

Quality Control of *Trichogramma atopovirilia* and *Trichogramma pretiosum* (Hym.: Trichogrammatidae) Adults Reared Under Laboratory Conditions

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ABSTRACT

The objective of this work was to assess the flight capacity, parasitism and emergence of *Trichogramma atopovirilia* and two strains of *T. pretiosum* (Hymenoptera: Trichogrammatidae) (L1 and L2). The flight capacity of these species was assessed in test units consisting of a plastic PVC cylinder with a rigid transparent plastic circle on the upper part and an extruded polystyrene disk closing its bottom. A tube was placed in each test unit containing a card with 300 *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs parasitised by *Trichogramma*. These cards were assessed to determine the parasitism rate and adult emergence of these natural enemies. *T. atopovirilia* and *T. pretiosum* L1 presented adequate flight capacity and parasitism, in addition to high percentages of adults emerged.

Key words: biological control, flight, parasitism, quality control, emergence

INTRODUCTION

The caterpillars of *Spodoptera frugiperda* (J.E. Smith) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) have great economic importance in corn crops in Brazil (Zanuncio et al. 2008; Rodriguez-del-Bosque et al. 2010; Barrera et al. 2011). These pests are generally controlled with insecticides that can cause negative impacts, such as pest resistance and environmental unbalance (Soares et al. 2009; Barrera et al. 2011). However, a range of natural enemies can perform

the biological control of these pests, such as Braconidae, Ichneumonidae, Eulophidae and Trichogrammatidae species parasitising eggs or larvae of *S. frugiperda* (Hoballah et al. 2004; Neto et al. 2004; Neto et al. 2005).

Biological control has been intensified, especially for *S. frugiperda* and *H. zea*, with releases of *Trichogramma atopovirilia* (Oatman and Platner) and *T. pretiosum* (Riley) (Hymenoptera: Trichogrammatidae), frequent parasitoids of eggs of these pests (Parra and Zucchi 2004). *T. pretiosum* is very common in many cultures,

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including avocado, cucumber and tomato (Miranda et al. 1998; Goring et al. 2003). These natural enemies can be easily multiplied with highly efficient and cheap methodologies (Oliveira et al. 2000; Oztemiz and Kornosor 2007). The *Trichogramma* species are released in the field after several generations of mass rearing in the laboratory that can reduce their biological characteristics (Prasad et al. 1999; Pratisoli et al. 2004).

Flight capacity is important for parasitoid dispersion and this attribute can vary during the colonization process or mass rearing. This was determined for two strains of *T. brassicae* (Bezdenko) (Hymenoptera: Trichogrammatidae) after two and 39 generations in the laboratory on *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (Dutton and Bigler 1995) and for *T. sibericum* (Sorkina) (Hymenoptera: Trichogrammatidae) reared at different temperatures (16, 21 and 26°C) on eggs of its natural host *Rhopobata naevana* (Hubner) (Lepidoptera: Tortricidae) (Prasad et al. 1999). The flight capacity of five *Trichogramma* species collected in eucalypt plantations was determined and *T. maxacalii* (Voegelé and Pointel) (Hymenoptera: Trichogrammatidae) showed the best flying ability (Soares et al. 2007).

Furthermore, rearing for successive generations can affect the ability to locate and to parasitize and parasitoid emergence of *Trichogramma* spp. (Pratisoli et al. 2004). The flight capacity, parasitism and emergence of *Trichogramma* spp. can differ depending on the species and/or strains of these parasitoids. Thus, these biological parameters may be used to select the species or strains as a simple, fast and cheap method of quality control of these natural enemies (Dutton and Bigler 1995; Prasad et al. 1999; Pratisoli et al. 2005a; Soares et al. 2007).

The objective of this work was to compare the flight capacity, parasitism and emergence of *T. atopovirilia* and *T. pretiosum* strains L1 and L2 adults reared on *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs.

MATERIALS AND METHODS

Trichogramma atopovirilia and *T. pretiosum* strain L1 were obtained from the rearing facility of EMBRAPA National Research Center for Corn and Sorghum-CNPMS in the municipality of Sete

Lagoas, Minas Gerais State, Brazil. Strain L2 was collected from the corn crops in the municipality of Montes Claros, Minas Gerais State, Brazil. The parasitoids were reared and multiplied at $25 \pm 2^\circ\text{C}$ and 12-h light period.

Eggs from the alternative host *A. kuehniella*, obtained according to the technique of Parra (1997) were fixed with Arabic gum diluted to 30% on 7.20 x 0.7 cm pieces of white card, placed for 60 minutes under an ultraviolet lamp at 25 cm high and used to maintain the parasitoids in the laboratory (Stein and Parra 1987). Each card, with 300 eggs of this host, was placed in a test tube (12.0 x 2.0 cm) with 30 newly emerged telitoc females of one of the *Trichogramma* species or strains (Stouthamer et al. 1993), at the proportion of one parasitoid for 10 eggs. The parasitism was permitted for five hours (Pratisoli and Parra 2000; Soares et al. 2007). A drop of pure honey was placed in each test tube to feed the parasitoids (Lundgren and Heimpel 2003).

The flight capacity of the *Trichogramma* species and strains was assessed in test units (Soares et al. 2007), consisting of a 18 cm high, 11 cm diameter PVC cylinder with its inside painted with black latex paint. The bottom of the cylinders was closed with black flexible plastic (larger in size than the tube diameter) firmly fitted by an extruded polystyrene disk that was approximately 1 cm thick and with the same diameter as the tube. The excess at the edges of the plastic on the outside of the tube after fitting were attached to it with elastic, for better sealing and to prevent parasitoid to escape.

Each card with 300 *A. kuehniella* eggs, parasitized by *Trichogramma* and near the adult emergence of these parasitoids was placed inside a test tube (7.5 x 1.0 cm) and fixed at the center of the bottom part of the test unit (extruded polystyrene disk) with adhesive tape (Prezotti et al. 2002). A ring of gum was applied to the inside wall of the cylinder at 4 cm from the lower end, as a barrier to parasitoid walking. A circle of rigid, transparent plastic, of a larger diameter than that of the PVC and brushed with glue 24 h prior to the beginning of the experiment, was placed on the top of the cylinder, as a trap for parasitoids in flight.

Four replications were carried out per *Trichogramma* species and strains at $25 \pm 2^\circ\text{C}$ and 24 h light period. The plots were randomly distributed on a counter under a continuous light source from the date of mounting (Prezotti et al. 2002). The parasitoids were kept in the test unit

for three days after the start of their emergence and then frozen.

The numbers of parasitoids caught in the glue ring “walkers” on the plastic circle “flyers” and on the bottom “non-flyers” were recorded by direct counting with a hand magnifying glass to calculate the percentage of each group in relation to the total adults emerged. The cards with parasitized *A. kuehniella* eggs were removed from the test tube after the death of the *Trichogramma* adults and assessed at three pre-determined points (superior, mean and inferior parts of the cards) under a magnifying glass (40X) to determine the number of eggs parasitized (Soares et al. 2007). These data were obtained by counting the number of blackened *A. kuehniella* eggs.

The emergence rate was calculated by counting the number of eggs with *Trichogramma* emergence,

observing the opening on the corium of the host *A. kuehniella* eggs. The data were transformed to arcsosen of x and submitted to analysis of variance and the means compared by the Scott-Knott test (Scott and Knott 1974) at 5% probability with the SAEG statistic program (Gomes 1985). Results were presented in percentage.

RESULTS AND DISCUSSION

The *Trichogramma* species and the strains presented similar flight capacity and both species had low percentage of individuals caught in the glue ring (walkers). The number of non-flyer individuals was low in all the treatments (Fig. 1).

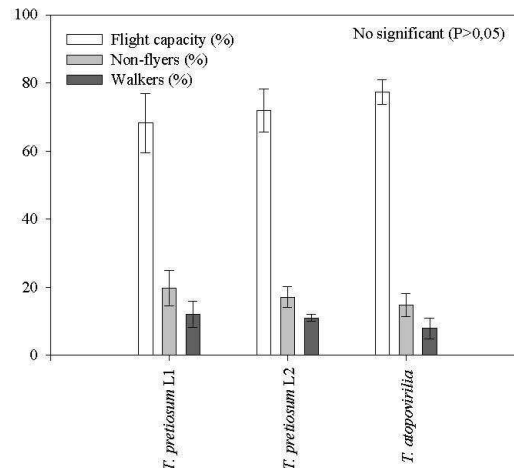


Figure 1 - Mean \pm standard error of the percentage of flyers, non-flyers and walkers adults of *Trichogramma pretiosum* L1, *Trichogramma pretiosum* L2 and *Trichogramma atopovirilia*. Municipality of Montes Claros, Minas Gerais State, Brasil.

The similar flight capacity of *T. atopovirilia* and *T. pretiosum* indicated that both the species could be released in the field but this might vary with the biological and behavioral characteristics and/or the mass rearing techniques of strains or species of these natural enemies. This was due to the fact that the percentage of adults with deformed wings and lower flight aptitude could increase in the laboratory (Prezotti et al. 2002). In this situation, the flight test performed showed sensitivity to detect the percentage of individuals flying reared under the laboratory conditions. Prezotti et al. (2002) observed that all the individuals who

reached the top of the test unit had wings fully expanded, indicating that the parasitoid reached the site flying and not walking.

Furthermore, the environmental temperature can affect *Trichogramma* flight capacity, as reported for *T. sibiricum* that presented variation in the proportion of adults with highest flight capacity ($51.74 \pm 2.3\%$) at 26°C and the lowest ($1.57 \pm 0.5\%$) at 16°C (Prasad et al. 1999). *T. pretiosum*, reared on *A. kuehniella* eggs presented $81.1 \pm 4.20\%$ individuals with flight capacity (Prezotti et al. 2002) while two *T. brassicae* strains had 72 and 61% individuals with flight capacity, with the

same host (Dutton and Bigler 1995). *T. acacioi* (Brun), *T. bruni* (Nagaraja), *T. demoraesi* (Nagaraja), *T. maxacalii* (Voegelé and Pointel) and *T. soaresi* (Nagaraja) (Hymenoptera: Trichogrammatidae) had respectively 39, 50, 45,

57 and 46% of flying individuals when reared with *A. kuehniella* eggs (Soares et al. 2007).

The *T. pretiosum* L2 strain presented lower parasitism rate on *A. kuehniella* eggs, than the L1 and *T. atopovirilia* (Fig. 2).

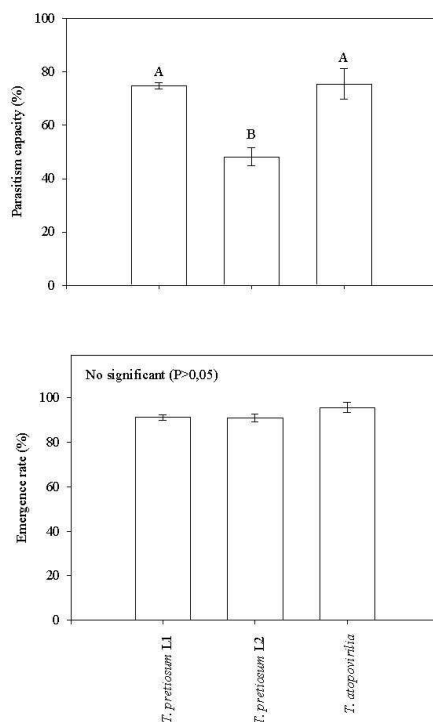


Figure 2 - Mean \pm standard error of the percentage of eggs parasitized and of emergence rate of *Trichogramma pretiosum* L1 and L2 and *Trichogramma atopovirilia*. Municipality of Montes Claros, Minas Gerais State, Brasil. Bars followed by the same letter do not differ by the Scott-Knott test at 5% probability.

The greater parasitism rates of *T. atopovirilia* and *T. pretiosum* strain L1 could be related to the fact that L2 strain was recently collected and it was still in an adaptation process to laboratory conditions and to the host used. Thus, the greater parasitism of *T. atopovirilia* and *T. pretiosum* L1 could be explained by the experience of the females after a certain number of generations parasitizing the alternative host, as reported for the associative learning of *T. atopovirilia* and *T. pretiosum* on *S. frugiperda* eggs after several generations (Bezerra and Parra 2003). This also indicated that the *Trichogramma* species could increase their preference for a determined host and *T. pretiosum* L2 might, after certain period of mass rearing, increase its parasitism rate on *A. kuehniella* eggs and thus to present similar results

of the other treatments. *Trichogramma* parasitism rate also varied with physical and chemical barriers and by the type and characteristics of host eggs such as their size, hardness, scales and cairomones (Bezerra and Parra 2004). Temperature could also affect the parasitism capacity, with many *Trichogramma* species presenting maximum fertility between 21 and 30°C that decreased in extreme temperatures (Prasad et al. 1999; da Fonseca et al. 2005; Zago et al. 2007). Life tables for *T. pretiosum* and *T. atopovirilia* reared with *H. zea* eggs showed higher specific fertility for the first species with all temperatures tested. The ideal temperature to rear *T. pretiosum* was 28°C with higher number of females produced per female of this parasitoid ($\Sigma mx = 104.48$). On the other hand, *T. atopovirilia* had better

development at 23°C ($\Sigma mx = 42.16$) and lower tolerance to high temperatures without development at 33°C (Navarro and Marcano 2000). Food absence (carbohydrates) could also affect the parasitism, such as reported for *T. pretiosum* (Bai et al. 1992) and *T. maxacalii* (Oliveira et al. 2003).

The two strains of *T. pretiosum* and *T. atopovirilia* presented adequate adult emergence rate (Fig. 2). The emergence rates showed higher values than those of *T. pretiosum* and *T. exiguum* (Pinter and Platner) (Hymenoptera: Trichogrammatidae), both with 86% of emergence at 28°C from *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) eggs (Pereira et al. 2004) and *T. pretiosum* (88.5%) from *S. cerealella* eggs at 30°C (Inoue and Parra 1998). The emergence rate of *Trichogramma* adults can also vary with the size and quality of the host egg, number of parasitoids that develop per egg, development period in host eggs and temperature (Doyon and Boivin 2005; Pratisoli et al. 2005a). Younger and larger diameter eggs may favor parasitoid development because of the greater use of the food reserves with a single parasitoid developing per egg, due to the lack of competition. However, the number of adults that develop per egg varies with the availability of host eggs for the parasitoid that increases as the egg availability decreases (Hohmann and Luck 2004). The biological control with *Trichogramma* spp. should use species with higher emergence rates from the host eggs even in adverse conditions because they present better performance for field releases and to colonize the area in biological control programs.

Species with high flight capacity and emergence rates can disperse quicker in the field to find the host and to produce high progeny numbers in shorter periods of time. *Trichogramma* spp. should present higher variability of these biological characteristics in the field due to environmental factors such as temperature, light and/or food (Lundgren and Heimpel 2003; Pratisoli et al. 2005a; Pratisoli et al. 2005b; Ulrichs and Mewis 2004). On the other hand, species of this genus with better performance in the laboratory could repeat it in the field. These studies can choose adequate species or strains of *Trichogramma* to be released for inundative biological control.

CONCLUSIONS

1. *T. atopovirilia* and *T. pretiosum* strain L1 presented adequate flight capacity, parasitism and high percentages of adults emerged in the laboratory.
2. *T. atopovirilia* and *T. pretiosum* strain L1 were suitable for multiplication and release in the field for biological control.

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