



## Assessment of *Trichogramma* species (Hymenoptera: Trichogrammatidae) for biological control in cassava (*Manihot esculenta* Crantz)

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**ABSTRACT.** Cassava is the sixth most important crop in the world, and it is attacked by many pests, such as *Erinnyis ello* (L.) (Lepidoptera: Sphingidae). This lepidopteran pest has natural enemies that can efficiently control its population, such as *Trichogramma* spp. (Hymenoptera: Trichogrammatidae). The objective of this research was to assess the flight capacity, parasitism and emergence of *Trichogramma pretiosum*, *T. marandobai* and *T. demoraesi* and to select the most efficient species among them for biological control programs. 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 portion of the cylinder and an extruded polystyrene disk to close the bottom of the cylinder. A tube was placed in each test unit containing a card with 300 *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs that had been parasitised by *Trichogramma*. These cards were later assessed to determine the parasitism rate and adult emergence of these natural enemies. *Trichogramma pretiosum* presented the highest flight capacity ( $68 \pm 5\%$ ), parasitism ( $74 \pm 2\%$ ) and percentage of adults emerged ( $91 \pm 3\%$ ) in the laboratory, making this species suitable for mass rearing and release in biological control programs.

**Keywords:** Lepidoptera, egg parasitoid, *Erinnyis ello*.

## Avaliação de espécies de *Trichogramma* (Hymenoptera: Trichogrammatidae) para o controle biológico em mandioca (*Manihot esculenta* Crantz)

**RESUMO.** A mandioca é a sexta cultura mais importante no mundo, sendo atacada por diversas pragas, incluindo *Erinnyis ello* (Linnaeus) (Lepidoptera: Sphingidae). Este lepidóptero apresenta inimigos naturais que podem controlar de forma eficiente sua população, destacando os parasitoides oófagos *Trichogramma* spp. (Hymenoptera: Trichogrammatidae). O objetivo deste trabalho foi avaliar *Trichogramma pretiosum*, *T. marandobai* e *T. demoraesi* quanto à sua capacidade de parasitismo, emergência e voo, visando selecionar a espécie mais eficiente para programas de controle biológico. Os parâmetros biológicos foram avaliados em unidades teste, consistindo de um cilindro de PVC com um círculo de plástico rígido e transparente no topo e um disco de poliestireno vedando a parte inferior. Um tubo de ensaio contendo 300 ovos de *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) parasitados por *Trichogramma* foi colocado no interior da unidade. *Trichogramma pretiosum* apresentou maior capacidade de voo ( $68 \pm 5\%$ ) e porcentagens de parasitismo ( $74 \pm 2\%$ ) e emergência de adultos ( $91 \pm 3\%$ ) em laboratório, sendo indicado para criação e liberação em programas de controle biológico.

**Palavras-chave:** Lepidoptera, parasitoide de ovos, *Erinnyis ello*.

### Introduction

Cassava (*Manihot esculenta* Crantz) is cultivated on tropical plains between 30° N and 30° S of the Equator in areas where the average annual temperature is greater than 18°C (COURSEY; HAYNES, 1970; NUWAMANYA et al., 2012). Cassava is considered the sixth most important

crop in the world behind rice, wheat, corn, potatoes, and sweet potatoes (FAO, 2012). Africa accounts for more than half of the world's cassava production (51%), and Asia and Latin America produce 29% and 19%, respectively. In addition, Nigeria and Brazil produce approximately one third of the global output (NASSAR; ORTIZ, 2007).

The cassava crop is attacked by several pests on the African continent, especially scale insects (Sternorrhyncha: Pseudococcidae) and drill roots *Stictococcus vayssierei* (Richard) (Hemiptera: Stictococcidae) (OERKE, 2006; TINDO et al., 2009). In Brazil, the main pest is *Erinnyis ello* (L.) (Lepidoptera: Sphingidae); others, such as the cassava fly (Diptera: Lonchaeidae), mites (Acari), thrips (Thysanoptera: Thripidae) and the white fly (Hemiptera: Aleyrodidae), may occur but with secondary importance (BARRIGOSI et al., 2002; BELLOTTI et al., 1999; GISLOTI; PRADO, 2011; LEITE et al., 2002, 2003).

*Erinnyis ello* infestations can result in the complete defoliation of plants, the destruction of thinner branches and the spread of bacterial diseases, reducing cassava production by 50% (BARRIGOSI et al., 2002). However, natural enemies can efficiently control the *E. ello* population, especially the egg parasitoids *Trichogramma pretiosum* (Riley) and *T. marandobai* (Brun, Moraes; Soares) (Hymenoptera: Trichogrammatidae) (BRUN et al., 1986; OLIVEIRA et al., 2010). Moreover, *T. demoraesi* (Nagaraja) (Hymenoptera: Trichogrammatidae), which was originally described as a parasitizing forest species in Minas Gerais State (NAGARAJA, 1983), also parasitizes the eggs of *E. ello* in the Amazonas State (RONCHI-TELES; QUERINO, 2005).

*Trichogramma* spp. can be easily multiplied in the laboratory with high efficiency and at low cost for biological control programs (ÓZTEMIZ; KORNOSOR, 2007). However, *Trichogramma* species are typically released onto crops after several generations of rearing in the laboratory, which may reduce their efficiency due to inbreeding and genetic erosion (PRATISSOLI et al., 2005).

Thus, quality control of *Trichogramma* production in the laboratory is important to ensure efficiency in the field (SOARES et al., 2012). The methodology used by several authors for the evaluation of natural enemies includes tests of parasitism, emergence and flight for different species or strains of *Trichogramma* (DUTTON; BIGLER, 1995; PRASAD et al., 1999; PRATISSOLI et al., 2008; PREZOTTI et al., 2002; SOARES et al., 2007; SOARES et al., 2012). These biological parameters can assist in choosing the individuals with the best characteristics for use in biological control programs of pests such as *E. ello*.

The objective of this research was to assess the capacity of parasitism, emergence and flight of *T. pretiosum*, *T. marandobai* and *T. demoraesi* and select the most efficient species for biological control programs.

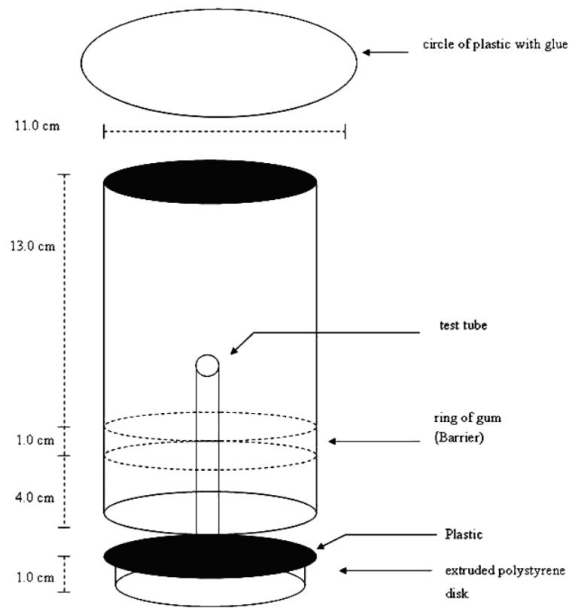
## Material and methods

The experiment was carried out in the Entomology Laboratory and the George Washington Gomez de Moraes Insectarium at the Agrarian Science Institute of the Federal University of Minas Gerais in the Municipality of Montes Claros, Minas Gerais State, Brazil. The parasitoids were reared and multiplied at  $25 \pm 2^\circ\text{C}$  with a 12-hours light period.

Eggs from the alternative host, *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae), which were obtained using the technique of Soares et al. (2012), were fixed with gum arabic diluted to 30% on  $7.20 \times 0.7$  cm pieces of white card and placed under an ultraviolet lamp that was 25 cm high for 60 min.; these eggs were used to maintain the parasitoids in the laboratory (SOARES et al., 2012). Each card, with 300 eggs of this alternative host, was placed in a test tube ( $12.0 \times 2.0$  cm) with 30 newly emerged thelytokous females of one of the *Trichogramma* species (STOUTHAMER et al., 1993) at a ratio of one parasitoid for every 10 eggs, and parasitism was permitted for five hours (PRATISSOLI; PARRA, 2000; SOARES et al., 2007). A drop of honey was placed in each test tube to feed the parasitoids (LUNDGREN; HEIMPEL, 2003).

The flight capacity of the *Trichogramma* species was assessed in test units (Figure 1) (SOARES et al., 2007) consisting of a PVC cylinder that was 18 cm long, 11 cm in diameter and painted with black latex paint on the inside. The bottom of the cylinder was closed with black flexible plastic (larger in size than the tube diameter) held firmly in place by an extruded polystyrene disk that was approximately 1 cm thick with the same diameter as the tube. The excess plastic that overlapped the edges of the tube after fitting the disk were secured to the tube with elastic to create a better seal and prevent the escape of the parasitoids.

Each card, with 300 *A. kuehniella* eggs parasitized by *Trichogramma* and near the adult emergence of these parasitoids (twenty days after parasitism), was placed inside a test tube ( $7.5 \times 1.0$  cm) and affixed to the center of the bottom portion of the test unit (the extruded polystyrene disk) with adhesive tape (PREZOTTI et al., 2002). A ring of gum was applied to the inside wall of the cylinder 4 cm from the lower end as a barrier to prevent the parasitoids from walking up the sides of the tube (SOARES et al., 2007; SOARES et al., 2012). A circle of rigid, transparent plastic of a larger diameter than the PVC was brushed with glue 24 hours prior to the beginning of the experiment and placed on top of the cylinder as a trap for flying parasitoids.



**Figure 1.** Schematic drawing of the test unit to assess *Trichogramma* flight capacity in the laboratory.

Four replicate experiments were conducted for each of the *Trichogramma* species at  $25 \pm 2^\circ\text{C}$  with a 24-hours light period. The plots were randomly distributed on a counter under a continuous light source from the date of mounting because *Trichogramma* species are phototropic positive (SOARES et al., 2007; SOARES et al., 2012). The parasitoids were kept in the test unit for three days after the start of their emergence and subsequently frozen.

The numbers of parasitoids caught in the glue ring, called 'walkers', on the plastic circle, called 'flyers', and on the bottom, called 'non-flyers', were recorded by direct counting with a handheld magnifying glass to determine 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 under a magnifying glass (40x) to determine the number of eggs that had been parasitized (SOARES et al., 2007; SOARES et al., 2012) 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 subjected to tests of the assumptions of the mathematical model (normality and homogeneity of variances). Subsequently, data were transformed to the arcsine of  $x$ , analysis of variance was performed, and the means were compared with the Scott-Knott test (SCOTT;

KNOTT, 1974) at 5% probability using the SAEG 9.1 statistics program (UFV) (GOMES, 1985). The data are presented as percentages.

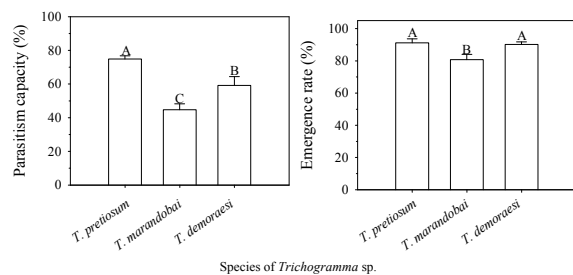
## Results and discussion

*Trichogramma pretiosum* showed a higher parasitism capacity than *T. marandobai*, followed by *T. demoraesi*, in the eggs of *A. kuehniella* (Figure 2). The parasitism rate can vary with physical and chemical barriers and by the type and characteristics of host eggs such as their size, hardness, scales and kairomones or differences in fertility among species of parasitoids (BESERRA; PARRA, 2004; SOARES et al., 2006; SOARES et al., 2009; SOARES et al., 2012). Temperature can also affect the parasitism capacity, with many *Trichogramma* species presenting maximum fertility between 21 and 30°C that decreases at extreme temperatures (DA FONSECA et al., 2005; PRASAD et al., 1999; ZAGO et al., 2007). Life tables for *T. pretiosum* and *Trichogramma atopovirilia* (Oatman; Platner) (Hymenoptera: Trichogrammatidae) reared with *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) eggs showed higher specific fertilities for the first species with all temperatures tested (NAVARRO; MARCANO, 2000). The ideal temperature to rear *T. pretiosum*, which had a higher number of females produced per female for this parasitoid ( $\Sigma m x = 104.48$ ), was 28°C. However, *T. atopovirilia* showed better development at 23°C ( $\Sigma m x = 42.16$ ) and a lower tolerance to high temperatures, with no development at 33°C (NAVARRO; MARCANO, 2000). The absence of food (carbohydrates) could also affect parasitism, as was reported for *T. pretiosum* (BAI et al., 1992) and *Trichogramma maxacalii* (Voegelé; Pointel) (Hymenoptera: Trichogrammatidae) (OLIVEIRA et al., 2003).

*Trichogramma* species may differ in biological characteristics, such as parasitism, as a result of being more aggressive or the adaptation of the species. Each species of parasitoid possesses an evolutionary history that shapes its particular adaptation route. Thus, great variability in biological characteristics can be observed among *Trichogramma* species due to their genotypic plasticity. Differences in the biological characteristics of strains of the same species are also observed due to phenotypic changes. When these changes occur, a genotype can produce different phenotypes, altering their physiology, morphology or development in response to changes in the environment (COLINET et al., 2007; PIGLIUCCI, 2005).

*Trichogramma pretiosum* and *T. demoraesi* showed a higher emergence of adults than *T. marandobai* (Figure 2). The emergence of these species is similar

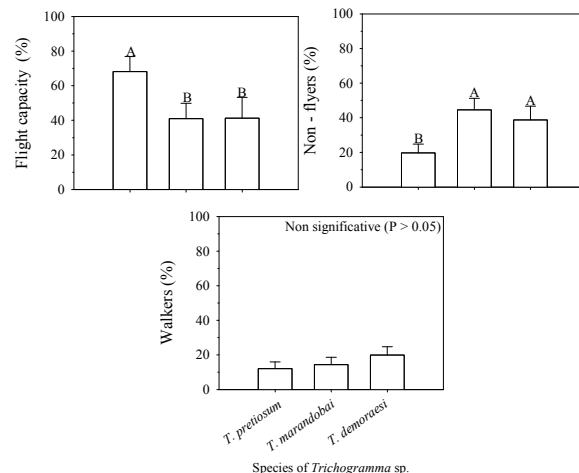
to that reported for *T. pretiosum* and *Trichogramma exiguum* (Pinter; Platner) (Hymenoptera: Trichogrammatidae), both with 86% emergence at 28°C in the eggs of *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (PEREIRA et al., 2004), indicating that emergence is usually high in this genus. *Trichogramma pretiosum* had rates of emergence of 100%, 100% and 99.8% at 25°C in the eggs of hosts *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and *P. xylostella*, respectively (VOLPE et al., 2006). The emergence rate of *Trichogramma* adults can also vary with the size and quality of the host egg, the number of parasitoids that develop per egg, the development period in the host eggs and the temperature (DOYON; BOIVIN, 2005; PRATISSOLI et al., 2005), but generally approaches 100%, as observed in this study.



**Figure 2.** Mean standard  $\pm$  error of the percentage of eggs parasitized and of emergence rate of *Trichogramma pretiosum*, *T. marandobai* and *T. demoraesi* 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.

*Trichogramma pretiosum* showed a higher flight capacity than *T. demoraesi* and *T. marandobai* (Figure 3). For *Trichogramma acacioi* (Brun), *T. bruni* (Nagaraja), *T. demoraesi*, *T. maxacalii* and *T. soaresi* (Nagaraja) (Hymenoptera: Trichogrammatidae), 39%, 50%, 45%, 57% and 46%, respectively, were flying individuals when reared with *A. kuehniella* eggs (SOARES et al., 2007). Two strains of *T. pretiosum*, in different geographical regions, had a similar flight capacity (69% and 73%) but different parasitism capacity (78% and 50%) (SOARES et al., 2012). The geographical origin of the parasitoid may have a strong influence on the biological parameters of the strain because the expression of several characteristics changes with the environment in which the organism is raised. With *T. pretiosum*, reared on *A. kuehniella* eggs,  $81.1 \pm 4.20\%$  of individuals showed flight capacity (PREZOTTI et al., 2002), while two *T. brassicae* lineages had 72% and 61% of individuals with flight capacity with the same host (DUTTON; BIGLER, 1995). Furthermore, the environmental temperature can affect *Trichogramma* flight capacity, as reported

for *Trichogramma sibericum* (Sorkina) (Hymenoptera: Trichogrammatidae) that presented variation in the proportion of adults, with the 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). Comparing the flight ability of various species of *Trichogramma* in the unit tests, it can be concluded that species with higher flight capacity are better able to disperse. The dispersal of *Trichogramma* species in the field is usually limited, but once in flight (flight capacity), the species can disperse more easily with the help of the wind. However, species with a high percentage of non-flying insects are less effective in the field because the parasitoid walking toward the bottom of the PVC is going against several intrinsic behavioral characteristics of species (negative geotropism and positive phototropism) and certainly has a reduced propensity for flight or develops deformed wings (PREZOTTI et al., 2002; SOARES et al., 2007).



**Figure 3.** Mean standard  $\pm$  error of the percentage of flyers, not-flyers and walkers adults of *Trichogramma pretiosum*, *T. marandobai* and *T. demoraesi* 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 results indicate that *T. pretiosum* is the most efficient species for biological control of *E. ello*, with a high capacity for parasitism, emergence and flight in adults. These results corroborate other studies that have reported improved biological characteristics for this species of parasitoid (PARRA; ZUCCHI, 2004). Furthermore, *T. pretiosum* is widely distributed throughout South America and is often found in studies that assess natural occurrence (BARBOSA et al., 2011; PARRA; ZUCCHI, 2004), which confirms these observations. *Trichogramma pretiosum* may be more likely to increase its population in the field in a shorter period of time and still disperse more easily, making the species a better option for biological control programs of *E. ello* in cassava plantations.

## Conclusion

*Trichogramma pretiosum* showed biological characteristics superior to the other species tested, being more efficient and suitable for mass rearing and release for biological control programs on cassava plantations.

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