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Performance of *Trichograma* spp in small tomato borer (*Neoleucinodes elegantalis* Guenée): Pest management with egg parasitoid

José Romário de Carvalho[1], Luis Moreira de Araujo Junior[1*], Dirceu Pratissoli[1], Débora Ferreira Melo Fragoso[1] and Amanda Carlos Túler[2]

[1]Centro de Ciências Agrárias e Engenharias, Universidade Federal do Espírito Santo, Alto Universitário, s/n, 29500-000, Guararema, Alegre, Espírito Santo, Brazil. [2]Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, São Paulo, Brazil. *Author for correspondence. E-mail: luisjrmoreira@gmail.com

ABSTRACT. The tomato is a crop of great importance for Brazilian agriculture. Among the most damaging pests, the small tomato borer, *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambiadae) has caused great losses, since they directly reach the fruits to be commercialized, being used for its handling a large volume of insecticides. In this way, the use of alternative techniques that help in the management of this pest becomes of great importance. Among them, the use of the egg parasitoid *Trichogramma* spp. has been promising. Thus, the present study aimed to evaluate the performance of *Trichogramma* species and/or strains in *N. elegantalis* by selection of strains. The selection was made based on four lineages maintained in the Nucleus of Scientific and Technological Development in Phytosanitary Management of Pests (NUDEMAFI), being three strains of the species *T. pretiosum* and one of *T. galloi* species. The parameters evaluated were percentage of parasitized eggs, egg viability, number of individuals per eggs, sex ratio and number of *Trichogramma* spp. to be released. The *T. galloi* (T. g1) showed the best parameters for selection of the strain**.** Estimating the optimal number of T. g1 in eggs of small-fruit-borer was 82 individuals per egg parasitoid. Therefore, this strain was selected for the management of the small tomato-borer, whose eggs presented favorable physicochemical characteristics for the development of the parasitoid.

Keywords: egg parasitoid; phytossanitary management; selection of parasitoid.

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Introduction

The tomato (*Solanum lycopersicum* L.) is cultivated in several regions of Brazil, being a high-risk crop, mainly due to the damages caused by the pests both in crops destined for the in natura consumption and for the industrialization (Souza & Reis, 2003). The pest complex that attacks the tomato crop in the state of Espírito Santo is composed of approximately 28 registered insect species (Fornazier, Pratissoli, & Martins, 2010). Among these, the Lepidoptera Tuta absoluta (Meyrick, 1917), Neoleucinodes elegantalis Guenée and Helicoverpa zea Boddie are the main ones. These pests, *N. elegantalis*, also known as the small tomato-borer, is one of the most important insects in the crop, causing damages directly on the fruit, causing 90% losses (Picanço, Bacci, Crespo, Miranda, & Martins, 2007; Pratissoli, 2015; Carvalho et al., 2017; Cagnotti, Virgala, Botto, & López, 2018).

The chemical control has been used in a large scale to control this pest, bringing risks to human health and imbalances to the environment, causing the death of natural enemies that could aid in pest management, as well as being ineffective in managing this pest, once that the larvae penetrate the fruit (Blackmer, Eiras, & Souza, 2001; Medeiros, Vilela, & França, 2006; Carvalho et al., 2017; Cagnotti et al., 2018).

New methods of management should be evaluated in order to reduce the amount of pesticides sprayed. Among these methods, the use of biological control with release of *Trichogramma* may be promising in the phytosanitary management of tomato-borer, because in tomato, this parasitoid has already been used on a large scale, because it is highly efficient and specialized. Another great advantage of these egg parasitoids is to control pests from different crops before they can cause damage (Kumar, Sekhar, & Kaur, 2013; Krishnamoorthy, Mani, & Visalakshy, 2013).

In Brazil, one of the most relevant studies in the field of biological control using egg parasitoids corresponds to the use of *Trichogramma pretiosum* Riley in the management of *T. absoluta* (Haji, Prezotti, Carneiro, & Alencar, 2002). Other works on tomatoes have been carried out with the aim of implementing alternative management methods that help to reduce the application of pesticides and reduce the initial population of the pest (Pratissoli & Parra, 2001; Díaz M. & Brochero, 2012).

For an effective management, using this parasitoid, can be applied in the field, it is necessary the previous study of the characteristics of the species/strains to be used, such as thermal requirements, biological aspects, egg age in relation to the parasitism rate parasitism and the influence of egg density on parasitism (Blackmer et al., 2001; Bakthavatsalam, Tandon, & Bhagat, 2013; Ahmed et al., 2018; Jalali, Omprakash, Srinivasa, Venkatesan, & Lalitha, 2018).

Material and methods

The *Trichogramma* spp. kept in the Nucleus of Scientific and Technological Development in Phytosanitary Management of Pests (NUDEMAFI) - Department of Entomology of the Center of Agricultural Sciences of the *Universidade Federal do Espírito Santo* (CCAE/UFES), came from different regions, such as the described in Table 1.

Table 1. *Trichogramma* spp. used for the study of strains selection in *Neoleucinodes elegantalis* eggs and their origin data.

Species	Code	Place of collection	City	Culture	Pests
T. pretiosum	T. p8	Farm Guandu	Afonso Cláudio - ES	Tomato	*H. zea*
T. pretiosum	T. p18	Business Line – BUG	-	-	-
T. pretiosum	T. p19	Experimental area - CCAUFES	Rive - Alegre-ES	Tomato	*N. elegantalis*
T. galloi	T. g1	Business Line – BUG	-	-	-

The alternative host Anagasta kuehniella Zeller was grown on a homogenized diet based on whole wheat flour (60%), maize (37%) and brewer's yeast (3%) according to the methodology described by Pratissoli and Parra (2001).

For the maintenance of the parasitoids, *A. kuehniella* eggs were unviable in germicidal light for 50 min. and fixed in rectangles of celestial blue paper (8.0 x 2.0 cm), with gum arabic diluted to 20%. These cards were inserted into glass tubes (8.5 x 2.4 cm) containing adults of newly emerged parasitoids. Subsequently, the tubes were sealed with PVC plastic film, in order to avoid the escape of the parasitoids. The cartons were kept in the tubes for 24 hours, later removed and stored in new tubes, being kept in an air conditioned room with a temperature of 25 ± 1ºC, relative humidity of 70 ± 10% and photophase of 14 hours.

The pest multiplication was carried out in an air conditioned room under the same conditions as the previous one. The adults were kept in acrylic cages and fed with 10% honey solution. For oviposition, tomato fruits of the cultivar F1 fowl were packed inside the cages. These fruits were collected daily and the eggs contained in them were counted and transferred to jilo fruits, placing an average of five eggs/fruit, which remained in plastic trays covered with TNT fabric, thus serving as a pupation site for the caterpillars. After this phase, the pupae were transferred to plastic pots or Petri dishes and conditioned in climatic chambers under the conditions mentioned until the emergence of the adults, which were again taken to the acrylic cages.

Twenty females (less than 24 hours old), individualized in eppendorf tubes (2 mL), containing on their wall honey droplets for feeding were used to conduct the experiment on the performance of females of *Trichogramma* spp. The eggs of *N. elegantalis* were collected from stock breeding, being removed from the fruits through the aid of a scalpel. For each female, 20 eggs were fixed in blue cardboard boxes (0.5 x 2.0 cm), aged of 0-24 hours, and parasitism was allowed for 24 hours in acclimatized chambers (25 ± 1ºC, UR of 70 ± 10% and 14 hours photophase). After this time, the females were removed from the tubes and the eggs remained in these chambers.

The sex of the individuals was determined by the sexual dimorphism presented in the antennas. The parasitized eggs were placed in plastic bags and kept at temperature (25 ± 2ºC, 70 ± 10% RH and 14 hours photophase) until emergence of the offspring. The parameters evaluated were: percentage of parasitized eggs, egg viability (number of eggs with orifice/number of parasitized eggs x 100), number of individuals per eggs and sex ratio (number of females/n ° of males + females).

This experiment was composed of a completely randomized design with 4 treatments (strains) and 20 replicates, each replicate being represented by a female of the strain tested. The data were submitted to

analysis of variance and the means were compared by the Tukey test at the 5% probability level, using the statistical program Assistat 7.7 (Silva, 2014).

The experiment to estimate the number of *Trichogramma* spp. to be released, was conducted in a greenhouse of the CCAE/UFES, where tomato seedlings of the Alambra F1 variety were transferred to plastic buckets of 20 kg containing substrate prepared in the proportion of 1/3 of subsoil, 1/3 of sand and 1/3 of manure from the tanned corral. The experiment was set up after 60 days of transplanting of the seedlings, where on the night before the experiment, tomato fruits were offered to the females of *N. elegantalis*. The next day 200 eggs were counted.

Females of *Trichogramma* spp. were released in the proportions of 1, 2, 4, 8, 16, 32, 64 and 128 per egg of the small drill, in a cage (60 x 60 x 150 m), made with anti-Aphid cloth, sealed in its base. The tomato containing the eggs was placed in the middle part of the tomato plant. Thus, according to the ratio, 200, 400, 800, 1600, 3200, 6400, 12800, 25600 females of *Trichogramma* spp. The parasitism was allowed for 24 hours, after which the fruits were identified and taken to the laboratory, arranged in an air-conditioned room (25 ± 2ºC, 70 ± 10% RH and 14 hours photophase).

In each proportion, 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 ideal number of the *Trichogramma* spp. to be released in the field. The evaluated parameter was the percentage of eggs parasitized in each proportion.

Results and discussion

Among the evaluated species, the species *T. galloi* (T. g1), due to the high percentage of parasitism (81.25%), differed from the others. For the biological parameter of sexual ratio only the T. p18 strain showed the presence of males in the population. Regarding egg viability, the selected T. g1 strain presented 100% viability, however, differing only from the T. p8 strain with 93.40% viability. As for the number of individuals per egg, the strains that stood out were T. p8 and T. p19 with 1.60 and 1.61 respectively, differing from the others, T. p18 and T. g1 with 1.24 and 1.17 individuals per egg, respectively (Table 2).

Table 2. Percentage of parasitism, sex ratio, viability and number of individuals per egg for the *Trichogramma* strains

Strains	% Parasitism[1]	Sex ratio[1]	Emergence (%)[1]	Number of individuals/egg[1]
T. p8	24. ± 0.39 c	1.00 ± 0.00 a	93.40 ± 0.27 b	1.60 ± 0.01 a
T. p18	67.25 ± 1.47 b	0.86 ± 0.01 b	100.00± 0.22 a	1.24 ± 0.01 b
T. p19	32.75 ± 0.37 c	1.00 ± 0.00 a	96.63± 0.19 ab	1.61 ± 0.02 a
T. g1	81.25 ± 0.89 a	1.00 ± 0.00 a	100.00± 0.00 a	1.17 ± 0.01 b

[1] Means followed by the same letter do not differ from one another by Tukey test ($p \leqslant 0.05$).

The selection of *Trichogramma* spp. and/or strain(s) has been one of the widely studied factors, as quality characteristics in biological control programs (Hassan, 1997; Venkatesan & Jalali, 2013; Paes, Lima, Pratissoli, Carvalho, & Bueno, 2018). Thus, the results obtained in the present work demonstrate the importance of selection of strains of this parasitoid for the management of tomato-borer, since there are differences for the parameters evaluated in the species and/or tested strains, since the host is a factor that can provide behavioral changes in this egg parasitoid (Pratissoli et al., 2003; Goulart, Bueno, Bueno, & Diniz, 2011; Hou et al., 2018). These variations occur mainly because of the nutritional and morphological characteristics of the egg, such as size, shape, thickness and rigidity of the chorion, posture behavior (Hassan, 1994; Bakthavatsalam et al., 2013). This fact shows that T. g1 had the highest rate of parasitism, being only progeny of females and emergence of descendants of all parasitized eggs. As for the number of individuals per egg, T. g1 statistically presented the lowest value, which does not really fit as a negative factor, since it is already a consensus among researchers that fewer individuals per egg leads to the development of more robust parasitoids.

Acceptance of tomato-borer eggs as a good host may be linked to the fact that the egg characteristics and the laying behavior, which is made in layers, are similar to that of their preferred host, which sugar cane drill *Diatraea saccharalis* (Fabr., 1794). This preference has been highlighted in several works (Zucchi, Parra, & Silveira Neto, 1991; Leite et al., 2014). The acceptance of hosts that have similar characteristics to the host preference was also highlighted in the work of Paes et al. (2018), where *T. galloi* had a better performance in the parasitism of *Duponchelia fovealis* Zeller eggs, compared to strains of *T. pretiosum*.

Thus, it can be stated that *T. galloi*, because it presents adequate qualitative and quantitative characteristics, can be an efficient parasitoid in the management of *N. elegantalis*. Other studies involving the characteristics of *T. galloi* in *N. elegantalis* eggs should be made to evaluate their favorable characteristics for the management of the pest in question, such as the ability of this strain to parasitize the eggs of the small drill of the fruit in different temperatures, data of thermal requirements and its life table, as well as the ideal estimate of the number of individuals of the parasitoid to be released in the field in relation to the number of eggs of the pest in question.

The estimation of the number of *T. galloi* per egg of *N. elegantalis* was determined by analyzing the number of parasitized eggs, where it was verified that the behavior of this parasitoid followed a quadratic function, demonstrating that a relationship between parasitism and number of parasitoids released, and the estimate of 82 individuals of the parasitoid per small-borer egg reached a parasitism peak of about 200 parasitized eggs (Figure 1).

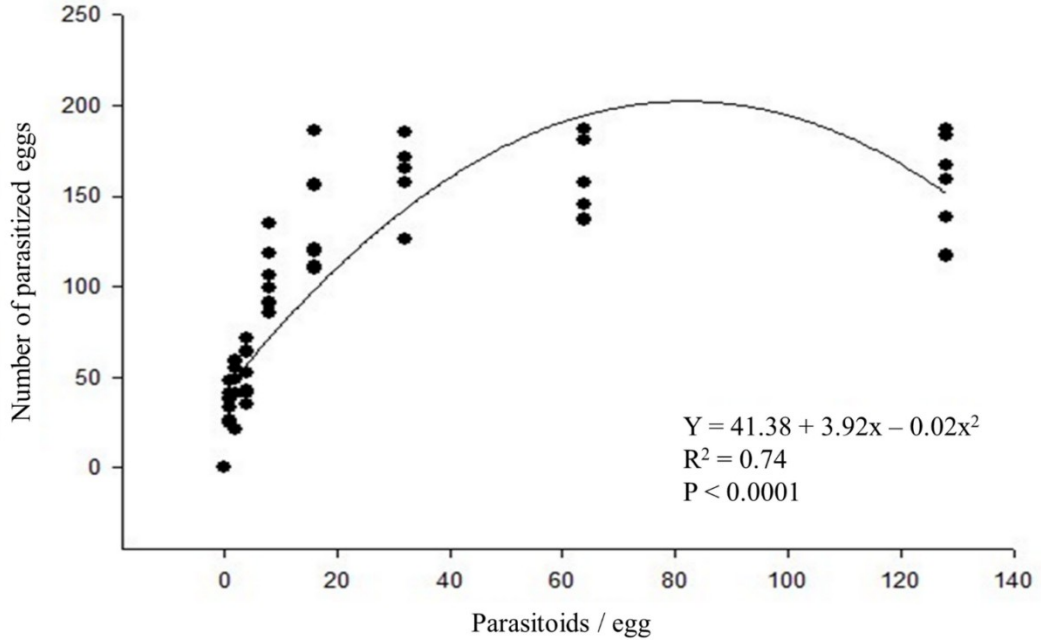

Figure 1. Relationship between number of eggs of *Neoleucinodes elegantalis* parasitized by *Trichogramma galloi* and Parasitoid/egg in tomato. plants.

In this way, the number of parasitoids to be released varies according to several factors, such as plant phenology, species and/or strain of the parasitoid and with the posture characteristic of the host (Bakthavatsalam et al., 2013; Pratissoli, Vianna, Zago, & Pastori, 2005).

Thus, success in pest control with the use of the *Trichogramma* parasitoid is related to the correct choice of species and strain to be used. Although it is classified as a generalist parasitoid, research results demonstrate that species and strains may have affinity due to the stimulus-driven search behavior, the nutritional and morphological characteristics of the egg, as well as abiotic factors such as climatic conditions that can affect, among other parameters, developmental duration, sex ratio, parasitism and longevity (Pratissoli & Parra, 2001).

In spite of the present large-scale study of T. g1 strain in *N. elegantalis* eggs in the laboratory and on the screen, it is of great importance the evaluation of this parasitoid in the field for the implantation of a phytosanitary management of the pest in question, taking into account the calculation of cost/benefit, since through the present work it was possible to observe that the release of a large numerous of parasitoids per pest egg is necessary.

Conclusion

The strain selected in *N. elegantalis* eggs was T. g1 belonging to the species *T. galloi* and the eggs of the small tomato-borer present physicochemical characteristics favorable to the parasitism by this strain.

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