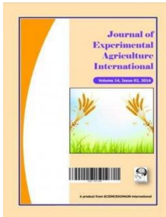

Journal of Experimental Agriculture International

38(5): 1-8, 2019; Article no.JEAI.49864
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Capacity for Parasitism of *Trichogramma* spp. in Tomato Fruit Borer under Different Temperatures

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Authors' contributions

This work was carried out in collaboration among all authors. Author DFMF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DP, JRC, LMAJ and RCOFB managed the analyses of the study. Author ACT managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2019/v38i530314
Editor(s):
(1) Dr. Lixiang Cao, Department of Biotechnology, Sun Yat-Sen University, Guangzhou, 510275, P. R. China.
Reviewers:
(1) P. Saravana Kumara, Subramaniam College of Arts and Science, India.
(2) Muhammad Indar Pramudi, Lambung Mangkurat University, Indonesia.
Complete Peer review History: http://www.sdiarticle3.com/review-history/49864

Original Research Article

Received 22 April 2019
Accepted 29 June 2019
Published 09 July 2019

ABSTRACT

The parasitoid in eggs of *Trichogramma* genre is the most studied in the world, being bred widely and used for flooding releases. This study aimed to evaluate the capacity for parasitism of *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) in *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) eggs at different temperatures. The experiment was developed at the Nucleus for Scientific and Technological Development in Phytosanitary Management (NUDEMAFI) in which the daily and accumulated biological parameters were

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assessed, as well as total parasitized eggs by female, sexual ratio (number of females/number of males + females), viability of the eggs (number of eggs with orifice/number of parasitized eggs x 100) and number of individuals per egg at temperatures 18, 21, 24, 27 and 30ºC. The eggs of the fruit borer were offered daily to each *T. galloi* female at each temperature until the death of the parasitoid could be verified. The larger number of parasitized eggs was found in the first 24 h, at temperatures 24 and 27ºC with 17 parasitized eggs. The accumulated parasitism in *N. elegantalis* eggs reached 80% of total parasitized eggs for each thermal range (18, 21, 24, 27 and 30ºC) on the 2nd, 3rd, 3rd, 1st and 2nd days respectively. The ideal parasitism conditions for this lineage vary between 24 and 27°C. Therefore, it is concluded that the studied *T. galloi* lineage has adequate biological parameters in *N. elegantalis* eggs, demonstrating promise in phytosanitary management of this pest.

Keywords: Egg parasitoid; Neoleucinodes elegantalis; phytosanitary management; tomato fruit; oviposition; Trichogrammatidae.

1. INTRODUCTION

Among the pests that attack the tomato culture, the tomato fruit borer *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) is considered one of the main pests for its preference for this culture and the damages caused directly in the fruit, making them inadequate for consumption and industrial processing, with significant loss [1,2,3,4,5,6,7].

Since this is a culture of high risk, with high intensity for pest attack, it is important to implement practices that aim to manage these pests. Among management methods, biological control is a viable technique, released in about 500,000 hectars every year in sugar cane especially when using parasitoids of the *Trichogramma* genre since it acts on the eggs avoiding the larvae to penetrate the fruit, reducing the loss caused by caterpillar feeding in its interior [8,9].

The egg parasitoid *Trichogramma* is the most often studied in the world, being greatly bred and used in flooding releases [10,11]. The advantage of its use is its capacity to control pests from different cultures. Moreover, they are highly specialized and efficient [12,13,14,15].

In Brazil, studies aiming at the use of *Trichogramma* were initiated over 30 years ago, with excellent results in many cultures, more recently *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) being the most often used species, released in about 500,000 hectares every year in sugar cane to control the cane borer *Diatraea saccharalis Fabricius, 1794* (Lepidoptera: Crambidae) [9,15, 16,17,18].

N. elegantalis studies have demonstrated its potential to use *Trichogramma* in its management [19]. Nonetheless, other studies must be conducted for better reliability on the use of these parasitoids in the management of *N. elegantalis*. These studies must involve the efficacy of the species, biological characteristics, thermal demands, ideal release numbers and dispersion, breeding of the alternative host *Anagasta kuehniella* Zeller (Lepidoptera) capacity [9].

Thus, the aim of this study was to evaluate the potential for parasitism of *T. galloi* in *N. elegantalis* eggs at different temperatures.

2. MATERIALS AND METHODS

The experiment was conducted in the Entomology Department at the Nucleus for Scientific and Technological Development in Phytosanitary Management (NUDEMAFI) at the Agronomic Sciences Center at the Federal University of Espirito Santo (CCAE-UFES), Alegre, ES (Brazil). A lineage Tg1of *T. galloi* species was used, provide by BUG Biological Agents.

2.1 Breeding of the Alternative Host *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae)

The alternative host *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) was bred in the homogenized diet of whole wheat flour (60%), corn (37%) and beer yeast (3%). The diet was disposed into plastic bins (30 x 25 x 10 cm) with corrugated cardboard strips (25 x 2 cm) on the inside, with the host eggs randomly selected for the diet. The adults were collected daily, with an adapted vacuum and transferred into PVC tubes (150 mm diameter x 25 cm height) with nylon strips folded in its interior for oviposition [20,21].

2.2 Breeding of *T. galloi*

For the maintenance of parasitoids, *Anagasta kuehniella* eggs were invisibilized in germicide lamp during 50 minutes and fixated in rectangles of sky blue cardboard (8.0 x 2.0 cm), with Arabic gum diluted to 20%. Those cards were inserted in glass tubes (8.5 x 2.4 cm), containing adult parasitoids recently emerged. Furthermore, the tubes were sealed with PVC plastic film to avoid parasitoid escape. The cards were kept in the tubes for 24 hours and later stored in clean glass tubes (9 x 3 cm) in an acclimatized room at 25 ± 1ºC, relative humidity 70 ± 10% and photophase of 14 h [21].

2.3 Breeding of *N. elegantalis*

Breeding of pests was conducted in an acclimatized room (25 ± 2ºC, RH 70 ± 10% and photophase of 12 h). Adults were kept in acrylic cages and fed with a solution of 10% honey. For oviposition, tomato fruit from the F1 wire was conditioned in the cages. Daily, the eggs were removed from the tomato fruit and distributed in African eggplant fruit (mean 5 eggs/fruit) which remained in plastic containers covered in non-woven fabric serving as places for pupation of caterpillars. Once this phase is finished, pupae were transferred into plastic containers or Petri dishes and stowed in acclimatized chambers in the above-mentioned conditions until adults emerge, then again taken to the acrylic cages.

2.4 Capacity of Parasitism

N. elegantalis eggs with up to 12 h of age were collected from the tomato fruit with the help of a scalpel and glued to sky blue cardboard (0.5 x 2.0 cm) with a brush and Arabic gum at 20%. For each temperature of the study, 20 recently emerged females were isolated in *Eppendorf* tubes (2.0 ml), containing drops of honey for feeding and sealed with the tubes' own lid. The cards with the 20 tomato fruit borer eggs were offered daily to each one of the *T. galloi* females at each temperature (18, 21, 24, 27 and 30ºC) until the death of the parasitoid was confirmed. The cards removed daily were identified and bagged (23.0 x 4.0 cm) and kept at its respective temperatures.

The following biological parameters were assessed: daily and accumulated parasitism, total parasitized eggs per female, sexual ratio (number of females/number of males + number of females), viability of the eggs (number of eggs with orifice/number of parasitized eggs x 100) and number of individuals per egg at different temperatures.

The experiment was conducted with a completely casual design, with five treatments (temperatures) and 20 repetitions, each repetition represented by a *T. galloi* female. For data analysis, a regression with test F was used at 5% probability level.

3. RESULTS

Daily parasitism decreased at all temperatures with the advance of age of the *T. galloi* female. At the different studied temperatures, higher rates of parasitism occurred in the first 24 hours, presenting between 13 and 17 parasitized eggs. The higher rates of parasitism were at temperatures 24ºC and 27ºC with around 17 eggs parasitized (Fig. 1).

In terms of longevity of the females, it was noted that lower temperatures (18ºC to 24ºC) females were able to live longer due to a reduction in the physiological activity of females when exposed to lower temperatures. At higher temperatures (27ºC and 30ºC), there is higher energy expenditure and, consequently, females lived for a shorter period of time (Fig. 1).

The parasitism (parasitism) period for *T. galloi* females was increased in the thermal range, of 18 to 24ºC (5, 7 and 8 days) and in the 27 to the 30ºC range there was a decrease (5 and 4 days). Therefore, the ideal conditions for the survival of *T. galloi* vary between 24ºC and 27ºC where better performance was observed.

Accumulated parasitism in *N. elegantalis* eggs in the studied thermal range reached 80% of total parasitized eggs in a maximum of three days. At extreme temperatures (18 and 30^{o}C) this condition was reached in two days. At milder temperatures (21 and 24^{o}C) the accumulated parasitism reached 80% in three days and at 27ºC was reached in the first day of parasitism (Fig. 1). Due to the 80% parasitism, it was noted that the potential for parasitism of this lineage occurs within the first days of parasitism, independent from temperature.

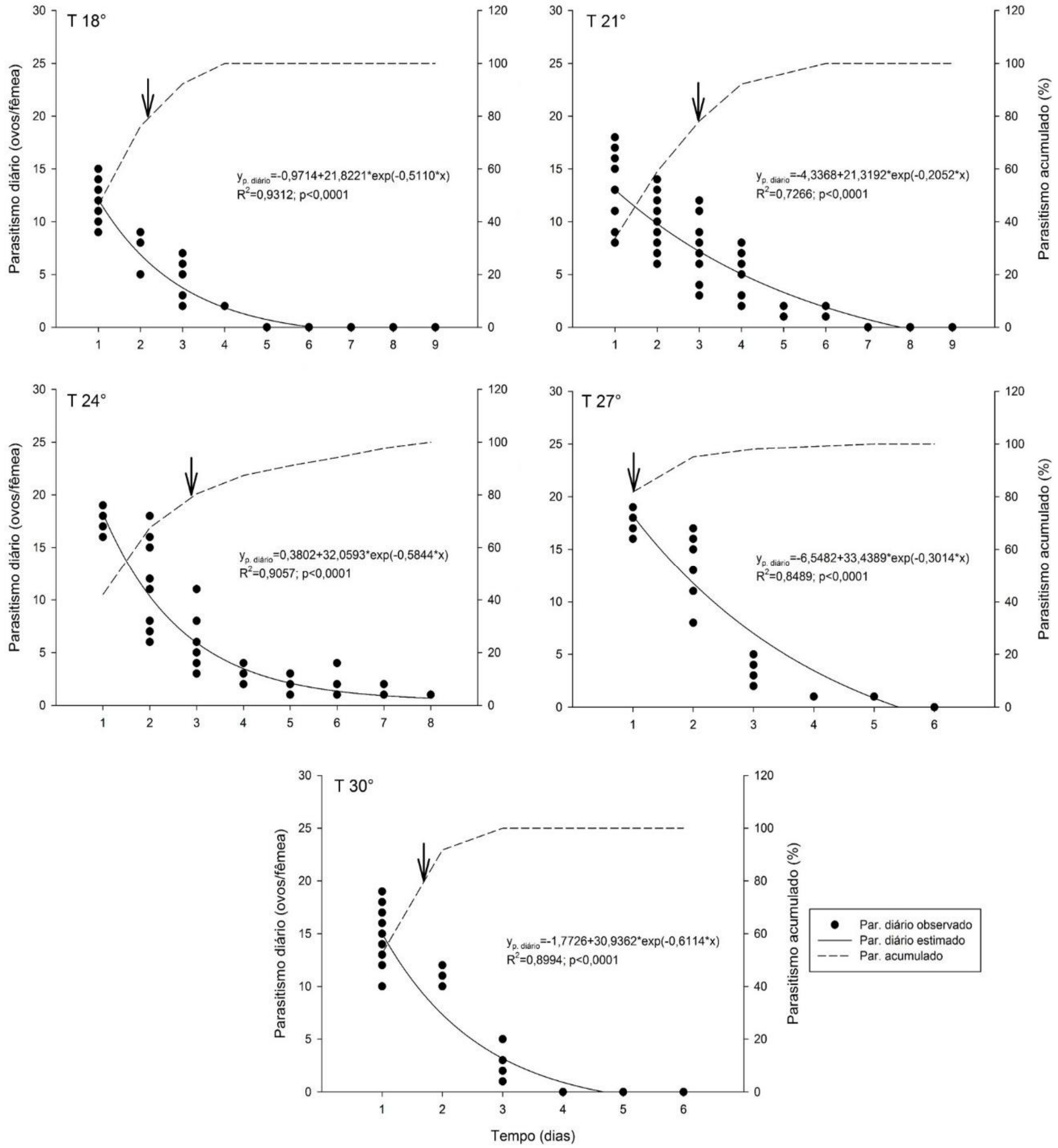

Fig. 1. Daily and accumulated parasitism for *T. galloi* in *N. elegantalis* eggs at different temperatures

For this lineage, the 24ºC temperature highlights the total amount of eggs parasitized per female reaching an average of 30 eggs. In terms of viability, as the temperature increased there was a reduction in viability, coming to 50% at 30ºC. For sexual ratio, it was observed that when temperature increased there was a higher number of males in the population, but the number of individuals per egg was constant (Fig. 2).

4. DISCUSSION

We verified that the temperature interferes in the potential for parasitism and biological characteristics of *T. galloi.*

Among the main factors affecting biological characteristics of species in the *Trichogramma* genre, temperature is highlighted since with its increase, there is lower performance by females causing metabolism to increase and, therefore, reducing parasitism [22,23,24,25]. This was demonstrated in the present study since extreme temperatures showed a decrease in parasitism with only 11 eggs parasitized on average at 18ºC, 10 eggs at 21ºC and 14 eggs parasitized at 30ºC (Fig. 2).

The potential for parasitism in the first days may be directly connected to the instinct of animal preservation once all species in the *Trichogramma* genre present this behavior [18,25,26]. This behavior may be related to the parasitism of 80% of the eggs as studies have confirmed this rate to be, in most cases, in the first few days of life in females [18,25].

Parasitism period may vary according to temperature and within each temperature. This fact may be related to the capacity of adaptability in each species and/or lineage of *Trichogramma* to the habitat in which it was collected [15,18,22,25,26].

Accumulated parasitism is another factor that may be related to the capacity of adaptability of each species and/or lineage of *Trichogramma* to the habitat in which it was collected since the necessary time to reach total percentage is variable [18,15]. The range of temperature in which species and/or lineage of *Trichogramma* present their higher potential for parasitism (number of parasitized eggs) is between 24 and 27°C [15,18,22,25].

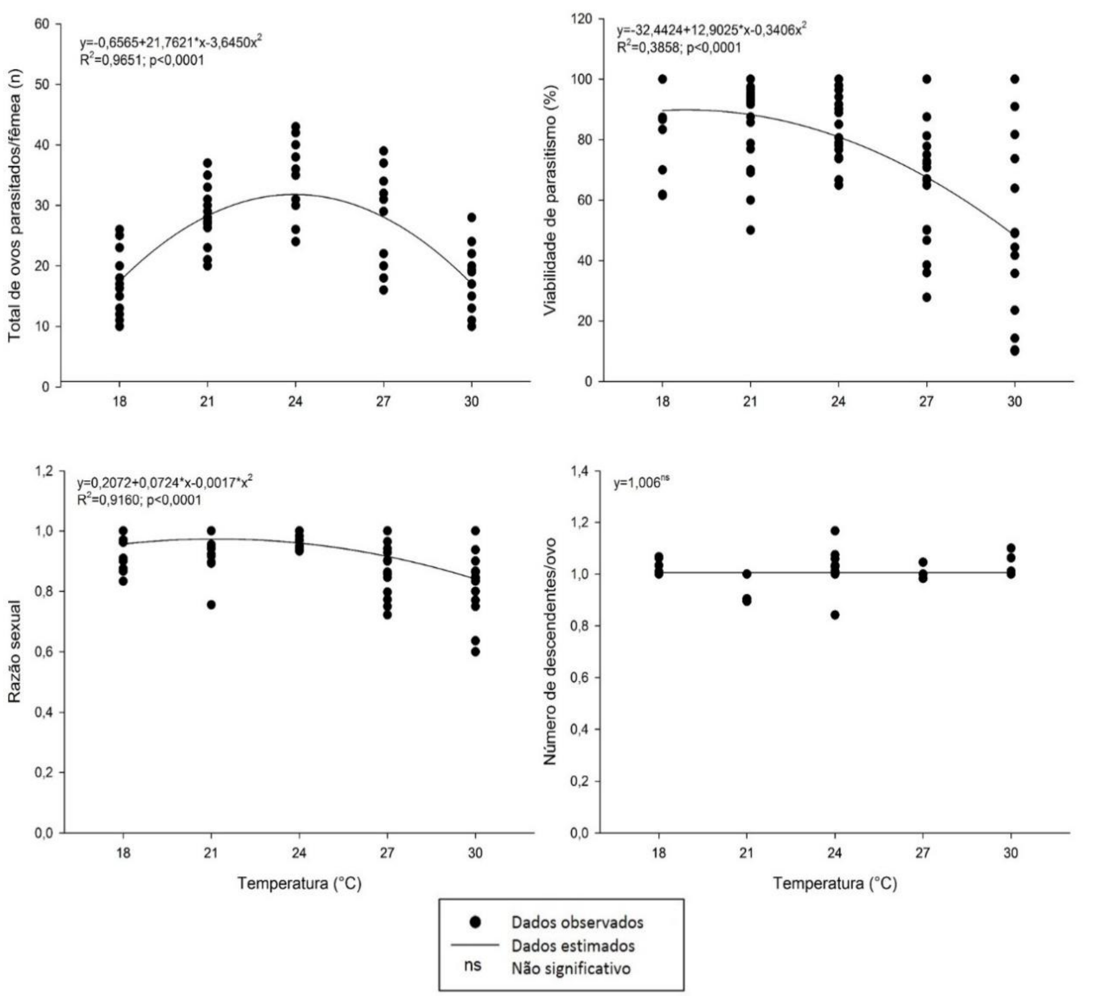

Fig. 2. Total *N. elegantalis* eggs parasitized by *T. galloi*, the viability of parasitism, sexual ratio and number of descendants per eggs at different temperatures

Through viability, there seems to also be direct interference from temperature. It is possible to verify that there is an inverse relationship between the percentage of the emergence of descendants and the increase in temperature. However, this was not found in any other studies.

The variation in sexual ration has been reported as influenced especially by temperature [24]. This was verified in extreme temperatures once the humidity, female age, and host were constant for all temperatures.

On the number of descendants per egg, it was verified that it was constant, that is, one individual per egg independent from temperature. The variation in this factor is directly related to nutritional and morphological characteristics of the egg such as size, shape, thickness, corion stiffness and lay behavior [10,26,27].

It was verified that the lineage studied for *T. galloi* holds true the adequate biological parameters for parasitism in *N. elegantalis* eggs, proving to be promising in phytosanitary management of this pest.

5. CONCLUSION

The studied *T. galloi* strain presents the appropriate biological parameters for parasitism in *N. elegantalis* eggs, showing promise in the phytosanitary management of this pest.

ACKNOWLEDGEMENTS

The authors appreciate the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Fundação de Amparo à Pesquisa e Inovação do Espírito Santo* (FAPES) and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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