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Tomato plant defence activation by *Nesidiocoris tenuis* and persistence of its effects against *Tuta absoluta*

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Abstract

Nesidiocoris tenuis (Reuter) (Hemiptera Miridae) is an omnivorous predator widely used in the control of tomato pests. This zoophytophagous mirid is capable of inducing plant defences in tomato due to its phytophagous behaviour with significant adverse effects on mites and whiteflies. However, the effects of plant defence induced by *N. tenuis* on *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae) have not been searched in detail. In this study, the density threshold of *N. tenuis* nymphs per plant for induction of defence on *T. absoluta* was searched by placing 3, 6 or 10 nymphs on each of the top and bottom leaf of young tomato plants for 4 days. The results showed that oviposition by *T. absoluta* females was significantly reduced on the plants punctured by the highest predator density in comparison to control plants. Further experiments showed that plants punctuated with the highest predator density were more repellent to *T. absoluta* for either period of 7 or 14 days after the removal of predators, compared to control plants. The systemic nature of the induced defences was also confirmed in all periods post treatment. The results offer valuable information in critical aspects of plant defence effects induced by *N. tenuis* on *T. absoluta* such as the number of predators required per plant, their systemic nature and the persistence of the effects induced.

Key words: tomato, systemic effects, oviposition, repellency, defence activation, Miridae, omnivorous.

Introduction

Nesidiocoris tenuis (Reuter) (Hemiptera Miridae) is an effective predator of several key pests of tomato. This predator is often released in protected tomato crops against whiteflies, aphids and the devastating tomato pest *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae) (Urbaneja *et al*., 2012; Zappalà *et al*., 2012; Soares *et al*., 2019). Plant feeding has been recognized as a key factor enhancing *N. tenuis* efficacy in biological control facilitating its populations to persist in the crop in periods of prey scarcity and suppress the pests when they emerge in the crop (Castañé *et al*., 2004; Perdikis *et al*., 2011).

Besides the major benefits of phytophagy to the efficacy of *N. tenuis* in pest control, plant feeding has recently attracted the scientific interest due to the evidence gained that it induces tomato plant defence against insect and mite pests. Plant feeding by *N. tenuis* activates defence pathways related to jasmonic acid (JA) and abscisic acid (ABA) (Pérez-Hedo *et al*., 2021). Tomato plants previously punctured by *N. tenuis* were less attractive to adults of *Bemisia tabaci* (Gennadius) (Hemiptera Aleyrodidae) but more attractive to its parasitoid *Encarsia formosa* Gahan (Hymenoptera Aphelinidae) (Pérez-Hedo *et al*., 2015; 2016; 2021). It has been also reported that *N. tenuis* elicits defensive response of tomato plant causing 35% reduction in the population of *Tetranychus urticae* Koch (Acari Tetranychidae) (Pérez-Hedo *et al*., 2018a). Moreover, survival and oviposition of *T. urticae* were significantly reduced on tomato plants punctured by females of another closely related mirid predator *Macrolophus pygmaeus* (Rambur) (Pappas *et al*., 2015). Sweet pepper plants punctured by adults of *M. pygmaeus* significantly reduced reproduction of the two-spotted spider mite but not that of *Myzus persicae* Sulzer (Hemiptera Aphididae) (Zhang *et al*., 2018).

These encouraging results indicate that defence activation by *N. tenuis* or *M. pygmaeus* may be exploited as a new strategy in the management of pest infestations on tomato plants (Pappas *et al*., 2015; Pérez-Hedo *et al*., 2016; Bouagga *et al*., 2020; Sarmah *et al*., 2021; Silva *et al*., 2021). Towards this direction a fundamental aspect that needs to be considered in relation to plant defence activation is the duration of persistence of the defence effects against pests. Repellence against *B. tabaci* and attraction to the parasitoid *E. formosa* were reported after 7 and 14 days of tomato plants punctured by *N. tenuis*, respectively (Bouagga *et al*., 2018). A significantly reduced oviposition and survival rate of spider mites on tomato plants punctured by *M. pygmaeus* 14 days earlier have been reported, too (Pappas *et al*., 2015).

An additional aspect critical in the evaluation of this pest control method is the potential of these plant defence responses to occur not only at or near the site of plant feeding by *N. tenuis* but throughout the plant, including the plant parts not punctured by the predator as well as the new plant leaves developed post treatment. These responses have been reported as systemic effects and have been considered important and essential for protecting plants by activating the defence before insects relocate to other parts of the plant (Fragoso *et al*., 2014; Erb *et al*., 2019; Malook *et al*., 2019). Systemic defence has been confirmed against *T. urticae* and *B. tabaci* for 14 days and 7 days after plant feeding by *M. pygmaeus* or *N. tenuis*, respectively (Pappas *et al*., 2015; Bouagga *et al*., 2018).

Aiming to exploit the application of the activation of plant defence responses as a sustainable new strategy in pest control it should be considered that *N. tenuis* plant feeding may cause plant injury characterized by the appearance of brown necrotic rings on stems, leaf or flower petioles (Castañe *et al*., 2011; Chinchilla-Ramírez *et al*., 2021). The damage severity depends on the population density of *N. tenuis* per plant (Calvo *et al*., 2009; Chinchilla-Ramírez *et al*., 2021). Therefore, it is essential to determine the lowest density of the predator able to initiate effective defence responses in tomato plants against the target pests, so that to achieve both an effective control level and reduce as much as possible the risk of plant damage.

Although *T. absoluta* is a serious pest of tomatoes worldwide little attention has been paid to the effects of plant defence responses on the performance of this pest. Pérez-Hedo *et al*. (2018b) reported that tomato plants previously punctured by *N. tenuis* were preferred less in comparison to unpunctured plants. Despite being proved as a potential tool for integrated pest management, the duration of the persistence of the defence effects, including their systemic nature and optimum density of *N. tenuis* required per plant for plant defence activation against *T. absoluta* are yet to be studied. This is particularly important for further steps in the development of this method because this pest uses a different feeding mode than all the other pests tested so far and that has been reported as potentially involved in the plant defence effects (Thaler *et al*., 2012; Zhang *et al*., 2018). Therefore, the specific objectives of the present study were to: a) compare the potential of several densities of *N. tenuis* in the defence induction of tomato plant b) assess of the systemic nature and c) evaluate the persistence of the plant defence effects against *T. absoluta*.

Materials and methods

Plant material

Tomato plants (cv Ace 55 and cv Elpida, Spirou House of Agriculture, Athens, Greece), were developed from seeds sown individually in plastic seed trays in the glasshouse. The seedlings were transplanted in plastic pots with compost (Bas Van Burren B.V, Netherlands) substrate. The potted plants were maintained in wooden cages of 75 × 68 × 68 cm kept in a ventilated glasshouse at the Agricultural University of Athens. Plants were kept pest-free and were not sprayed with any pesticide.

Insects rearing

T. absoluta rearing was initiated from adults collected from a tomato crop located in Marathon, Greece and maintained on tomato plants (cv. Elpida). Rearing of *N. tenuis* (Nesibug, Koppert, The Netherlands) was maintained on tomato plants (cv. Elpida) with "Entofood" (Koppert B.V., The Netherlands), a mixture of *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) eggs and dried cysts of *Artemia* sp. offered *ad libitum*.

Tomato plant defence activation by *N. tenuis*

Five-week-old tomato plants (cv. Ace 55) with three fully expanded leaves were used in the experiments. The plant defence activation was investigated using third- instar nymphs of *N. tenuis* (less than 12 hours in that instar). Before their use, the nymphs were deprived of prey for 2 hours in dishes with tomato leaflets. On each bottom and the top leaf of each plant nymphs were placed at the following densities: 1) three, 2) five and 3) ten. No food for *N. tenuis* was added and each leaf was enclosed into an organdy bag (12 × 15 cm). The middle leaf of each plant was also enclosed in organdy bag but without any predator inside. Each leaf of the control (unpunctured) tomato plants was caged individually without any predator. All the experiments were conducted at 25 ± 1 °C, 65 ± 5% RH, and photoperiod of 16:8 L:D. After four days of exposure, the predators were removed and their survival was found to be always higher than 80%. The plant damage caused by the predators was recorded as a surrogate of their damage potential by counting the brown necrotic rings in bottom and top leaves of each punctured tomato plant. Ten replications (plants) were used for each predator density and control and the experiments were repeated twice in 2 months interval.

Predator's density effects on life traits of *T. absoluta* through plant defence induction

After the removal of the predators (0-day post treatment, 0-dpt), a *N. tenuis*-punctured plant was introduced in a cage (60 × 35 × 35 cm) (BioQuip CA, USA) together with an unpunctured (control) tomato plant. Then, three pairs of *T. absoluta* adults (less than 48 hours old) were introduced into the cage and were allowed to oviposit for the next 24 hours. *T. absoluta* adults were provided with sugar solution (10% sucrose) via a piece of cotton in one plastic cup (30 mL) placed inside the cage. After 24 hours, the number of eggs oviposited on each leaf of both plants was recorded. Experiments were conducted at 25 ± 1 °C, 65 ± 5% RH, and photoperiod of 16:8 L:D. Ten replications (cages) were used for each predator density and the experiments were repeated twice in 2 months intervals. In addition, on 0-dpt, the time required by newly emerged 1st instar larvae of *T. absoluta* to initiate tunnelling was also recorded on the bottom, middle and top leaf of plants punctured by the highest density of *N. tenuis*, as described above. This was done by placing an egg with a ready to hatch larva on bottom, middle and top leaf and recording under stereomicroscope the time required by the emerged larva to insert its cephalic capsule in the leaf tissue and initiate a mine, at 25.0 ± 2.0 °C. Ten replications (larvae) were used per leaf category for the punctured and unpunctured (control) plants. The experiments were repeated twice in 2 months interval.

Persistence of the plant defence induction effects

The oviposition preference of *T. absoluta* was further tested on plants punctured by the highest nymphal density (10 nymphs of *N. tenuis* enclosed per bottom and top leaf), 7 and 14-dpt to record the persistence of the induced defence against *T. absoluta*. For this reason, the same procedure was followed as above described and at the same conditions, but after the removal of the predators at 0-dpt, the punctured plants were maintained at 25 ± 1 °C, 65 ± 5% RH, and 16:8 L:D photoperiod and kept without exposure to any insect for either 7-dpt or 14-dpt. After 7-dpt, the plants had four fully expanded leaves. Three pairs of *T. absoluta* adults (less than 48 hours old) were introduced into a cage with a punctured and an unpunctured plant and were allowed to oviposit for the next 24 hours. After this period, the number of eggs oviposited on each leaf of both plants was recorded. Similarly, another group of plants 14-dpt was exposed to the pest, under the same conditions. Fourteen days post treatment the plants had five fully expanded leaves. Ten replications (cages) were used for each post treatment period. The experiments were repeated twice in 2 months interval.

Statistical analysis

The data of the number of necrotic rings produced by *N. tenuis* per leaf at 0-dpt were analysed with a 2-way ANOVA with the "predator density" and the "leaf position, bottom vs top tomato leaves", and their interaction as fixed factors, the "time interval" as random factor and aiming to control for plant and leaf position variation we included in the model "plant" × "leaf position" as nested random effects. The data of the total number of eggs laid per plant by each *T. absoluta* female at 0-dpt were analysed by fixed factors the "predator density" and the "treatment (punctured or control plant)", including the "time interval" as a random factor. The data of the number of *T. absoluta* eggs laid per leaf at 0-dpt were analysed with a 3-way ANOVA with factors the "predator density", the "treatment" (punctured and unpunctured plant) and the "leaf position" (bottom, middle or top leaf). The "time interval" and "plant" were included as random factors.

The time required by each *T. absoluta* larva to initiate tunnel mining in plants punctured by the highest predator density were analysed with factors the "leaf position" and the "treatment" after data were $\log(x+1)$ transformed. In the model, the "time interval" as a random factor and "plant" × "leaf position" as nested random effects were included.

The data on the total number of eggs laid per cage with a control and a punctured plant on 0-dpt, 7-dpt and 14-dpt were analysed with fixed factor "period post treatment, i.e. 0-dpt, 7-dpt and 14-dpt" and with random factors the "time interval" and the "cage". The data on the number of eggs laid per plant were analysed with factors the treatment (punctured or control plant) and the period after treatment (0, 7, 14-dpt), with random factor the "time interval". The number of eggs laid in plants either 7-dpt or 14-dpt were analysed with factors the "leaf position" and the "treatment". In the model, "time interval" as a random factor and "plant" × "leaf position" as nested random effects were included.

In all cases the means were compared using the Tukey's HSD test ($\alpha = 0.05$). All the analyses were performed by the statistical package JMP 14.1.0. (SAS Institute, 2016).

Results

Damage caused on tomato plants by *N. tenuis*

The effect of the "predator density" ($F_{2,59} = 70.04$, $P < 0.0001$), the "leaf position" (bottom vs top tomato leaves) ($F_{1,59} = 29.12$, $P < 0.0001$), and their interaction ($F_{2,59} = 29.12$, $P < 0.0001$) were significant on the number of necrotic rings recorded per leaf. The interaction was due to the fact that the mean number of necrotic rings was significantly higher in the top than the bottom leaves at the highest predator density (i.e., 20 *N. tenuis* per plant in total), however, at the lower predator densities there was no significant difference (figure 1).

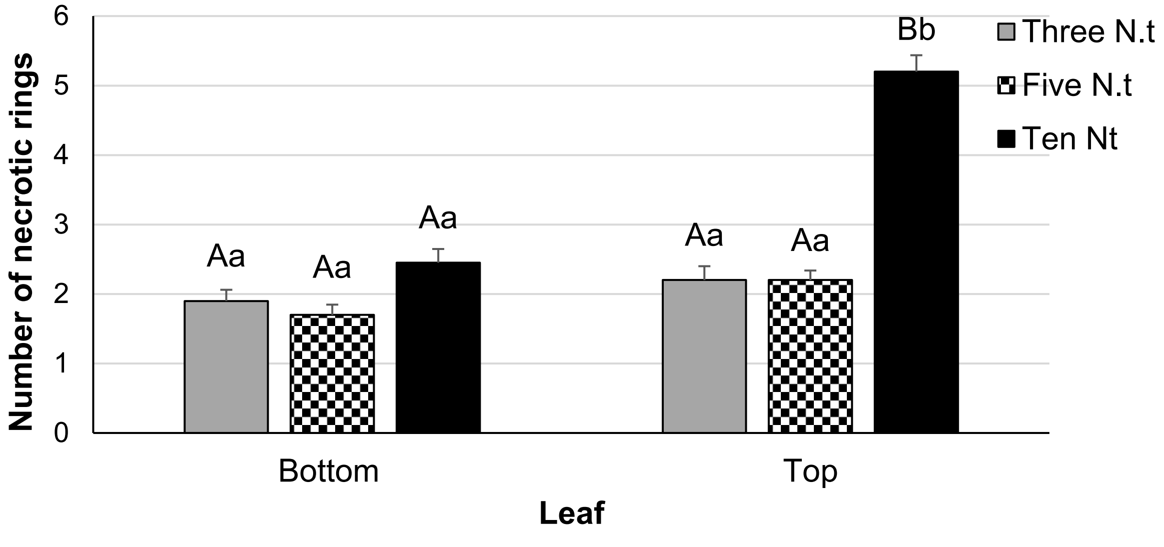

Figure 1. Number (mean ± SE) of necrotic rings produced by different densities of *N. tenuis* third- instar nymphs enclosed on L1 (bottom) and L3 (top) leaves of tomato plants for 4 days. Three, five or ten nymphs of *N. tenuis* had been enclosed for 4 days on each bottom and top leaf of each plant, while middle leaves remained without *N. tenuis*. Columns with the same capital letter are not significantly different between leaves within each predator density treatment, and columns followed by the same small letter are not significantly different among treatments within each leaf category (Tukey HSD, $P < 0.05$).

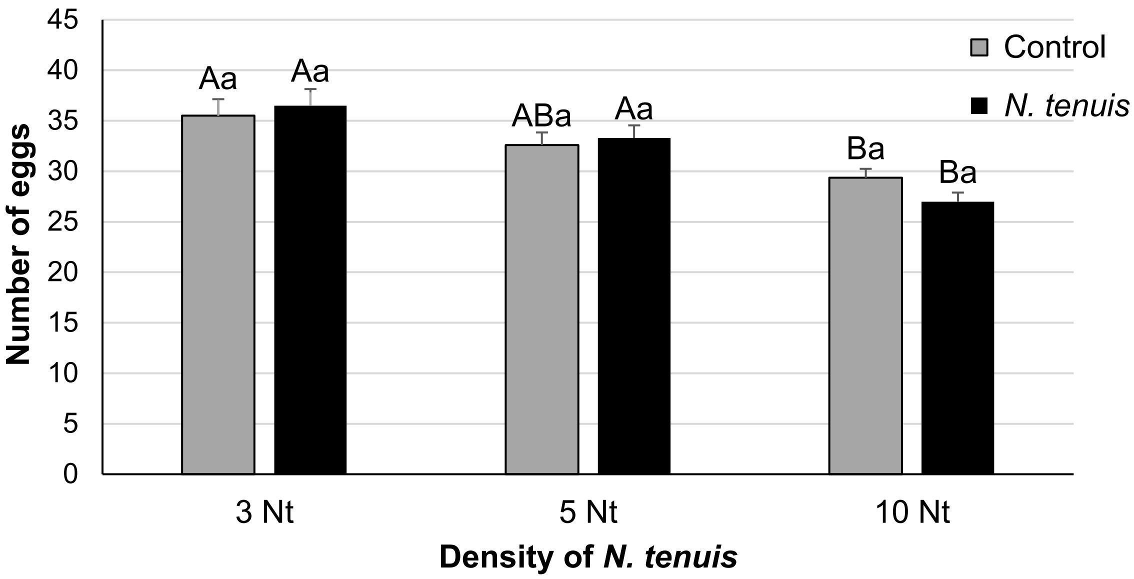

Figure 2. Number (mean ± SE) of eggs per female of *T. absoluta* oviposited on tomato plants punctured by different densities of *N. tenuis* in comparison to unpunctured (control) tomato plants at 0-dpt. Three, five or ten nymphs of *N. tenuis* had been enclosed for 4 days on each bottom and top leaf of each plant, while middle leaves remained without *N. tenuis*. On the leaves of the control plants no *N. tenuis* had been enclosed. Columns with the same capital letter are not significantly different among predator density levels at each treatment and columns followed by the same small letter are not significantly different between treatments at each predator density (Tukey HSD, $P < 0.05$).

Oviposition preference of *T. absoluta* between unpunctured and punctured tomato plants by different *N. tenuis* densities

According to the statistical analysis, the total number of eggs laid per plant by each *T. absoluta* female at 0-dpt was significantly affected only by the predator density ($F_{2,113} = 18.73$, $P < 0.001$). The number of eggs laid on either the punctured or the unpunctured plant was significantly reduced at the highest density in comparison to that recorded at the lowest predator density (figure 2). The number of eggs laid was not different between the punctured and the unpunctured plant in each predator density level.

Regarding leaf oviposition preference of *T. absoluta* females at 0-dpt, a significant interaction was recorded among the factors "predator density" (i.e. *N. tenuis*-punctured with 6, 10 or 20 nymphs and control) and "leaf position" (bottom vs middle vs top leaf) ($F_{4,332} = 4.32$, $P < 0.002$). In all cases a significantly higher number of eggs had been oviposited on the top in comparison to the middle and the bottom leaf. Comparisons showed that a significantly reduced number of eggs of *T. absoluta* laid in the top leaves when plants were punctured by 20 third-instar nymphs of *N. tenuis* as compared to the corresponding leaf of unpunctured plants (figure 3).

Time required for larval tunneling initiation of *T. absoluta* on *N. tenuis*-punctured and unpunctured plants

The time required by a newly hatched first- instar larva of *T. absoluta* at 0-dpt to locate a suitable location on the tomato leaflet to insert its cephalic capsule and initiate feeding on plants exposed to 20 *N. tenuis* nymphs was significantly affected by the factor "treatment" ($F_{1,104} = 156.57$, $P < 0.0001$). The amount of time required on bottom, middle and top leaf of unpunctured plants was always significantly lower than that in the punctured plants and did not differ significantly among leaves in the punctured or the unpunctured plants (figure 4).

Persistence of the plant induction effects

The total number of eggs of *T. absoluta* laid per cage on both punctured and control plant by 20 *N. tenuis* nymphs was significantly affected by the "period post treatment" ($F_{2,47} = 38.11$, $P < 0.001$). It was higher on 0-dpt than the longer periods post treatment, without significant difference between the latter (57.85 ± 0.74, 48.80 ± 0.89 and 48.95 ± 0.83 eggs laid per cage on 0, 7, 14-dpt, respectively).

The total number of eggs laid on each plant (punctured or unpunctured) in the cage on 0-dpt, 7-dpt and 14-dpt, was affected by a significant interaction between "treatment" and "post treatment period" ($F_{2,113} = 4.92$, $P < 0.009$) whereas the main effects of "treatment" and "post treatment period" were significant, too ($F_{1,113} = 206.41$, $P < 0.0001$ and $F_{2,113} = 40.35$, $P < 0.0001$, respectively) (figure 5). In all periods, the number of eggs laid on the control plant was significantly higher than in the punctured plant and the respective percentage of reduction was 15%, 26% and 28%. The number of eggs laid on 7-dpt and 14-dpt on either the control or the punctured plants, was significantly reduced in comparison to the respective number laid on 0-dpt.

The factor "treatment" (i.e. 20 nymphs of *N. tenuis* or without nymphs), the "leaf position" and their interaction

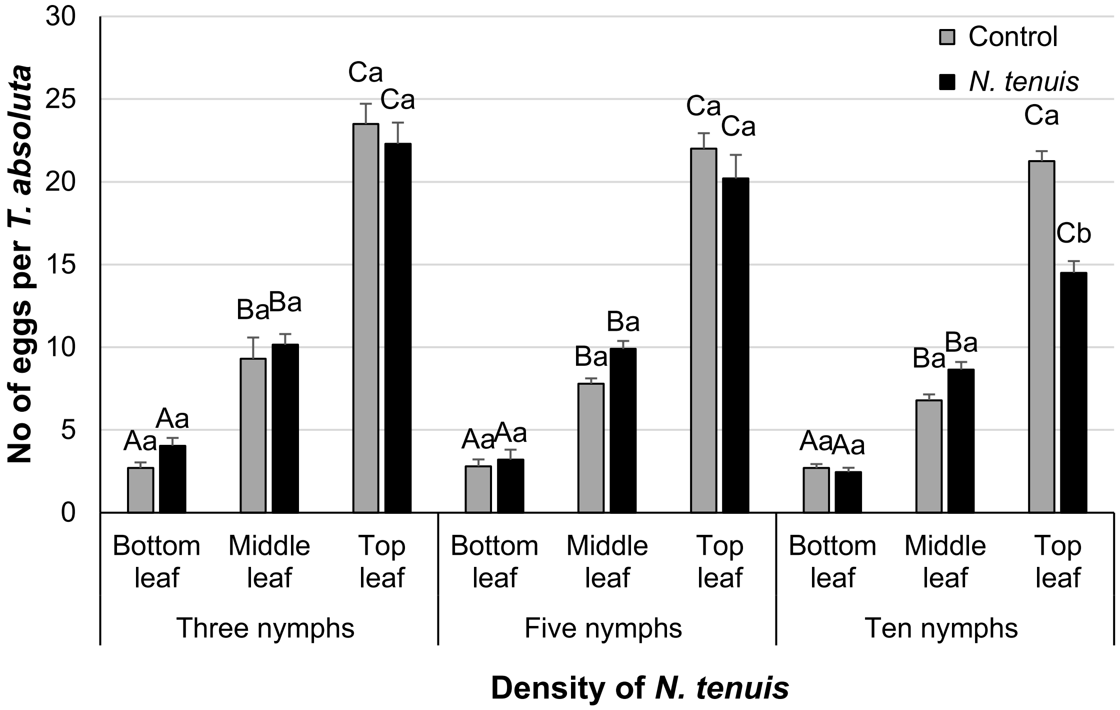

Figure 3. Number (mean ± SE) of eggs per female of *T. absoluta* oviposited on tomato plants punctured with different densities of *N. tenuis* in comparison to unpunctured tomato plants on bottom, middle and top leaves of tomato. Three, five or ten nymphs of *N. tenuis* had been enclosed for 4 days on each bottom and top leaf of each plant, while middle leaves remained without *N. tenuis*. On the leaves of the control plants no *N. tenuis* had been enclosed. Columns followed by the same capital letter are not significantly different among leaves in each treatment (i.e. density of *N. tenuis*) and columns followed by the same small letter are not significantly different between treated and control plants in each leaf category within each treatment (Tukey HSD, $P < 0.05$).

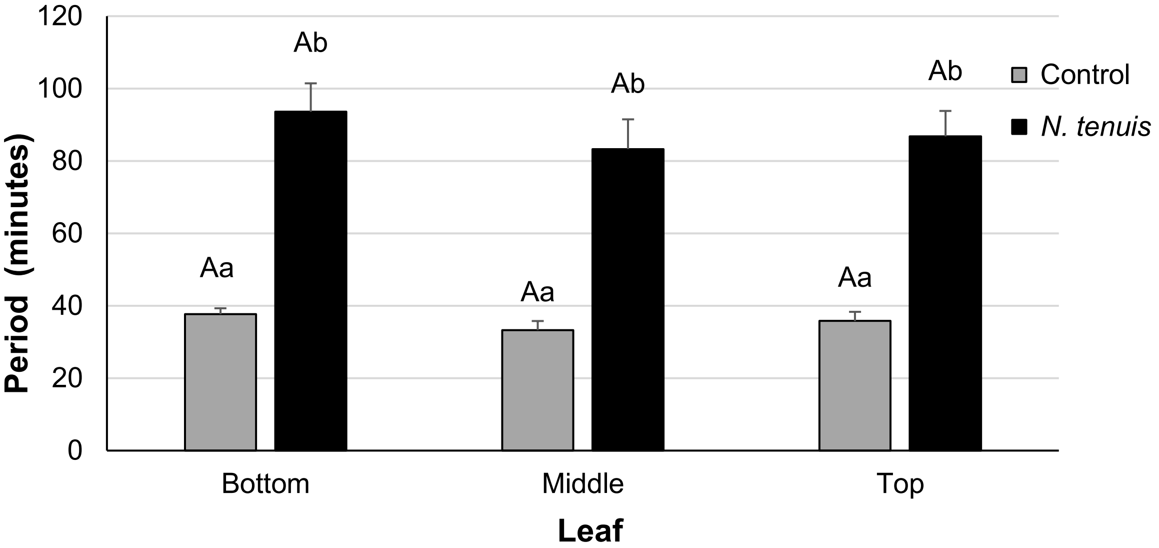

Figure 4. The length of the period required (mean ± SE) by 1st instar larvae of *T. absoluta* to initiate tunnel mining on punctured by *N. tenuis* tomato plants in comparison to unpunctured tomato plants. Ten nymphs of *N. tenuis* had been enclosed for 4 days on each bottom and top leaf of the punctured plants, while the middle leaf remained without *N. tenuis*. On the leaves of the control plants no *N. tenuis* had been enclosed. Columns with the same capital letter are not significantly different among bottom, middle and top leaves at each treatment, and columns followed by the same small letter are not significantly different between treatments within each leaf category (Tukey HSD, $P < 0.05$).

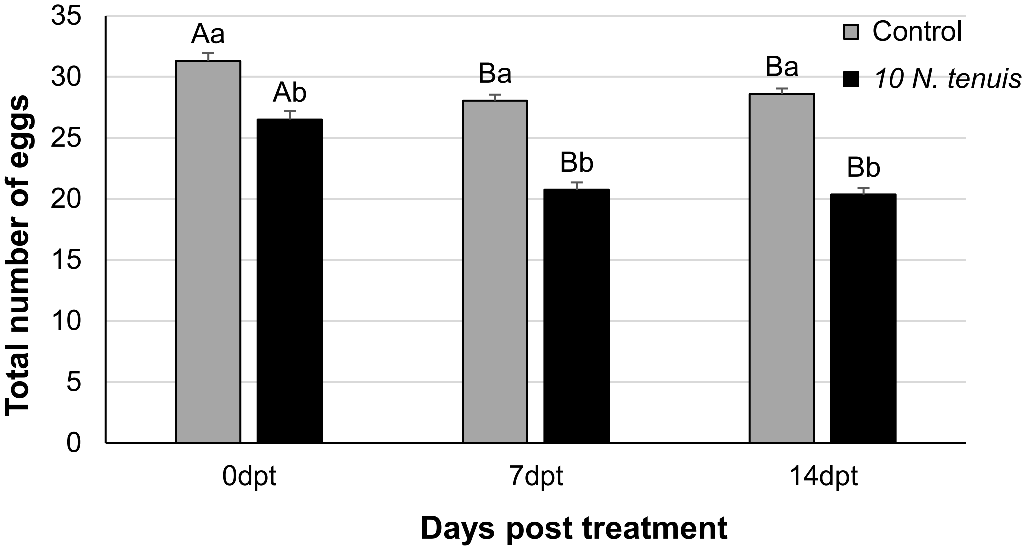

Figure 5. Number (mean ± SE) of *T. absoluta* eggs oviposited on a tomato plant at 0, 7 and 14-dpt after punctured by *N. tenuis* and a control (unpuctured) plant, when placed together in a cage. Ten nymphs of *N. tenuis* had been enclosed for 4 days on each bottom and top leaf of the punctured plants, while the middle leaf remained without *N. tenuis*. On the leaves of the control plants no *N. tenuis* had been enclosed. Columns with the same capital letter are not significantly different among the three post treatment intervals within each treatment (puntured or unpunctured plant) and columns followed by the same small letter are not significantly different between the two treatments within each time interval (Tukey HSD, $P < 0.05$).

had significant effects on persistence of plant defence after one week period from predators' removal ($F_{1,115}$ = 122.86, $P < 0.0001$, $F_{3,115}$ = 207.79, $P < 0.0001$ and $F_{3,115}$ = 18.15, $P = 0.001$, respectively). Significantly reduced number of eggs was laid by *T. absoluta* on top (L4) and L3 leaf of punctured plants in comparison to their corresponding leaves of unpunctured plants. There was no significant difference in number of eggs laid on the bottom or middle leaves (L1 or L2) (table 1).

The factor "treatment" (i.e. 20 nymphs of *N. tenuis* or without nymphs) and the "leaf position" had significant effect on persistence of resistance after two-week time lag and their interaction was also significant ($F_{1,144}$ = 113.18, $P < 0.0001$, $F_{4,144}$ = 396.44, $P < 0.0001$ and $F_{4,144}$ = 16.77, $P < 0.0001$). The number of eggs laid was significantly higher on the top leaves in both punctured and unpunctured plants. Significantly reduced number of eggs was laid on the L3, L4 and L5 (top) leaves in comparison to their corresponding leaves of unpunctured plant (table 2).

Table 1. Number of eggs laid by *T. absoluta* (mean ± SE) on each leaf of tomato plants punctured by *N. tenuis* 7-days earlier, in comparison to unpunctured plants. Ten nymphs of *N. tenuis* had been enclosed for 4 days on each L1 (bottom) and L3 (top) leaves of the plants, while L2 (middle) leaves remained without *N. tenuis*. The newly grown leaves were designated as L4 which were also not punctured by *N. tenuis.* Means followed by the same capital letter are not significantly different in a row and means followed by the same small letter are not significantly different in each column (Tukey HSD, $P < 0.05$).

Treatment	Leaf position			
	L1	L2	L3	L4
Unpunctured plant	2.82 ± 0.24 Aa	3.05 ± 0.26 Ba	9.40 ± 0.38 Ca	13.35 ± 0.41 Da
Punctured plant by *N. tenuis*	1.75 ± 0.20 Aa	2.61 ± 0.25 Ba	7.55 ± 0.36 Cb	10.60 ± 0.33 Db

Table 2. Number of eggs laid by *T. absoluta* (mean ± SE) on each leaf of tomato plants punctured by *N. tenuis* 14-days earlier, in comparison to unpunctured plants. Ten nymphs of *N. tenuis* had been enclosed for 4 days on each L1 (bottom) and L3 (top) leaves of the plants, while L2 (middle) leaves remained without *N. tenuis*. The newly grown leaves were designated as L4 and L5 which were also not punctured by *N. tenuis.* Means followed by the same capital letter are not significantly different in a row and means followed by the same small letter are not significantly in each column (Tukey HSD, $P < 0.05$).

Treatment	Leaf position				
	L1	L2	L3	L4	L5
Unpunctured plant	0.65 ± 0.16 Aa	1.5 ± 0.18 Ba	5.4 ± 0.35 Ca	7.95 ± 0.33 Da	12.0 ± 0.36 Ea
Punctured plant by *N. tenuis*	0.85 ± 0.16 Aa	1.05 ± 0.15 Ba	3.95 ± 0.25 Cb	6.75 ± 0.21 Db	10.15 ± 0.36 Eb

Discussion

The results showed that the defence effects of tomato plants induced by *N. tenuis* on *T. absoluta* were dependent on the predator density per plant. Tomato plants punctured by 6 or 10 third-instar nymphs in total, for 4 days were not able to repel adults of *T. absoluta*. However, *T. absoluta* females laid significantly reduced number of eggs on tomato plants punctured by 20 nymphs of *N. tenuis*. Previous studies showed that tomato plants punctured by 20 adults of *N. tenuis* for 24 hours induced defence against *B. tabaci* or *T. urticae* (Naselli *et al.*, 2016; Pérez-Hedo *et al.*, 2018). Pappas *et al.* (2015) reported significantly lower oviposition and survival rate of spider mites on tomato plants punctured by 2 females of *M. pygmaeus* for 4 days, but such an effect was not recorded in the case of whiteflies. Zhang *et al.* (2018) showed that on tomato plants punctured by 10 adults of *M. pygmaeus* for 4 days, *T. urticae* reproduction was significantly reduced but this was not observed for *M. persicae*. These results show that plant defence responses against different pests may be dependent on the pest species under study, the density of the predator and the period of predators' presence on the plant. Our results showed that a higher number of *N. tenuis* may be required for the induction of plant defence against *T. absoluta* compared to that against mites or whiteflies. This could be associated with different feeding mode of *T. absoluta* in comparison to mites or whiteflies and suggests that species specific studies should be undertaken.

The adverse effects on the oviposition of *T. absoluta* recorded at 14-dpt after the exposure of plants to the predator, indicate a long persistence of the mediated response effects. Pérez-Hedo *et al.* (2018) showed that at 14-dpt the number of *T. urticae* individuals was reduced by 35% on tomato plants punctured by twenty fourth-instar nymphs of *N. tenuis* for 24 hours. Pappas *et al.* (2015) showed that the number of eggs laid and the survival of spider mites were adversely affected at 14-dpt by 41% and 20% respectively, when two young females of *M. pygmaeus* had been used per plant for a period of 4 days. Bouagga *et al.* (2018) using a Y-tube olfactometer assay showed that sweet pepper plants punctured by 25 *N. tenuis* adults per plant for 24 hours significantly repelled *B. tabaci* 7 days after exposure of the plant to the predator but this effect was raised 14-dpt. Therefore, it seems that the persistence of the effects depends on the target pest species, the predator density and the length of the period of plant exposure to predators. In our study, the adverse effects on the oviposition of *T. absoluta* were comparable at 7-dpt and 14-dpt indicating that may persist over longer periods of time and future research therefore should be directed at evaluating further the persistence of the defence responses.

Our results provide evidence that the persistence of plant defence responses against *T. absoluta* occur not only on the leaves punctured by the predator but also on neighbouring unpunctured leaves and new leaves. This persistence of the systemic defensive responses may have important practical implications due to the effective protection against *T. absoluta* on entire plants, including their new leaves. Pappas *et al.* (2015) reported analogous to our results systemic adverse effects on the oviposition and survival of *T. urticae* on tomato plants exposed to only two virgin females of *M. pygmaeus* for a period of 4 days. These effects lasted for a similarly long post treatment period (14-dpt). It is likely that systemic defence response and its persistence against different pest species may be associated with different predator density and the period of the plant exposure to predators.

The period required for a newly hatched larva of *T. absoluta* to mine into leaves was significantly increased at 0-dpt in all the leaves of a punctured compared to an unpunctuated tomato plant. Such an effect may increase larval susceptibility to predation or insecticides indicating another aspect of the protective effect of the plant defence activation by *N. tenuis* (Sarmah *et al.*, 2021). In addition, considering that the middle leaf was not punctured by the predator, this confirms further the effective systemic plant defence responses against young larvae of *T. absoluta*. It was not included in the aims of this paper to investigate the mechanisms responsible for the adverse effects recorded on the oviposition or tunnelling of *T. absoluta*. However, in another study of our group (Sarmah *et al.* submitted), the expression of the defence-related genes and metabolomics showed that the JA defence pathway had been activated on tomato plants punctured by *N. tenuis*.

The number of eggs laid by *T. absoluta* females on an unpunctured (control) plant was significantly reduced at the 7-dpt and 14-dpt compared to that recorded at the 0-dpt, in a cage where it was placed together with a punctured plant. Similarly, the total number of eggs laid per cage with one punctured and one unpunctured plant was reduced at 7-dpt and 14-dpt. These findings suggest that the induced defence effects produced by plant feeding of *N. tenuis* may provide protection to neighbouring tomato plants increasing the potential of this method in pest control. This supports the results of Pérez-Hedo *et al.* (2021) reported that the release of herbivore induced plant volatiles (HIPV) in greenhouse environment could activate defence mechanisms in healthy tomato plants. However, specific research is required to clarify further whether a defence activated tomato plant can activate defences in neighbouring healthy plants and the mechanisms involved.

The density of 20 *N. tenuis* nymphs per plant, where plant defence effects against *T. absoluta* were recorded, produced 7.65 necrotic rings per plant after 4 days of exposure. This finding is in accordance with results reported by Urbaneja-Bernat *et al.* (2019) (i.e. 7.8 necrotic rings per 30 cm high plant exposed to twelve third instar nymphs of *N. tenuis* for seven days). Further research is needed to determine whether these effects may cause significant adverse effects on plant growth. Alternatively, persistence of plant defence responses induced by spraying with metabolites or by releasing volatiles may be investigated, too (Esmaeily *et al.*, 2020; Pérez-Hedo *et al.*, 2021).

The present study explored the density level of the predator *N. tenuis* required to induce tomato plant defence response against the serious tomato pest *T. absoluta*, and the persistence of the protective effects produced. The results showed that the effects depend on the density of the predator, are systemic and persisted at least as long as 14-dpt. Therefore, plant defence induced by plant feeding of *N. tenuis* may have a long persistence and should be assessed in the control of *T. absoluta*. Thus, towards a more sustainable management of *T. absoluta*, further studies are required to explore the persistence of plant defence effects for longer periods including effects on other biological traits of *T. absoluta* such as its development or reproduction, under laboratory and field conditions.

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References

Bouagga S., Urbaneja A., Rambla J. L., Flors V., Granell A., Jaques J. A., Pérez-hedo M., 2018.- Zoophytophagous mirids provide pest control by inducing direct defenses, antixenosis and attraction to parasitoids in sweet pepper plants.- *Pest Management Science*, 74: 1286-1296.

Bouagga S., Urbaneja A., Depalo L., Rubio L., Pérez-hedo M., 2020.- Zoophytophagous predator-induced defenses restrict accumulation of the tomato spotted wilt virus.- *Pest Management Science*, 76: 561-567.

Calvo J., Bolckmans K., Stansly P. A., Urbaneja A., 2009.- Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato.- *BioControl*, 54: 237-246.

Castañé C., Alomar Ò., Goula M., Gabarra R., 2004.- Colonization of tomato greenhouses by the predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*.- *Biological Control*, 30: 591-597.

Castañé C., Arnó J., Gabarra R., Alomar O., 2011.- Plant damage to vegetable crops by zoophytophagous mirid predators.- *Biological Control*, 59: 22-29.

Chinchilla-ramírez M., Garzo E., Fereres A., Gavara-Vidal J., Ten Broeke C. J., Van Loon J. J., Urbaneja A., Pérez-Hedo M., 2021.- Plant feeding by *Nesidiocoris tenuis*: quantifying its behavioral and mechanical components.- *Biological Control*, 152: 104402.

Erb M., Reymond P., 2019-. Molecular interactions between plants and insect herbivores.- *Annual Review of Plant Biology*, 70: 527-557.

Esmaeily S., Amin Samih M., Izadi H., 2020.- Induced eggplant resistance against *Trialeurodes vaporariorum* triggered by jasmonic acid, abscisic acid, and *Nesidiocoris tenuis* feeding.- *Bulletin of Entomological Research*, 110: 285-292.

Fragoso V., Rothe E., Baldwin I., Kim S-G., 2014.- Root jasmonic acid synthesis and perception regulate folivore-induced shoot metabolites and increase *Nicotiana attenuata* resistance.- *New Phytologist*, 202: 1335-1345.

Malook S., Qi J., Hettenhausen C., Xu Y., Zhang C., Zhang J., Lu C., Li J., Wang L., Wu J., 2019.- The oriental armyworm (*Mythimna separata*) feeding induces systemic defence responses within and between maize leaves.- *Philosophical Transactions Royal Society B*, 374: 20180307.

Naselli M., Urbaneja A., Siscaro G., Jaques J. A., Zappalà L., Flors V., Pérez-Hedo M., 2016.- Stage-related defense response induction in tomato plants by *Nesidiocoris tenuis*.- *International Journal of Molecular Sciences*, 17: 1210.

Pappas M. L., Steppuhn A., Geuss D., Topalidou N., Zografou A., Sabelis M. W., Broufas G. D., 2015.- Beyond predation: the zoophytophagous predator *Macrolophus pygmaeus* induces tomato resistance against spider mites.- *PLoS ONE*, 10: 0127251.

Perdikis D., Fantinou A., Lykouressis D., 2011.- Enhancing pest control in annual crops by conservation of predatory Heteroptera.- *Biological Control*, 59: 13-21.

Perez-Hedo M., UrbanejA A., 2016.- The zoophytophagous predator *Nesidiocoris tenuis*: a successful but controversial biocontrol agent in tomato crops, pp. 121-138. In: *Advances in insect control and resistance management* (Horowitz A., Ishaaya I., Eds).- Springer, Cham, Switzerland.

Pérez-Hedo M., Bouagga S., Jaques J. A, Flors V., Urbaneja A., 2015.- Tomato plant responses to feeding behavior of three zoophytophagous predators (Hemiptera: Miridae).- *Biological Control*, 86: 46-51.

Pérez-Hedo M., Arias-Sanguino Á., Urbaneja A., 2018a.- Induced tomato plant resistance against *Tetranychus urticae* triggered by the phytophagy of *Nesidiocoris tenuis*.- *Frontiers of Plant Science*, 9: 1419.

Pérez-Hedo M., Rambla J. L., Granell A., Urbaneja A., 2018b.- Biological activity and specificity of Miridae-induced plant volatiles.- *BioControl*, 63: 203-213.

Pérez-Hedo M., Riahi C., Urbaneja A., 2021.- Use of zoophytophagous mirid bugs in horticultural crops: current challenges and future perspectives.- *Pest Management Science*, 77: 33-42.

Sarmah N., Kaldis A., Taning C. N., Perdikis D., Smagghe G., Voloudakis A., 2021.- dsRNA-mediated pest management of *Tuta absoluta* is compatible with its biological control agent *Nesidiocoris tenuis*.- *Insects*, 12: 274.

SAS Institute, 2016.- *JMP version 14.0*.- SAS Institute Inc., Cary, NC, USA.

SilvA D. B., Urbaneja A., Pérez-Hedo M., 2021.- Response of mirid predators to synthetic herbivore-induced plant volatiles.- *Entomologia Experimentalis et Applicata*, 169: 125-32.

Soares M. A., Campos M. R., Passos L. C., Carvalho G. A., Haro M. M., Lavoir A. V., Biondi A., Zappalà L., Desneux N., 2019.- Botanical insecticide and natural enemies: a potential combination for pest management against *Tuta absoluta*.- *Journal of Pest Science*, 92: 1433-1443.

Thaler J. S., Humphrey P. T., Whiteman N. K., 2012.- Evolution of jasmonate and salicylate signal crosstalk.- *Trends in Plant Science*, 17: 260-270.

Urbaneja A., Gonzalez-Cabrera J., ARnó J., Gabarra R., 2012.- Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin.- *Pest Management Science*, 68: 1215-1222.

Urbaneja-Bernat P., Bru P., González-Cabrera J., Urbaneja A., Tena A., 2019.- Reduced phytophagy in sugar-provisioned mirids.- *Journal of Pest Science*, 92: 1139-1148.

Zappalà L., Bernardo U., Biondi A., Cocco A., Deliperi S., Delrio G., Giorgini M., Pedata P., Rapisarda C., Tropea Garzia G., Siscaro G., 2012.- Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy.- *Bulletin of Insectology*, 65: 51-61.

Zhang N. X., Messelink G. J., Alba J. M., Schuurink R. C., Kant M. R., Janssen A., 2018.- Phytophagy of omnivorous predator *Macrolophus pygmaeus* affects performance of herbivores through induced plant defenses.- *Oecologia*, 186: 101-113.

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