NATURAL OCCURRENCE AND ECOLOGY OF *Trichogramma pretiosum* (HYMENOPTERA: TRICHOGRAMMATIDAE) IN COTTON PLANTATIONS WITH INSECTICIDES SPRAYING IN MINAS GERAIS STATE, BRAZIL

OCURRENCIA NATURAL Y ECOLOGÍA DE *Trichogramma pretiosum* (HYMENOPTERA: TRICHOGRAMMATIDAE) EN CULTIVOS DE ALGODÓN CON ROCIAMIENTO DE INSECTICIDAS EN EL ESTADO DE MINAS GERAIS, BRASIL

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ABSTRACT

Pest damage is one of the greatest problems in cotton production in the world. The objective of this study was to assess the natural occurrence, parasitism rate, number of adults of *Trichogramma* emerged and coexistence with predators species in cotton plantations with insecticide spraying in Brazil. Parasitoids were collected in two areas of two-hectares each with the Dp 4049 and Delta Opal cotton varieties, using 12.0 x 2.5 cm pieces of white cards with an average of 3.000 *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs each. The predation percentage of *A. kuehniella* eggs in the field and parasitism occurrence was estimated based on the cards with eggs of this prey brought from the field. All parasitoids obtained were identified as *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae). The predators collected were identified as belonging to the families Chrysopidae (Neuroptera), Coccinelidae (Coleoptera) and Sirphidae (Diptera). The average number of eggs parasitized per cardboard, during the cycle of this culture was 16.34 ± 5.1 in the area with Dp-4049 and 23.38 ± 4.3 in that with Delta opal. The predation average during the cycle of this culture was 42.58 ± 3.8 e 35.58 ± 2.9% in these areas, respectively. It is necessary to preserve and to increase the performance of *T. pretiosum* in cotton plantations.

Key words: biological control; parasitoid; predator

RESUMEN

Los daños causados por plagas son uno de los problemas más graves de la producción de algodón en el mundo. El objetivo de este estudio fue determinar la ocurrencia natural, el grado de parasitismo, número de adultos de *Trichogramma* emergidos y coexistencia con predadores, en cultivos de algodón de diferentes variedades con rociamiento de insecticida. Se utilizaron pedazos de cartulina blanca de 12 x 2.5 cm, cada uno con un promedio de 3.000 huevos de *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae). El porcentaje de predación de huevos de *A. kuehniella* en condiciones de campo y la ocurrencia de parasitismo se estimó con base en el estado de los huevos de esta especie, que se considera presa, expuestos a condiciones de campo en los pedazos de cartulina. Todos los parasitoides obtenidos fueron identificados como *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae). Se identificaron los predadores como individuos de las familias Chrysopidae (Neuroptera), Coccinelidae (Coleoptera) y Sirphidae (Diptera). El número promedio de huevos parasitados por cada pedazo de cartulina, durante el ciclo de este cultivo fue de 16.34 ± 5.1% para la variedad Dp-4049 y de 23.38 ± 4.3% en la variedad Delta opal. La tasa de predación estimada durante el ciclo del cultivo corresponde con 42.58 ± 3.8 para Dp-4049 y 35.58 ± 2.9% para Delta opal. Es necesario preservar e incrementar la actividad biológica de *T. pretiosum* en cultivos de algodón.

Palabras clave Control biológico; parasitoides; predador

INTRODUCTION

Lepidoptera species are included as one of the greatest problems for cotton production in

the world and their control has been made traditionally with insecticides (Avilla & Gonzalez-Zamora, 2010). However, the application of insecticides can eliminate natural enemies and causes pest resurgence. As a result, many non-target secondary pests become uncontrollable (Hegazi *et al,* 2005; Soares *et al,* 2009).

The ecological environment of cotton plantations is complex and includes beneficial soil – plant – pest – natural enemy relationships that should be better interpreted in the management of this crop (Wu & Guo, 2005).

Trichogramma species are eggs parasitoids and they are important for biological control of cotton pests. These natural enemies are used in more than 30 countries for insect pest control of various crops (Van Lenteren & Bueno, 2003; Olson & Wäckers, 2007) such as cotton, sugarcane, corn, tomato, orchard and reforested areas (Oliveira *et al,* 2000; Oliveira *et al*, 2003; Pratissoli *et al*, 2004; Pratissoli *et al*, 2005; Soares *et al*, 2007, Ksentini *et al*, 2010).

However, successful bio-control of *Trichogramma* spp. depends on the pre-introductory assessment with studies of its natural occurrence and ecology during the crop cycle where these natural enemies are found (Pratissoli *et al*, 2003; Hegazi *et al,* 2005). It is important to choose the best species to be released in the fields. Furthermore, abundance, ecology and interaction among the species of these parasitoids and the crop should be studied (Barnay *et al*, 2001; Hegazi *et al,* 2005).

The objective of this study was to assess the natural occurrence, parasitism rate, number of adults of *Trichogramma* emerged and coexistence with predators species in cotton plantations with insecticide spraying in Brazil.

MATERIALS AND METHODS

This experiment was carried out at the Agrarian Science Institute (ICA) at the Federal University of Minas Gerais (UFMG) in Montes Claros, Minas Gerais State, Brazil from January to April 2004 in two areas of two-hectares each. The first cultivated with the Dp 4049 cotton variety and the second one cultivated with the Delta Opal cotton variety in 0.95 m inter-row spacing with seven plants/m^2. A randomized block design was used in a factorial 2 x 4 x 7 design (varieties x quadrants x replications).

A culture of the flour moth, *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) was reared on wheat flour in the Laboratory of Entomology of UFMG (Soares *et al*, 2007). Eggs of this alternative host were used in this work by your easy rearing in laboratory and low cost. The *Trichogramma* lines were collected on pieces of white cards (12.0 x 2.5 cm), with an average of 3000 *A. kuehniella* eggs each exposed to ultraviolet light for 60 minutes (Stein & Parra, 1987). Another card with the same size was stapled over the first to protect the eggs from rain and sunlight (Pratissoli *et al*, 2003).

The experimental areas were divided into North, South, East and West quadrants with seven pieces of cards with *A. kuehniella* eggs distributed per quadrant, representing seven replications. These card pieces were hung on the upper third of the cotton plants, with 28 pieces of cards per experimental area, one in each plant. They were left in the field during three days when they were removed and taken to the laboratory. These cards were then individualized in glass tubes closed with PVC plastic film and kept in an acclimatized room at 25 ± 2°C and 12 hours light period.

Month/Active Ingredient	Dosage	Frequency	Pest objective
February			
Betacyflutrin	80ml ha^{-1}	one application	*Anthonomus grandis*
Methamidophos	40 ml ha^{-1}	one application	*Alabama argillacea*
Methamidophos	500 ml ha^{-1}	one application	*Alabama argillacea*
March			
Betacyflutrin	80ml ha^{-1}	two applications	*Anthonomus grandis*
Methamidophos	40 ml ha^{-1}	two applications	*Alabama argillacea*
April			
Endosulfan	1,0 l ha^{-1}	two applications	*Anthonomus grandis*
Endosulfan	2,0 l ha^{-1}	one application	*Pectinophora gossypiella*
Endosulfan	1,0 l ha^{-1}	three applications	*Anthonomus grandis*

Table 1. Insecticides applied on cotton plantations for pest control during the crop cycle.

Parasitism occurrence by *Trichogramma* was assessed four days after the cards were removed from the field by counting the number of darkened eggs. The parasitoids emerged were counted in the laboratory after their death to determine their emergence rate. Part of these adults was sent to Dra. Ranyse Barbosa Querino da Silva (Embrapa Meio Norte, Teresina, Piauí, Brazil) in 70% alcohol for identification.

The predation percentage of *A. kuehniella* eggs in the field was evaluated using the following visual scale: 0%= cards without predation; 10%= cards with few eggs predated … 50%= cards with half of the eggs predated… 100%= cards with all eggs predated. The predators found in the cards feeding of the eggs were captured and conditioned in glass tubes containing 70% alcohool for identification in the Laboratory of Entomology of UFMG.

The total period of cultivation of the cotton in Brazil is of five months. This experiment was initiated 30 days after the cotton crop was sown and repeated weekly until harvest. Means of the weekly data of parasitism and predation were calculated per month to present the data during the cultivation period of the cotton.

Insecticides were applied on cotton plants from the second month for pest control (Table I).

Data were submitted to the analysis of variance (ANOVA) and the means of parasitsm and predation were compared by the Scott-Knott test at 5% probability with the SAEG Statistical Analysis System (Scott & Knott, 1974).

RESULTS

All parasitoids collected in the experimental area were identified as *Trichogramma*

pretiosum (Riley) (Hymenoptera: Trichogrammatidae). The predators collected were identified as belonging to the families Chrysopidae (Neuroptera), Coccinelidae (Coleoptera) and Sirphidae (Diptera).

The parasitism and predation rates on *A. kuehniella* eggs were similar in the two cotton varieties and in the North, South, East and West orientations. The average number of eggs parasitized per card board, during the cycle of this culture was 16.34 ± 5.1 in the area with Dp-4049 and 23.38 ± 4.3 in that with Delta Opal. The average predation during the cycle of this culture was 42.58 ± 3.8 e 35.58 ± 2.9% in these areas, respectively.

Trichogramma pretiosum showed parasitism activity during the cotton cultivation period even after insecticide spraying in the area (Figure 1). The predation rate of *A. kuehniella* eggs was higher in the first months and decreased in the last two, while the parasitism rate performed inversely (Figure 1).

An average of 1.5 *T. pretiosum* adults emerged per *A. kuehniella* egg.

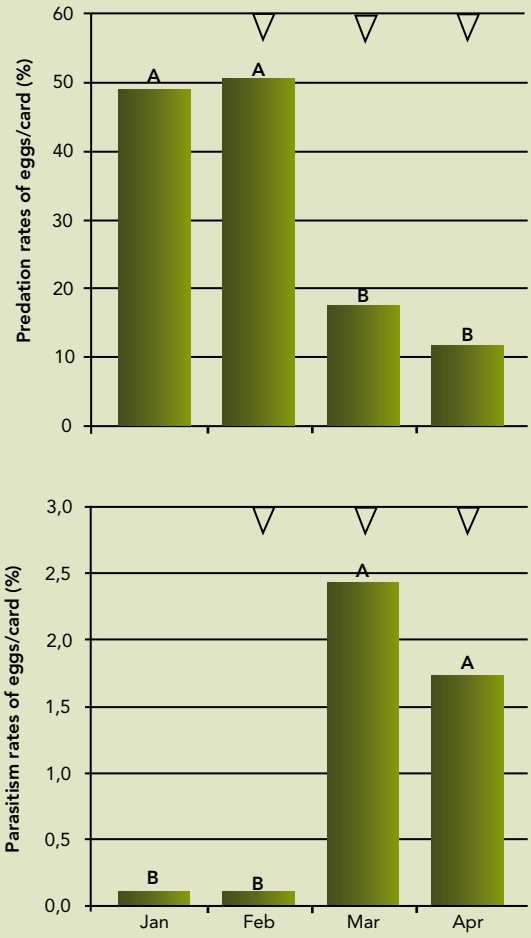

Figure 1. Predation and parasitism rates of eggs/card (%) and insecticide sprayings () in two areas cultivated with cotton from January to April 2004. Municipality of Montes Claros, Minas Gerais State, Brazil. Bars followed by the same letter in the figure do not differ between them by the test of Scott-Knott at 5% probability.

DISCUSSION

The north region of Minas Gerais State, Brazil presents peculiarities as high average temperatures, scattered rains and savannah vegetation. *Trichogramma pretiosum* seems to be the only species of this group adapted to these conditions and it naturally occurs in the cotton culture. This represents an important aspect for biological control programs with parasitoids that is the selection of species or lineages adapted and efficient in a specific environment. However, *T. pretiosum*, even being an endemic species, has been recorded in numbers not enough to control pests. Other studies showed the presence of few species and number of *Trichogramma* parasitoids in semi-arid areas. *Trichogramma bourarschae* (Westwood) (Hymenoptera: Trichogrammatidae) was the only species of this genus collected in semi-arid regions of Egypt with great

adaptation potential to this habitat. On the other hand, areas with arid climate presented higher number of species to be released with *Trichogramma cordubensis* (Vargas & Cabello), *T. pretiosum* and *Trichogramma cacociae* (Marchal) (Hymenoptera: Trichogrammatidae) as endemic ones (Hegazi *et al*, 2005).

The Dp 4049 and Delta Opal are commercial varieties of cotton with similar plant architecture and stand, that may have explained the similar parasitism by *T. pretiosum*. Furthermore, the spacing was similar in the two cotton varieties and no phenological changes occurred during the experiment that might alter leaf mass and the size and complexity of plant architecture. If this occurred, the dispersion of the natural enemies and the parasitism and predation efficiency might have been affected in this crop (Gingras *et al*, 2002).

The number of *A. kuehniella* eggs parasitized by *T. pretiosum* was low and similar in the first two months, because the parasitoids that are at the third trophic level presented retarded effect compared to the pest, because they depend on it. *Alabama argillacea* (Huebner) (Lepidoptera: Noctuidae) and *Pectinophora gossypiella* (Saud.) (Lepidoptera: Noctuidae) were not common in this habitat because this was the first year of cotton cultivation in the area. Thus the low incidence of *T. pretiosum* may be due to the fact that this parasitoid was still migrating to the cotton cropping area. Besides *T. pretiosum* population with low number of individuals in the field, during the first two months, may have been affected by the adaptation of this parasitoid to the environmental conditions of the cotton crop (Bleicher & Parra, 1990) and by interspecific competition with predators (Babendreier *et al*, 2003; Crist *et al*, 2006).

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The predation percentage in the same period was high by generalist predators Chrysopidae (Neuroptera), Coccinelidae (Coleoptera) and Sirphidae (Diptera) families that installed themselves in the crop, possibly attracted by aphid population, the initial cotton pest (Men *et al*, 2004). Predators feeding on eggs would decrease its availability in the

field or, even those parasitized by *Trichogramma*. This agrees with reports of an inverse correlation between predation and parasitism rates in the corn crop (Hoballah *et al*, 2004). However, the massive release of *Trichogramma* may offer no risk to predator populations, such as observed for *Trichogramma brassicae* (Bezdenko) (Hymenoptera: Trichogrammatidae), with low parasitism on Chrysopidae, Coccinellidae and Syrphidae eggs in the field (Babendreier *et al,* 2003). Furthermore, *T. brassicae* releases against leaf eating Lepidoptera could be associated with the predators such as *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) that preferred non parasitized eggs compared to those with *T. brassicae* pupa (Oliveira *et al*, 2003).

The decrease on the predation rates in the last two months may have been caused by the impact of the insecticides applied on the cotton crop or by migration of predators seeking other prey. The greater parasitism rate of *A. kuehniella* eggs in the last two months showed the establishment of *T. pretiosum* in the cotton crop. This was beneficial because the increase on the *Trichogramma* populations could reduce infestation of pests when they are more severe, that is, after cotton flowering (Quirino & Soares, 2001).

The high population growth of *T. pretiosum* after insecticide spraying shows good tolerance of this parasitoid to these products. Insecticides are generally less toxic to parasitoids, because the juvenile stage of this natural enemy develops in the host egg that provides greater protection, while the nymph stage of predators are more susceptible to contact insecticides (Bartlett, 1963). Other hypothesis to explain the good resistance of the *T. pretiosum* to insecticides could be the fact that this natural enemy parasites a single host during its cycle, that reduces bioaccumulation or it presents good metabolic rate of toxic compounds, thus enabling a lower sensitivity of the action site of the insecticides (Moura *et al*, 2000). Spinosad, 48 g/100 l water; tebufenozide, 12 g/100 l water; lufenuron, 15 g/100 l water; triflumuron, 25 g/100 l water had a greater impact on the first instar and decreased during the later ones (pre-pupa and pupa) of *Trichogramma galloi* (Zucchi) (Hymenoptera: Trichogrammatidae). However, the parasitism capacity and survival of adults of these parasitoids from eggs treated with spinosad, tebufenozide, lufenuron and triflumuron were not affected and they did not present morphological anomalies (Cônsoli *et al*, 2001). The insecticides diflubenzuron 25 WP (12.5 g a.i./ha), monocrotophos 400 EC (120.0 g a.i./ha), endosulfan EC 350 (525 g a.i./ha) and betacyfluthrin 125 CS (7.5 g a.i./ha) were sprayed for control of cotton pests in Juarez Tavora, Paraiba State, Brazil and didn't affect the parasitism of *Trichogramma* species liberated simultaneously (de Almeida, 2001). This showed that the performance of *Trichogramma* adults was not affected when their larvae had contact with these chemical products.

Parasitoids of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) such as *Encarsia lutea* (Masi) (Hymenoptera: Chalcidoidea) and *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae) had high parasitism levels (24 and 59%, respectively), in cotton fields treated with the insecticides buprofezin (2.5 g/l), buprofezin (20 g/l) and diafenthiuron (20 g/l) (Gerling & Naranjo, 1998). Furthermore,

Trichogramma cacoeciae (Marchal), *Trichogramma daumalae* (Dugast & Voegele), *Trichogramma evanescens* (Westwood) and *Trichogramma principium* (Sugonjaev & Sorokina) (Hymenoptera: Trichogrammatidae) populations were reported in vineyards in the same year as the insecticides were applied regularly in the crop (Barnay *et al,* 2001).

The number of emerged *T. pretiosum* adults per *A. kuehniella* egg was 1.5, that is in line with reports of more than one individual emerging from the same egg of the alternative host, *Sitotroga cerealella* (Olivier) (Lepidoptera: Pyralidae) (Alencar *et al*, 2000). The number of *T. pretiosum* adults developing in the same host egg can vary with temperature (Maceda *et* al, 2003). This natural enemy presented a greater number of individuals per egg at 25°C and fewer at 18°C in the laboratory (Pratissoli & Parra, 2000) and emergence rate of 1.41 with a maximum of two individuals per *S. cerealella* egg at 25.9 ± 0.9°C (Alencar *et al*, 2000).

CONCLUSIONS

1. *Trichogramma pretiosum* naturally occurs throughout the cotton crop cycle even after spraying with insecticides.

2. *Trichogramma pretiosum* was the only egg parasitoid collected in the Dp 4049 and Delta Opal cotton varieties in the north of Minas Gerais State, Brazil and thus it is indicated for biological control programs on this culture.

3. Generalist predators Chrysopidae (Neuroptera), Coccinelidae (Coleoptera) and Sirphidae (Diptera) occurs throughout the cotton crop cycle and reduced your population number in the last two months.

4. *Trichogramma pretiosum* colonized the area slowly and its action needs to be preserved and enhanced by massive releases in cotton plantations.

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