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Parasitism of *Tuta absoluta* in tomato plants by *Trichogramma pretiosum* Riley in response to host density and plant structures

Parasitismo de *Tuta absoluta* por *Trichogramma pretiosum* Riley em resposta a densidade do hospedeiro e estruturas da planta

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ABSTRACT

One important factor determining the efficacy of parasitoids is the way they exploit different host patch. This study evaluated the response of females of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) to the oviposition sites of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on processing tomato plants. In fully developed caged tomato plants *T. absoluta* moths were released, followed by the release of *T. pretiosum* females 12h later. After 24h of parasitoid release, the moth oviposition sites were mapped according to the plant canopy, and levels of parasitism assessed. The parasitism rate varied from 1.5 to 28%. There was not influence of plant structures on parasitism, except for the absence of parasitism on the plant apex. Levels of both *T. absoluta* oviposition and parasitism by *T. pretiosum* were higher on the upper third of the plant, decreasing downward along the plant canopy.

Key words: biological control, density dependent parasitism, tomato leafminer, egg parasitoid.

RESUMO

Um dos fatores que podem determinar a eficácia de parasitóides é como esses agentes de controle biológico exploram o habitat de seus hospedeiros. Este estudo avaliou a resposta de fêmeas de *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) à oviposição da praga *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) em tomate industrial. Mariposas de *T. absoluta* foram liberadas em gaiolas contendo plantas de tomate e, após 12h, foi realizada a liberação de fêmeas de *T. pretiosum*. Após 24h da liberação do parasitóide, a oviposição da praga foi mapeada de acordo com as estruturas no dossel da planta de tomate e, posteriormente, foram determinados os níveis de parasitismo. O parasitismo de ovos da praga variou de 1,5 a 28%. Não

houve influência significativa das estruturas da planta na taxa de parasitismo dos ovos da praga, exceto pela ausência de parasitismo na parte apical da planta. Ambos os níveis de oviposição de *T. absoluta* e parasitismo por *T. pretiosum* foram maiores no terço superior da planta de tomate.

Palavras-chave: controle biológico, parasitismo densidade dependente, traça-do-tomateiro, parasitóide de ovos.

INTRODUCTION

The ecological theory of biological control predicts that the most efficient natural enemies have to show a high attack rate and allocate a greater proportion of their searching time to areas with high host densities. However, they must also be able to find their host at low densities (WAAGE, 1983). This is called an aggregative response (HASSELL, 1978). Theoretically, the aggregative response can affect the total proportion of parasitized hosts and the spatial distribution of parasitism, which consequently can affect the impact on host population growth and host-parasitoid population dynamics (ROYAMA, 1971). Therefore, the way that parasitoids exploit different host densities coupled to host distribution is one important factor determining their efficiency as a biological control agent.

The parasitism distribution is affected by several factors, and much of the variation in parasitism levels is due to direct and indirect influence of the host

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plant (BOTTRELL et al., 1998). The effect of plant characteristics should be studied in each relevant plant-host-parasitoid system. For this study, the system was: the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), and one of its important natural enemies, the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) and processing tomato plants. *T. absoluta* is one of the most important lepidopteran pests associated with the processing tomato crop in Brazil. Losses are due to direct damage by caterpillars on leaves, terminal buds, flowers and fruits, and indirect damage to fruit caused by scorching after defoliation (HAJI et al., 1988; SOUZA & REIS, 1992).

Concerning the distribution of tomato leaf miner eggs within processing tomato plants, TORRES et al. (2001) showed that females lay their eggs preferentially on the upper part of the plant, followed by the middle and lower parts, respectively. The relationship between oviposition along the plant and parasitism by *T. pretiosum* has not yet been studied. The pattern of *Trichogramma* spp. parasitism is species-specific and dependent upon the release method and crop (SMITH, 1988). Moreover, one of the factors influencing the efficiency of *Trichogramma species* in inundative releases is their distribution pattern within the crop and host plant (SAAVEDRA et al., 1997), and if there is a preference for specific niches they must coincide with those of the host (BIGLER et al., 1997). By understanding the oviposition site preference of *T. absoluta* on the plant and its relationship with *T. pretiosum* parasitism it will be possible to develop a decision threshold incorporating egg parasitism by *T. pretiosum*, such as the one proposed by HOFFMANN et al. (1991) for *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) in tomatoes. Therefore, this study was conducted to determine how *T. pretiosum* exploit the egg distribution of *T. absoluta* on the plant canopy, and the effect of plant morphology on parasitism.

MATERIAL AND METHODS

Plant material and experimental conditions

Processing tomato plants, *Lycopersicon esculentum* Mill. (var. IPA-5), were cultivated in an open-sided greenhouse using pots (20cm height by 20cm diameter) filled with a mixture of soil and humus (4:1) under natural photoperiod of ~ 12h, and temperature of 28±3°C, and relative humidity (RH) of 61±23%. Plants at the beginning of the flowering stage for the experiments with approximately 35cm height from the soil surface and with seven developed leaves were used to run the experiment.

Insect material

A colony of *T. absoluta* was established from insects collected in a tomato (var. IPA-5) commercial plantation in Petrolina (Pernambuco State, Brazil). Adults were maintained in a nylon cage (100 x 40 x 40cm) and fed with a 10% honey solution. Tomato terminal buds and leaves were provided for egg laying and the caterpillars later transferred to fresh leaves. Leaves were kept in water in glass vials, on a tray containing a 1cm layer of sterilised sand, as *T. absoluta* pupation may occur on different parts of the plant but also in the ground. Pupae were then transferred to the adult cages.

Anagasta (=Ephestia) kuehniella (Zeller) (Lepidoptera: Pyralidae) was used as the alternative host for *T. pretiosum* colony maintenance. The colony was established with eggs provided by the Entomology Laboratory of the 'Centro de Ciências Agrárias da Universidade Federal do Espírito Santo', Alegre – Espírito Santo State, Brazil), and maintained using the methodology proposed by TORRES et al. (1995).

A colony of *T. pretiosum* was established with parasitoids provided by Embrapa/Semi-Árido, Petrolina (Pernambuco, Brazil). For the experiments, 1-day-old females were used, after being mated and fed with a droplet of honey inside the rearing vials. All the insect rearing was conducted in the Biological Control laboratory at 'Universidade Federal Rural de Pernambuco' at 26±1.5°C, 50-75% RH, and a 14:10h (L:D) photoperiod.

Effect of host location on parasitism

The experiments were carried out in an open-sided greenhouse under the same environmental conditions as the tomato plants growth. Plants in the flowering stage were isolated in 39 cylindrical cages (80 by 100cm) and covered with organdie cloth. Five to 15 *T. absoluta* moths (ca. 3-days-old) were released at 6:00pm in each cage followed by the release of 15 *T. pretiosum* females at the base of the plant 12h later (ca., in the next morning). Between 24 and 48 hours after parasitoid's release the eggs of *T. absoluta* were counted and mapped according to their position on the plant. To determine egg distribution and its parasitism according to the egg density per plant and plant structure, tomato plants were harvested and transferred to the laboratory for examination. The specific location of eggs was recorded in reference to leaves on the principal stem (leaf 7 to leaf 1 from the bottom to the apex). Following mapping of the eggs, each egg was removed carefully by cutting a small piece of the substratum and kept individually in empty gelatine capsules (size '0' Sintética Distribuidora, SP,

Brazil). Then, they were incubated in the laboratory at $26 \pm 1.5^\circ\text{C}$. Four days after the beginning of the tests the parasitism level by *T. pretiosum* was determined by counting the number of parasitized eggs, recognized by their dark coloration due to the parasitoid developing inside.

Data analysis

The counts of *T. absoluta* eggs and parasitism along the plant axis and among different plant structures were submitted to ANOVA and the means were compared using the Scott-Knott test ($P < 0.05$). The plant structures used as categories for the ANOVA were: plant apex (A); individual leaves from first to seventh (L1-L7); and main stem (MS). The top-most leaf was considered as the first one. However, apex was not considered as factor to analysis since no parasitism was observed on eggs collected in this structure.

The effect of the leaf position along the plant axis and its relation with the number of *T. absoluta* eggs and number of parasitized eggs were assessed using linear regression. All analyses were performed using Statistica 3.2 (STATSOFT, 1993).

RESULTS

The number of *T. absoluta* parasitized eggs was positively correlated with the number of this pest eggs on the plant (Figure 1). The rate of parasitism

ranged from 1.5 to 28% and only occurred on plants with more than six eggs, despite the fact that the egg density varied from 2 to 146 eggs per plant.

Parasitism as a function of plant structure

Both, *T. absoluta* oviposition ($F_{df=8, 883}=6.94$, $P < 0.0001$) and the parasitism ($F_{df=7, 845}=3.67$, $P=0.0007$) by *T. pretiosum* differed among the tomato plant structures (Figure 2A and B). Most *T. absoluta* eggs were located on the leaves, followed by the main stem and apex. No eggs of *T. absoluta* were found on flowering structures in this study. Egg parasitism was observed on all plant structures with moth oviposition, except for host eggs located on the plant apex, which were never parasitized (Figure 2B).

Distribution of the host and its parasitism within plant canopy

Moth oviposition behaviour on leaves showed similar pattern between upper and lower leaf surface and greater than on petioles (Figure 3). *T. absoluta* oviposition was highest on the upper part of the plant, and decreased significantly down the plant axis ($y=38.9-6.28x$, $r=0.60$, $P=0.0012$, $n=814$ eggs). A similar pattern was observed for parasitism rate by *T. pretiosum* ($y=29-4.03x$, $r=0.83$, $P=0.0001$, $n=814$ eggs). Together, these results generated a positive correlation between *T. absoluta* oviposition and parasitism by *T. pretiosum* along the plant axis ($r=0.86$, $P=0.0001$, $n=814$ eggs) (Figure 3).

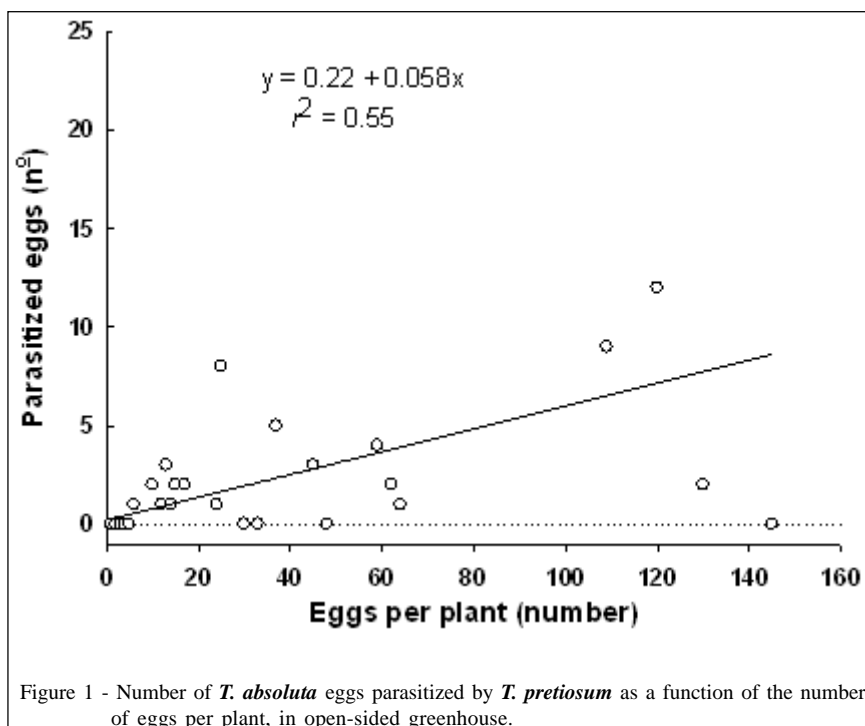
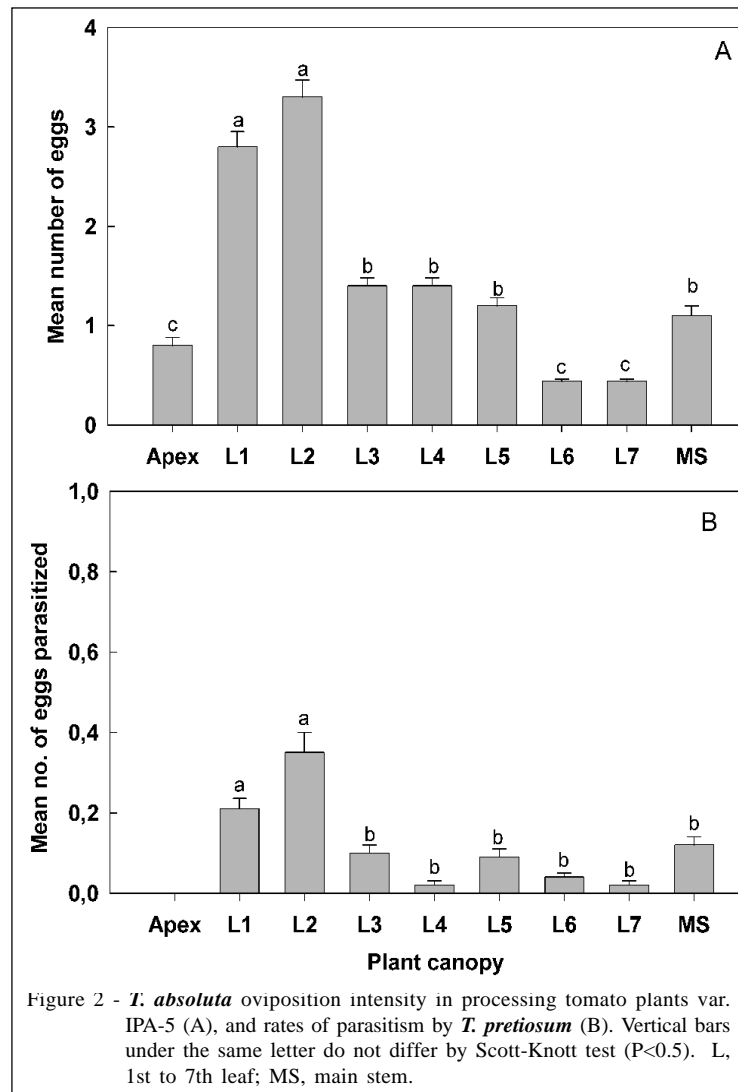


Figure 1 - Number of *T. absoluta* eggs parasitized by *T. pretiosum* as a function of the number of eggs per plant, in open-sided greenhouse.



DISCUSSION

The results show that parasitism by *T. pretiosum* was related to the number of eggs deposited by *T. absoluta* on the different parts of the plant canopy, but that eggs on the plant apex escaped parasitism. The level of oviposition by *T. absoluta* was greatest on the upper third of the plant, a finding that agrees with previous results (LEITE et al., 1999; TORRES et al., 2001). This suggests that selection by the moth of that site provides a better food source for the caterpillars. When considering the different plant structures, the level of oviposition by *T. absoluta* was the same on both the upper and lower leaf surfaces. The same was true for the parasitism rate. A similar pattern is discussed by ROMEIS et al. (1998) for other species of *Trichogramma* on corn, cotton and pigeon pea. YU et al. (1984) reported higher parasitism rates

by *Trichogramma* spp. on the upper leaf surface of apple trees, where most eggs of the host were located. These results suggest that *Trichogramma* adjusts its parasitism to the distribution of the host.

One important plant characteristic known to greatly influence parasitoid performance is the presence of non-glandular and glandular trichomes. This is true especially for small parasitoids like *Trichogramma* spp. that can be trapped by trichome exudates, or have their walking speed reduced on such surfaces (ROMEIS et al., 1998; KENNEDY, 2003). However, *L. esculentum* has primarily non-glandular trichomes (CHANNARAYAPPA et al., 1992), which do not inhibit parasitoid searching behaviour as much as glandular trichomes. Despite this, the apex (buds and young leaves) is the structure with the highest non-glandular trichome densities in *L. esculentum*, and it decreases as leaves expand (WILKENS et al., 1996).

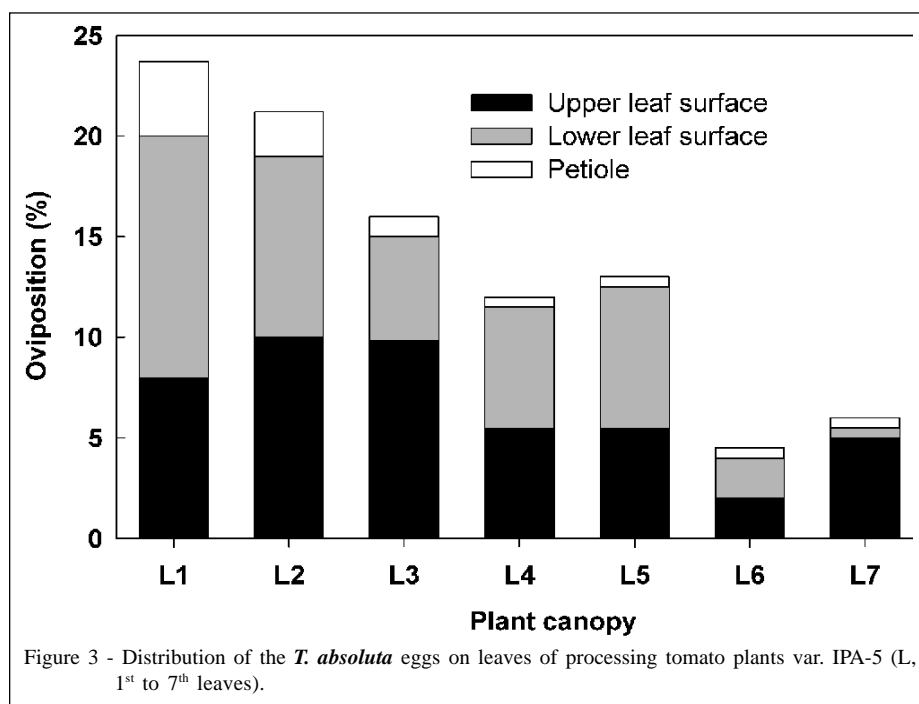


Figure 3 - Distribution of the *T. absoluta* eggs on leaves of processing tomato plants var. IPA-5 (L1 to L7 leaves).

Another indication that high density of non-glandular trichomes impairs the parasitism on this part of the plant is the fact that parasitism did occur on leaves, which had egg numbers similar to those on the plant apex. The fact that the plant apex is less accessible for the parasitoids, suggests that it might be an 'enemy-free' space (PRICE et al., 1980) for the tomato leaf miner.

The fact that the upper third of the plant is the most preferred site for oviposition of both, *T. absoluta* and *T. pretiosum*, makes this parasitoid a good candidate to control the tomato leafminer. As *T. pretiosum* showed some level of aggregative response it will probably be a better control agent in situations of high pest populations as observed with natural parasitism of *T. absoluta* in tomato fields (MIRANDA et al., 2005), because the released parasitoids might not stay in the fields with low host densities. Other complementary strategies aiming to control small populations of *T. absoluta* and targeting the individuals that escape parasitism should also be used. These strategies could be the preservation and implementation of biological control agents that have better success on the plant apex, and particularly predators and parasitoids that would attack the caterpillars escaping from parasitism.

With our data alone it is not possible to determine if the higher parasitism rates on the leaves where most of the oviposition by the pest occurred is due to a density dependent response of the parasitoid to the host, or if *T. pretiosum* concentrates its parasitism

on the upper part of the plant. The results of ABLES et al. (1980) help to clarify this question as they found that when host eggs are equally distributed on cotton plants (lower, middle and upper thirds) and *T. pretiosum* is released on the base of the plant, most of the parasitism is located on the lower and middle thirds of the plant. Based on these results, we can suggest that in our study when the parasitoid females were released at the base of the plant, they first searched for hosts on the lower part of the plant, but as they were scarce, the females moved towards the upper part and remained longer in this area due to the fact that more hosts were encountered. This assumption can be supported by the fact that *T. pretiosum* tends to remain longer in host patches after an oviposition (GROSS et al., 1981). In the context of biological control, the display of an aggregative response is especially desirable from parasitoids used in inoculative biological control. By providing a refuge for hosts, this type of response allows the coexistence of both, host and parasitoid over time. For parasitoids used in inundative biological control, such as *Trichogramma* spp., the coexistence of the host and parasitoid at low density is not essential.

In conclusion, this study showed that *T. pretiosum* is not strongly influenced by the morphology of commercial processing tomato plants. Only the higher density of non-glandular trichomes present on the plant apex could have affected parasitoid behaviour, leaving the host eggs on this structure free of parasitism. Additionally, parasitism was spatially

correlated with the pest oviposition, making this species a strong candidate for the biological control of the tomato leaf miner. The understanding of the pattern of parasitism by *T. pretiosum* on this pest is also important for further development of threshold tables that incorporate egg parasitism, and strategies for the use of complementary methods for the control of *T. absoluta*.

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