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Screening of two *Trichogramma* species, native to southeastern Brazil, for the control of tobacco budworm

Estudio de dos especies de *Trichogramma*, nativas del suroeste de Brasil, para el control del gusano cogollero del tabaco

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Abstract: The biological control of insect pests using parasitoids may have limited success without an understanding of the effect of temperature on natural enemies. The objective was to determine the parasitism, egg viability and sex ratio of *Trichogramma exiguum* and *Trichogramma acacioi* (Hymenoptera: Trichogrammatidae) to control *Heliothis virescens* (Lepidoptera: Noctuidae) eggs after two generations on eggs of *Anagasta kuehniella* at 18, 20, 25, 30 and 33ºC. The increase in temperature favored the parasitism of *T. exiguum*. The egg viability of *T. exiguum* ranged between 70.1 and 97.1% and from 81.5 to 98.4% for *T. acacioi* in *H. virescens* eggs. The sex ratio of *T. acacioi* was constant at all temperatures studied, with only females in the progeny. *Trichogramma exiguum* reared in *H. virescens* eggs met the standards of quality control for mass production. *Trichogramma exiguum* showed higher parasitism than *T. acacioi* at high temperatures. However, both species had low parasitism on *H. virescens* eggs.

Keywords: Egg parasitoid. Trichogrammatidae. Temperature. Cotton insect pest. *Heliothis*.

Resumen: El control biológico de insectos con parasitoides podría tener éxito limitado sin entender el efecto de la temperatura sobre los enemigos naturales. El objetivo fue determinar el parasitismo, la viabilidad de los huevos y la razón sexual de *Trichogramma exiguum* y *Trichogramma acacioi* (Hymenoptera: Trichogrammatidae) para controlar los huevos de *Heliothis virescens* (Lepidoptera: Noctuidae) después de dos generaciones sobre huevos de *Anagasta kuehniella* a 18, 20, 25, 30 y 33ºC. El aumento en temperatura favoreció el parasitismo de *T. exiguum*. La viabilidad de los huevos de *T. exiguum* osciló entre 70.1 y 97.1% y entre 81.5 a 98.4% para *T. acacioi* en los huevos de *H. virescens*. La razón sexual de *T. acacioi* fue constante en todas las temperaturas estudiadas con solamente hembras en la progenie. Criado en los huevos *H. Virescens*, *T. exiguum* alcanzó las normas de control de calidad para la producción masiva. *Trichogramma exiguum* presentó un mayor parasitismo que *T. acacioi* a las temperaturas altas. No obstante, ambas especies presentaron bajo parasitismo de huevos de *H. virescens*

Palabras clave: Parasitoide de huevos. Trichogrammatidae. Temperatura. Insectos plaga del algodón. *Heliothis*.

Introduction

Cotton plants can host a number of insects that cause significant damage to this crop. The use of insecticides is a widely used method to control pests, which generally contributes to the selection of resistant insect populations (McCafferry 1998).

Biological interactions between the pest and the environment can be used as a strategy to reduce the use of insecticides (Ghini and Bettiol 2000; Waage 2001). For example, egg parasitoid species of the genus *Trichogramma* can control insect pests in different agroecosystems (Pratissoli *et al*. 2005a; Bueno *et al*. 2008; Faria *et al*. 2008). However, research is necessary to predict the impact of natural enemies on the target pest prior to mass release programs (Parra *et al*. 1991; Pastori *et al*. 2007). Furthermore, highly specific parasitoids must be selected to control their host insect pest (Molina and Parra 2006; Yong and Hoffmann 2006).

Both the temperature and host species may impact the flight capacity, parasitism, longevity, viability and sex ratio of parasitoids (Gutierrez *et al*. 2007; Soares *et al*. 2007; Pandey and Tripathi 2008), which can modify the population dynamics of *Trichogramma* spp. and their role in biological control strategies (Pratissoli *et al*. 2005b). Thus, studies evaluating the impact of temperature on *Trichogramma* biology can help improve the establishment and efficacy of natural enemies in agroecosystems (Zilahi-Balogh *et al*. 2007). The phenotypic plasticity and physiological status of natural enemies may result in varying levels of performance.

The objective of this research was to study the parasitism, viability and sex ratio of *Trichogramma exiguum* Pinto & Platner, 1978 and *Trichogramma acacoi* Brun, Moraes & Soares, 1984 (Hymenoptera: Trichogrammatidae) under different thermal conditions to control *Heliothis virescens* (F., 1777) (Lepidoptera: Noctuidae).

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Material and Methods

The experiment was conducted at the Department of Scientific and Technological Development in Pest Management of the Center for Agrarian Sciences, Universidade Federal do Espírito Santo, Alegre, Espírito Santo state, Brazil, in acclimated chambers at temperatures of 18, 20, 25, 30 and 33ºC, with a relative humidity of 70 ± 10% and a photophase of 12 hours.

Trichogramma exiguum (T.e strain 2) were collected in corn fields (Oliveira *et al*. 2005) and *T. acacoi* (T.ac) from avocado. Both parasitoids were captured with eggs of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae). Parasitoids were reared in eggs of the factitious host *Anagasta kuehniella* attached on cardboard paper (4.0 x 2.5 cm; central area of 2.5 cm^2) and exposed to UV light for 50 minutes. Parasitism was allowed for 24 hours, and the parasitized eggs were kept in glass tubes (8.5 x 2.5 cm) (Parra 1997). Species were identified by Dr. B. Ranyse Querino from Brazilian Agricultural Corporation-Embrapa Roraima, Roraima state, Brazil.

Heliothis virescens (Lepidoptera: Noctuidae) was established from insects provided by the Laboratory of Insects Biology from Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, State of São Paulo, Brazil. Insects were reared in an acclimatized room (temperature 25 ± 1ºC, RH 70 ± 10% and photophase of 12 hours). The adults of *H. virescens* were kept in cages made with PVC tubes (20 cm diameter x 25 cm in height), internally lined with sheets of standard A4 white paper for oviposition. The top of the cage was closed with fabric. Adult moths were fed with a 10% honey solution.

Heliothis virescens eggs were collected and maintained in acclimated chambers (25 ± 1ºC, RH 70 ± 10% and photophase of 12 h) until emergence. Larvae were then individually placed in sterilized (100ºC for 1.5 h) glass tubes (8.5 x 2.5 cm) until pupation with ¼ of its volume with artificial diet based on wheat bran and beans adapted to *H. virescens* (Greene *et al*. 1976). *Trichogramma* species were maintained for two generations in eggs of *A. kuehniella* at temperatures of 18, 20, 25, 30 and 33ºC for temperature adaptation.

Newly emerged mated females species of *Trichogramma* were individually placed in glass tubes (4.5 x 0.7 cm). Each one received 30 *H. virescens* eggs on blue cardboard (3.0 x 0.5 cm). The eggs were fixed with 10% arabic gum. Parasitism was allowed for 24 hours. The cards with parasitized *H. virescens* eggs were maintained in glass tubes (8.5 x 2.5 cm) sealed with PVC plastic.

The percentage of parasitism, egg viability, sex ratio and number of parasitoids per egg were recorded. The treatments were arranged in a completely randomized design with 15 replications. Each replicate was represented by a parasitoid female. Regression equations were used to explain the variations in those parameters due to temperature. In order to achieve the most accurate predictive equations, the data were fitted based on the coefficient determination (R^2), and the significance of the coefficients regression (SSI), and the regression F test (≤ 0.05).

Results and Discussion

Trichogramma acacioi showed higher parasitism in *H. virescens* eggs than *T. exiguum* at 18ºC (Fig. 1). However, at temperatures above 20°C, the percentage of parasitism was higher for *T. exiguum* than for *T. acacioi*. Different thermal requirements were apparent between the two Trichogrammatidae species. The greatest parasitism potential of *T. exiguum* was at 25°C. Similar results were found by Gomes-Torres *et al*. (2008) for *Trichogramma atopovirilia* Oatman y Platner, 1983 (Hymenoptera: Trichogrammatidae) in eggs of *Gymnandrosoma aurantianum* Lima, 1927 (Lepidoptera: Tortricidae). On other hand, the potential parasitism of *T. acacioi* was reduced at that temperature.

Such variations of parasitism can be related to the adaptation of the species to the climate conditions where they were collected. Differences between *T. exiguum* and *T. acacioi* indicate that the biology of these species have variations, probably, due to the host *H. virescens* or temperature. This is supported by studies on the biological and thermal requirements of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) and *T. acacioi* on factitious hosts (Pratissoli *et al*. 2005b).

The higher parasitism of *T. acacioi* at lower temperatures is likely explained by the occurrence of this species in a milder climate than *T. exiguum* (Pratissoli and Fornazier 1999). Similarly, *T. pretiosum* (Hymenoptera: Trichogrammatidae) strain on *Bonagota salubricola* (Meyrick, 1931) (Lepidoptera, Tortricidae) had better parasitism rate at 18ºC (Pastori *et al.* 2007). Thus, interspecific genetic variations are probably involved in the response of insects to temperature changes (Hercus *et al*. 2000).

Egg viability at different temperatures ranged between 70.1 and 97.1% and from 81.5 to 98.4% in *H. virescens* eggs for *T. acacioi* and for *T. exiguum*, respectively (Fig. 2). Differences between parasitoid species were observed only above 25°C. Moreover, for both species of parasitoids, the egg viability was reduced at temperatures greater than 25ºC. However, *T. acacioi* developing in *H. virescens* showed higher egg viability that the same egg parasitoid in *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) (Pratissoli *et al.* 2008),

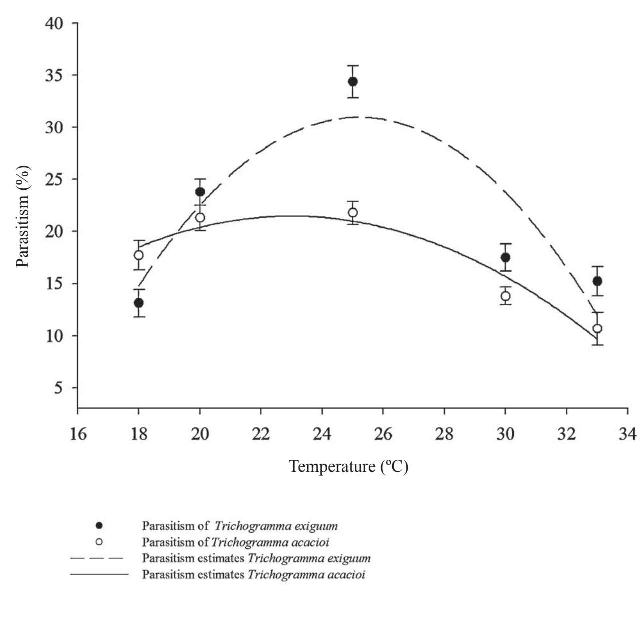

Figure 1. Parasitism (±SE) of *Trichogramma exiguum* ($Y = -0.3122x^2 + 15.733x - 167.25$; $R^2 = 0.77$) and *Trichogramma acacoi* ($Y = -0.118x^2 + 5.427x - 40.91$; $R^2 = 0.92$) on *Heliothis virescens* eggs at different temperatures.

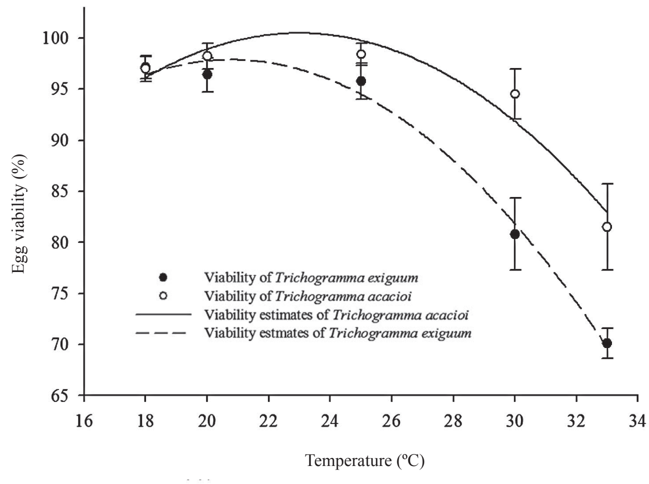

Figure 2. Viability (±SE) of *Trichogramma exiguum* (Y = -0.1749 x^2+8.037x+8.18; R^2 = 0.94) and *Trichogramma acacoi* (Y = -0.188 x^2+7.82x+16.674; R^2 = 0.99) on *Heliothis virescens* eggs at different temperatures.

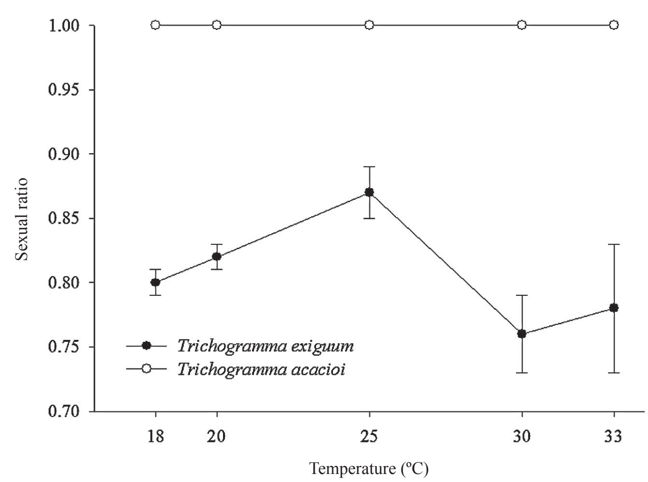

Figure 3. Sex ratio (±SE) de *Trichogramma exiguum* and *Trichogramma acacoi* on *Heliothis virescens* eggs at different temperatures.

indicating the influence of developing host. The egg viability of *T. exiguum* and *T. acacioi* was satisfactory between 18 and 30°C, with values above the minimum required for quality control in mass rearing of parasitoids (Navarro 1990). The results found on the present study for *T. exiguum* and *T. acacioi* were similar to these for *Trichogramma maxacalii* Vogelé y Pointel, 1980 (Hymenoptera: Trichogrammatidae) in eggs of *Oxydia vesulia* (Cramer, [1779]) (Lepidoptera: Geometridae) at different embryonic stages (Oliveira *et al*. 2003).

The sex ratio of *T. acacioi* was constant at all temperatures studied with only females in the progeny. Similar results were found with *Trichogramma pratissolii* Querino & Zucchi, 2003 (Hymenoptera: Trichogrammatidae) reared in *Anagasta kuehniella* (Lepidoptera: Pyralidae) and *Corcyra cephalonica* (Stainton, 1865) (Lepidoptera: Pyralidae) (Zago *et al*. 2006). Despite showing variation in sex ratio, *H. virescens* eggs were appropriate as a host for *T. exiguum* such the standards of quality control in mass production (Lenteren *et al*. 2003).

Both southeastern species *Trichogramma* showed low parasitism with better fitness of *T. exiguum* than *T. acacioi*. The selection of *Trichogramma* to achieve the control of insect pests is necessary also to determine other features such as biological and behavioral aspects of the pests and parasitoids, pest population dynamics, release techniques, selectivity studies, and efficiency evaluation (Smith 1996; Parra and Zucchi *et al.* 2004). Higher rates of parasitism with *Trichogramma* species have been achieved by preimaginal conditioning, but in practice the natural enemies are reared and released with alternative hosts. Thus, the results with *T. exiguum* and *T. acacoi* suggest a possible effect without retention of learning, which can induce a change in adult behavior.

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