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Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato

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Abstract

An early release system developed for *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) could provide a good control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato. *Tuta absoluta* and the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) often appear simultaneously in tomato crops and this might affect control capacity. Therefore, the new approach needs to be tested in a situation with both pests present. In addition, *Bacillus thuringiensis* Berliner and *Trichogramma achaeae* Nagaraja & Nagarkatti (Hymenoptera: Trichogrammatidae) have been shown to be effective against *T. absoluta* and could be a supplement to *N. tenuis*. Two experiments were carried out to evaluate the potential of this approach and its combination with supplementary control agents against *T. absoluta*. In the first experiment four treatments were compared (*T. absoluta*, *B. tabaci*, *T. absoluta* + *N. tenuis*, and *T. absoluta* + *B. tabaci* + *N. tenuis*) and *N. tenuis* was able to control *T. absoluta* and *B. tabaci* either alone or together. In the second experiment, five treatments were compared: *T. absoluta*, *T. absoluta* + *N. tenuis*, *T. absoluta* + *N. tenuis* + *T. achaeae*, *T. absoluta* + *N. tenuis* + *B. thuringiensis*, and *T. absoluta* + *N. tenuis* + *T. achaeae* + *B. thuringiensis*. *Nesidiocoris tenuis* again proved capable of significantly reducing *T. absoluta* populations, and the implementation of additional agents did not increase its effectiveness.

Introduction

The tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), one of the most devastating pests of tomato in South America (Miranda et al., 1998), was first detected in Europe in Spain at the end of 2006 and it has spread quickly thereafter throughout the Mediterranean Basin (Desneux et al., 2010), causing very serious damage to tomato crops.

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) is a key pest of tomato worldwide, in part due to its role in transmission of plant viruses (Jones, 2003). Although resistant cultivars offer a partial solution to this problem, *B. tabaci* is a pest in its own right, by debilitating the plant, producing honeydew that serves as a substrate of sooty mould, and inducing the physiological disorder of tomato irregular ripening (Schuster, 2001). Therefore, suppression of *B. tabaci* is a high order objective in most greenhouse tomato production. The mirid bug *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) commonly appears in tomato and other agricultural crops and natural vegetation in the Mediterranean region and the Canary Islands (Goula & Alomar, 1994; Carnero et al., 2000; Trottin-Caudal et al., 2006). *Nesidiocoris tenuis* is known as an effective natural enemy of whitefly (Sánchez & Lacasa, 2008; Calvo et al., 2009) and more recently as potential biological control agent of *T. absoluta* (Urbaneja et al., 2009). The mirid is mass reared commercially and typically released augmentatively after transplanting to establish in 5–8 weeks (Calvo & Urbaneja, 2004; Calvo et al., 2009). This system may allow the target pests time to increase to damaging levels before the predator has reached sufficient numbers to provide control. Urbaneja et al. (2009)

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concluded that successful biocontrol of *T. absoluta* with *N. tenuis* would require the presence of the predator in the crop before the appearance of the pest.

Lenfant et al. (2000) developed an early release system for the predaceous mirid *Macrolophus caliginosus* Wagner in the nursery prior to transplanting to shipment and transplantation by the vegetable grower. The predator adults are released in the seedlings, fed with *Ephestia kuehniella* Zeller eggs and allowed several days for egg laying. Nymphs eclose from these eggs once the crop has been transplanted so that the predator is available in the event of early pest infestation. The system allows the predator to establish just after transplanting and therefore could provide good control of the target pest, usually whiteflies. *Nesidiocoris tenuis* is biologically comparable to *M. caliginosus* and could well adapt to the same system. Both *T. absoluta* and whitefly are suitable preys of *N. tenuis* (Calvo et al., 2009; Urbaneja et al., 2009) which may appear simultaneously or individually in greenhouses. Therefore, one objective of the present study was to evaluate the effectiveness of pre-plant releases of *N. tenius* under different scenarios of pest entry into the crop.

Recent studies have demonstrated that *Bacillus thuringiensis* Berliner (Bt) and the *T. abosluta*-egg parasite *Trichogramma achaeae* Nagaraja & Nagarkatti (Hymenoptera: Trichogrammatidae) can also provide good control of *T. absoluta* in tomato greenhouses (Giustolin et al., 2001; Desneux et al., 2010; González-Cabrera et al., 2011; Mollá et al., 2011). However, these agents do not establish in the crop and they must therefore be reapplied frequently. Moreover, the use of *T. achaeae* for *T. absoluta* control may not be economically sustainable unless combined with other biological control methods, especially with mirid predators (Desneux et al., 2010). Combination with *N. tenuis* which can establish on the crop could provide the desired short- and long-term control. However, interactions between natural enemies can also be antagonistic and thus impede herbivore suppression (Straub et al., 2008). Therefore, interactions among natural enemies should be assessed before recommending a particular combination of predators for general use. In some cases, successful implementation of biocontrol can be assigned to a single agent, even when more than one species was introduced (Myers et al., 1989). Better understanding of these relationships among available natural enemies will facilitate optimal decisions on what to use and when to use it. Therefore, a second objective of this study was to evaluate a combination of pre-plant application of *N. tenuis* with release of *T. achaeae* and applications of Bt to control *T. absoluta*.

Materials and methods

Greenhouse

Two experiments were conducted in a multi-tunnel greenhouse located in Vicar (Almeria, Andalusia, Spain). Walk-in cages were constructed inside the greenhouse to accommodate plants and isolate treatments. Each walk-in cage (5 × 3.5 × 4 m) was constructed of 'anti-thrips' polyethylene screen with 220 × 331 μm interstices and supported by heavy wires. Floors were covered with woven 2-mm-thick polyethylene cloth and access to each cage was through a zippered doorway. Sixteen of these cages were used for the first experiment (referred to as 'whitefly + *T. absoluta*') and 20 for the second experiment (referred to as 'strategies'). The greenhouse was equipped with Climatec^{TM} system (Novedades Agrícolas, Murcia, Spain) for temperature and relative humidity control. Temperature and relative humidity were monitored in four randomly selected walk-in cages with a HOBO H8 r.h./Temp Loggers (Onset Computer, Bourne, MA, USA).

Experimental design and procedure

Experiment 1: whitefly + Tuta absoluta. Four treatments were compared in a complete randomized block design with four replicates: (1) *T. absoluta* (one couple *T. absoluta* per plant released every week for 3 weeks beginning the week of planting); (2) *B. tabaci* (10 *B. tabaci* per plant released every week for 3 weeks beginning the week of planting); (3) *T. absoluta* + *N. tenuis* (one *N. tenuis* per two plants released in transplant trays 5 days before planting and one couple *T. absoluta* per plant released every week for 3 weeks beginning the week of planting); and (4) *T. absoluta* + *B. tabaci* + *N. tenuis* (one *N. tenuis* per two plants released in transplant trays 5 days before planting; one couple *T. absoluta* and 10 *B. tabaci* per plant released every week for 3 weeks beginning the week of planting). Timing and rate for pests and predator releases are summarized in Table 1.

Seeds of tomato, *Solanum lycopersicum* L. (Solanaceae) cv. 'Razymo' (Rijk Zwaan, De Lier, The Netherlands), were first sown into 5.4-cm^2 peat moss root cubes. When seedlings reached the five-leaves stage, they were moved to 'inoculation' cages (1 × 1 × 1.5 m). Each inoculating cage contained 40 seedlings, all of which were destined for one treatment. Adult *N. tenuis* were then cooled briefly in a cold room at 8 °C for counting before being released into designated inoculation cages at a sex ratio of 1:1 and a rate of one predator per two plants (Table 1). Four paper strips (3 × 1 cm) with eggs of *E. kuehniella* glued to one side were also placed inside the inoculating cages to serve as a

Table 1 Timing and rates [no. per plant (pl)] of whitefly and *Tuta absoluta* for infestation and natural enemy releases during the whitefly + *T. absoluta* experiment. Tabs: *T. absoluta*; Btab: *Bemisia tabaci*; Nten: *Nesidiocoris tenuis*

	Treatment			
Days from planting	Tabs	Btab	Tabs + Nten	Tabs + Btab + Nten
−5			0.5 Nten/pl	0.5 Nten/pl
0	1 couple Tabs/pl	10 Btab adults/pl	1 couple Tabs/pl	1 couple Tabs/pl 10 Btab adults/pl
7	1 couple Tabs/pl	10 Btab adults/pl	1 couple Tabs/pl	1 couple Tabs/pl 10 Btab adults/pl
14	1 couple Tabs/pl	10 Btab adults/pl	1 couple Tabs/pl	1 couple Tabs/pl 10 Btab adults/pl

food source for the mirids. Plants were maintained inside the inoculating cages for 5 days, after which adult *N. tenuis* were removed. Seedlings were then transplanted on 22 September 2009 into 25 l coco peat fibre bags placed inside the designated walk-in cage, 10 per cage providing a plant density of 2 plants m^{-2}. Plants were trained by the main stem to a black polyethylene string tied to a stainless steel overhead wire. Secondary shoots were removed and water and fertilizers were supplied as required through the drip irrigation system (Mithra™; Novedades Agrícolas, Murcid, Spain).

Eggs of *E. kuehniella* were sprinkled on all plants at a rate of 0.01 g in each walk-in cage with *N. tenuis*, beginning at transplanting and for 2 weeks thereafter. Adult pests were cooled briefly in a cold room at 8 °C for counting and then released in designated walk-in cages on the same schedule as *E. kuehniella* eggs at a rate of 1 *T. absoluta* couple and 10 *B. tabaci* per plant (Table 1). This release schedule was intended to simulate gradual but heavy immigration of both pests into the greenhouse.

Experiment 2: strategies. A complete randomized block design was used with four replicates and five treatments: (1) *T. absoluta* (one couple *T. absoluta* per plant released every week for 3 weeks beginning the week of planting); (2) *T. absoluta* + *N. tenuis* (one *N. tenuis* per two plants released in transplant trays 5 days before planting and one couple *T. absoluta* per plant released every week for 3 weeks beginning the week of planting); (3) *T. absoluta* + *N. tenuis* + *T. achaeae* (one *N. tenuis* per two plants released in transplant trays 5 days before planting, one couple *T. absoluta* per plant released every week for 3 weeks beginning the week of planting, and 50 *T. achaeae* m^{-2} released weekly for 6 weeks beginning the week of planting); (4) *T. absoluta* + *N. tenuis* + *B. thuringiensis* (one *N. tenuis* per two plants released in transplant trays 5 days before planting, one couple *T. absoluta* per plant released every week for 3 weeks beginning the week of planting, and 0.5 g l^{-1} Bt sprayed on plants weekly for 6 weeks beginning 1 week after planting when larvae were first observed); and (5) *T. absoluta* + *N. tenuis* + *T. achaeae* + *B. thuringiensis* (one *N. tenuis* per two plants released in transplant trays 5 days before planting, one couple *T. absoluta* per plant released every week for 3 weeks beginning the week of planting, 50 *T. achaeae* m^{-2} released weekly for 6 weeks beginning the week of planting, and 0.5 g l^{-1} Bt sprayed on plants weekly for 6 weeks beginning the week after planting when larvae were first observed) (Table 2).

Transplanting date was 2 June 2010. Procedures for *N. tenuis* and *T. absoluta* releases, supplemental food use, and plant management and settlement were the same as those described for the whitefly + *T. absoluta* experiment. Timing and rate for *T. achaeae* was established in accordance with Desneux et al. (2010) who mentioned that weekly releases at a rate of 50 adults m^{-2} is the recommended approach for *T. absoluta* control in combination with mirid predators. The frequency of *B. thuringiensis* applications conformed to recommendations of Gonzalez-Cabrera et al. (2011) for *T. absoluta* at the highest dose recommended on the labels.

Pests, control agents, and supplemental food

Bemisia tabaci adults to infest the tomato plants were collected from a colony originally obtained from field samples from several locations within the Region de Murcia, Spain (37°59′10″N, 1°7′49″W) and identified with polymerase chain reaction (PCR) as biotype 'Q' and maintained on tobacco plants, *Nicotiana tabacum* L. *Tuta absoluta* adults for infestation were collected from a mass-rearing maintained on tomato plants and originally obtained in greenhouses grown tomatoes from the Region de Murcia. They belonged to a single colony cohort and sex ratio of releases was always 1:1. *Nesidiocoris tenuis* was provided in bottles containing 500 adults (Nesibug™; Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands). The parasitic

Table 2 Timing and rates [no. per plant (pl)] of *Tuta absoluta* for infestation and supplementary agent releases or applications during the strategies experiment. Tabs, *T. absoluta*; Nten, *Nesidiocoris tenuis*; Tach, *Trichograma achaeae*; Bthu, *Bacillus thuringensis*

Days from planting	Treatment				
	Tabs	Tabs + Nten	Tabs + Nten	Tabs + Btab + Nten	Tabs + Nten + Tach + Bthu
−5		0.5 Nten/pl	0.5 Nten/pl	0.5 Nten/pl	0.5 Nten/pl
0	1 couple Tabs/pl	1 couple Tabs/pl	1 couple Tabs/pl	1 couple Tabs/pl 50 Tach m^{-2}	1 couple Tabs/pl 50 Tach m^{-2}
7	1 couple Tabs/pl	1 couple Tabs/pl	1 couple Tabs/pl 0.5 $g\,l^{-1}$ Bthu	1 couple Tabs/pl 50 Tach m^{-2}	1 couple Tabs/pl 50 Tach m^{-2} 0.5 $g\,l^{-1}$ Bthu
14	1 couple Tabs/pl	1 couple Tabs/pl	1 couple Tabs/pl 0.5 $g\,l^{-1}$ Bthu	1 couple Tabs/pl 50 Tach m^{-2}	1 couple Tabs/pl 50 Tach m^{-2} 0.5 $g\,l^{-1}$ Bthu
21			0.5 $g\,l^{-1}$ Bthu	50 Tach m^{-2}	50 Tach m^{-2} 0.5 $g\,l^{-1}$ Bthu
28			0.5 $g\,l^{-1}$ Bthu	50 Tach m^{-2}	50 Tach m^{-2} 0.5 $g\,l^{-1}$ Bthu
35			0.5 $g\,l^{-1}$ Bthu	50 Tach m^{-2}	50 Tach m^{-2} 0.5 $g\,l^{-1}$ Bthu
42			0.5 $g\,l^{-1}$ Bthu		0.5 $g\,l^{-1}$ Bthu

wasp *T. achaeae* was provided in cards with adhered parasitized eggs from a rearing colony maintained on eggs of *E. kuehniella* and originally obtained from several locations within the Canary Islands, Spain (27°57′31″N, 15°35′33″W) where they parasitized eggs of *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae). Eggs of *E. kuehniella* used as supplemental food during the experiment were supplied in bottles containing 10 g of eggs (Entofood™; Koppert Biological Systems). *Bacillus thuringienesis* var. kurstaki was obtained as the commercial product Costar™ (Syngenta Agro, Madrid, Spain) which contains 90.4 MIU mg^{-1} (Millions of International Units per mg).

Sampling

Plants were monitored weekly for 7 weeks after transplanting in the whitefly + *T. absoluta* experiment and 9 weeks in the strategies experiment, beginning on 29 September 2009 and 9 June 2010, respectively. For both experiments, five plants were randomly selected in each experimental cage. Whitefly nymphs were counted on three leaves from each of the five selected plants. One leaf was selected at random from the upper, middle, and bottom third of the plant. Nymphs and adults of *N. tenuis* were counted on five leaves from each of these five plants. In this case, three of these leaves were selected at random from the upper, one from the middle, and one from the bottom third of the plant. *Tuta absoluta* eggs were counted on five leaves selected from the upper third of each of the five selected plants. In each case, leaves were turned carefully to count first whitefly and *N. tenuis* adults and then the other insect stages using a 15× hand lens. Finally, fruits from all plants were collected at the end of the experiment, counted, and classified as damaged or not by *T. absoluta*. In addition, 10 leaves were selected along each of the five selected plants and rated as 0, 1, 2, 3, 4, or 5 when the mined area was 0, 1–25%, 26–50%, 51–75%, 76–99%, or 100% of the leaf surface, respectively.

Ambient conditions

Temperature and relative humidity during the whitefly + *T. absoluta* experiment averaged 21.1 ± 3.3 °C and 68.1 ± 8.6%, and during the strategies experiment 24.4 ± 4.4 °C and 62.1 ± 7.7%.

Statistical analysis

Treatment effects on whitefly, *N. tenuis*, and *T. absoluta* during the whitefly + *T. absoluta* and strategies experiments were analysed with linear mixed effects models, using time as random factor nested in blocks to correct for pseudo replication due to repeated measures (Crawley, 2002). Thereafter, treatments were compared through model simplification by combining treatments (Crawley, 2002). Percentage of damaged fruits by *T. absoluta* were compared among treatments using a one-way ANOVA and Tukey's test for mean separation ($\alpha = 0.05$). The number of whiteflies, *N. tenuis*, and eggs of *T. absoluta* and percentages of affected area and damaged fruits by *T. absoluta* were $\log(x + 1)$ and $\arcsin\sqrt{(x + 1)}$ transformed, respectively, prior to analysis to stabilize error variance; untransformed values are given in tables and figures. Temperature and humidity were initially included, but proved not significant and were therefore removed from further analysis.

Results

Whitefly + *Tuta absoluta* experiment

Whitefly. Numbers of whitefly nymphs per leaf increased during the first 2 weeks and decreased progressively afterwards until the end of the experiment in the treatment with *N. tenuis*, whitefly, and *T. absoluta* (Figure 1). In the absence of *N. tenuis*, whitefly numbers increased through week 3, remained high thereafter, and finished 30-fold greater by the end of the experiment, providing a significantly different result ($F_{1,54}$ = 67.445, P<0.001).

Tuta absoluta. Numbers of *T. absoluta* eggs peaked 3 weeks after first release of the moths in all the treatments (Figure 2A), and were much higher in the treatment with *T. absoluta* alone compared to either treatments with *N. tenuis* alone, or *N. tenuis* plus whitefly ($F_{1,54}$ = 10.309 and 12.003, P = 0.004 and 0.002, respectively). No significant effect of whitefly was observed on the suppression of *T. absoluta* eggs ($F_{1,54}$ = 0.714, P = 0.41).

Leaf area damaged by *T. absoluta* increased constantly and reached more than 80% at the end of the experimental period in the treatment with the pest only (Figure 2B). Greater plant damage in the absence of *N. tenius* was observed compared to treatments with *N. tenuis* (*T. absoluta* vs. *T. absoluta* + *N. tenuis*: $F_{1,54}$ = 22.209, P<0.001; *T. absoluta* vs. *T. absoluta* + *B. tabaci* + *N. tenuis*: $F_{1,54}$ = 24.615, P<0.001). No significant effect on leaf damage was seen from the presence of *B. tabaci* (*T. absoluta* + *N. tenuis* vs. *T. absoluta* + *B. tabaci* + *N. tenuis*: $F_{1,54}$ = 2.442, P = 0.13). Fruit damage by *T. absoluta* was also reduced from more than 90% to ca. 5% when *N. tenuis* was released ($F_{3,9}$ = 71.877, P<0.001; Figure 3).

Nesidiocoris tenuis. Abundance of *N. tenuis* nymphs plus adults was always similar with or without whiteflies (*T. absoluta* + *N. tenuis* vs. *T. absoluta* + *B. tabaci* + *N. tenuis*: $F_{1,54}$ = 2.442, P = 0.13) (Figure 4). In both treatments, the first predators were observed 1 week after first pest release. Population density decreased during the first 3 weeks, increased strongly during the following 2 weeks, then levelled off at more than two predators per leaf until the end of the experiment.

Strategies experiment

Tuta absoluta. Numbers of *T. absoluta* eggs per leaf remained below one in treatments including *N. tenuis* (Figure 5A) with no statistical differences among these treatments ($F_{3,140}$ = 0.400, P = 0.75). In contrast, more than 60 *T. absoluta* eggs per leaf were observed 7 weeks after when *T. absoluta* was released alone, significantly higher compared to treatments with *N. tenuis* (*T. absoluta* vs. *T. absoluta* + *N. tenuis*: $F_{1,70}$ = 5.189, P = 0.027; *T. absoluta* vs. *T. absoluta* + *N. tenuis* + *T. achaeae*: $F_{1,70}$ = 5.163, P = 0.032; *T. absoluta* vs. *T. absoluta* + *N. tenuis* + *B. thuringiensis*: $F_{1,70}$ = 5.203, P = 0.027; *T. absoluta* vs. *T. absoluta* + *N. tenuis* + *T. achaeae* + *B. thuringiensis*: $F_{1,70}$ = 5.199, P = 0.031).

Where released alone, *T. absoluta* rapidly reduced healthy leaf area significantly more than treatments with *N. tenuis* (*T. absoluta* vs. *T. absoluta* + *N. tenuis*: $F_{1,70}$ = 32.558, P<0.001; *T. absoluta* vs. *T. absoluta* + *N. tenuis* + *T. achaeae*: $F_{1,70}$ = 27.412, P<0.001; *T. absoluta* vs. *T. absoluta* + *N. tenuis* + *B. thuringiensis*: $F_{1,70}$ = 32.389, P<0.001; *T. absoluta* vs. *T. absoluta* + *N. tenuis* + *T. achaeae* + *B. thuringiensis*: $F_{1,70}$ = 31.016, P<0.001), with no significant differences among the last ($F_{3,140}$ = 0.732,

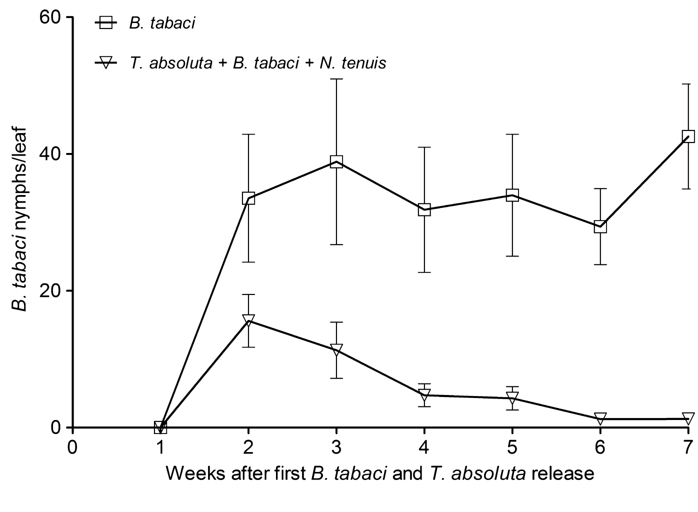

Figure 1 Mean (± SE) number of *Bemisia tabaci* nymphs per leaf per week in each treatment receiving the pest during the whitefly + *Tuta absoluta* experiment. *Nesidiocoris tenuis* was released 5 days before transplanting and the first whitefly release was carried out just after transplanting (week 0).

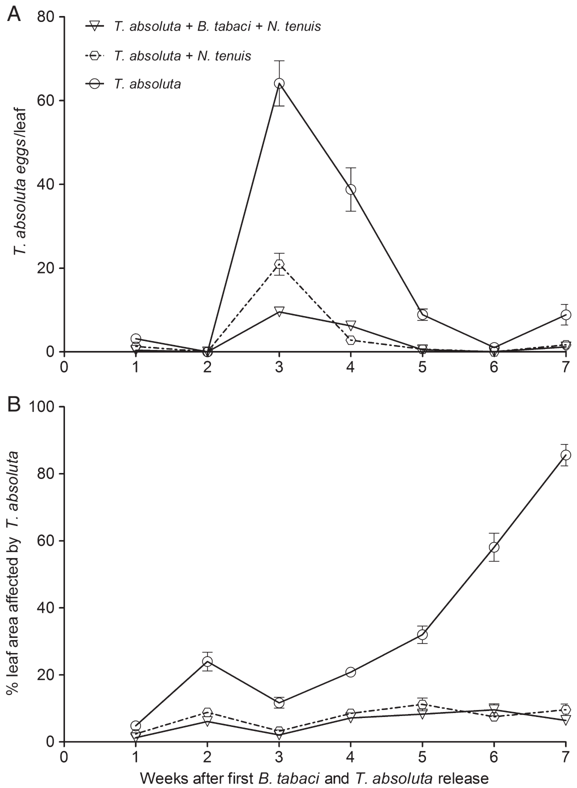

Figure 2 Mean (± SE) number of *Tuta absoluta* eggs per leaf (A) and affected leaf area by *T. absoluta* (B) per week in each treatment receiving the pest during the whitefly + *T. absoluta* experiment. *Nesidiocoris tenuis* was released 5 days before transplanting and the first *T. absoluta* release was carried out just after transplanting (week 0).

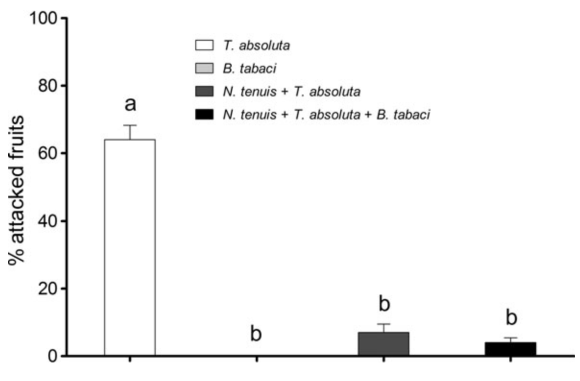

Figure 3 Mean percentage (+ SE) of fruits affected by *Tuta absoluta* in the whitefly + *T. absoluta* experiment in each treatment: (1) *T. absoluta*; (2) *Bemisia tabaci*; (3) *Nesidiocoris tenuis* + *T. absoluta*; and (4) *N. tenuis* + *T. absoluta* + *B. tabaci*. Columns with the same letter are not significantly different (Tukey: P>0.05).

P = 0.41). The affected area was around 10 times less at the end of the experiment in treatments with *N. tenuis* than that observed on plants receiving the pest only. All fruits,

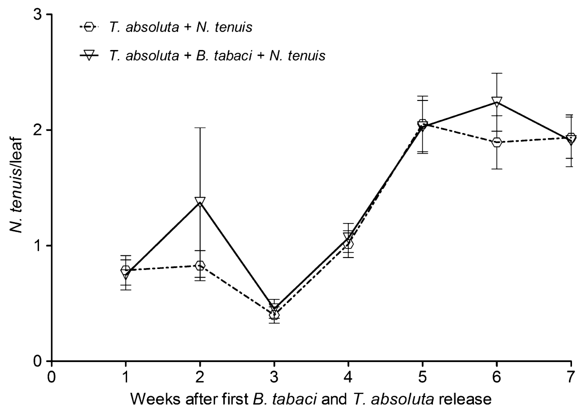

Figure 4 Mean (± SE) number of *Nesidiocoris tenuis* per leaf per week in each treatment receiving the predator during the strategies experiment. *Nesidiocoris tenuis* was released 5 days before transplanting and the first *Tuta absoluta* release was carried out just after transplanting (week 0).

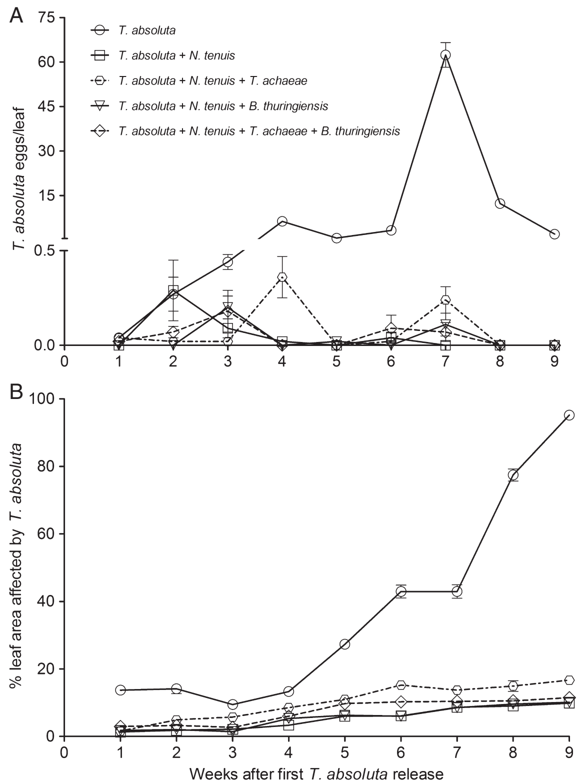

Figure 5 (A) Mean (± SE) number of *Tuta absoluta* eggs per leaf and (B) affected leaf area by *T. absoluta* per week in each treatment receiving the pest during the strategies experiment. *Nesidiocoris tenuis* was released 5 days before transplanting and the first *T. absoluta* release was carried out just after transplanting (week 0).

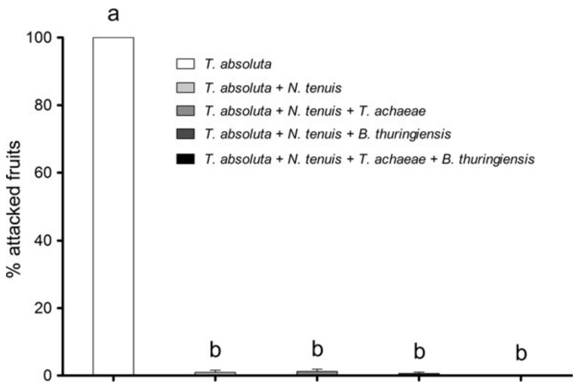

Figure 6 Mean percentage (+ SE) of fruits affected by *Tuta absoluta* in the strategy experiment in each treatment: (1) *T. absoluta*; (2) *T. absoluta* + *Nesidiocoris tenuis*; (3) *T. absoluta* + *N. tenuis* + *Trichogramma achaeae*; (4) *T. absoluta* + *N. tenuis* + *Bacillus thuringiensis*; and (5) *T. absoluta* + *N. tenuis* + *T. achaeae* + *B. thuringiensis*. Columns with the same letter are not significantly different (Tukey: P>0.05).

collected from walk-in cages belonging to the treatment with *T. absoluta* only, were damaged (Figure 6), compared to 1.2 ± 0.70% which was the highest percentage in treatments receiving *N. tenuis* ($F_{4,12} = 106.1$, $P<0.001$).

Nesidiocoris tenuis. The average number of nymphs plus adults of *N. tenuis* per leaf was similar and not significantly different among treatments receiving the predator ($F_{3,140} = 1.630$, $P = 0.19$; Figure 7). Numbers of predators per leaf decreased slowly the first 4 weeks, then increased to a peak of more than three predators per leaf in the 5th week. The mirid population then declined gradually but maintained an average greater than 2 per leaf until the end of the experiment.

Discussion

We first evaluated the potential of a pre-plant application of *N. tenuis* in a situation with whitefly and *T. absoluta* present and its combination with supplementary control agents against *T. absoluta*, afterwards. In the first experiment, the abundance of whitefly and *T. absoluta* was significantly lower where *N. tenuis* was released, confirming that pre-plant release of *N. tenuis* can suppress populations of the tobacco whitefly or *T. absoluta* either alone or simultaneously in tomato greenhouses. This result is in agreement with earlier investigations that demonstrated the capacity of *N. tenuis* to suppress whitefly and *T. absoluta* infesting tomato crops alone (Sánchez & Lacasa, 2008; Calvo et al., 2009; Urbaneja et al., 2009).

The second experiment confirmed again that pre-plant release of *N. tenuis* in tomato resulted in significant suppression of the *T. absoluta* population. It further demonstrated that the additional release of *T. achaeae* alone, or in combination with applications of Bt, did not improve control of *T. absoluta*. Moreover, the release of *N. tenuis* reduced yield losses due to *T. absoluta* in both experiments. In contrast, releases of *T. achaeae* with or without applications of Bt did not further reduce the percentage of damaged fruits. Thus, pre-plant release of *N. tenuis* in the present experiment obviated the need for additional control of *T. absoluta* from either *T. achaeae* or Bt.

Previous studies concluded that supplementary releases of *T. achaeae* to *N. tenuis* improved control of *T. absoluta* (Desneux et al., 2010). However, rates, timing, methods, and frequency of natural enemy release, synchronization between prey and predator, abiotic factors (humidity, photoperiod, temperature, etc.), and pesticide use can

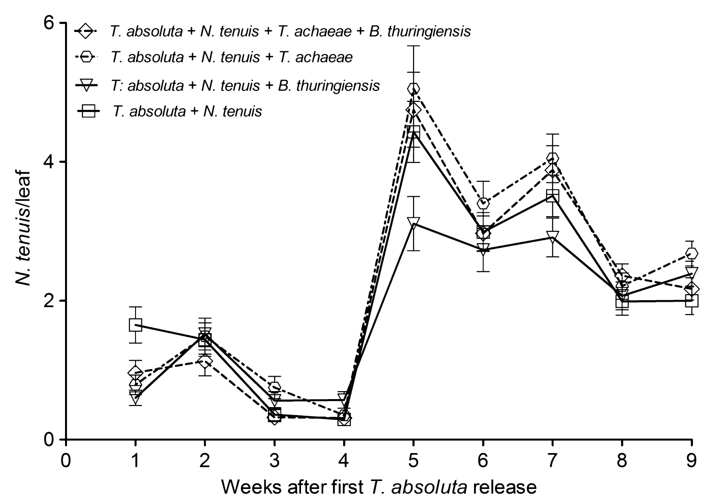

Figure 7 Mean (± SE) number of *Nesidiocoris tenuis* per leaf per week in each treatment receiving the predator during the strategies experiment. *Nesidiocoris tenuis* was released 5 days before transplanting and the first whitefly release was carried out just after transplanting (week 0).

modify the control capacity of a natural enemy (Collier & van Steenwyk, 2004; Stiling & Cornelissen, 2005; Crowder, 2006; Desneux et al., 2007). In trials combining *N. tenuis* and *T. achaeae* (Desneux et al., 2010), the mirid was released after transplanting, which probably permitted *T. absoluta* increase early in the season before the predator could build up sufficiently to provide control, necessitating additional suppression from *T. achaeae*. González-Cabrera et al. (2011) demonstrated that regular applications of Bt alone suppressed *T. absoluta* populations, although at twice the dose we tested, which was also twice that permitted by the label. Furthermore, these authors did not include *N. tenuis* in their system. Mollá et al. (2011) combined *N. tenuis* with three different periods of weekly applications of Bt but at the same high dose and observed a significant reduction of *T. absoluta* populations in respect to the untreated plots. However, they did not observe differences between treatments combining Bt and *N. tenuis* with *N. tenuis* alone, which agrees with our results. Unfortunately, they did not include a Bt alone treatment, which would have clarified the role of both the entomophatogen and predator.

In conclusion, *N. tenuis* was able to establish in tomato on a diet of *T. absoluta* or *B. tabaci*, either alone or in combination, and was able to control both pests under all three scenarios. The option of targeting two pests with a single natural enemy reduces the complexity and costs of the biological control. This, and the capacity to utilize other pests as alternate food sources, increases the likelihood of establishment. *Nesidiocoris tenuis* is able to feed and reach maturity on other food sources that are common in tomato crops, including spider mites and thrips (Urbaneja et al., 2003). All of this has positive implications for biocontrol. Overall, the present experiment provides guidelines for successful control of *T. absoluta* – either alone or in presence of whitefly – in greenhouse-grown tomato, based exclusively on a strategy of early augmentative biological control with *N. tenuis*.

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