32°C, it took only 6.1 and 5.9 days for the development of *T. pretiosum* TM on eggs of *H. armigera*, respectively (Table 1, Figure 1 A).

The variation in temperature affected the biological characteristics of *T. pretiosum* TM, when reared on eggs of *H. armigera*. The necessary time for the complete development of the parasitoid decreased as the temperature increased. The development may affect the suitability of the parasitoid, as well as the handling and foraging time, interfering with the host behavior of searching (Carvalho et al., 2014; Krechemer & Foerster, 2015).

The reduced development found in low temperatures may also be associated with the adaptive characteristics of the *T. pretiosum* TM strain. It is a strain collected in a high temperature region, providing a greater adaptation of the parasitoid at specific temperatures of their origin region (Altoé et al., 2012; Lessard & Boivin, 2013).

The parasitism of *T. pretiosum* on eggs of *H. armigera* had the optimum temperature of 25°C, resulting in higher percentage in relation to other temperatures (Table 1, Figure 1 B). Similar results were found by Bari et al. (2015), who verified low percentage of parasitism at the temperatures of 18°C and 34°C, when evaluating the performance of *Trichogramma zahiri* (Polaszek, 2002) (Hymenoptera: Trichogrammatidae) on eggs of *Dicladispa armigera* (Oliver, 1808) (Coleoptera: Chrysomelidae). Foerster et al. (2014) obtained a higher percentage of parasitism of *T. pretiosum* and *T. atopovirilia* on eggs of *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Noctuidae) at the temperature of 26°C. Marchioro et al. (2015) observed the highest *T. pretiosum* and *T. atopovirilia* parasitism rates on eggs of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), at the temperatures of 20 and 25°C. In extreme temperatures, the low occurrence of parasitism may be associated with a reduction in the turgor of *H. armigera* eggs, which makes it difficult to insert the oviduct of the parasitoid into the host chorion, compromising its efficiency in the field.

There was 100% emergence at the temperature of 25°C, being higher than at the temperatures of 18, 20, 22, 30 and 32°C, and being statistically the same only at 28°C (Table 1, Figure 1 C). The emergence percentage was different due to the temperature increment, being significantly higher at 25°C. Cabezas et al. (2013) found significant values for emergence when evaluating three strains of *T. pretiosum* on eggs of *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera: Noctuidae) at 25°C. As observed in the study herein, Bari et al. (2015) also observed smaller emergence rates in extreme temperatures for *T. zahiri* (Polaszek, 2002) (Hymenoptera: Trichogrammatidae) on eggs of *Dicladispa armigera* (Oliver, 1808) (Coleoptera: Chrysomelidae).

Regarding the number of emerged individuals per egg, the highest proportion was obtained at 18°C with 1.84 individual, which was different from the other evaluated temperatures, even though, in the other temperatures, they did not differ significantly from each other, showing more than one individual per *H. armigera* egg (Table 1, Figure 1 D). Despite this difference, the values were always higher than one, at all evaluated temperatures, highlighting that the quality of the host directly influences the development of the parasitoids (Krechemer & Foerster, 2015).

For the sex ratio of *T. pretiosum* developed on eggs of *H. armigera*, in virtually all the tested temperatures, a satisfactory proportion of females was found, with a percentage higher than 70%. However, at the temperature of 18ºC, the sex ratio was lower than at the other temperatures (Table 1).

Table 1. Means of life cycle, parasitism, emergence, number of *Trichogramma pretiosum* emerged by egg, sex ratio, viability, and longevity of *Trichogramma pretiosum* TM strain reared on *Helicoverpa armigera* eggs at different temperatures[(1)].

Temperature[(2)] (°C)	Cycle	Longevity	Parasitism	Emergence	Viability	Individuals per egg	Sex ratio
	(days)		(%)				
18	14.78±0.11a	23.2±0.19a	33.9±0.56d	58.50±0.94bc	79.05±0.70c	1.84±0.07a	0.74±0.03b
20	14.80±0.10a	11.25±0.18c	38.00±0.55d	54.50±0.92bc	88.15±0.68bc	1.40±0.07b	0.88±0.03ab
22	12.60±0.10b	10.90±0.18c	50.75±0.55bc	71.90±0.92bc	86.70±0.68bc	1.36±0.06b	0.84±0.04ab
25	10.25±0.10c	12.35±0.18b	85.00±0.55a	100.00±0.92a	80.30±0.68c	1.19±0.06b	0.78±0.03ab
28	7.85±0.10d	7.85±0.18e	57.50±0.55b	76.30±0.92ab	94.34±0.68a	1.13±0.06b	0.91±0.03a
30	6.10±0.10e	9.00±0.18d	45.50±0.55cd	62.00±0.92bc	88.52±0.68bc	1.27±0.07b	0.89±0.03ab
32	5.90±0.10e	6.55±0.18f	40.00±0.55cd	52.13±0.92c	90.62±0.68ab	1.36±0.07b	0.87±0.03ab
CV (%)	4.56	7.43	24.65	36	35.16	23.2	18.69
F	2,327.85	704.07	38.85	8.71	16.44	11.35	58.03
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
df	132	132	132	132	132	132	132

[(1)]Means (±standard error) followed by equal letters in the columns do not differ by Tukey test, at 5% probability. [(2)]RH 70±10% and photophase of 14 hours.

The study of temperature has often been reported as a relevant factor in determining the sex ratio in many species of *Trichogramma* (Moiroux et al., 2014). The results for the sex ratio parameter did not fit in the regression model determined by the R^2 value.

The sex ratio can also vary according to the species or strain, as well as the environmental condition. In

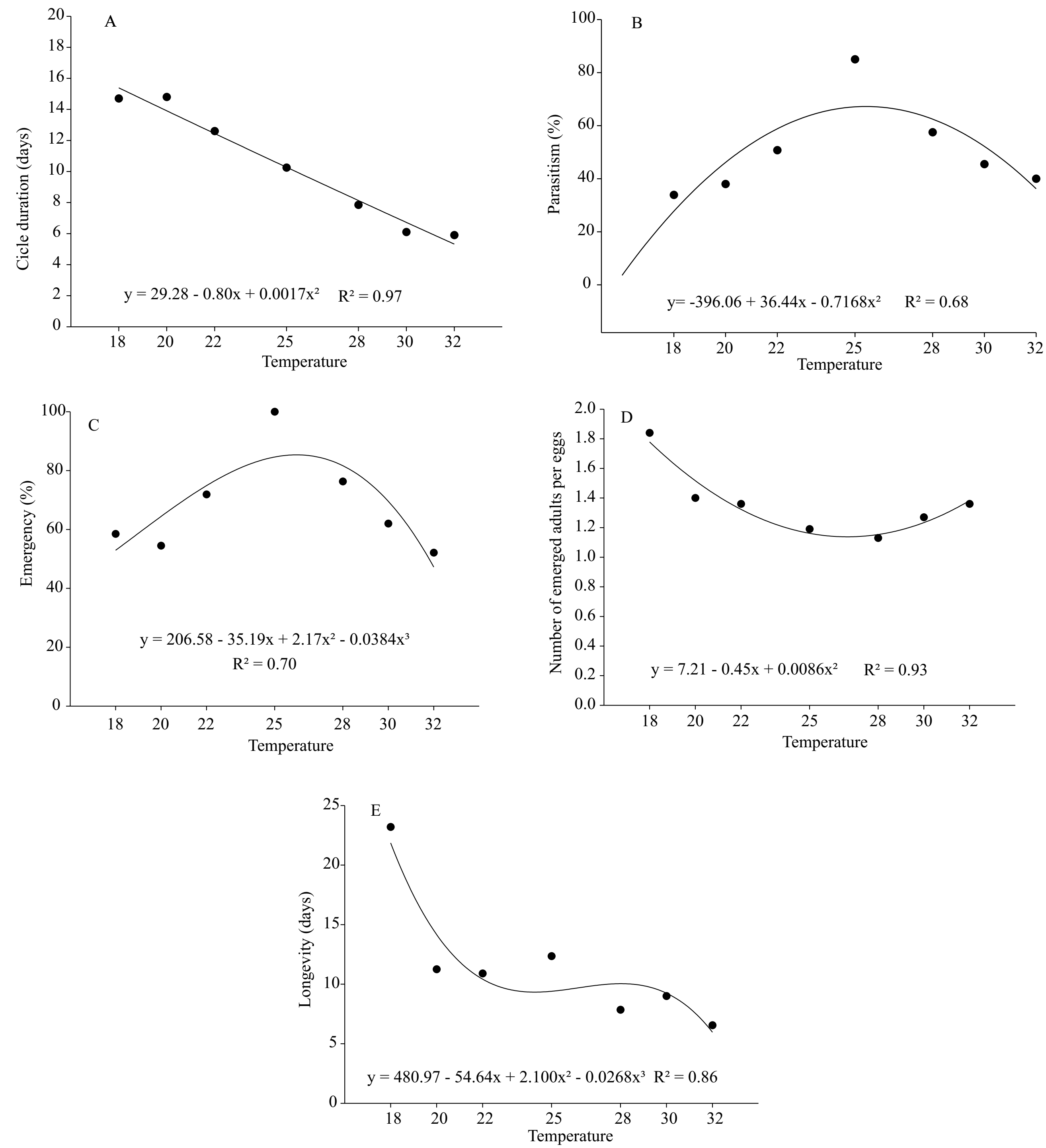

Figure 1. Duration of the cycle (A), parasitism (B), emergence (C), number of emerged individuals per egg (D), longevity (E) of *Trichogramma pretiosum* TM strain reared on *Helicoverpa armigera* eggs at different temperatures.

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this study, the highest proportion was observed at 28°C, differing only from the temperature of 18°C, with percentage lower than the other temperatures. However, with the sex ratio above 70% at all temperatures, it indicates the actual quality of the host *H. armigera* in the development of *T. pretiosum* TM, where the values obtained represent a greater number of females than males, which is an important fact for the biological control.

The viability of parasitism of *T. pretiosum* on eggs of *H. armigera* varied depending on the tested temperatures (Table 1). The highest results were obtained at the temperatures of 28 and 32°C, with viability of 94.3 and 90.6%, respectively, while the temperature of 18°C was the one that had the lowest value, of 79.0%. The results of viability parameter did not fit in the regression model determined by the R^2 value.

The percentage of viability was influenced by temperatures, ranging between 79 and 94%. The viability values were close to or above 80% at all temperatures, demonstrating the potential of egg parasitoid *T. pretiosum* on *H. armigera*, with effective development of the parasitoid, even under different thermal regimes. This characteristic is extremely important for parasitoids as biological control agents, since it is efficient in the management of these species in regions with large thermal variations throughout the harvest period.

The longevity of the parasitoid was significantly influenced by the tested temperatures. An indirect relationship between temperatures and longevity was observed, with 6.5 to 23.2 survival days at the temperatures of 32 and 18°C, respectively (Table 1 and Figure 1 E). Similar results were found by Firake & Khan (2014), who observed the same behavior when assessing the biology of *T. poliae* and *T. chilonis* on eggs of *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae). These results are contrary to the ones reported by Pastori et al. (2007), who claim that the reduction in temperature promotes an increase on the parasitoids longevity due to a reduction in metabolic rate when submitting the parasitoids to reduced thermal shocks.

According to the biological parameters tested at different temperatures, the lowest thermal threshold was determined by the lowest threshold temperature (Tb) and thermal requirement (K) for *T. pretiosum* TM, on eggs of *H. armigera*. It was possible to verify that the embryonic development of the parasitoid started when the temperature remained above 10.82°C (Tb), and the accumulated heat necessary for the complete development of the parasitoid was 134.55 degrees-days (K) (Figure 2).

Through these lower threshold temperature (Tb) and thermal requirement (K) results, it is possible to observe the direct influence of temperature, both in the host and in the parasitoid. Results similar to those

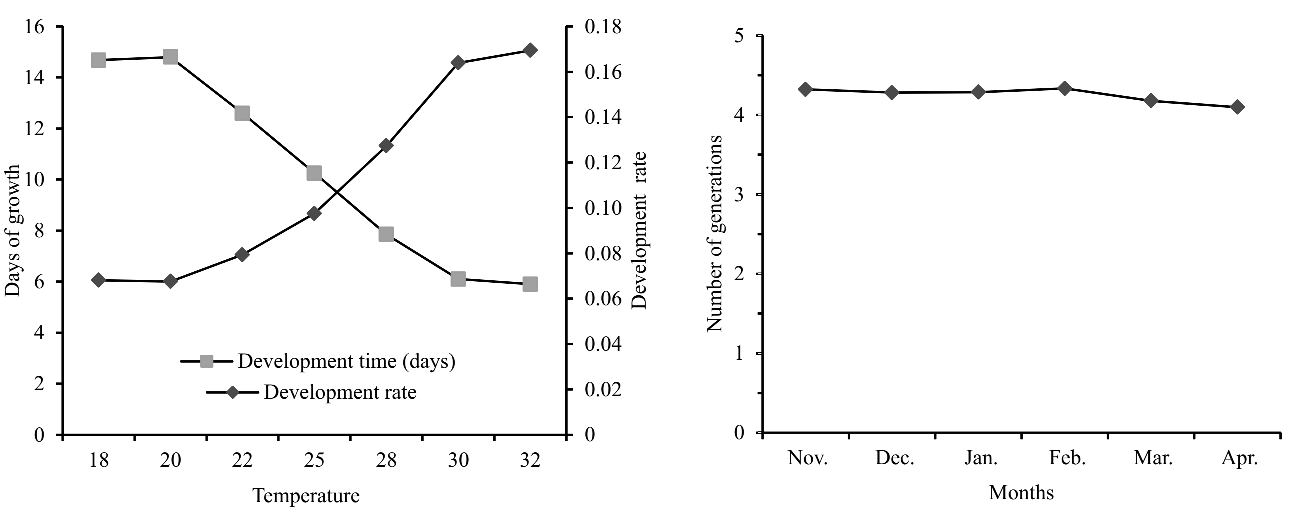

Figure 2. Duration (days) and development speed of *Trichogramma pretiosum* TM reared on *Helicoverpa armigera* eggs subjected to different temperatures (RH=70±10% and photophase=14 hours) (A); and estimated number of generations per month of *T. pretiosum* TM, reared on eggs of *Helicoverpa armigera* (B).

found in this study were found by Pastori et al. (2008), who observed a base temperature of 11.9ºC, and thermal requirement of 153.4 degrees per day for *T. pretiosum* LM strain on eggs of *Bonagota salubricola* (Meyrick, 1931) (Lepidoptera: Tortricidae). Altoé et al. (2012), when evaluating *T. pretiosum* on eggs of *Trichoplusia ni*, found 11.84ºC for Tb and 128.37 degrees per day, confirming that this characteristic may vary depending on the host.

The estimate of the number of generations of *T. pretiosum* TM on *H. armigera*, using the average temperatures corresponding to the soy productive period in the municipality of Bom Jesus was obtained based on the duration of the parasitoid cycle, studied at different temperatures, and an average of four generations per month was found (Figure 2), which may reach approximately 20 generations throughout the soybean crop cycle.

Extreme temperatures in the field affect parasitoid efficiency in biological control programs (Foerster et al., 2015). The optimal temperature range for the development of a parasitoid is an important tool for programming the breeding in order to synchronize the parasitoid emergence with the presence of the interest target host stage in the field and improve the efficiency of breeding and mass releasing programs, according to the environmental conditions (Bueno et al., 2009; Foerster et al., 2015; Wuet al., 2016). Thus, the results of this study provide subsidies to choose the ideal time to release the parasitoid in an integrated *H. armigera* management program. In addition to this, the results indicate that *T. pretiosum* had satisfactory performance in *H. armigera* eggs at the temperatures studied, suggesting the potential of its successful use as a control agent of this pest in regions where they show the same range of temperatures as the ones evaluated in the study herein. However, further studies are necessary at the semi-field and field level to confirm the potential of *T. pretiosum* to maintain the pest population at lower levels.

Conclusions

1. The temperature of 25°C provides a better performance of *Trichogramma pretiosum* reared on *Helicoverpa armigera* eggs, with a high percentage of parasitism, longevity and parasitoid emergence.

2. The highest proportion of individuals emerged per egg is observed at 18°C, and the major proportion of females and percentage of viability of *T. pretiosum* parasitism occurs at 28°C.

3. The lowest thermal threshold for total preimaginal development and thermal requirements of *T. pretiosum* are 10.82°C and 134.55 degree-days, respectively, and its estimated number of generations is on average four per month.

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