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Thermal requirements of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) lines in *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) eggs

Requerimientos térmicos de las líneas de *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) en huevos de *Neoleucinodes elegantalis* (Lepidoptera: Crambidae)

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Abstract: This study aimed to evaluate the impact of temperature on the development of two lines of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), to determine the thermal requirements of this parasitoid wasp on *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) eggs. The experiment was carried out with two lines ("Ubajara" and "Guaraciaba", Ceará State) of *T. pretiosum* collected naturally parasitizing eggs of *N. elegantalis* in tomato (*Solanum lycopersicum*) fruits. In this experiment, 40 eggs of the host *N. elegantalis* and 4 females of the parasitoids were used, with exposure to parasitism for 24 hours at 25 ± 1 °C, relative humidity of 70 ± 10 %, and 12-h photophase. At the end of this period, females were removed and the tubes were transferred to incubators (RH = 70 ± 10 %; 12-h photophase), exposed to 15, 20, 25, 30, or 35 °C, until emergence of the following generation of the parasitoids. The percentage of emergence, sex ratio, the number of parasitoids that emerged per egg, and cycle duration were assessed. The experiments were set up in a completely randomized experimental design with 5 treatments (temperatures) and 12 replicates. ANOVA was conducted and the means were compared by Tukey test ($P < 0.05$). The base temperatures were 10.77 °C and 10.86 °C and the number of generations per year were 33.29 and 35.63 for "Ubajara" and "Guaraciaba", respectively. The study showed that temperature changed the biological parameters, and cycle duration of the "Ubajara" and "Guaraciaba" lines decreased as temperature increased.

Keywords: Tomato fruit borer, egg parasitoids, biological parameters, base temperature, *Trichogramma pretiosum*, *Neoleucinodes elegantalis,* Trichogrammatidae, Crambidae.

Resumen: El objetivo de este estudio fue evaluar el impacto de la temperatura en el desarrollo de dos líneas de *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), para determinar los requisitos térmicos de este parasitoide en huevos de *Neoleucinodes elegantalis* (Lepidoptera: Crambidae). El experimento se llevó a cabo con dos líneas ("Ubajara" y "Guaraciaba", Ceará) de *T. pretiosum* a partir de huevos parasitarios de *N. elegantalis* en frutos de tomate (*Solanum lycopersicum*). En este experimento, se usaron 40 huevos del huésped *N. elegantalis* y 4 hembras de los parasitoides, con exposición al parasitismo durante 24 horas (a 25 ± 1 °C, 70 ± 10 % de HR y fotofase de 12 h). Al final de este período, se retiraron las hembras y los tubos se transfirieron a incubadoras (HR = 70 ± 10 % y fotofase de 12 h, a 15, 20, 25, 30 y 35 °C, hasta la aparición de la generación siguiente de parasitoides. Los parámetros evaluados fueron el porcentaje de emergencia, la proporción de sexos, el número de parasitoides que surgieron por huevo y la duración del ciclo. Los experimentos se establecieron en un diseño experimental completamente al azar con cinco tratamientos (temperaturas) y 12 repeticiones. Se realizó ANOVA y se compararon las medias mediante la prueba de Tukey ($P < 0.05$). La temperatura base fue de 10,77 y 10,86 °C y el número de generaciones por año fue de 33,29 y 35,63 para "Ubajara" y "Guaraciaba", respectivamente. La temperatura cambió los parámetros biológicos, y la duración del ciclo de las líneas "Ubajara" y "Guaraciaba" disminuyó a medida que aumentaba la temperatura.

Palabras clave: Barrenador de la fruta del tomate, parasitoides de huevo, parámetros biológicos, temperatura base, *Trichogramma pretiosum*, *Neoleucinodes elegantalis*, Trichogrammatidae, Crambidae.

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Introduction

Tomato (*Solanum lycopersicum* L.) is a vegetable crop of economic and social importance, due to the scale of production and job creation (Barros *et al.* 2014; Gomes and Castro 2017). Tomato is grown in all regions of Brazil, occupying a total area of 63,300 hectares (Instituto Brasileiro de Geografia e Estatística 2018). Tomato production requires a high financial investment, due to broad susceptibility to physiological disorders, infection from plant pathogens, a high application in inputs and services, and attack from insect pests (Loos *et al.* 2008).

One of the most prominent pests is the tomato fruit borer *Neoleucinodes elegantalis* (Guenée, 1854) (Lepidoptera: Crambidae) due to the damages it brings about in fruit, leaving it unsuitable for consumption and industrial processes (Gravena and Benvenga 2003; Picanço *et al.* 2007).

Females of *N. elegantalis* lay eggs on the calyx or green fruit of 2 cm diameter. After emergence, larvae penetrate and develop within the fruit, thus remaining protected, making the adoption of several control methods unfeasible (Benvenga *et al.* 2010; Fornazier *et al.* 2010; Moura *et al.* 2014).

In accordance with the habit of the insect pest, a control alternative is the use of parasitoid wasps *Trichogramma* (Hymenoptera: Trichogrammatidae), because they parasitize the eggs of *N. elegantalis*, impeding the larval emergence and, consequently, the injuries they cause (Berti and Marcano 1991; Oliveira *et al.* 2020).

Species of *Trichogramma* are biological control agents associated mainly with parasitizing eggs of Lepidoptera pests. In Brazil, *Trichogramma pretiosum* Riley, 1879 is widely distributed species, and is often found worldwide (Goulart *et al.* 2008; Wajnberg 2010).

The success of biological control programs with *Trichogramma* species depends on several factors, including, particularly, the control potential of the species and/or line, the insect pest, and the effect of climate conditions (Pastori *et al.* 2008; 2012a; Moreira *et al.* 2009).

The choice of the species/line is of utmost importance since parasitoids of the genus *Trichogramma* may have inter- and intraspecific genetic variations, leading to differences in search strategy, host selection, and tolerance to environmental conditions (Pastori *et al.* 2008; Siqueira *et al.* 2012).

Thus, to ensure the success of inundative releases of *Trichogramma*, it is essential to select the species/line most adapted to the target host and to the environmental adversities (Vianna *et al.* 2011; Foresti *et al.* 2012).

Temperature is one of the most important abiotic factors, and it can affect parameters such as parasitism, life cycle duration, sex ratio, and parasitoid emergence (Pastori *et al.* 2008; Silva Júnior 2009; Paes 2015). Knowing thermal requirements of the parasitoid species makes it possible to predict and control parasitoid production (Pastori *et al.* 2012b). Knowledge of these thermal requirements allow for the determination of optimum temperatures for development of this natural enemy, as well as the estimation of the number of generations. This allows for planning and production/multiplication of hosts and parasitoids, making for synchronization and efficiency in production (Haddad *et al.* 1999; Pratissoli and Parra 2000).

Storing natural enemies at low temperatures is also a factor made possible through knowledge of the effect of temperature on the development of the parasitoid. This method promotes success in mass rearing, constant material for use in laboratory experiments, as well as release in the field at times of greatest occurrence of the insect pest targeted (Chen *et al.* 2008; Rodrigues and Sampaio 2011).

In this context, this study aimed to evaluate the impact of temperature on the biology of two lines of *T. pretiosum*, thus determining the thermal requirements of the parasitoids in eggs of *N. elegantalis*.

Materials and methods

The experiment was performed in the 'Laboratorio de Entomologia Aplicada (LEA)' of the 'Universidade Federal do Ceará (UFC)', Fortaleza, Ceará State, Brazil. Two lines of *T. pretiosum* ("Ubajara" and "Guaraciaba") were used, which were collected naturally parasitizing *N. elegantalis* eggs in tomato fruit in commercial areas in the region of 'Serra da Ibiapaba', Ceará State, Brazil (Oliveira *et al.* 2020). The first line was collected in the rural area in the municipality of Ubajara under a conventional tomato growing system, and the second line was collected in the municipality of Guaraciaba do Norte under an organic tomato growing system. The municipality of Ubajara is at 3º51'S, 40º55'W, and 847.5 masl, with mean maximum temperature of 27.3 °C and mean minimum of 16.0 °C. The municipality of Guaraciaba do Norte is at 4º10'S, 40º44'W, and 902.4 masl, with mean maximum temperature of 26.6 °C and mean minimum of 15.6 °C.

Fabricio Fagundes Pereira, *D. Sc.*, of the 'Universidade Federal da Grande Dourados (UFGD)' (Dourados, Mato Grosso do Sul State, Brazil) identified the parasitoid wasps and subsequently was confirmed by Ranyse Barbosa Querino, *D. Sc.*, (Empresa Brasileira de Pesquisa Agropecuária, Secretaria de Inovação e Negócios', Distrito Federal, Brazil).

Biology of two lines of *T. pretiosum* exposed to different temperatures in eggs of *N. elegantalis.* To conduct the experiment, *N. elegantalis* eggs (n = 40) were removed from scarlet eggplant (*Solanum aethiopicum* L.) using a moistened brush and glued onto sheets of sky-blue construction paper (8.0 × 2.0 cm) using arabic gum (30 %). The sheets were inserted in glass tubes (8.5 × 2.5 cm) and then 4 females of *T. pretiosum* of 24 h-old were released. Females were fed with a drop of pure honey placed on the wall of the tubes. The tubes were closed with PVC® plastic film to prevent the parasitoid wasps from leaving. The eggs were exposed to parasitism for 24 h at 25 ± 1 °C, relative humidity of 70 ± 10 %, and 12-h photophase. After parasitizing the eggs, females were removed and the tubes were transferred to incubators under the same conditions cited above and exposed to 15, 20, 25, 30, or 35 °C, remaining until the emergence of the following generation of parasitoid wasps.

The percentage of emergence [(number of dark eggs with orifice/total number of parasitized eggs) × 100], sex ratio [number of females emerged/(number of females + males)], number of parasitoids emerged per egg (number of parasitoids emerged/total number of parasitized eggs), and life cycle duration (egg-adult) were the parameters evaluated. The experiment was set up in a completely randomized experimental design, with 5 treatments (temperatures) and 12 replicates (each tube constituting one replicate). An ANOVA one-way was conducted on the data and the means were compared by Tukey test ($P < 0.05$). To biological parameters data, thermal requirements of the *T. pretiosum* lines were determined and the probable number of generations per year was calculated using the following equation: $NG = \{T(T_m - Tb)/K\}$, where K = thermal constant, T_m = mean monthly temperature for each location studied, Tb = base temperature (°C), and T = time (days). Relationship between temperature, time, and developmental rate of the "Guaraciaba" and "Ubajara" lines were determined by regression models.

Results

At 35 °C, development of *T. pretiosum* was not observed and, thus, continuity of the analyses using this temperature, aiming to evaluate the biological parameters and later determination of thermal requirements, was not possible.

The emergence of the two lines of *T. pretiosum* was affected by the temperatures, such that for the "Ubajara" line, a lower rate (51.6 %) was observed at the temperature of 15 °C. The other temperatures led to percentages higher than 80.0 % (Table 1). For the "Guaraciaba" line, 25 °C led to a viability rate of 98.9 %. The lowest viability was observed at 15 °C (64.9 %). There was no difference for the temperatures among the lines examined (Table 1).

The sex ratio of the "Guaraciaba" line ranged from 0.54 to 0.73, and the largest number of females was observed at 25 °C. Effects of temperature were not observed on the sex ratio of the *T. pretiosum* "Ubajara" line (Table 1).

Regardless of the temperature and line used, emergence of 1.0 parasitoid per egg was observed (Table 1). The mean duration of the biological cycle (egg-adult) of the two lines was affected by the temperatures; there was reduction in this parameter with the increase in temperature. The longest duration cycle was observed at the temperature of 15 °C (37.3 days and 36.6 days) and the shortest at 30 °C (8.3 days and 7.7 days) for the "Ubajara" and "Guaraciaba" lines, respectively. The mean time for development of the parasitoids at 30 °C was 4.49, 2.14, and 3.84 times less than at 15, 20, and 25 °C for the "Ubajara" line; and 4.75, 2.34, and 3.97 times less under the same temperature conditions for the "Guaraciaba" line. The "Guaraciaba" line was superior to "Ubajara" regarding cycle duration in the temperature ranges studied (Table 1). From the results obtained for duration of the egg-adult phase of the *T. pretiosum* lines (Table 1), the lower thermal threshold for development (Tb), the thermal constant (K), and the number of generations per year in *N. elegantalis* eggs were calculated (Fig. 1).

For the lines tested, the thermal requirements were Tb of 10.77 °C and 10.86 °C, K for the egg-adult phase of 153.85 and 142.86 GD, and 33.29 and 35.63 generations per year for the "Ubajara" and "Guaraciaba" lines, respectively. The values obtained for coefficients of determination (R^2) were 0.97 for the "Ubajara" line and 0.98 for "Guaraciaba", which therefore meet the value recommended by the hyperbolic method, which is $R^2 \geq 0.90$ (Table 2).

Discussion

When the parasitoids of the two lines were exposed to 35 °C, the lines did not develop because the metabolism and survival of the parasitoids are affected when, for example, the temperature is above that considered "optimal" for the species (Pastori *et al.* 2008).

The highest percentage of emergence was observed when the two lines of *T. pretiosum* were maintained at 25 °C, and the lowest percentage at 15 °C. The use of temperatures lower than 20 °C result in a reduction in percent of emergence (Pratissoli and Parra 2000). The emergence rates of the "Ubajara" and "Guaraciaba" *T. pretiosum* lines was less than 70 % at 15 °C, denoting that this temperature range is not suitable for rearing the parasitoid in the laboratory aiming to achieve this parameter most effectively.

Other authors also found effects of temperature on the percentage of emergence of species of *Trichogramma*. The emergence of *T. pretiosum* in *Sitotroga cerealella* (Oliver, 1789) (Lepidoptera: Gelechiidae) eggs was lower (63.6 %) at 18 °C than at 25 °C (93.5 %) (Inoue and Parra 1998). The percentage of emergence of *T. pretiosum* in Brazilian-apple-leafroller, *Bonagota salubricola* (Meyrick, 1937) (Lepidoptera: Tortricidae) eggs was 66.2 % at 22 °C (Pastori *et al.* 2008). The temperature of 25 °C was most adequate for percentage of emergence of *T. atopovirilia* Oatman & Platner, 1983 (Hymenoptera: Trichogrammatidae) and *T. pretiosum* in citrus borer *Ecdytolopha aurantiana* (Lima, 1927) (Lepidoptera: Tortricidae) eggs (Molina *et al.* 2005).

A sex ratio greater than 0.50 was found in all treatments, and this value is considered appropriate for the quality control in mass rearing of *Trichogramma* spp. (Navarro 1998).

Table 1. Emergence (%) (mean ± standard error), sex ratio (mean ± standard error), number of individuals (parasitoids) emerged per egg (mean ± standard error), and duration (days) (mean ± standard error) of the development cycle (egg-adult) of two *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) lines in eggs of *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) at different temperatures.

	Temperature (ºC)			
Line	**15**	**20**	**25**	**30**
		Emergence (%)		
"Ubajara"	51.65 ± 5.62 Ab[1]	85.25 ± 3.62 Aa	92.49 ± 4.39 Aa	86.29 ± 9.53 Aa
"Guaraciaba"	64.94 ± 3.22 Ac	81.00 ± 4.11 Ab	98.88 ± 1.11 Aa	82.52 ± 4.38 Ab
		Sex ratio		
"Ubajara"	0.51 ± 0.06 Aa	0.65 ± 0.03 Aa	0.67 ± 0.05 Aa	0.56 ± 0.06 Aa
"Guaraciaba"	0.54 ± 0.04 Ab	0.66 ± 0.02 Aab	0.73 ± 0.03 Aa	0.68 ± 0.04 Aab
		Parasitoid/egg		
"Ubajara"	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
"Guaraciaba"	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
		Cycle duration		
"Ubajara"	37.30 ± 0.15 Bd	17.40 ± 0.16 Bc	9.70 ± 0.15 Bb	8.30 ± 0.15 Ba
"Guaraciaba"	36.60 ± 0.16 Ad	15.60 ± 0.16 Ac	9.20 ± 0.13 Ab	7.7 ± 0.15 Aa

[1] Means followed by the same lowercase letter in the line and uppercase letter in the column do not differ from each other by Tukey test at the level of 5 % probability.

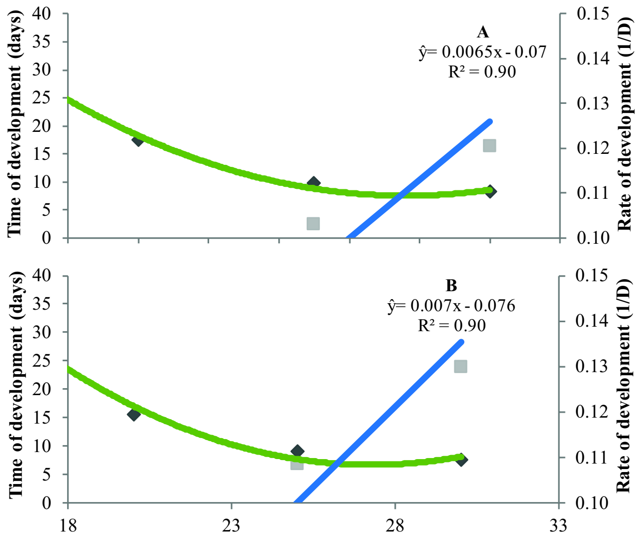

Figure 1. Relationship between temperature, time, and rate of development of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) lines (**A**) "Ubajara", and (**B**) "Guaraciaba", in eggs of *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae).

The greater number of females in the present study may be attributed to the good development conditions provided by the host *N. elegantalis*. Among the thermal ranges studied, 25 °C for the "Guaraciaba" line, can be considered the ideal for obtaining the greatest number of females.

Other authors also confirmed the effect of temperature on sex ratio of *T. pretiosum*. Generally, there is a greater occurrence of males of *Trichogramma* spp. at temperatures greater than 30 °C (Bowen and Stern 1966). The sex ratio of the *T. pretiosum* LM line exhibited the highest number of females at 28 °C (Fonseca *et al.* 2005).

The number of parasitoids emerged per egg of *N. elegantalis* was 1 at all the temperatures studied, showing that the eggs of *N. elegantalis* have adequate size and nutritional quality for development of only one parasitoid per egg. The emergence of more than one individual per egg of the host may lead to parasitoids of smaller size and with inferior nutritional quality, due to intraspecific competition. This fact can compromise the performance of parasitoids under laboratory and field conditions (Moreira *et al.* 2009).

The life cycle (egg-adult) duration of the *T. pretiosum* lines "Ubajara" and "Guaraciaba" was inversely related to temperature increase. In addition to temperature, variation in cycle duration is affected by adaptation of the parasitoid species/line and host (Pereira *et al.* 2004).

An increase in degree-days at the higher temperature (30 °C) is observed where a shorter development cycle is found. Cumulative degree-days are the number of degrees above a determined temperature that the organism needs for its development. Thus, it can be suggested that higher temperatures accelerate the biological cycle of the insects, such that they pass from one stage to another in shorter time compared to lower temperatures. However, lower temperatures reduce the insect metabolic processes, making for longer life cycle duration (Iowa State University 1987).

An inverse relationship between development time and temperature was also found by Paes (2015) in an experiment performed with *T. pretiosum* and *T. galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) in European

Table 2. Lower thermal threshold for development (Tb), thermal constant (K), number of generations (per year), and coefficient of determination (R^2) of the egg to adult phase of two lines of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) in eggs of *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) at different temperatures.

Line	Tb (°C)	K (GD)	Number of generations (per year)	R^2
"Ubajara"	10.77	153.85	33.29	0.97
"Guaraciaba"	10.86	142.86	35.63	0.98

pepper moth *Duponchelia fovealis* Zeller, 1847 (Lepidoptera: Crambidae) eggs, and by Silva Júnior (2009) in an experiment with *T. pretiosum*, *T. acacioi* Brun, Moraes & Soares, 1984 (Hymenoptera: Trichogrammatidae), and *T. atopovirilia* in eggs of the Brazilian-poplar-moth *Condylorrhiza vestigialis* (Guenée, 1854) (Lepidoptera: Crambidae) host. Life cycle duration ranged from 6.8 to 23.1 days for *T. pretiosum* in eggs of the diamondback moth *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) at 32 °C and from 6.9 to 22.0 days for *T. exiguum* Pinto & Platner, 1978 (Hymenoptera: Trichogrammatidae) at 18 °C (Pereira *et al.* 2004).

Determination of the temperature suitable for insect development is fundamental for rearing insects in the laboratory, as well as for adoption of control methods that make studies about biological aspects of the pests possible (Prezotti and Parra 2002). Through comparison of the thermal requirements of the "Ubajara" and "Guaraciaba" *T. pretiosum* lines obtained in this study, it can be predicted that throughout the year, the number of generations of the "Guaraciaba" line will be greater than that of the "Ubajara" line, a characteristic which is important for a biological control program.

The results obtained in the present study confirm the effect of temperature on the *T. pretiosum* lines collected in the "Serra da Ibiapaba" region and emphasize the importance of checking of this on biological parameters. This also indicates that temperature range most adequate for development of the *T. pretiosum* lines in *N. elegantalis* eggs was from 25 to 30 °C, in which the shortest egg-adult cycle was obtained.

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Author contribution

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