Original Research Article

Behavior of *Trichogramma pretiosum* exposed to extreme temperatures for different periods on Ephestia kuehniella (Zeller)

.

ABSTRACT

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| Biological control is essential in modern agriculture, as it suppresses pest populations, minimizing dependence on chemical pesticides, and safeguarding ecosystem services. Multiple biological control strategies can be combined with agronomic pest management measures. Among them, parasitoids are a viable alternative, as they prevent insect pest eggs from hatching, preventing damage from a new population. To evaluate the control potential of parasitic wasps under adverse environmental conditions in the field, this study examined the biological characteristics of trichogrammatids under different temperatures. Therefore, the objective of this study was to evaluate the biological characteristics of a species/strain of *Trichogramma pretiosum* (Hymenoptera, Trichogrammatidae) under different temperature regimes for different periods. At extreme temperatures of 18°C and 30°C, female Trichogrammatidae showed inactivity for at least 4 hours, and continued to be inactive for a period of time after returning to the ideal temperature (25°C), indicating that their adaptability to temperature changes is weak. The *T. pretiosum* needs time to adapt to changing environmental conditions. Taken together, the results indicate that abrupt climate-related temperature variations can temporarily impair parasitoid performance, highlighting the need to adjust release strategies to maintain effective biological control in the face of ongoing global warming. |

*Keywords: Egg Parasitoid; Trichogrammatidae; Biological Control; Control Methods.*

1. INTRODUCTION

Climate projections for Central and South America indicate that mean temperatures will rise faster than the global average and that heat-wave days (>30 °C) will occur more frequently across the main tomato-growing belts of south-eastern Brazil by mid-century, with average warming of 2 – 4 °C expected under intermediate-to-high emission scenarios (Ortega et al 2021; Reboita et al 2021; Veiga et al 2023; Ramarao et al. 2024). Because chemical insecticides are less reliable at higher temperatures and raise concerns about resistance, residues and environmental risk, sustainable alternatives that remain effective under a wider thermal envelope are urgently required (Verheyen & Theys 2022).

Biological control therefore constitutes a pivotal component of modern integrated pest-management (IPM) programs: it curbs pest populations efficiently while minimizing contamination of soil, water, workers and harvested fruit, and it can be combined with complementary tactics, including selective chemicals (Van Lenteren 2018; Pratissoli et al. 2019). Among the available natural enemies, egg-parasitoids in the genus *Trichogramma* are the most widely studied and deployed: more than 200 pest species—mainly Lepidoptera—have been recorded as hosts, and releases covering over 15 million ha in 40 countries (Pratissoli et al. 2019; Zang et al. 2021). In Brazil, 25 *Trichogramma* species have been described to date, with *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae) being the most abundant and commercially exploited (Noyes 2021).

Despite their operational success, each species or even strain of *Trichogramma* can differ markedly in reproductive capacity, host preference and tolerance to abiotic stress. Temperature is the dominant abiotic factor shaping these attributes: it modulates development time, fecundity, sex ratio and adult longevity, thereby affecting field efficacy (Pastori et al. 2017; Milanez et al. 2018; Santana et al. 2021). While several studies have modeled *Trichogramma* performance across constant or gradually fluctuating temperatures, there is still no predictive framework for the short-term thermal shocks (4–6 h excursions below 18 °C or above 30 °C) that typify tropical tomato systems during cold fronts and midday heat spikes. This gap hinders the fine-tuning of release schedules under the increasingly erratic thermal regimes projected for Brazilian horticulture.

Against this background, the present study evaluated key biological traits of a commercially used strain of *T. pretiosum* exposed to brief episodes of low (18 °C) and high (30 °C) temperatures, followed by return to the optimum (25 °C). By quantifying inactivity periods, parasitism capacity and progeny quality after each thermal shock, we aim to generate parameters that can be incorporated into climate-smart IPM decision tools and thus maintain the reliability of biological control in a warming world.

2. material and methods

The experiment was conducted in the Entomology Laboratory of the Center for Scientific and Technological Development in Phytosanitary Management of Pests and Diseases (NUDEMAFI) of the Center for Agricultural Sciences and Engineering of the Federal University of Espírito Santo (CCAE-UFES) in Alegre, Espírito Santo, Brazil.

**2.1 Rearing of the alternative host,** Ephestia kuehniella **(Zeller) (Lepidoptera: Pyralidae)**

The alternative host was reared in the laboratory at a temperature of 25 ± 1°C, relative humidity of 70%, and photoperiod of 14 hours. A diet based on whole wheat flour (60%), corn flour (37%), and brewer's yeast (3% m/m) was used as feed. Each kilo of feed was placed in a plastic tray, wherein 0.4 grams of alternative host eggs were distributed. When the adults emerged, they were collected with the help of a trap adapted to a vacuum cleaner and placed in 1.7-liter plastic jars with strips of plastic mesh inside, which served as spawning sites. Eggs were collected daily, and stored in a refrigerator set to 3±1°C.

**2.2 Parasitoid maintenance and multiplication**

The maintenance and multiplication of the *Trichogramma pretiosum*genus parasitoids occurred in the Entomology sector of NUDEMAFI, CCA-UFES, in a climatic-controlled chamber, set to a temperature of 25 ± 1°C, relative humidity (RH): 70 ± 10%, and 14-hour photoperiod.

Eggs from the alternative host stock were used and fixed with 20% gum Arabic on blue cardboard (8.0 x 2.5 cm). Subsequently, they were submitted to an infeasibility process – a 50-minute exposure to a germicidal lamp with ultraviolet (UV) radiation of 15 watts at 40 cm from the eggs. After this process, the cardboard pieces were transferred to flat-bottomed glass tubes (8.5 x 2.5 cm) containing newly emerged parasitoid adults. Parasitism was allowed to occur for 24 hours.

**2.3 Experimental Development**

To evaluate the effect of submitting females to different temperatures for different periods of time, 2 cm2 cardboard pieces containing unviable alternative host eggs were placed in flat-bottomed glass tubes (8.5 x 2.5 cm), each containing five newly mated females.

Four extreme (18°C, 21°C, 27°C, and 30°C) and one ideal (control) (24°C) temperatures were selected. For each condition, 100 tubes were placed in each of the climatic chambers, regulated at the temperatures listed; every 2 hours, twenty tubes were removed and placed in a climatic chamber set to 25°C, where they remained until completing a 24-hour period. Thus, the treatments were combinations of the 5 temperature conditions (18°C, 21°C, 24°C, 27°C and 30°C) and the five exposure periods at these temperatures (2h, 4h, 6h, 8h and 10h), with 20 replicates for each treatment. The control treatment was the temperature of 24°C, considered the ideal temperature for parasitoid development. Females were subsequently removed from the tubes, which remained in the chamber until the emergence of individuals.

Evaluated parameters include number of parasitized rate, viability, and sex ratio.

**2.4 Statistical Analysis**

Response surface analysis (comparing everything (response as a function of temperature and exposure time) within each situation: before or after temperature exposure) and t-test (comparing each composition, before and after, within each period and temperature) were performed. All analyses and graphics were conducted in R v4.3.0 (R Core Team, 2024).

3. results

The rate of parasitism shows an increase in the number of parasitized eggs as a function of the amount of time the eggs were exposed to T. pretiosum females (Figure 1A and B). At the lower studied temperatures, the parasitism rate increased regardless of the amount of time females were exposed to these temperatures (Figure 1A). The parasitism rate of females exposed to a 25°C temperature exhibited increased as a function of the amount of time they were left in contact with the eggs (Figure 1B).

Gráfico, Gráfico de superfície

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**Fig. 1.** *Trichogramma pretiosum* **parasitism on** *Ephestia kuehniella* **eggs response surfaces at different exposure times under alternating temperature conditions. Parasitism at extreme temperatures (A) and corresponding conditions at 25°C (B).**

Females exposed to a temperature of 18°C for up to 4 hours showed no parasitism rate; when transferred to a temperature of 25°C, parasitism was low. A minimal increase in the rate of parasitism was observed at 6 and 10 hours of exposure; no parasitism was observed at 8 hours of exposure. When these females were transferred to a temperature of 25°C, an increased parasitism was observed for all exposure times to this temperature, significant higher when the contact time with eggs reached 8 and 10 hours (Figure 2A). When subjected to a temperature of 21°C, the parasitism rate increased after 4 hours of exposure. However, when these females were transferred to the temperature of 25°C, egg parasitism was observed for all exposure times, and at 8 and 10 hours of exposure the parasitism rate was significantly higher than at 21°C (Figure 2B). At 24°C, regarded as close to the ideal temperature (25°C), the rate of parasitized eggs was always higher, even when females were kept at 25°C, at all egg exposure time intervals, being significantly higher at 4 and 6 hours (Figure 2C). Females, when subjected to parasitism for up to 10 hours at a temperature of 27°C, had a significantly higher parasitism rate when kept for the same time at the ideal temperature (25°C) (Figure 2D). On the other hand, females submitted to an extreme temperature of 30°C, and subsequently maintained at 25°C, showed parasitism rates when exposed to the eggs for a period between 4 and 10 hours (Figure 2E).

Uma imagem com mesa

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*Not tested*

**Fig. 2. Comparison of** *Trichogramma pretiosum* **parasitism on** *Ephestia kuehniella* **eggs at initial and final temperatures at each exposure time. Alternating thermal regime 18°C-25°C (A); 21°C-25°C (B); 24°C-25°C (C); 27°C-25°C (D); and 30°C-25°C (E).**

*Bars accompanied by an asterisk (\*) differ significantly by paired t-test (p = .05).*

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**Fig. 3. Response surfaces of** *Trichogramma pretiosum* **viability in** *Ephestia kuehniella* **eggs at different exposure times under alternating temperature conditions. Parasitism at the extreme temperatures (A) and the corresponding conditions at 25°C (B).**

Figure 3A shows an extremely low viability rate when females were exposed to lower extreme temperatures, regardless of exposure time. At 27°C and 30°C, offspring emergence was observed at all exposure times; the highest rates were observed for females exposed to 25°C (Figure 3B).

At 18°C, offspring emergence was observed only when females were exposed for 6 and 10 hours (Figure 4A), whereas at 21°C offspring emergence was not observed only when females were exposed for 2 hours (Figure 4B). The viability rate was almost similar when females were exposed to 24°C and 25°C regardless of the exposure time, with statistical differences only at 6 hours of exposure (Figure 4C). When females were exposed at 27°C and 25°C, offspring emergence occurred at all exposure times, but at the first temperature the viability rate up to 6 hours of exposure was significantly higher than at the second temperature (Figure 4D). In the extreme temperature of 30°C, when compared to 25°C, viability showed a significant difference when females were exposed for up to 6 hours (Figure 4E).

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*Not tested*

**Fig. 4. Comparison of** *Trichogramma pretiosum* **viability rate on** *Ephestia kuehniella* **eggs at initial and final temperatures at each exposure time. Alternating thermal regime 18°C-25°C (A); 21°C-25°C (B); 24°C-25°C (C); 27°C-25°C (D); and 30°C-25°C (E).**

*Bars accompanied by an asterisk (\*) differ significantly by paired t-test (p = .05).*

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**Fig. 5. Response surfaces of** *Trichogramma pretiosum* **sex ratios on** *Ephestia kuehniella* **eggs at different exposure times under alternating temperature conditions. Parasitism at the extreme temperatures (A) and the corresponding conditions at 25°C (B).**

Regarding the sex ratio parameter, at the temperatures of 18°C, 21oC and 30°C, when parasitism by females was observed, the female ratio in the offspring increases with the exposure time to these temperatures; nevertheless, the value is approximately 0.5, i.e., the proportion of males and females are similar (Figure 5A). However, when females were exposed to temperatures in the 24°C-27°C range, a direct relationship between the increase in exposure time and the ratio of females in the offspring was observed, reaching a value of 1.0, i.e., 100% of females (Figure 5B).

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O conteúdo gerado por IA pode estar incorreto.

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**Fig. 6. Comparison of the** *Trichogramma pretiosum* **sex ratio on** *Ephestia kuehniella* **eggs at initial and final temperatures at each exposure time. Alternating thermal regime 18°C-25°C (A); 21°C-25°C (B); 24°C-25°C (C); 27°C-25°C (D); and 30°C-25°C (E). t initial and final temperatures at each exposure time. Alternating thermal regime 18°C-25°C (A); 21°C-25°C (B); 24°C-25°C (C); 27°C-25°C (D); and 30°C-25°C (E).**

*Bars accompanied by an asterisk (\*) differ significantly by paired t-test (p = .05).*

When parasitism occurred at a temperature of 18°C a low sex ratio was observed, regardless of the time of exposure, indicating a greater proportion of males in the offspring. When these females were exposed to the temperature of 25°C, there was an increase in the sex ratio rate, i.e., a higher proportion of females as the exposure time at this temperature increased, which was statistically significant for the 8- and 10-hour time intervals (Figure 6A). The sexual ratio parameter for females exposed and parasitized at 21°C and later exposed to 25°C exhibited similar tendencies to the 18°C-25°C temperature pair (Figure 6B).

Females exposed to a temperature of 24°C showed the highest sex ratio rates between 6 and 10 hours, being statistically superior at 6 hours, when compared to exposure to a temperature of 25°C. At this temperature, the highest sex ratio rates occurred at 8 and 10 hours of exposure (Figure 6C).

At 27°C the sex ratio rate was extremely high, regardless of the exposure time, showing that the offspring were almost 100% females, and were statistically superior when compared to those at the temperature of 25°C, after 2, 4, and 6 hours of exposure. However, when these females were exposed to the temperature considered to be ideal (25°C), the highest female ratios were observed after 8 and 10 hours.

When the females were exposed to 30°C and later to 25°C, one could observe that the highest sex ratio rates (0.6) were found after exposure for 6 and 10 hours; for the former temperature, this rate was statistically superior when compared to 25°C. When the females were transferred to the climatic chamber set to 25°C, the highest sex ratio rates (0.6) were observed after 4 and 10 hours of exposure, and at this shorter exposure time the rate was statistically significantly higher than at 30°C.

4. discussion

The use of *Trichogramma* on a commercial scale has been verified for the management of various pests in different agroecosystems and in several countries. Since it is an egg parasitoid, it has become a satisfactory option, either alone or associated with other management techniques, as seen in several other pest and agroecosystems, highlighting its potential for commercial use, which has been explored in other countries (Parra & Zucchi, 2004; Costa et al., 2011). When well implemented, biological control using parasitoids of the *Trichogramma* genus can constitute an alternative to obtain more satisfactory and longer-term results compared to chemical control recommendations.

The success of the use of biological control with *Trichogramma* species in pest insect management depends on the survival of this parasitoid and its reproduction in the field. Temperature is one of the factors that affects the behavior of *Trichogramma* the most, as it can interfere with survival biological characteristics and parasitism rate (Pratissoli et al., 2019; Carvalho et al., 2020; Pratissoli et al., 2021).

Data from this study shows that the 24ºC -27ºC temperature range yielded the best results for all biological characteristics evaluated (parasitism, viability, and sex ratio), regardless of the exposure time. However, the species, or even their strains, can present great variability regarding the optimal development temperature (Pratissoli et al., 2007; Pizzol et al., 2010; Pratissoli et al., 2019), as well as the collection site, which can influence insect adaptation - natural habitats pose an important conditioning factor of the species (Pratissoli et al., 2019). These correlations have also been found in several studies; Fragoso et al. (2019a) determined that the same temperature range (24°C-27°C) yielded the maximum percentage when evaluating the *Trichogramma galloi* parasitism rate (Figures 2, 4, 6 - C, D). However, Pratissoli et al. (2021) determined that temperature does not interfere in the sex ratio and number of individuals per egg parameters when evaluating a different *Trichogramma pretiosum* strain in the 18ºC-27ºC temperature range. Waghmare et al. (2021) showed that that the best percentage of parasitism and offspring emergence rate for *Trichogramma chilonis* occurred at 25 ºC.

When this parasitoid was subjected to temperatures above 27ºC, it remained inactive when exposed for at least 4 hours; the same happens when it returned to the ideal temperature (25ºC) (Figures 2E, 4E, 6E). This suggests that the *Trichogramma pretiosum* strain needs time to adapt to changing environmental conditions (temperatures). For the 30-35ºC temperature range, *Trichogramma* species and/or strains can perform after exposure to these temperatures for at least 24 hours (Pratissoli et al., 2021; Waghmare et al., 2021; Pratissoli et al., 2022).

Between the lower temperatures (21ºC and 18ºC) and the preferential range (24ºC -27ºC), it was observed that females remained inactive after up to 4 hours of exposure to 18ºC; from 6 hours of exposure on, females began to adapt to this temperature, showing signs of parasitism – nevertheless, all analyzed parameters’ rates were extremely low. When these females were transferred to a temperature of 25ºC, they were observed to move when exposed for at least 2 hours, but the rates of the parameters evaluated were only significant when these females were exposed to this temperature for at least 8 hours (Figures 2A, 4A, 6A). At 21ºC the females remained inactive for only 2 hours of exposure, with parasitism rates and the other parameters analyzed. When the females returned to a temperature of 25ºC, indices for all analyzed parameters were observed, but the rates were significant only after 8 hours of exposure (Figures 2B, 4B, 6B).

At temperatures below the preferred range, it was shown that all biological traits of *Trichogramma pretiosum* show low rates, and that the development rate is slow (Fragoso et al., 2019a; Fragoso et al., 2019b; Pratissoli et al., 2019; Araujo Júnior et al., 2021; Pratissoli et al., 2021).

5. Conclusion

The sex ratio for *Trichogramma pretiosum* females reaches 1.0, that is 100%, when exposed to the ideal temperature, between 24 and 27°C, indicating maximum use in the field, since females are responsible for parasitism. of eggs. Subjected to extreme temperatures, a strain of *Trichogramma pretiosum* needs time to adapt to the changes and return to its normal development. This discovery demonstrates the importance of planning the release of this parasitoid, considering field climate variations and synchronizing their release under favorable conditions, in addition to adopting complementary practices such as shading and microclimate management in the field.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**DISCLAIMER** (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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