

## Biological characteristics of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) under temperature stress by successive generations

Luis Moreira de Araujo Junior<sup>a</sup>, Hugo Bolsoni Zago<sup>a</sup>, Alixelhe Pacheco Damascena<sup>a\*</sup>, Luana Viana Faria<sup>b</sup>, Aléxia Gonçalves Pereira<sup>c</sup>, Fernando Zanotti Madalon<sup>b</sup>, José Romário de Carvalho<sup>d</sup>, Dirceu Pratissoli<sup>e</sup>

<sup>a</sup> Programa de Pós-Graduação em Produção Vegetal, Universidade Federal do Espírito Santo, Alegre, 29500-000, Espírito Santo, Brasil.

<sup>b</sup> Programa de Pós-Graduação em Entomologia, Universidade Federal de São Paulo Luiz de Queiroz, Piracicaba, 13418-900, São Paulo, Brasil.

<sup>c</sup> Programa de Pós-Graduação em Genética e Melhoramento, Universidade Federal do Espírito Santo, Alegre, 29500-000, Espírito Santo, Brasil. \*xellydamascena@hotmail.com

<sup>d</sup> Secretaria de Educação do Estado do Espírito Santo, Jerônimo Monteiro, 29550-000, Espírito Santo, Brasil.

<sup>e</sup> Programa de Pós-Graduação em Agronomia, Universidade Federal do Espírito Santo, Alegre, 29500-000, Espírito Santo, Brasil.

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### Abstract

The objective of this study was to evaluate the biological characteristics of *Trichogramma pretiosum* (Riley 1879) under room temperature and temperature stress by successive generations. The evaluation was carried out in split-plot in the time. The temperature (25 °C – room temperature and 33 °C – temperature stress) was the Factor 1 and the Generations (n = 15) were the Factor 2. The biological characteristics evaluated were: longevity; posterior tibia length; number of parasitized eggs; emergence; egg-adult period; sexual ratio and deformed individuals. The increase in temperature, from the first generations of *T. pretiosum*, caused an influence in all observed variables. However, for the emergence and sex ratio variables, there was no significant difference between heat stress and control (room temperature) as of the 14th generation, which suggests an adaptability of the species. This adaptability may imply obtaining more aggressive natural enemies in relation to their host (insect pest) and consequently in the greater efficiency of biological control.

**Keywords:** Acclimation; biological control; climatic factors; egg parasitoid.

## Características biológicas de *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) sob estresse de temperatura por gerações sucessivas

### Resumo

O objetivo deste estudo foi avaliar as características biológicas de *Trichogramma pretiosum* (Riley 1879) submetido a temperatura ambiente e estresse térmico por sucessivas gerações. A avaliação foi realizada em parcelas subdividida no tempo. A Temperatura (25 °C – temperatura Ambiente e 33 °C – estresse térmico) foi o Fator 1 e as gerações (n = 15) foram o Fator 2. As características biológicas avaliadas foram: longevidade; comprimento da tibia posterior; número de ovos parasitados; emergência; período ovo-adulto; razão sexual e indivíduos deformados. O aumento da temperatura, provocou, desde as primeiras gerações de *T. pretiosum*, influência em todas as variáveis observadas. Entretanto, para as variáveis emergência e a razão sexual, não houve diferença significativa entre o stress térmico e o controle (temperatura ambiente) a partir da 14 geração, o que sugere-se uma adaptabilidade da espécie. Essa adaptabilidade podem implicar na obtenção de inimigos naturais mais agressivos em relação ao seu hospedeiro (inseto praga) e consequentemente na maior eficiência do controle biológico.

**Palavras-chave:** Aclimação; controle biológico; fatores climáticos; parasitoide de ovo.

### Introduction

The set of mutualistic biotic interactions of natural enemies is as relevant as any other factor in agricultural productivity,

because it regulates deleterious actions in the plant, caused by herbivorous organisms (De Bach, 1964; Van Den Bosh, Messenger, & Gutierrez, 1982). In this context, the egg

parasitoids of the genus *Trichogramma* Westwood, 1833 (Hymenoptera Trichogrammatidae) are widely used as natural enemies in biological control of pests, either by inundative or inoculative release of the parasitoid in the area of cultivation, where they are exposed to climatic factors.

The effect of global warming in recent decades has led to an increase in the frequency and intensity of high temperatures, which tend to increase further and to affect communities of natural occurrence of *Trichogramma* spp., in the world's major agricultural areas (Wu, Hoffmann, & Thomson, 2016). The species *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) was considered the most polyphagous and distributed in Brazil (Querino, Mendes, Costa, & Zucchi, 2017). In the Espírito Santo state, the presence of wild *T. pretiosum* was observed in regions of different altitudes, across different temperature conditions (Pratissoli et al., 2003).

Among the climatic factors, the temperature is the one that exerts more influence on the parasitoids, being one of the main regulators of insects metabolism (Angilletta, Niewiarowski, & Navas, 2002). In laboratory conditions, the constant temperature of 25 °C was shown to be the most favorable for the maintenance of *T. pretiosum* activities, but when subjected to extremes between 28 °C and 33 °C there was a significant reduction in longevity, parasitism, emergence, developmental cycle and sexual ratio (Bueno, Parra, & Freitas Bueno, 2012; Carvalho et al., 2017; Oliveira, de Oliveira, Breda, França, & Duarte, 2017).

The acclimation consists of physiological adjustments, due to exposure to a climatic stressor (Moyes & Schulte, 2010). The possible acclimation of *T. pretiosum* at high temperatures might be relevant to the understanding of the mechanism of tolerance of the insect, in biological control of agricultural pests under adverse conditions. Therefore, the objective of this work was to evaluate the biological characteristics of *T. pretiosum* under room temperature and temperature stress by successive generations.

## Materials and Methods

The *T. pretiosum* was originally obtained from an area cultivated with corn (S 20°52'28.75" W 41°29'1.65") at Alegre, Espírito Santo, Brazil in September 2016. For this work, the *T. pretiosum* was raised on *Anagasta kuehniella* (Zeller, 1879) eggs in laboratory.

### Experiment

The essay consisted of the evaluation of the biological characteristics of *T. pretiosum* exposed to 25 ± 1 °C (room temperature) and 33 ± 1 °C (temperature stress) by 15 generations, in regulated chambers (Eletrolab, serial number: 080336, potency: 280 W, voltage: 220 V, model: EL202) to 70±10% RH and photophase of 14 h (L:D). The temperature 33 ± 1 °C was chosen for being the upper limit for the development of *T. pretiosum* (Oliveira et al., 2017). The parental generation (G0) was composed of females from the ninth generation of the population established in the standard laboratory condition. Therefore, it was used only for the pre-imaginal conditioning of females due to changes in the environment.

### Biological characteristics analysis

In order to standardize the samples in each generation, the 20 females with larger size, without deformation and emerged up to six hours old were individualized in glass tubes of 8.5 cm x 2.5 cm, closed with PVC plastic film, contained by a droplet of honey, to feed the parasitoid, and a carton with 40 eggs of *A. kuehniella* of 24 h, offered daily. The individualization of the 20 females in the following generations occurred only in the cartons of the first day.

In the females, the longevity (days) and the posterior tibial length (mm) were evaluated. In the posture, the cumulative number and the emergence rate of parasitized eggs were evaluated. In the progenies, the duration of the development cycle (egg-adult period), the sexual ratio and the percentage of deformed individuals were evaluated.

The longevity of the females was determined by daily observations, from emergence to death. The measurement of the posterior tibia was performed in a micrometric eyepiece. The cumulative number of parasitized eggs was obtained by counting and summing all days of parasitism. The emergency percentage was calculated based on the number of eggs that had exit orifice in relation to the total number of parasitized eggs.

In the evaluation of the egg-adult period, the observations were made daily, always at the same time. The sex ratio was calculated by dividing the number of females by the total number of individuals (males + females) and the dimorphism determined by the antennas (Bowen & Stern, 1966). The percentage of deformed individuals was calculated based on the number of individuals that presented some type of deformation in relation to the total number of individuals emerged.

The biological characteristics were evaluated in split-plot in the time. The temperature (25 °C and 33 °C) was the Factor 1 and the generation (15 generation) was the Factor 2. The data were submitted to analysis of variance. The averages were compared to the level of 5% probability in the test of Scott Knott due to the high number of levels of Factor 2. The statistical analyzes were performed in the R Development Core Team (2019) program.

## Results and Discussion

In general, all variables showed better values for the *T. pretiosum* generations exposed to room temperature, except for the last generations (generations 13 to 15) in the emergence variable, where there were no significant differences between the temperatures (Tables 1 and 2).

Within the room temperature, the values of all generations in all variables were statistically equal. Within the Temperature 33 °C, there was a gradual increase in the values of all variables over the generations.

Heat stress caused a high presence of individual deformations in all generations. This difference in values between the temperatures confirms how stressful the Temperature 33 °C was for the *T. pretiosum*.

**Table 1.** Longevity, posterior tibia length, number and viability (emergence) of the parasitized eggs by *Trichogramma pretiosum* females in successive generations at 25 °C and 33 °C.

G	Longevity (Days)		Posterior tibia length (mm)		Number of parasitized eggs		Emergence (%)	
	25 °C	33 °C	25 °C	33 °C	25 °C	33 °C	25 °C	33 °C
1	7.8±0.79 <sup>a</sup>	1.3±0.36 <sup>d</sup>	0.1322±0.0007 <sup>a</sup>	0.1140±0.0030 <sup>e</sup>	197.85±25.04 <sup>a</sup>	38.35±13.02 <sup>d</sup>	80.54±2.87 <sup>a</sup>	45.08±2.82 <sup>g</sup>
2	7.8±0.89 <sup>a</sup>	1.6±0.48 <sup>d</sup>	0.1323±0.0005 <sup>a</sup>	0.1179±0.0028 <sup>d</sup>	187.75±47.78 <sup>a</sup>	42.10±15.99 <sup>d</sup>	80.66±3.21 <sup>a</sup>	44.99±3.12 <sup>g</sup>
3	7.9±1.21 <sup>a</sup>	2.3±0.47 <sup>c</sup>	0.1323±0.0005 <sup>a</sup>	0.1184±0.0040 <sup>d</sup>	189.45±55.61 <sup>a</sup>	39.50±11.90 <sup>d</sup>	79.74±2.56 <sup>a</sup>	43.05±4.28 <sup>g</sup>
4	8.6±1.47 <sup>a</sup>	1.9±0.48 <sup>d</sup>	0.1325±0.0005 <sup>a</sup>	0.1242±0.0033 <sup>c</sup>	220.10±44.95 <sup>a</sup>	62.15±15.65 <sup>c</sup>	79.22±4.98 <sup>a</sup>	45.30±2.51 <sup>g</sup>
5	8.1±1.05 <sup>a</sup>	2.6±0.60 <sup>c</sup>	0.1323±0.0005 <sup>a</sup>	0.1240±0.0029 <sup>c</sup>	206.75±30.10 <sup>a</sup>	58.25±17.89 <sup>c</sup>	80.05±2.17 <sup>a</sup>	45.20±3.16 <sup>g</sup>
6	8.0±1.08 <sup>a</sup>	2.7±0.50 <sup>c</sup>	0.1324±0.0004 <sup>a</sup>	0.1249±0.0029 <sup>c</sup>	204.70±29.75 <sup>a</sup>	70.40±12.63 <sup>c</sup>	80.07±2.24 <sup>a</sup>	52.60±3.70 <sup>f</sup>
7	7.7±1.03 <sup>a</sup>	3.5±0.50 <sup>b</sup>	0.1323±0.0005 <sup>a</sup>	0.1246±0.0028 <sup>c</sup>	196.65±31.04 <sup>a</sup>	67.25±13.18 <sup>c</sup>	80.08±2.59 <sup>a</sup>	56.61±3.30 <sup>e</sup>
8	7.9±0.72 <sup>a</sup>	3.5±0.50 <sup>b</sup>	0.1324±0.0004 <sup>a</sup>	0.1246±0.0028 <sup>c</sup>	202.35±25.59 <sup>a</sup>	68.65±12.39 <sup>c</sup>	79.80±2.96 <sup>a</sup>	71.19±2.92 <sup>d</sup>
9	7.9±0.76 <sup>a</sup>	3.2±0.48 <sup>b</sup>	0.1324±0.0004 <sup>a</sup>	0.1258±0.0030 <sup>c</sup>	206.70±22.78 <sup>a</sup>	70.80±14.08 <sup>c</sup>	79.09±3.41 <sup>a</sup>	74.85±3.14 <sup>c</sup>
10	8.4±1.57 <sup>a</sup>	3.0±0.40 <sup>c</sup>	0.1325±0.0005 <sup>a</sup>	0.1274±0.0031 <sup>b</sup>	192.95±62.06 <sup>a</sup>	81.95±16.68 <sup>c</sup>	79.45±2.45 <sup>a</sup>	75.97±2.96 <sup>b</sup>
11	8.2±1.93 <sup>a</sup>	4.2±0.51 <sup>a</sup>	0.1324±0.0005 <sup>a</sup>	0.1287±0.0034 <sup>b</sup>	177.45±64.44 <sup>a</sup>	88.95±15.39 <sup>b</sup>	80.19±2.43 <sup>a</sup>	74.61±3.40 <sup>c</sup>
12	8.5±1.85 <sup>a</sup>	4.4±0.40 <sup>a</sup>	0.1325±0.0004 <sup>a</sup>	0.1282±0.0034 <sup>b</sup>	188.25±61.70 <sup>a</sup>	95.65±17.03 <sup>b</sup>	79.65±2.08 <sup>a</sup>	74.32±2.93 <sup>c</sup>
13	8.7±1.87 <sup>a</sup>	4.6±0.43 <sup>a</sup>	0.1326±0.0004 <sup>a</sup>	0.1302±0.0034 <sup>a</sup>	186.95±64.22 <sup>a</sup>	111.80±13.45 <sup>a</sup>	80.14±2.68 <sup>a</sup>	77.00±3.37 <sup>b</sup>
14	7.8±3.32 <sup>a</sup>	4.8±0.41 <sup>a</sup>	0.1323±0.0005 <sup>a</sup>	0.1304±0.0039 <sup>a</sup>	176.70±69.05 <sup>a</sup>	122.30±11.26 <sup>a</sup>	79.46±7.13 <sup>a</sup>	78.70±2.09 <sup>a</sup>
15	8.3±1.07 <sup>a</sup>	4.6±0.43 <sup>a</sup>	0.1325±0.0004 <sup>a</sup>	0.1308±0.0034 <sup>a</sup>	211.35±22.84 <sup>a</sup>	125.30±11.34 <sup>a</sup>	80.55±2.87 <sup>a</sup>	78.91±2.14 <sup>a</sup>

\* Averages followed by the same lowercase in the column, do not differ significantly at 5% probability by the Scott Knott test.

However, it was observed that *T. pretiosum* showed better values in all variables throughout its generations. Although these values did not exceed the values reported by *T. pretiosum* exposed to room temperature and the number of deformities was 53% lower in generation 7, reducing from 71 to 81% in the last generations (11 to 15). This tendency suggests that the species demonstrates the evolutionary and adaptive potential to this thermal stress.

The temperature at which insects are exposed directly influences the rate of development. Oliveira *et al.* (2017) found that the longevity of *T. pretiosum* on eggs of *Neoleucinodes*

*elegantalis* Guen., 1854 (Lepidoptera: Pyralidae) was reduced from, approximately, 5 days at 25 °C to 1 days at 33 °C. This last value was similar to the first generation at 33 °C of this research, which was 1.33 ± 0.36 days. Also in the study by Oliveira *et al.* (2017) there was also a reduction in the values of parasitism, emergence, adult egg period and sexual ratio, when the temperature ranged from 25 °C to 33 °C. Similar tendency were also observed in other studies, regardless of the host (Carvalho *et al.*, 2017; Milanez, Carvalho, Lima, & Pratisoli, 2018).

**Table 2.** Duration of the biological cycle (egg-adult period), sexual ratio and percentage of deformed individuals of *Trichogramma pretiosum* females in successive generations (G) at 25 °C and 33 °C.

G	Sexual ratio		Egg-adult period (Days)		Number of parasitized eggs	
	25 °C	33 °C	25 °C	33 °C	25 °C	33 °C
1	0.77±0.04 <sup>a</sup>	0.61±0.04 <sup>e</sup>	9.90±0.31 <sup>a*</sup>	6.30±0.57 <sup>c</sup>	1.78±0.44 <sup>a</sup>	20.28±1.93 <sup>a</sup>
2	0.78±0.03 <sup>a</sup>	0.64±0.04 <sup>d</sup>	9.92±0.28 <sup>a</sup>	6.50±0.61 <sup>c</sup>	1.83±0.52 <sup>a</sup>	14.63±1.60 <sup>c</sup>
3	0.78±0.04 <sup>a</sup>	0.63±0.04 <sup>c</sup>	9.95±0.22 <sup>a</sup>	6.50±0.61 <sup>c</sup>	1.95±0.86 <sup>a</sup>	16.24±2.22 <sup>b</sup>
4	0.80±0.04 <sup>a</sup>	0.65±0.03 <sup>d</sup>	10.00±0.00 <sup>a</sup>	6.50±0.51 <sup>c</sup>	1.89±0.40 <sup>a</sup>	15.86±2.17 <sup>b</sup>
5	0.79±0.03 <sup>a</sup>	0.64±0.04 <sup>d</sup>	9.95±0.22 <sup>a</sup>	6.75±0.55 <sup>b</sup>	1.83±0.56 <sup>a</sup>	13.30±1.91 <sup>d</sup>
6	0.78±0.03 <sup>a</sup>	0.65±0.05 <sup>d</sup>	9.95±0.22 <sup>a</sup>	6.80±0.52 <sup>b</sup>	1.67±0.79 <sup>a</sup>	11.78±1.47 <sup>e</sup>
7	0.78±0.03 <sup>a</sup>	0.64±0.04 <sup>d</sup>	9.80±0.41 <sup>a</sup>	6.60±0.50 <sup>c</sup>	1.71±0.43 <sup>a</sup>	9.62±1.43 <sup>f</sup>
8	0.78±0.03 <sup>a</sup>	0.61±0.05 <sup>d</sup>	9.95±0.22 <sup>a</sup>	6.75±0.55 <sup>b</sup>	1.80±0.30 <sup>a</sup>	8.18±1.54 <sup>g</sup>
9	0.78±0.04 <sup>a</sup>	0.66±0.05 <sup>d</sup>	9.95±0.22 <sup>a</sup>	7.45±0.60 <sup>a</sup>	1.84±0.28 <sup>a</sup>	7.55±1.80 <sup>g</sup>
10	0.79±0.02 <sup>a</sup>	0.67±0.03 <sup>d</sup>	10.00±0.32 <sup>a</sup>	7.55±0.51 <sup>a</sup>	1.89±0.84 <sup>a</sup>	7.98±1.69 <sup>g</sup>
11	0.77±0.08 <sup>a</sup>	0.70±0.03 <sup>c</sup>	10.00±0.46 <sup>a</sup>	7.65±0.59 <sup>a</sup>	1.88±1.38 <sup>a</sup>	5.88±1.70 <sup>h</sup>
12	0.79±0.03 <sup>a</sup>	0.70±0.04 <sup>c</sup>	10.10±0.31 <sup>a</sup>	7.70±0.57 <sup>a</sup>	1.46±0.85 <sup>a</sup>	4.21±1.38 <sup>i</sup>
13	0.79±0.05 <sup>a</sup>	0.74±0.03 <sup>b</sup>	10.10±0.31 <sup>a</sup>	7.75±0.55 <sup>a</sup>	1.57±0.97 <sup>a</sup>	4.25±1.16 <sup>i</sup>
14	0.76±0.06 <sup>a</sup>	0.77±0.03 <sup>a</sup>	10.00±0.32 <sup>a</sup>	7.80±0.52 <sup>a</sup>	1.96±0.66 <sup>a</sup>	4.25±1.20 <sup>i</sup>
15	0.80±0.03 <sup>a</sup>	0.78±0.03 <sup>a</sup>	10.10±0.55 <sup>a</sup>	7.85±0.59 <sup>a</sup>	1.78±0.58 <sup>a</sup>	3.92±1.28 <sup>i</sup>

\* Averages followed by the same lowercase in the column, do not differ significantly at 5% probability by the Scott Knott test.

However, despite the temperature stress condition of *T. pretiosum* females in this study, it was observed that the longevity, tibial length, number of parasitized eggs and adult emergence rate increased. The size of the females of *Trichogramma* spp., based on the length of the tibia, has been used as an index of performance and quality of the biological control agent, due to the significant relationship between the

size of the individual and biological fitness (Kazmer & Luck, 1995). Therefore, it is possible that the growth of longevity, parasitism and egg viability values occurred due to the increase of the *T. pretiosum* size.

Probably, the exhaustion of thermal capacity has compromised the development of individuals, as has occurred in organisms of different species (Owen & Stern,

1966; Kingsolver & Huey, 2008; Zuo, Moses, West, Hou, & rown, 2012; Nielsen & Papaj, 2015). Therefore, certainly for this reason, in the early generations more males of *T. pretiosum* were produced due to the need to select genotypes that were more tolerant to thermal stressors, which led to a tendency to regulate the duration of the biological cycle and reduce the number of deformed individuals.

The biological characteristics of *T. pretiosum*, under temperature stress, tends to evolve over generations. These data can be used for future acclimatization and / or genetic improvement tests for *T. pretiosum*, to be used in places whose release has been impracticable due to the occurrence of high temperatures.

## Conclusion

*T. pretiosum* showed negative effects on its development under heat stress. However, over fifteen generations, individual deformities were significantly reduced and all values for longevity; posterior tibia length; number of parasitized eggs; emergence; egg-adult period; sexual ratio improved.

This improvement of *T. pretiosum* under temperature stress for successive generations may suggest that the species demonstrates an adaptive potential to this thermal stress.

The adaptive evolution of the *T. pretiosum* may imply greater aggressiveness of the parasitoid in relation to its host. Then, as an environmental impact, greater efficiency in biological pest control is expected, but without major risks to nature, because it is a specific action organism, that is, they parasitize only certain groups of insects and do not leave toxic residues in the environment.

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