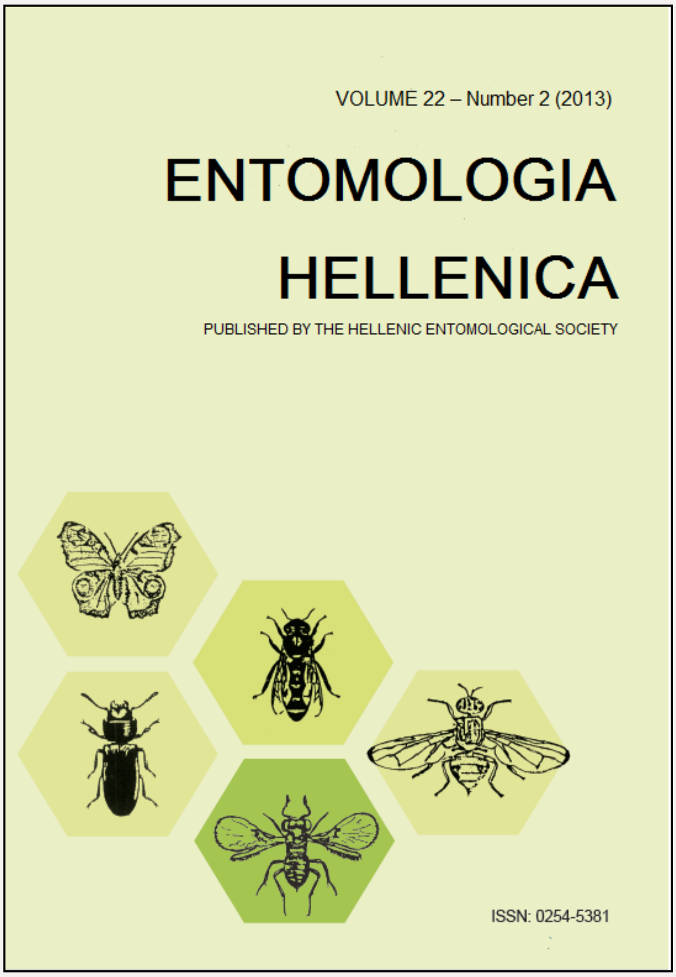
ENTOMOLOGIA HELLENICA

Vol. 22, 2013



Trichogramma cacoeciae as a biological control agent of the tomato pinworm Tuta absoluta in Northeastern Tunisia

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https://doi.org/10.12681/eh.11527

To cite this article:

Cherif, A., & Kaouthar, L. (2013). Trichogramma cacoeciae as a biological control agent of the tomato pinworm Tuta absoluta in Northeastern Tunisia. *ENTOMOLOGIA HELLENICA, 22*(2), 35-42. doi:https://doi.org/10.12681/eh.11527

Trichogramma cacoeciae as a biological control agent of the tomato pinworm *Tuta absoluta* in Northeastern Tunisia

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ABSTRACT

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is considered as key pest attacking tomato in Tunisia and in many other countries around the world. In order to investigate the efficacy of *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) for biological control of this pest in Northeastern Tunisia, releases were performed in open field conditions. *T. cacoeciae* showed a good efficacy on reducing the number of *T. absoluta* eggs and larvae when releasing 30 adults/plant in plots covered with insect-proof netting in May 2012. The results showed that this parasitoid may be a promising agent for *T. absoluta* biological control in Tunisia.

KEY WORDS: biological control, *Trichogramma cacoeciae, Tuta absoluta*.

Introduction

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is considered to be a major pest on tomato and other solanaceous crops (EPPO 2005). *T. absoluta* is reported oligophagous (Siquiera et al. 2000), exclusively feeding on plants of the family Solanaceae (Vargas 1970, Lietti et al. 2005). *T. absoluta* larvae can significantly reduce both yield and fruit quality by direct feeding and the secondary pathogens which may enter through the wounds caused (Moore 1983, Oliveira et al. 2009, Silva et al. 2011). Severe damages have been reported from different regions. For example, in Italy, Sannino and Espinosa (2010) reported losses of up to 100% in protected tomato in 2009.

Since its initial detection, chemical control has been the main method used against *T. absoluta* in all production regions in the world. However, chemical control may not be effective, firstly due to the pest biology, i.e. the mine-feeding behavior of larvae (Siquiera et al. 2000, EPPO 2005, Lieitti et al. 2005) and secondly, due to insecticide resistance development (Cabello et al. 2009).

Various predators (*Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae)), parasitoids (*Trichogramma* sp., Braconidae) and entomopathogens attack *T. absoluta* in tomato crops in Europe and in Northern Africa (Desneux et al. 2010). Among the different entomopathogens that act against *T. absoluta*, *Bacillus thuringiensis* subsp. *kurstaki* (BtK) seems to carry exceptional promise for use in Brazil (Desneux et al. 2010). Biological control, especially by *Trichogramma* parasitoids is considered to be promising as a management tool for this pest (Chailleux et al. 2012, 2013).

In Tunisia, the pest has spread rapidly and in the absence of control strategies, larval feeding can result in up to 100% crop losses by attacking leaves, flowers, stems and fruits (Apablaza 1992, Estay 2000). For this reason, implementing effective control tactics is of primary importance.

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The objective of this work was to evaluate the efficacy of *T. cacoeciae* (Marchal) (Hymenoptera: Tricho-grammatidae) to control *T. absoluta* in open field tomato crops.

Materials and Methods

Experimental site

The study was conducted in an open fresh market field over one growing season, spring-summer, in an area of intensive open field tomato cultivation located in Takelsa (Cap-Bon region, North-Eastern of Tunisia). The field covered an area of 1 ha with 16.000 plants (cv. Ferrinz) with a distance between tomato plants on the row of 0.50 m and the tomato rows were separated by 1.50 m. The tomato plants were drip irrigated.

Effectiveness of *T. cacoeciae* to control *T. absoluta* in open field tomato

The rearing of *T. cacoeciae* in the laboratory was realized using ultraviolet-irradiated eggs of a substitute host, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Colonies of parasitoids were reared under constant conditions of temperature (27±1°C) and relative humidity (70±5%). Rearing was carried out in glass tubes and the parasitoids were fed on honey droplets. *E. kuehniella* eggs were glued on a piece of cardboard (1.5 by 7.5 mm) with water.

To evaluate the efficacy of *T. cacociae*, a test was conducted on two plots (each plot contained 90 plants) covered with insect-proof on May, 3rd, 2012.

A total of 3 releases (30 parasitized eggs / plant / release) were carried out on 03/05/2012, 10/05/2012 and 15/05/2012. Two thousand and seven hundred eggs of *E. kuehniella* parasitized by *T. cacoeciae* were released on the plants of the first plot (3 pieces of cardboard, each with 300 parasitized eggs, per 30 plants) in each release date. The plants of the second plot were used as a control. In order to detect the number of *T. absoluta* parasitized eggs and to define if releases of *T. cacoeciae* in the plot had decreased the number of *T. absoluta* eggs and larvae, forty young tomato leaves were collected at random on 3rd, 10th, 17th, 24th and 31st May 2012 from 30 treated plants and another 40 leaves from 30 control plants. So, the total number of harvested leaves from the 90 plants of each plot was 120/plot/week. The leaves were inspected under binocular microscope (Leica® model MS5).

Pieces of cardboard containing eggs of *E. kuehniella* parasitized with *T. cacoeciae* were recuperated after each release in order to determinate the emergence rate of *T. cacoeciae* on 10/05/2012, 15/05/2012 and 24/05/2012. The infestation level of *T. absoluta* (i.e. number of *T. absoluta* eggs per plant) was equal in both plots in the start of the experiments. In fact, the two plots had the same method of crop management (same date of treatments, irrigation, fertilization).

The emergence rate of the parasitoids from the cardboards and the parasitism rate of *T. absoluta* eggs by the parasitoid on the tomato plants were calculated as follows:

Emergence rate = (number of hatched eggs of *E. kuehniella*/total number of released eggs)*100

Parasitism rate = (total number of parasitized eggs of *T. absoluta*/total number of eggs recorded)*100

Climatic data

The meteorological data were given by the Tunisian Meteorological Institute. In Takelsa, the mean relative humidity ranged from 88.25% (March) to 70% (July) and the mean temperature ranged from 15°C (March) to 31°C (July) (Table 1).

Statistical analyses

The obtained data corresponding to the *T. cacoeciae* emerged adults from *E. kuehniella* eggs, the number of *T. absoluta* parasitized eggs by *T. cacoeciae* and the effect of releases of *T. cacoeciae* on *T. absoluta* were analyzed using one-way ANOVA. Means of treatments were separated using Duncan's multiple range test at P=0.05. All statistical analyses were performed using the software SPSS 17 (SPSS Inc. 2009).

TABLE 1. Average monthly temperature (°C) and relative humidity (%) in Takelsa, Tunisia, in 2012.

	March	April	May	June	July
Temperature	15	17	18	29	31
Relative humidity	88.25	83.33	76.75	71.52	70

Results and Discussion

Emergence rate of *T. cacoeciae*

The number of emerged adults was similar among the release dates (F= 46.85, df=17, P<0.001). The emergence rate of *T. cacoeciae* was, on average, more than 63% in the open field plot during this assay (Table 2). Blibech et al. (2010) demonstrated that under laboratory conditions, the emergence rate of *T. cacoeciae* was 84.2%. This difference of *T. cacoeciae* emergence rates between the open field and the laboratory conditions may be explained by two factors. Firstly, it may be linked to abiotic conditions such as temperature and relative humidity. In fact, *T. cacoeciae* was reared on alternative host (*E. kuehniella*) at optimal temperature (25°C) and relative humidity (70%). However during the experiment in May the temperature and relative humidity values averaged 18°C and 76.75% respectively. Russo and Voegelé (1982) evaluated in a previous study the effect of different constant temperatures ranging from 10° to 35°C on the development of four *Trichogramma* species: *Trichogramma nubilale* (Ertle and Davis), *T. nwidis* (Pintureau and Voegele), *T. rhenana* (Voegeld and Russo) and *T. schuberti* (Voegelé and Russo) in the eggs of *E. kuehniella* and showed that *T. schuberti* and *T. rhenana* had a maximum percentage of emerged adults at a temperature range of 17 to 25°C and 19 to 26°C, respectively.

In a previous study, Tezze and Botto (2004) demonstrated that low temperature (4°C) caused a low emergence rate with deformation of emerging *T. nerudai* (Pintureau) individuals and reduced mobility. Secondly, the emergence rate of *T. cacoeciae* may be influenced by the quality of *E. khueniella* eggs used to rear this parasitoid (Blibech et al. 2010).

Parasitism rates of *T. absoluta eggs*

T. cacoeciae caused an average rate of parasitism of 54.7% (Table 3). From 10/05/2012 till 24/05/2012, a similar number of parasitized eggs were recorded. On 31/05/2012, the number of parasitized eggs had a slight decrease, which may be explained by the fact that the number of *T. absoluta* eggs had decreased after releases of *T. cacoeciae*.

TABLE 2. Mean number (± SE) of *T. cacoeciae* adults emerged from parasitized eggs of *E. kuehniella* and the respective emergence rate (%) after one week of each of three subsequent releases of 900 eggs, in the area of Takelsa on May, 2012, on tomato plants.

Date of release	Adults emerged	Emergence rate (%)
10/05/2012	621.0±172.8a*	69.0
15/05/2012	568.6±143.3a	63.2
24/05/2012	576.0±147.2a	64.0

* Means followed by the same letter do not differ significantly at the 5% level of significance (Duncan's multiple range test)

TABLE 3. Mean number (±SE) of *T. absoluta* parasitized eggs and parasitism rate (%) per 120 leaves by *T. cacoeciae* on tomato plants in the area of Takelsa on May, 2012.

Sampling date	Parasitized eggs	Parasitism rate (%)
03/05/2012	0	0
10/05/2012	2.6±1.1a*	43.3
15/05/2012	2.3±0.5a	53.4
24/05/2012	2.6±1.1a	78.7
31/05/2012	1.0±1.0a	43.4

*Means followed by the same letter do not differ significantly at the 5% level of significance (Duncan's multiple range test).

The parasitism rate was as high as that recorded in other studies, i.e. *T. achaeae* showed a parasitism rate of 35.4% of *T. absoluta* eggs on tomato plants obtained on sentimental leaflets placed in greenhouse cage (Chailleux et al. 2013). However, Cabello et al. (2009) showed that under laboratory conditions *T. achaeae* showed a parasitism rate of 83.3%. A previous study in palm groves of southern Tunisia showed that *T. cacoeciae* was able to control the carob Moth *Ectomyelis ceratoniae* Zeller (Lepidoptera: Pyralidae). A dose of 20.000 *Trichogramma* per date palm parasitized 80% of *E. ceratoniae* eggs (Khoualdia et al. 2008). The reasons for the comparatively low parasitism rates of *T. absoluta* eggs by the parasitoid on tomato plants could be associated with reduced parasitoid mobility because of the dense trichomes of tomato plants (Chailleux et al. 2013). Similarly, previous studies mentioned that tomato trichomes have negative effects on *Trichogramma* efficiency (Kauffman and Kennedy 1989, Farrar et al. 1994). In addition, *Trichogramma* spp. are known to be poor flyers and to forage primarily by walking and jumping (Chailleux et al. 2013). Another reason could be the size of the egg host. It has been shown that *Trichogramma* parasitoids prefer hosts with relatively large size eggs; i.e. the eggs of *T. absoluta* are 3-fold narrower than the eggs of *E. kuehniella* used in the rearing (Roriz et al. 2006, Chailleux et al. 2012).

The effectiveness of *T. cacoeciae* against *T. absoluta*

The effectiveness of the parasitoid was recorded by weekly sampling started at the second release (10/05/2012) and lasting to two weeks (31/05/2012) after the last (3rd) release (Table 4).

Indeed, the number of eggs in the treated plot decreased from 6 to 2 eggs/120 leaves while this number in the control plot increased from 6 to 11 eggs/120 leaves during the assay (F= 12.43, df=9, P<0.008) (Table 4). Similarly, the number of alive larvae found in the treated plot decreased considerably. For example, on May 10th, the number of alive larvae was 5-fold lower on treated plot compared with the control (Table 4). In fact, the decrease of the number of *T. absoluta* eggs after releases of *T. cacoeciae* resulted in a decrease in the infestation level of the plot.

This result shows that *T. cacoeciae* is able to reduce the number of *T. absoluta* eggs and larvae and therefore may reduce the infestation in open field crops. Under greenhouses, in southern Tunisia, a high efficacy (75.54% of damage reduction) was obtained when releasing 40 adults of *T. cacoeciae*/tomato plant in 3-4 day intervals in February and March of 2009 (Zouba and Mahjoubi 2009). Cabello et al. (2009) showed that *T. achaeae* at a rate of 750.000 adults per ha released every 3 or 4 days was highly efficient in lowering *T. absoluta* infestation in experimental and commercial tomato greenhouses in southern Spain.

TABLE 4. Density (mean±SE) of *T. absoluta* eggs and larvae per tomato plant after three releases of *T. cacoeciae* on 03/05/2012, 10/05/2012 and 15/05/2012 (treated plot) vs plants without parasitoids (control) in open tomato field (Takelsa, May 2012).

	***T. absoluta* densities (mean±SE) per 120 leaves**			
	Eggs		**Larvae**	
	control plot	**Treated plot**	**control plot**	**Treated plot**
03/05/2012	6.0±1.7a*	6.0±1.7a	4.0±2.6 a*	4.0±2.6a
10/05/2012	11.0±3.6a	6.0±2.0b	5.0±1.0a	1.0±1.0b
15/05/2012	9.0±1.0a	4.3±1.5b	7.3±3.7a	3.3±0.5b
24/05/2012	7.0±1.7a	3.3±0.5b	9.0±3.4a	2.3±0.5b
31/05/2012	9.0±1.7a	2.3±1.1b	8.0±2.0a	3.0±1.0b

*Means followed by a different letter in each row differ significantly at the 5% level of significance (Duncan's multiple range test).

These are quite important results which encourage the further use of the indigenous *T. caceociae*. Its effectiveness may be promising for *T. absoluta* control particularly if used in a combination with *N. tenuis*. In fact, *T. cacoeciae* could be used at the onset of the tomato growing cycle (low infestation), when the predator *N. tenuis* is not yet established. During the period of infestation increase, releases of the mirid predator, which needs more time for establishment, in combination with releases of *T. cacoeciae* can reduce *T. absoluta* populations (Cabello et al. 2009*)*. Furthermore, *N. tenuis* attacks *T. absoluta* eggs and larval instars, although it prefers first instar larvae. This predator is able to consume more than 100 eggs per individual per day (Arnó et al. 2009). Mollă et al. (2009) showed that when *N. tenuis* was well established in the crop, it was able to reduce leaflets or fruit infestations up to 97% and 100% respectively.

In conclusion, the present study showed that *T. cacoeciae* may be a promising agent to control *T. absoluta* but further research should be performed to evaluate its efficacy in open field conditions. It has to be searched the required release rates and frequency for establishment of this parasitoid according to the density of the pest and the growth stage of the crop. Likely, weekly releases of the parasitoid could give an effective control of the pest as proved in the current study. Further studies should be realized to identify other native efficient parasitoids and predators of *T. absoluta* and combine releases with other biological control agents.

Acknowledgements

The authors would like to express their gratitude to the farmers (Khira, Ahmed and Anwer) for their ongoing availability and collaboration.

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Το *Trichogramma cacoeciae* ως παράγοντας αντιμετώπισης του *Tuta absoluta* στη Βορειανατολική Τυνησία

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ΠΕΡΙΛΗΨΗ

Το *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) αποτελεί ένα πολύ σημαντικό έντομο-εχθρό της τομάτας στην Τυνησία και σε πολλές άλλες χώρες σε όλο τον κόσμο. Στη μελέτη αυτή αξιολογήθηκε η αποτελεσματικότητα του παρασιτοειδούς *Trichogramma cacoeciae* στην αντιμετώπιση του *T. absoluta* στη βορειοανατολική Τυνησία. Σε συνθήκες πεδίου, το *T. cacoeciae* έδειξε καλή αποτελεσματικότητα στη μείωση του αριθμού των ωών του *T. absoluta* αλλά και των προνυμφών μετά την απελευθέρωση 30 ενηλίκων του παρασιτοειδούς / φυτό τον Μάιο του 2012. Το αποτέλεσμα δείχνει ότι αυτό το *T. cacoeciae* μπορεί να αποτελέσει έναν σημαντικό παράγοντα για την αντιμετώπιση του *T. absoluta* στην Τυνησία.