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ZOOLOGY

# Selection of parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) and parasitism at different eggs ages of *Duponchelia fovealis* (Lepidoptera: Crambidae)

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**ABSTRACT.** The European pepper moth *Duponchelia fovealis* (Zeller, 1847) (Lepidoptera: Crambidae), despite being among the principal pests of strawberries in Brazil. However, biological control methods are rarely documented for this pest. In the current study, the goal was to select promising *Trichogramma* strains to enable the control of *D. fovealis* and to determine the acceptance of different ages of host eggs by the parasitoid. In the first bioassay, evaluations were done on the biological parameters of five strains of *Trichogramma pretiosum* Riley and one of *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae). The best performance came from the strains, Tp18 (*T. pretiosum*) and Tg (*T. galloi*), showing parasitism and viability higher than 50 and 90%, respectively. Another bioassay revealed the acceptance of both these species (Tp18 and Tg) for different ages of *D. fovealis* eggs. All host ages were accepted by *T. pretiosum* (Tp18) and *T. galloi*, with a preference for younger eggs (65% parasitism). Adult emergence, number of individuals per egg and sex ratio revealed no significant differences with respect to the host age. The current study provides pertinent data on the selection and performance of *Trichogramma* species on *D. fovealis* eggs of different ages.

**Keywords:** biological control; egg parasitoid; parasitism percentage; European pepper moth; host age.

## Seleção de parasitoides do gênero *Trichogramma* (Hymenoptera: Trichogrammatidae) e parasitismo em ovos de diferentes idades de *Duponchelia fovealis* (Lepidoptera: Crambidae)

**RESUMO.** A traça do morangueiro *Duponchelia fovealis* (Zeller, 1847) (Lepidoptera: Crambidae) é uma das principais pragas da cultura do morango no Brasil. Entretanto, métodos de controle biológico desta praga são raramente documentados. Este trabalho teve como objetivo selecionar linhagens de *Trichogramma* e avaliar o parasitismo de diferentes idades de ovos de *D. fovealis*. Foram avaliados os parâmetros biológicos de cinco linhagens de *Trichogramma pretiosum* Riley e uma de *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae). As espécies/linhagens com melhor desempenho foram Tp18 (*T. pretiosum)* e Tg (*T. galloi*), com taxas de parasitismo e viabilidade acima de 50 e 90%, respectivamente. Em seguida, foi avaliada a suscetibilidade das duas espécies (Tp18 e Tg) em diferentes idades de ovos de *D. fovealis*. Todas as idades do ovo hospedeiro avaliadas foram aceitas pelos dois parasitoides e houve uma maior tendência a parasitar ovos mais jovens. Em todas as idades do hospedeiro houve emergência de parasitoides adultos, e sem diferença significativa entre idades. As variáveis número de indivíduos por ovo e razão sexual não apresentaram diferença significativa quanto à idade do hospedeiro. O atual estudo fornece informações sobre a seleção e o desempenho de espécies de *Trichogramma* em diferentes idades de ovos de *D. fovealis*.

**Palavras-chave:** controle biológico; porcentagem de parasitismo; parasitoide de ovos; traça-do-morangueiro; idade do hospedeiro.

## Introduction

The European pepper moth, *Duponchelia fovealis* (Zeller, 1847) (Lepidoptera: Crambidae), ranks among the major greenhouse pests across Europe (Efil, Özgür, & Efil, 2014; Gonzalez et al., 2016), ornamental plants in certain parts of North America (Bethke & Bates, 2014; Brambila & Stocks, 2010) and on strawberry crops in Brazil (Zawadneak et al., 2016). It is a polyphagous species recorded earlier for damaging the roots, leaves, flowers and fruits of 38 plant families (Atay & Oğur, 2011; Brambila & Stocks, 2010; Efil, Efil, & Atay, 2011; Efil et al., 2014). The cryptic behavior of the caterpillars and the difficulty in identifying the presence of eggs and pupae in their hosts, are among the main disadvantages found in the control of the European pepper moth, because it is difficult to monitor and apply pesticides (Ahern, 2010; Bethke & Bates, 2014).

Although the potential for damage by the European pepper moth can be devastating, only a few studies have been done using alternative techniques to replace insecticides (Blok & Messelink, 2009; Efil et al., 2014; Messelink & Van Wensveen, 2003). As strawberry is consumed in vast quantities as fresh fruit, the application of insecticides poses a high risk of toxicity to humans, non-target organisms, as well as the environment (Fenik, Tankiewicz, & Biziuk, 2011), and the employment of the natural enemies in *D. fovealis* control is highly recommended as a safe management method (Zawadneak et al., 2016).

Parasitoids of the genus *Trichogramma*, which are among the natural enemies of *D. fovealis* are the popular choice in biological pest control programs (Zawadneak et al., 2016). Ranking among the most prominent biotic agents, parasitizing the eggs of more than 200 insect species across the globe, these parasitoids hold out great promise in pest control of the Lepidoptera, including *Helicoverpa armigera* (Hübner), *Spodoptera frugiperda* (J.E. Smith) and *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae) (Bueno, Bueno, Parra, Vieira, & Oliveira, 2010; Bueno, Parra, & Bueno, 2012; Jalali, 2013). These parasitoids are extensively used because they are wide in geographic distribution, have high efficiency, are amenable to mass production and involve low production costs (Jalali, 2013; Pratissoli et al., 2010; Zago, Pratissoli, Barros, Gondim Jr., & Santos Jr., 2007).

For egg parasitoids to be successfully used in biological control programs extensive knowledge of their biological features and host interactions is mandatory (Carvalho et al., 2014). Because *Trichogramma* is a cosmopolitan parasitoid, most of them exhibit interspecific and intraspecific variations in host preference (Hassan, 1989). However, the acceptance of certain hosts may be due to the search behavior and nutritional and morphological characteristics of the host egg (Farahani, Ashouri, Zibaee, Abroon, & Alford, 2016; Mansfield & Mills, 2004). One of the significant characteristics of the parasitoid-host interaction is the correct understanding of parasitoid acceptance when exposed to the host eggs of different ages (Pratissoli, Polanczyk, Pereira, Furtado, & Cocheto, 2007; Song et al., 2015; Zhang et al., 2014). The age of the host egg is crucial to its acceptance by the parasitoid, as advanced egg age can change the nutritional values and modify the parasitism rate (Farahani et al., 2016).

In the current study, the goal was to select promising *Trichogramma* strains to enable the control of *D. fovealis* and to determine the acceptance of *Trichogramma pretiosum* Riley and *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae) at different ages of *D. fovealis* eggs.

## Material and methods

### Parasitoid and Host Rearing

*Duponchelia fovealis*, *T. pretiosum* and *T. galloi* are the species utilized in the bioassays drawn from the insects rearing of the Entomology Laboratory of the *Universidade Federal do Espírito Santo* (UFES-CCAE), in Alegre, Espírito Santo, Brazil.

*Duponchelia fovealis* was raised by placing freshly emerged adults in PVC tube cages (20 x 20 cm). At the base of each cage, a paper-coated styrofoam sheet was spread and a piece of voile fabric (30 x 30 cm) was placed at the cage top and fixed with a rubber strip. Sulfite paper was used to line the inside of the tube to facilitate the egg collection. Then, honey solution soaked in cotton was provided as food for the adults. The paper containing *D. fovealis* eggs was collected every day and disinfection with 0.5% formaldehyde solution and 17% copper sulfate was done for 10 seconds. The paper holding the eggs was then carefully packed in an acrylic box (11 x 11 x 3 cm) until the caterpillars emerged. These caterpillars were transferred to glass tubes (8.5 cm height x 2.5 cm diameter) and provided with the artificial diet according to King and Hartley (1985). Pupae were placed in pots having moist paper until the adults emerged. *Duponchelia fovealis* were laboratory reared at 25 ± 1°C, 70 ± 10% RH and 14 hours photoperiod.

*Trichogramma* species were reared on eggs of the alternative host *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae), according to Pratissoli et al. (2010). Host eggs were sterilized under a germicidal lamp for 50 minutes and glued to the surface of the cardboard pieces (8 x 2 cm) by a solution of "Arabic glue" (20%). Then, each cardboard piece was inserted into a glass tube (8.5 cm height x 2.5 cm diameter) and a newly emerged parasitoid was added to each tube, closed with PVC plastic film.

### Selection of the *Trichogramma* strains

In the selection bioassay, six *Trichogramma* strains were assessed (Table 1). Two strains (Tp18 and Tg) were supplied by BUG *Agentes Biológicos*, São Paulo, Brazil, and the other four were indigenous strains (Tp 1, 8, 15 and 19) collected from different hosts and sites in the field (Table 1). After the morphological identification of the strains based on the protocol of Querino and Zucchi (2012), they were deposited in the Entomology Laboratory collection in the *Universidade Federal do Espírito Santo* (UFES-CCAE), Alegre, Espírito Santo, Brazil. From each *Trichogramma* strain, a female (age 0-5 hours) was selected and placed in individual glass tubes (8.5 x 2.5 cm). For each female, thirty *D. fovealis* eggs (up to 24 hours old) were glued on a cardboard piece (8 x 2 cm) using arabic glue solution (20%). The parasitism was allowed without interruption for 24 hours. After that, using a fine bristle brush, the females were removed from the tube, and the cardboards carrying the parasitized eggs were maintained in a climate chamber (25 ± 1°C, 70 ± 10% RH and 14 hours photoperiod). The variables percentage of parasitism of eggs, number of individuals per egg, emergence rate and sex ratio were assessed after ten days.

### Effect of host egg age on the parasitism by *Trichogramma* strains

For this bioassay, the best strains of the previous bioassay (Tp18 and Tg) were used. The newly emerged *T. pretiosum* (Tp18) and *T. galloi* (Tg) females were isolated in glass tubes (8.5 x 2.5 cm). Each female was offered thirty *D. fovealis* eggs with 24, 48 and 72 hours, glued with arabic glue solution on one piece of cardboard (8 x 2 cm). A drop of honey was deposited on the inside of the tube wall to provide a carbohydrate source to the wasp. After 24 hours of parasitism, the parasitoids were removed. The cardboard pieces bearing the parasitized eggs remained undisturbed in the same glass tubes, which were once again sealed with PVC plastic film and placed in a climate chamber (25 ± 1°C, 70 ± 10% RH and 14 hours photoperiod). Once the parasitoids had emerged, the number of parasitized eggs, emergence rate, number of individuals per egg and sex ratio, were quantified.

### Data analysis

### Selection of the *Trichogramma* strains

Adopting the completely randomized experimental design, the choice of the *Trichogramma* strains was performed involving six strains with 15 replicates. The data were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test ($p < 0.05$), applying the Package *ExpDes* (Ferreira, Cavalcanti, & Nogueira, 2018) of the computer application R version 3.4.0 (R Development Core Team, 2017).

To confirm which of the *Trichogramma* strains exhibited more similar characteristics, multivariate procedures were done. Using the averages of the variables of the strains in the study a 6 x 4 matrix (strains x explanatory variables) was built. First, this matrix was subjected to scalar and linear transformations of centralization and normalization to enable the Euclidean distance to be calculated later, and a matrix of dissimilarities 6 x 6 was obtained, in which the higher values between two lines were indicative of greater degree of distance, while the lower values implied greater degree of closeness (Borcard, Gillet, & Legendre, 2011; Mingoti, 2013). The dissimilarity matrix was thus utilized to identify the number of binding groups. To accomplish this, the Single Linkage method was employed to establish groups produced from closer (similar) strains. This procedure used 26 criteria (Charrad, Ghazzali, Boiteau, & Niknafs, 2014) to select and validate the number of binding groups for the Agglomerative Hierarchical Methods (Borcard et al., 2011; Mingoti, 2013). From the number of groups thus defined, the dendrogram was constructed using the matrix of dissimilarities. Therefore, to understand the effect of the explanatory variables on cluster formation, one principal coordinate analysis (PCoA) was performed using the matrix of dissimilarities. Subsequently, a "Biplot" graph was drawn to interpret the behavior of the variables (Borcard et al., 2011).

All the analyses were performed utilizing the computational application R version 3.4.0 (R Development Core Team, 2017). For the multivariate analysis procedures, the *vegan* package was employed (Oksanen et al., 2017), and the number of clusters was determined by applying the *NbClust* package (Charrad et al., 2014).

**Table 1.** *Trichogramma* strains, hosts, crops and collection site/company.

| Code | Specie | Host | Crops | Company/Collection site |
|---|---|---|---|---|
| Tg | *Trichogramma galloi* | Alternative host | - | Commercial strain BUG |
| Tp18 | *Trichogramma pretiosum* | Alternative host | - | Commercial strain BUG |
| Tp15 | *Trichogramma pretiosum* | Alternative host | - | Jaciara, Mato Grosso, Brazil |
| Tp1 | *Trichogramma. pretiosum* | *Helicoverpa zea* | Tomato | Alegre, Espírito Santo, Brazil |
| Tp19 | *Trichogramma pretiosum* | *Neoleucinodes elegantalis* | Tomato | Alegre, Espírito Santo, Brazil |
| Tp8 | *Trichogramma pretiosum* | *Helicoverpa zea* | Tomato | Afonso Cláudio, Espírito Santo, Brazil |

### Effect of host egg age on the parasitism by *Trichogramma* strains

Using the completely randomized design, the host age bioassay was performed in a 2 x 3 factorial scheme, with two *Trichogramma* species, three egg ages (24, 48 and 72 hours) and ten replicates. Data were submitted to analysis of variance (ANOVA). In the case of significant interaction, the interaction between the factors "species" and "egg ages of *D. fovealis*" was performed, with the means of each combination (treatments) compared by Tukey's test ($p < 0.05$). However, if the interaction was not significant, the factors "species" and "ages of eggs" would be analyzed in isolation. Thus, as the factor "species" has only two levels would be the decisive ANOVA analysis (F test). As for the factor "age of eggs", because it has three levels, the average would be compared by Tukey test ($p < 0.05$) utilizing the computational application R version 3.4.0 (R Development Core Team, 2017).

## Results

### Selection of the *Trichogramma* strains

All the *Trichogramma* strains tested parasitized the *D. fovealis* eggs. The Tg (15.36) and Tp18 (15.30) strains revealed the highest value of parasitized eggs ($F_{5,\ 84} = 13.548$; $p < 0.001$; Table 2), which corresponded to a parasitism above 50%. The emergence of parasitoids in the treatments range between 92.93 to 97.57%, showing no significant difference ($F_{5,\ 84} = 2.061$; $p = 0.0783$; Table 2). In the case of number of the individuals per host egg, no significant difference was observed among the strains tested ($F_{5,\ 84} = 2.207$; $p = 0.0610$), showing values close to one individual per host egg (Table 2). A statistical difference among the means of the sex ratio was noted for the *Trichogramma* strains ($F_{5,\ 84} = 6.075$; $p < 0.0001$). Sex ratio values higher than 0.9 were observed, except in the case of the Tp1 strain, which exhibited the lowest sex ratio (0.79) (Table 2).

From the multivariate analysis the clear evidence of four groups emerged, based on the dissimilarity matrix and Single Linkage method (Figure 1A). The dendrogram constructed showed that the groups thus formed included the following strains: group 1 - Tp15; group 2 - Tp19 and Tp8; group 3 - Tp1; and group 4 - Tg and Tp18 (Figure 1B).

The PCoA analysis revealed five explanatory dimensions, in which the first two dimensions were consistent with 81.76% of the variation observed (Figure 2A). The PCoA indicates the placement of the groups and explanatory variables (Figure 2B).

On analysis of the first dimension (greater percentage of variation) group 4 (Tg and Tp18) was confirmed to have the highest parasitism value. Groups 2 (Tp19 and Tp8) and 3 (Tp1), however, showed lower parasitism values. Group 1 (Tp15) revealed an intermediate parasitism value, although it was placed near to group 4. For the first dimension, the variables number of individuals per egg, emergence and sex ratio did not exhibit significant influence. However, for the second dimension, the effect of the variables parasitism, number of individuals per egg and sex ratio, was clearly evident, which enabled the distinction between groups 3 and 4, respectively, to be made. The distinction between groups 1 and 2 resulted from the combined interpretation of both dimensions, as these groups exhibited closely similar characteristics, with the strong expression of the variable parasitism, in the grouping.

**Table 2.** Biological parameters (±SE) of *Trichogramma* strains reared on *Duponchelia fovealis* eggs. Temperature 25 ± 1°C, RH 70 ± 10% and photoperiod 14 hours.

| Strain codes | Parasitized eggs | Emergence (%)* | Number of individuals per egg* | Sex ratio |
|---|---|---|---|---|
| Tg | 15.36±1.26 a | 92.93±2.39 | 1.08±0.03 | 0.99±0.08 a |
| Tp18 | 15.30±0.96 a | 94.89±1.08 | 1.03±0.03 | 0.99±0.39 a |
| Tp15 | 12.92±0.82 ab | 95.98±1.92 | 0.99±0.04 | 0.90±0.66 bc |
| Tp1 | 10.87±1.01 bc | 92.96±1.66 | 1.10±0.05 | 0.79±0.92 c |
| Tp19 | 7.76±1.35 cd | 97.57±0.85 | 0.95±0.07 | 1.00±0.00 a |
| Tp8 | 6.12±0.79 d | 96.72±1.54 | 1.05±0.02 | 0.93±0.54 ab |
| *F* | 13.548 | 2.061 | 2.207 | 6.075 |
| *p* | < 0.0001 | 0.0783 | 0.0610 | 0.0001 |

Means followed by the same letter do not differ statistically by the Tukey test (p > 0.05). * Variables are statistically the same by the F test (p > 0.05).

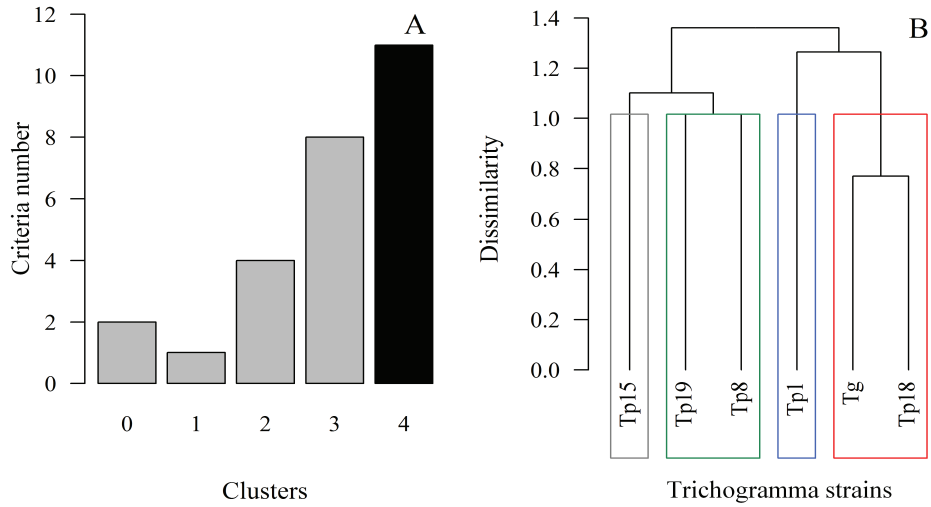


**Figure 1.** Number of clusters based on 26 criteria for group selection (A) and cluster analysis (B) of the different *Trichogramma* strains using Euclidean distance and the Single Linkage method. Strains belonging to the same group are the same rectangle.

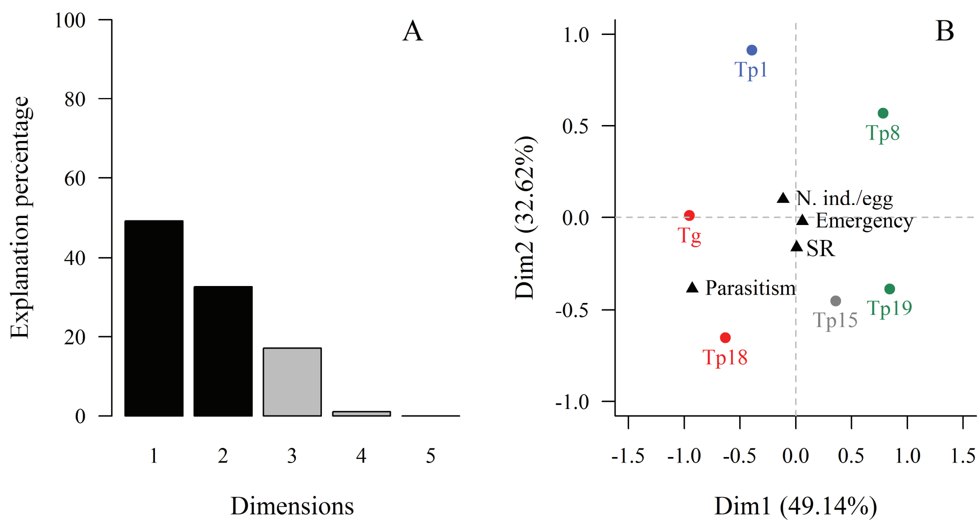


**Figure 2.** Percentage of explanation of the dimensions of the principal coordinate analysis (PCoA) of the *Trichogramma* strains (A) and "Biplot" graph (B) showing the effect of the explanatory variables on the clusters formed. Groups of lineages: 1: gray, 2: green, 3: blue and 4: red.

## Effect of host egg age on the parasitism by *Trichogramma* strains

No significant interaction between the factors of the "*Trichogramma* species" and "egg age" was observed for any variable studied: parasitized eggs ($F_{2,\,54}$ = 1.011, p = 0.370), number of individuals per egg ($F_{2,\,54}$ = 0.157, p = 0.854) and emergence ($F_{2,\,54}$ = 0.659, p = 0.521). The sex ratio also did not show variation, since all the progenies were female, therefore it was not submitted to ANOVA. In this way, the factors were analyzed separately.

For the percentage of parasitized eggs, again no significant difference was observed between *T. pretiosum* (Tp18) and *T. galloi* (Tg) ($F_{1,\,54}$ = 1.586; p = 0.213; Table 3). However, the variable egg age showed a strong influence on the parasitized eggs, with 24 hours eggs revealing higher rates of parasitism compared to 72 hours eggs (19.6 and 16.8 parasitized eggs, respectively viz., $F_{2,\,54}$ = 4.055; p = 0.022; Table 3). Regarding the emergence, no significant difference was observed among the species ($F_{1,\,54}$ = 0.048; p = 0.944) and host egg age ($F_{2,\,54}$ = 1.775; p = 0.179; Table 3). *Trichogramma pretiosum* presents a higher number of individuals per egg (1.06) than *T. galloi* (1.04) ($F_{1,\,54}$ = 6.806; p = 0.011). However, such differences were not evident among the host egg ages ($F_{2,\,54}$ = 0.963; p = 0.388; Table 3). The *T. pretiosum* and *T. galloi* sex ratios were significantly the same for all *D. fovealis* egg ages (Table 3).

**Table 3.** Biological parameters (±SE) of *Trichogramma pretiosum* (Tp18) and *Trichogramma galloi* (Tg) reared on *Duponchelia fovealis* eggs of different ages. Temperature 25 ± 1°C, RH 70 ± 10% and photoperiod 14 hours.

| Variable | Species | Egg age (hours) | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | Mean $^{ns}$ |
| Parasitized eggs | Tp18 | 20.00±0.97 A | 17.90±1.25 A | 18.10±1.16 A | 18.66±0.65 |
| | Tg | 19.30±0.75 A | 18.10±0.84 A | 15.50±0.98 A | 17.63±0.56 |
| | Mean* | 19.65±0.44 a | 18.00±0.86 ab | 16.80±0.57 b | - |
| Emergence (%) | Tp18 | 96.19±1.51 A | 95.84±2.74 A | 99.44±0.56 A | 97.15±1.06 |
| | Tg | 94.37±3.21 A | 98.27±1.29 A | 98.50±1.01 A | 97.04±1.21 |
| | Mean $^{NS}$ | 95.28±1.52 | 97.05±1.53 | 98.97±0.70 | - |
| Number of individuals per egg | Tp18 | 1.07±0.02 A | 1.08±0.04 A | 1.04±0.02 A | 1.06±0.02 |
| | Tg | 1.02±0.02 A | 1.01±0.01 A | 1.00±0.01 A | 1.04±0.02 |
| | Mean $^{NS}$ | 1.04±0.01 | 1.04±0.02 | 1.01±0.01 | - |
| Sex ratio[1] | Tp18 | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 |
| | Tg | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 |
| | Mean $^{NS}$ | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 | - |

Means followed by same uppercase letter for interaction do not differ by Tukey's test ($p < 0.05$). *Means followed by same lowercase letter for factors do not differ by Tukey's test ($p < 0.05$). $^{ns}$Not significant by F test (ANOVA) ($p > 0.05$). $^{NS}$Not significant by Tukey test ($p > 0.05$). [1]It was not analyzed; all values were equal.

## Discussion

In this study, a selection of the *Trichogramma* strains on the *D. fovealis* eggs was first performed. All strains parasitized *D. fovealis* eggs, with higher outcomes for the Tp18 and Tg strains. Later on, the best strains (Tp18 and Tg) were employed in a bioassay to the parasitism at the different egg ages of *D. fovealis*. It was evident that the Tp18 and Tg strains parasitized the different egg ages of *D. fovealis*, showing a clear preference for the younger eggs.

Four of the six strains assessed in this study are indigenous (Tp 1, 8, 15 and 19) while two are commercial (Tp18 and Tg). From the multivariate analyses performed, Tp18 and Tg were identified as the most effective strains on *D. fovealis*, which corresponded to the *T. pretiosum* and *T. galloi* species. Normally, these commercial strains are subjected to several selection and quality control tests, and possess the potential to overpower populations of diverse host species (Lenteren, 2003). However, the findings regarding the indigenous strains in this study appear to be promising.

The indigenous strains, Tp15 and Tp1, exhibited parasitism of 12.92 and 10.87 eggs, respectively, in 24 hours of host exposure. Parasitism would probably increase if the time of exposure to the host was higher, because parasitoids of the genus *Trichogramma* can normally parasitize 10 or more days, based on the prevailing environmental status (Carvalho et al., 2014; Pratissoli, Bueno, Bueno, Zanúncio, & Polanczyk, 2009). In addition, indigenous strains can tolerate much better adverse climatic conditions and thus be more effective against their hosts (Herz & Hassan, 2006).

The results showed that the age of *D. fovealis* eggs significantly influenced the number of eggs parasitized by the *T. pretiosum* (Tp18) and *T. galloi* (Tg). Higher parasitism was noted on the 24 and 48 hours eggs, with no significant difference between the 48 and 72 hours eggs. Besides, parasitism was observed also in more than 50% of the *D. fovealis* eggs presented to the parasitoids, regardless of age of the host egg. However, the decline in parasitism observed among the treatments in both the *Trichogramma* species highlights the preference exhibited by the parasitoid for the *D. fovealis* eggs in the initial phase of embryonic development. Other studies have also that the older host egg can reduce *Trichogramma* parasitism in many host species, such as *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) (Ko et al., 2014; Zhang et al., 2014), *Bonagota salubricola* (Meyrick) (Lepidoptera: Tortricidae) (Pastori, Monteiro, Botton, & Pratissoli, 2010) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Pratissoli et al., 2007). Such preference for younger eggs may result from an alteration in the egg reserve nutrients. As the embryonic development advances, the egg reserve nutrients are transformed into chemically more complex tissues which render them less appealing to the parasitoid (Vinson, 1997). Another factor that may have exerted some influence on the number of parasitized eggs is the chorion hardening in the older eggs, which may limit its acceptance by the parasitoid, posing a hindrance in penetrating the ovipositor of the parasitoid (Mellini, 1987).

In terms of the viability of the parasitized eggs, an emergence rate above 95% was observed for the different ages of the *D. fovealis* eggs. Therefore, even in the more advanced embryonic developmental phases, *D. fovealis* eggs were suitable for the development of *T. pretiosum* and *T. galloi*. The high viability rates, over 85%, are acceptable for biological control programs (Almeida, Silva, & Medeiros, 1998). Besides, in this study the emergence rate recorded were higher than those in other studies, such as *T. galloi* on eggs of *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) (Oliveira, Santana, Bellon, & Oliveira, 2014) and *T. pretiosum* on the eggs of *B. salubricola* (Pastori et al., 2010) with an emergence rate below 50 and 60%, respectively, for the eggs of different ages. Therefore, the findings of the high emergence rate in this study indicate the potential of *T. pretiosum* and *T. galloi* as possible and effective control agents of *D. fovealis*. This factor is significant for success in the establishment of inoculative biological control programs, as even in the event of high parasitism, the low emergence rate can influence the biological control outcomes (Bueno, Parra, Bueno, & Haddad, 2009).

*Trichogramma pretiosum* and *T. galloi*, did not present significant differences in the number of individuals per host egg, however, with values near to one individual per egg. The *D. fovealis* eggs were observed to supply adequate nutritional support irrespective of egg age. To arrive at the number of one individual per egg can be considered a good finding for the establishment of biological control programs for *D. fovealis*. The emergence rate of more than one individual per host egg can give rise to smaller sized and poorer quality parasitoids, which can affect the parasitoid performance caused by intraspecific competition (DaSilva, Morelli, & Parra, 2016; Moreira, Santos, Beserra, Torres, & Almeida, 2009).

*Trichogramma pretiosum* and *T. galloi* species produce female individuals only as the descendants. This emergence rate of solely females for both the *Trichogramma* species may be linked to the favorable developmental conditions provided by the host, because factors like age and host quality, size of the postures and superparasitism affect the development and therefore the sexual ratio (Vinson, 1997). Besides, the progeny of this parasitoid may result from the behavioral decisions taken by the females during host acceptance, thus enabling the parasitoids to optimize the sexual ratio of the offspring to suit the local conditions (Luck, Janssen, Pinto, & Oatman, 2001). However, other factors that could influence the sex ratio in the *Trichogramma* populations, include the presence of the bacterium *Wolbachia*, which produces reproductive alterations in many arthropods, inducing thelytokous parthenogenesis (Boivin et al., 2014) and the presence of an extra chromosome called PSR (Paternal Sexual Reason) or the selfish genetic element, which can convert an egg destined to be a diploid female into a haploid male with PSR (Russell & Stouthamer, 2010).

The European pepper moth *D. fovealis* is one of the principal strawberry pests in Brazil. At present, there is neither record of any plant protection products available in Brazil to control or manage this insect, nor any effective measures of biological control. Of the *Trichogramma* species assessed in this study, *T. pretiosum* strain Tp 18 and *T. galloi* showed great promise, revealing high rates of parasitism, emergence rate and production of female offspring, which are the fundamental characteristics to selection of parasitoids for biological control programs. Besides, the two *Trichogramma* species parasitized *D. fovealis* eggs of all ages, showing a sharp preference for the younger eggs.

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