Thermal requirements, life table and estimate of number of *Trichogramma galloi* in eggs of *Neoleucinodes elegantalis*

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***Abstract*— *The Neoleucinodes elegantalis is considered one of the main tomato pests, as it directly damages the fruit. The use of the parasitoid Trichogramma galloi in the management of N. elegantalis requires studies for knowledge of parasitoid behavior regarding the pest to be managed. The objective of this research was to evaluate the behavior of T. galloi for the management of N. elegantalis, through thermal requirements and of life table. According to the results, the fetal develop of T. galloi starts when the temperature remains above 8.02 ± 0.2°C and the heat buildup for complete development is of 159.74 ± 3.88°C degree days (DD). The number of generations of T. galloi was estimated to the five largest municipalities from the Espírito Santo state (Venda Nova do Imigrante, Domingos Martins, Conceição do Castelo, Vargem Alta and Santa Maria do Jetibá), checking that T. galloi may have 24.72 to 35.37 generations per year according to the temperatures reached in each region studied.*

Keywords*— *biological control, parasitoid, pest management, small fruit borer, tomato.

I. INTRODUCTION

The small fruit borer, *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) is considered one of the main tomato pests, as it directly damages the fruit, making them unfit for consumption and industrial processing, with lossy that vary of 20 to 90% (Picanço *et al.,* 2007; Moraes & Foerster, 2015; Silva *et al.,* 2017). This species occurs, mainly, in the neotropical area, reaching the Central America and the South America (Querino, 2002; Moraes & Foerster, 2015; Silva *et al*., 2017).

In addition to causing serious damage to tomato fruit, this pest still has other host plants like solanaceae (scarlet eggplant, bell pepper and eggplant), besides weeds like joa and the jurubeba (Fornazier *et al.,* 2010; Pratissoli, 2015).

There are few studies to indicate alternative tactics for the management of *N. elegantalis*, because caterpillars penetrate the newly hatched fruit and remain inside the fruit until the pupal phase. This fact induces the producer to make constant pesticide applications (Plaza *et al*., 1992; Oliveira *et al*., 2017).

The Phytosanitary Pest Management is the establishment of control strategies that involve all the knowledge related to the biological cycle of culture, insect pest and of environmental factors. This kind of pest management involves pest control methods aimed at reducing producer costs over time, optimize production and reduce environmental impacts, mainly through lower use of chemical insecticides (Pratissoli *et al*., 2007; França *et al*., 2015).

Currently, the biological control assumes great importance in the pest management context, especially in the face of discussions for more sustainable agriculture, with less aggression to the environment and human health. A viable option, for the management of this pest, is the use of the egg parasitoid of the genre *Trichogramma* because the control of *N. elegantalis* is performed in the egg phase (Querino, 2002; Diaz & Brochero, 2012 Zucchi *et al*., 2015).

The use of this parasitoid in the management of *N. elegantalis* requires studies for knowledge of the parasitoid behavior regarding environmental conditions, the characteristic of culture, and the plague to be managed (Plaza *et al*., 1992; Milanez *et al*., 2018; Wang *et al*., 2018; Paes *et al*., 2018).

Therefore, the objective of this work was to evaluate the behavior of *Trichogramma galloi* for the management of *N. elegantalis*, through thermal requirements, fertility life table and the optimal number of parasitoids to be released in the field.

II. MATERIAL AND METHODS

The laboratory creation of *Neoleucinodes elegantalis* occurred in air conditioned room (25 ± 2 ºC, 70 ± 10% of relative humidity (RH) and 12 h of photophase). The adults *N elegantalis* were kept in acrylic cages and fed with honey solution at 10%. The tomato fruits were packed in oviposition cages. Daily, the fruits of tomatoes were removed and the eggs of *N elegantalis* were distributed in fruits of Scarlet eggplant (on average five eggs/fruit) that remained in plastic trays, covered with non woven fabric, for pupation.

In the creation of *Trichogramma galloi*, eggs of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) were made unfeasible in germicidal lamp for 50 minutes and set in rectangles of blue cardstock (cartouches of 8.0 x 2.0 cm), with gum arabic at 20%. The cartouches were inserted in glass tubes (8.5 x 2.4 cm), with newly emerged adult parasitoids. Posteriorly, the tubes were sealed with plastic film of polyvinyl chloride, in order to prevent parasitoids from escaping. The cartouches were kept in the tubes for 24 h. Posteriorly, the cards were stored in clean glass tubes (9 x 3 cm) in air-conditioned room with temperature of 25 ± 1ºC, 70 ± 10% RH and 14h of photophase.

2.1 Thermal requirements and life table of *Trichogramma galloi* in eggs of *Neoleucinodes elegantalis*

The eggs of *N. elegantalis* were grouped into 10 eggs per female *Trichogramma galloi*. Three females *T. galloi* were used by repetition. The eggs of *N. elegantalis* were exposed to parasitism for 5 h, in order to prevent the occurrence of superparasitism. The eggs of *N. elegantalis* were transferred daily to blue cardstock (0.5 x 2.0 cm). These were inserted into eppendorf tubes (2 mL) along with the parasitoids (age 0 – 12 h) and a honey droplet for food. The tubes were sealed with the container lid, and packed in Climatic chamber at 25 ± 1°C, 70 ± 10% RH and 14 h of photophase. After 5 h, the female parasitoids were killed with the aid of a brush; and the parasitized eggs were transferred to climate chambers at temperatures of 18, 21, 24, 27 e 30 ºC ± 1 °C, 70 ± 10% RH and 14 h of photophase. These temperatures correspond to the optimal range (24°C and 27°C) and the extreme (18, 21 e 30 ºC) for parasitoid development (Melo *et al.*, 2007).

From the emergence of the adult *T. galloi*, 15 females (age 0 - 6 h), from each temperature (18; 21; 24; 27 and 30 ± 1ºC), were individualized in Eppendorf tubes (2.0 mL) and returned to their respective temperatures. The cartouches of blue cardstock (0.5 x 2.0 cm) with 20 eggs of *N. elegantalis* (age 0 - 24 h) were offered daily, until the death of female *T. galloi.* The cartouches with parasitized eggs, from each treatment, were packed in plastic bags (23.0 x 4.0 cm) and kept under the same conditions.

For the assessment of thermal requirements, the calculation of base temperature (bT) and thermal constant (K), were obtained by the hyperbole method (Haddad *et al.,* 1999), in the SAS program, version 9.0 (SAS Institute 2001), based on cycle length (egg-adult) at temperatures tested.

The estimated number of annual generations of *T. galloi* was estimated based on monthly average temperature of the municipalities of the highland region from Espírito Santo state, which were: Venda Nova do Imigrante; Domingos Martins; Conceição do Castelo; Vargem Alta and Santa Maria do Jetibá. The data of monthly average temperature of municipalities were obtained by the National Institute of Meteorology.

For the elaboration of fertility life tables, the methodology proposed by Silveira Neto *et al*. (1976) was used. The fertility life table of the parasitoid was made based on age interval values (x), specific fertility (m_x), probability of survival (l_x) in estimating Jackknife (Maia; Luiz & Campanhola, 2000; Maia; Luiz, 2006). The parameters determined were: net reproduction rate (Ro), time interval between each generation (T), innate ability to increase in number (r_m) and finite rate of increase (λ).

The design used was the entirely randomized with five treatments (temperatures) being the quantitative data submitted to regression analysis. The choice of equation what se ajustou that fit to the data was based on the phenomenon under study and on the significance of regression coefficients (β_i) and regression by the test F at 5% probability level of error and in the coefficient of determination (R^2) (Pimentel-Gomes & Garcia, 2002; Souza, 1998; Gujarati & Porter, 2011).

2.2 Estimated number of *Trichogramma galloi* to be released

The experiment was conducted in a greenhouse. The tomato seedlings were transferred for 20 Kg plastic buckets with prepared substrate in proportion to 1:1:1 (soil: sand: tanned manure). After 60 days of transplanting, tomato fruits were offered to females *N. elegantalis*. Posteriorly, 200 eggs of *N.* elegantalis were accounted. The females *Trichogramma galloi* were released in the proportions of 1, 2, 4, 8, 16, 32, 64 and 128 per egg of the *N. elegantalis*, in a cage (60 x 60 x 150 m) made with anti-aphid screen and sealed at the base. The tomato infested with eggs of *N. elegantalis* was placed in the middle part of the tomato plant. Thus, according to the proportion, 200, 400, 800, 1600, 3200, 6400, 12800 and 25600 females *T. galloi* were released. Parasitism occurred for 24 h. Posteriorly, the fruits were taken to the laboratory, arranged in a climate chamber (25 ± 2 °C, 70 ± 10% RH and 14 h of photophase).

The experiment was repeated six times, in a completely randomized experimental design. The results were submitted to analysis of variance and regression in order to determine the optimal number of *T. galloi* to be released in the field. The percentage of parasitized eggs in each proportion was the evaluated parameter.

III. RESULTS AND DISCUSSION

3.1 Thermal requirements and fertility life table

The embryonic development of *Trichogramma galloi* in eggs of *Neoleucinodes elegantalis* started from the temperature 8.02 ± 0.2 °C. The heat buildup for the complete development of *T. galloi* was of 159.74 ± 3.88 °C degree days (DD) (Figure 1).

The number of generations of *T. galloi* in eggs of *N. elegantalis* for the municipalities of Venda Nova do Imigrante, Domingos Martins, Conceição do Castelo, Vargem Alta and Santa Maria do Jetibá, tomato producing localities of the Mountain Region from the Espírito Santo state, were estimated through the thermal requirement with respectively 28.21, 24.72, 35.37, 30.96 and 28.72 generations per year (Table 1).

The highest number of generations per month of *T. galloi* in eggs of *N. elegantalis* occurred in the hottest periods of the year in all municipalities evaluated (Figure 2). The municipality of Domingos Martins presented the lowest number of generations in all months. The municipalities of Conceição do Castelo and Vargem Alta got bigger highlights with the most number of generations in every month, over three generations between the months of December and March.

In the life table, the average duration of one generation (T) of *T. galloi*, raised on eggs from *N. elegantalis*, demonstrated an inverse relationship with the increased temperature of 18 to 30 ºC with values of 16.06 and 8.11 days, respectively (Table 2). The net reproductive rate (Ro), which indicates the number of times that population multiplied by generation, ranged from 11.37 to 66.58 depending on temperature, with the maximum increase of the population in the temperature of 24 ºC and lowest net reproduction rate at temperatures of 30 and 27 ºC, with 11.37 and 28.28, respectively. The infinitesimal rate of increase (r_m) of *T. galloi* increased in the range of 18 to 24ºC (0.22 to 0.35). The finite ratio of increase (λ), which indicates the number of females *T. galloi* added to population by females parasitoid per day was greater at 24 ºC and smaller at 18 ºC, with 1.42 and 1.25 females / females / day, respectively.

3.2 Estimate Number of *T. galloi* per egg of *N. elegantalis*

In the analysis of the number of parasitized eggs, the behavior of *T. galloi* followed a quadratic function, which demonstrated a relationship between parasitism and the number of parasitoids released. The estimated 82 individuals of *T. galloi* per egg of *N. elegantalis* reached a parasitism peak of about 200 parasitized eggs (Figure 3).

The variables analyzed in the present study may present different values in other species of *Trichogramma*, since these are individual parameters related to adaptive capacities to various hosts and room temperature (Dias *et. al*., 2013; Arruda *et al*., 2014; Carvalho *et al*., 2017; Milanez *et al*., 2009; Zuim *et al.* 2013; Paes *et al*., 2018; Mansour, 2019).

The life table has been relevant for understanding the population dynamics of a species (Silveira Neto *et al.,* 1976; Melo *et al*., 2007; Pratissoli *et al*., 2007). In the present experiment, the temperature of 24 ºC was the most appropriate for the parameters evaluated on *T. galloi*.

The number of parasitoids to be released varies depending on several factors, such as plant phenology, the parasitoid species and with the host posture characteristic (Pratissoli *et al*., 2005; Bakthavatsalam, *et al*., 2013; Milanez *et al*., 2009; Wang *et al*., 2018; Paes *et al.,* 2018).

Therefore, the success in pest control, with the use of the parasitoid *Trichogramma*, is related to the correct choice of the species to be used. This because although it is classified as a generalist parasitoid, research results show that species may have affinity for certain hosts. This affinity can occur by stimulus-driven search behavior, of the nutritional and morphological characteristics of the egg. Abiotic factors, as weather conditions, also can affect other parameters as: the duration of development, sexual rate, parasitism and adult longevity (Pratissoli & Parra, 2001; Mansfield & Mills, 2004; Krishnamoorthy, 2013; Carvalho *et al.,* 2017; Hou *et al.,* 2018; Jalali, 2018, Paes *et al*., 2018).

https://dx.doi.org/10.22161/ijaers.612.39 ISSN: 2349-6495(P) | 2456-1908(O)

IV. FIGURES AND TABLES

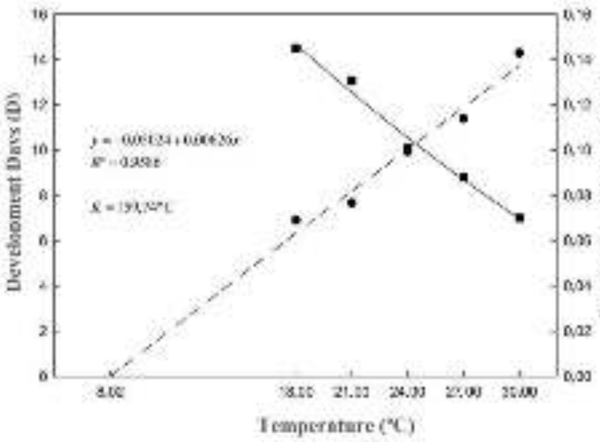

Fig.1. Duration (days) and development velocity of Trichogramma galloi, in eggs of Neoleucinodes elegantalis, under to different temperatures. RH: 70 ± 10% and Photophase: 14 h.

Table 1. Number of Generations of *Trichogramma galloi* in eggs of *Neoleucinodes elegantalis* in different municipalities of the Mountain Region from the Espírito Santo state, Brazil.

Municipality	**Number of Generations / year**
Venda Nova do Imigrante	28.21
Domingos Martins	24.72
Conceição do Castelo	35.37
Vargem Alta	30.96
Santa Maria do Jetibá	28.72

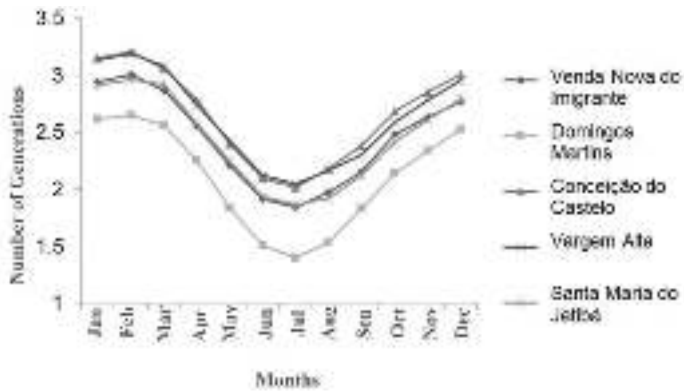

Fig.2. Estimate of number of generations of T. galloi in eggs of n. elegantalis in the different months of the year, to four tomato producing municipalities on Espírito Santo state.

Table 2. Duration of a generation (T), net reproduction rate (Ro), infinitesimal rate of increase (r_m) and finite rate of increase (λ) of Trichogramma galloi (Hymenoptera: Trichogrammatidae) in eggs of Neoleucinodes elegantalis at different temperatures. RH: 70 ± 10% and Photophase: 14 h.

Temperatures	T (days)	Ro	r_m	λ
18	16.06	36.81	0.22	1.25
21	14.91	61.14	0.27	1.32
24	11.91	66.58	0.35	1.42
27	10.14	28.28	0.32	1.39
30	8.11	11.37	0.30	1.35

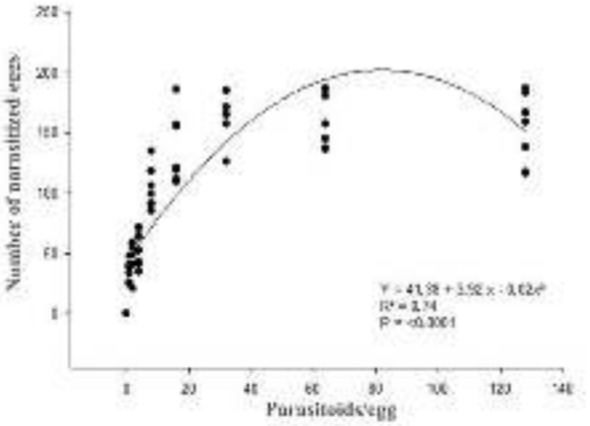

Fig.3. Number of eggs of Neoleucinodes elegantalis parasitized by Trichogramma galloi in tomato plants.

V. CONCLUSION

It can be concluded that the estimated number of *T. galloi* generations in the five municipalities of Espírito Santo (Venda Nova do Imigrante, Domingos Martins, Conceição do Castelo, Vargem Alta and Santa Maria do Jetibá) can vary from 24.72 to 35, 37 generations per year, according to the temperatures reached in each region studied.

ACKNOWLEDGEMENTS

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES).

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