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Biological Control of *Tuta absoluta* (Lepidoptera: Gelechiidae) with Release of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) in Tomato Greenhouse in Tunisia

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ABSTRACT

The egg parasitoid *Trichogramma cacoeciae* has been identified as a candidate for biological control of the tomato pinworm, *Tuta absoluta*. Under laboratory conditions 74.28% of the parasitized eggs developed until the blackhead stage (apparent parasitism). Under greenhouse conditions a high efficacy (75.54% of damage reduction) was obtained when releasing 40 adults of *Trichogramma cacoeciae*/ tomato plant every 3-4 days on February and March of 2009. This shows that this parasitoid can be a good weapon to control *T. absoluta* in greenhouses of southwestern Tunisia.

Keywords: oophagous parasitoid, releases, tomato, tomato pinworm, *Trichogramma*

INTRODUCTION

The tomato pinworm, *Tuta absoluta* (Meyrick), is a devastating pest of tomato and other Solanaceous crops. It originated from South America, and it has spread to most South America regions and presently to most Mediterranean regions (EPPO 2005). *T. absoluta* is a very challenging pest to control. Effectiveness of chemical control is limited due to the insect's biology (the larvae make feeding galleries in the leaves, stems, the apical buds, and fruits that protect them from chemicals) as well as its rapid capability of development of insecticide resistant (Siqueira *et al.* 2000; EPPO 2005; Lietti *et al.* 2005; Urbaneja *et al.* 2007). Consequently, alternatives to chemical control and particularly biological control have been attempted in several South American countries, especially using the oophagous parasitoids *Trichogramma pretiosum* (Riley) and *T. nerudai* Pintureau and Gerding (Parra and Zucchim 2004; Faria *et al*. 1994). In Tunisia, *T. absoluta* was reported for the first time in October 2008 (Chermiti *et al*. 2009), and since then it has caused extensive damage on tomatoes in open fields and greenhouses.

Trichogramma cacoeciae (section *exiguum*; order Hymenoptera; family Trichogrammatidae) is not a *Wolbachia*-infected species, and its thelytoky (males come from fertilized eggs and females come from unfertilized eggs) is genetically fixed (Pintureau *et al.* 1999). *T. cacoeciae* is reported to be omnipresent in Europe (Pintureau *et al.* 1999). It was found in the Netherlands (Vavre *et al.* 2004), in France on the eggs of *Lobesia botrana* (Volkoff and Daumal 1994) and in Germany on baits hung in plum trees (*Prunus pissardii*) (Schöller and Hassan 2001). It was also collected in olive groves in Portugal and Greece (Herz *et al.* 2007).

In Tunisia, *T. cacoeciae* was the species that mostly parasitized carob moth *Ectomyeloïs ceratoniae* Zell (Lepidoptera, Pyralidae) eggs on pomegranate (*Punica granatum* L.). When released, it can settle easily and spreads well in a pomegranate orchard where no parasitism has been detected before (Ksentini *et al*. 2010). It was collected on host baits hung in olive orchards in Sfax (Herz *et al*. 2007). A local oasis strain of *T. cacoeciae* is currently used in biological control against *Ectomyeloïs ceratoniae* Zell on date palm (*Phoenix dactylifera* L.) and pomegranate (Khoualdia 2006). The objectives of the present study were to investigate whether a local strain of *T. cacoeciae* could effectively reduce the incidence of *T. absoluta* in tomato greenhouses.

MATERIALS AND METHODS

Rearing of insects

T. absoluta individuals used in the trials were obtained from a laboratory rearing them on tomato leaves. The rearing of a local strain of *T. cacoeciae* was conducted using *Ephestia kuehniella* Zeller eggs as an alternative host, according to the methodology described by Cabello (1985).

Laboratory bioassay

A preliminary test under laboratory conditions (25 ± 1°C and 16: 8 h, L: D) was carried out to evaluate the acceptance and the parasitism of *T. absoluta* eggs by females of the parasitoid. The methodology was adapted from Brotodjojo and Walter (2006), with the exception that 35 eggs of *T. absoluta* (< 24 h of age) were fixed to carton cards and offered to each female. There were 3 repetitions per treatment. All the parasitized eggs were developed until adult emergence.

The capacity of *T. cacoeciae* to detect and reach the eggs of *T. absoluta* on tomato (*Solanum lycopersicum*, cultivar 'Naziha') leaves was also evaluated. To perform this test, 90 eggs of *T. absoluta* was collected and randomly placed on the leaves of three young tomato plants at a rate of 30 eggs per plant. On each of three plants 25 females of *T. cacoeciae* were released.

Greenhouse assay

To evaluate the efficacy of *T. cacoeciae* under greenhouse conditions a test was conducted in a compartmented glass greenhouse in the Regional Centre of Research on Oasis Agriculture -Tunisia, between the 15th March and 8th April, 2010. Nine cages (3 m^2) were placed into the greenhouse with 12 tomato plants (var.

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Research Note

'Naziha') per cage arranged on pots. Plants were infested with *T. absoluta* (20 adults/cage) before releasing parasitoids. Three treatments of *T. cacoeciae* were evaluated: 25 adults of *T. cacoeciae*/ tomato plant (T1); 40 adults of *T. cacoeciae*/tomato plant (T2); control without T. *cacoeciae* (T3). Release of the parasitoids was repeated every 3-4 days for 25 days. The treatments were arranged in a complete randomized block design with three replications to assess the damage caused by this pest. The number of *T. absoluta* larva and leaf mines were counted alter 25 days. Data was analyzed using STATISTICA v. 5.0. and the comparison of averages was done by a Newman-Keul's test (P = 0.05).

RESULTS AND DISCUSSION

Laboratory bioassay

T. absoluta eggs were accepted as hosts by *T. cacoeciae*, 74.28% of the offered eggs developed to the blackhead stage (prepupa of the parasitoid: apparent parasitism) (**Table 1**). Similar results have been found by Cabello when testing the performance of *T. achaea* as a biological control agent against *T. absoluta* in tomato greenhouses in Spain (Cabello *et al*. 2009).

The size of the rearing host eggs seems to affect the parasitism rate of natural hosts (Brotodjojo and Walter 2006), female parasitoid usually accepts host eggs with about the same size as their natal host or larger ones (Nurindah *et al*. 1999). The eggs of *T. absoluta* are smaller than those of *E. kuehniella*, which may explain why a percentage of *T. absoluta* eggs offered to *T. coccociae* females was not parasitized. In addition, this test was conducted under optimal conditions for *T. absoluta* which accelerates the embryonic development of this pest (once the embryo of the pest is formed in the egg, female parasitoid does not accept it as host). Females of *Trichogramma* also kill host eggs by feeding on them; the host egg is stung and the female feeds on the drop of liquid appearing at the site of the sting. The host egg dies, leaving no evidence of parasitism (Cabello *et al*. 2009). In some species of *Trichogramma*, host feeding contributes significantly to pest control (Vasquez *et al*. 1997). In the present work host feeding by female parasitoids was not observed.

This study showed that *T. coccociae* females is able to search for *T. absoluta* eggs on the tomato leaves and it can give a high rate of parasitism (75.55%) despite the difficult texture characterizing the leaves of this plant (**Table 2**). The capacity of *T. coccociae* to parasitize and search for *T. absoluta* eggs suggests that this parasitoid can be an efficient biological control agent against this pest on tomato plants.

Greenhouse assay

After 25 days from the beginning of the trial, it was found that the number of *T. absoluta* larvae and the number of mined leaves are significantly lower where parasitoids had been released (**Fig. 1**). It seems clear that the release of *T. cacoeciae* has minimized the damage caused by *T. absoluta*.

Table 1 Percentage of parasitized eggs of *Tuta absoluta* by females of *Trichogramma cacoeciae*.

Egg cards	C1	C2	C3
Number of eggs/card	35	35	35
Number of parasitized eggs	21	24	33
% parasitism	60	68.57	94.28
Mean % parasitism		74.28	

Table 2 Percentage of parasitized eggs of *Tuta absoluta* by females of *Trichogramma cacoeciae*/tomato plant.

Plant number	PL1	PL2	PL3
Number of eggs/card	30	30	30
Number of parasitized eggs	15	25	28
% parasitism	50	83.33	93.33
Mean % parasitism		75.55	

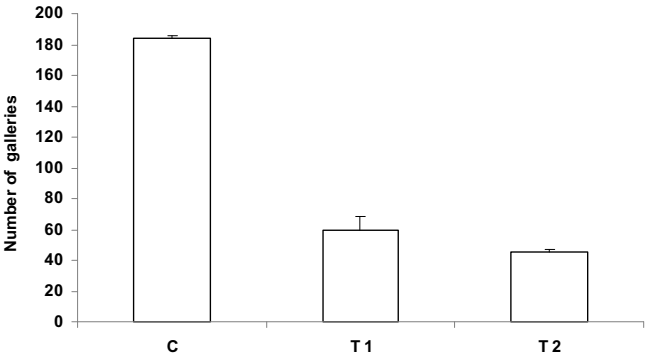

Fig. 1 Number of *Tuta absoluta* galleries on tomato plants according to the treatment. C: control; T1: first treatment; T2: second treatment. Each mean is an average of three repetitions. The error bars represent the standard deviation (ECARTYPE).

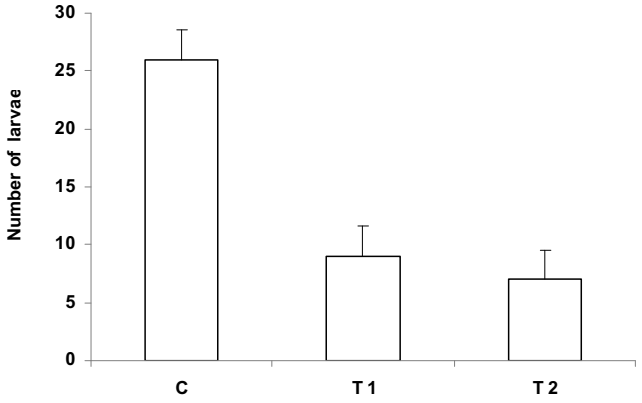

Fig. 2 Number of *Tuta absoluta* larvae on tomato plants according to the treatment. C: control; T1: first treatment; T2: second treatment. Each mean is an average of three repetitions. The error bars represent the standard deviation (ECARTYPE).

When applying 40 and 25 adults of *T. cacoeciae*/tomato plant the number of galleries mined by *T. absoluta* larvae was respectively 4 and 3 times less than that of the control (**Fig. 1**), this corresponds to an efficacy of 75.54 and 67.39%, respectively. The application of 40 parasitoids has reduced the number of *T. absoluta* larvae by 4 times (73.07% efficacy) as compared to the control (**Fig. 2**).

Statistical analysis revealed that there is no significant difference between the number of larvae survived with T1 and T2 treatments (**Fig. 2**), while there is a significant difference between the numbers of galleries mined by *T. absoluta* larvae, T2 treatment gave the best protection of tomato culture by reducing significantly the number of galleries (**Fig. 1**).

This work has demonstrated that the local strain of *T. cacoeciae* could be used as an effective control measure against the tomato pinworm. Our results are similar to those obtained in Spain with *T. achaea* (Cabello *et al.* 2009) and in Brazil with *T. pretiosum* (Freitas *et al*. 1994; Parra *et al*. 2004). However, this work must be completed with field studies (now in course), mainly to establish the needed releases based on the density of the pest and the growth stage of the tomato crop.

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