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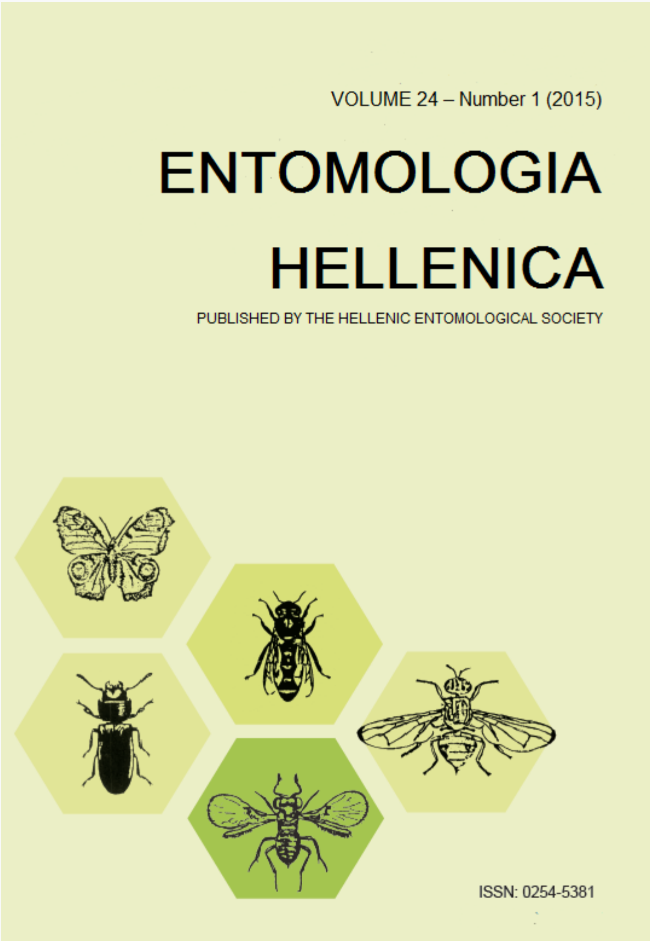

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Pre-plant release enhanced the earlier establishment of *Nesidiocoris tenuis* in open field tomato

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ABSTRACT

The invasive pest of the tomato crops, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), soon after its establishment became a major problem of outdoor and greenhouse tomato crops across the Mediterranean countries. The pre-plant release of the predator *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae) has been found to substantially contribute to its establishment on the crop and efficient control of *T. absoluta* in greenhouses. The aim of the current study was to assess whether the pre-plant release of *N. tenuis* could contribute to its earlier establishment. It was concluded that the release of *N. tenuis* in the nursery contributed substantially in the earlier increase of its numbers in the field in comparison to the control plots. The native populations of *M. pygmaeus* appeared on the tomato plants before the native populations of *N. tenuis*. Thus, the conservation of both predators should be taken into consideration in the integrated management strategies against *T. absoluta*. Therefore, the application of this method in open field tomato crop enhances the earlier establishment of *N. tenuis* and should be further evaluated in the control of *T. absoluta*.

KEY WORDS: conservation biological control, Gelechiidae, Miridae, omnivorous predator, pest control, tomato borer.

Introduction

Tomato (*Solanum lycopersicum* L.) crops are damaged by a wide range of pests such as whiteflies, thrips, leafminers, species of Lepidoptera and aphids. In 2006 a new pest, the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was recorded in Europe. After its detection, *T. absoluta* was rapidly spreading in the Afro-Eurasian continent (Desneux et al. 2011, Tropea-Garzia et al. 2012).

Damage by this pest is due to larval feeding-mining in the leaves, the terminal buds, the stem and the fruits. Damage on fruits is the most serious as damaged fruits become unmarketable. The pest may cause yield losses as high as 80-100% (Lopez 1991). Soon after its establishment in the Mediterranean countries *T. absoluta* has become a major threat for the tomato crops (Desneux et al. 2010).

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Control of *T. absoluta* under IPM strategies should include an array of measures such as sanitation, the use of insect exclusion nets on the greenhouse openings, monitoring by sexual pheromone traps (Benvenga et al. 2007), mass trapping (Witzgall et al. 2010), biological control (Desneux et al. 2010, Trottin-Caudal et al. 2012, Terzidis et al. 2014, Luna et al. 2015, Cabello et al. 2015) and application of insecticides (Roditakis et al. 2012, Biondi et al. 2013). The wide application of insecticides may cause adverse health effects to consumers and the environment but also may lead to inconsistent results and failures in control as resistance development in *T. absoluta* has been reported for abamectin, certain pyrethroids and chitin synthesis inhibitors (Siqueira et al. 2001, Lietti et al. 2005, Silva et al. 2011, Campos et al. 2015, Roditakis et al. 2015).

Biological control is a valuable strategy against *T. absoluta* populations since several natural enemies of this pest have been reported (Desneux et al. 2010, Garcia del Pino et al. 2013, Gabarra et al. 2014, Luna et al. 2015). The native of the Mediterranean region polyphagous predator *Macrolophus pygmaeus* (Rambur) and the cosmopolitan predator *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae) may highly contribute to the control of whiteflies and other greenhouse pests on tomatoes (Albajes and Alomar 1999, Perdikis et al. 2011, Itou et al. 2012). For this reason, studies on the potential of both predators to control *T. absoluta* were prioritized. Urbaneja et al. (2009) showed that a 24h starved female of *N. tenuis* or *M. pygmaeus* can consume almost 60 eggs in 24h, when 60 eggs were available, whereas their predation rate on larvae was much lower, feeding mostly on the younger instars. Arnó et al. (2009) reported that the mirid predators can highly contribute to the control of *T. absoluta* when protected from insecticide sprayings.

In management schemes aiming to take benefit from the activity of the mirid predators the use of *Bacillus thuringiensis* (Berliner) (Bt) may provide a compatible strategy. *Bacillus thuringiensis* effectively controls young larvae (González-Cabrera et al. 2011) and the predators control mainly eggs (Urbaneja et al. 2009, Jaworski et al. 2013, Queiroz et al. 2015). This potential was evaluated with positive results in greenhouse tomato crops where Bt was applied weekly for long periods (extending from 2 months to the entire crop period) combined with the release of *N. tenuis* (Mollá et al. 2011). Similar results were obtained in an open field study where Bt was applied weekly for 2.5 months that also permitted the complementary activity of native mirid predators (González-Cabrera et al. 2011).

Although these studies showed the high efficacy of the combined use of mirid predators and Bt applications, there is a well justified need to search if effective control might be achieved by a less intensive schedule of Bt applications to reduce control costs and the risk for resistance development to Bt (Ferre and Van Rie 2002; Gassmann et al. 2009).

Furthermore, the control of this pest in open field tomatoes may be a challenge since the threat it poses is anticipated to be much more severe than that in the greenhouses where exclusion measures can be adopted. Aiming to obtain a higher efficacy of native predators in pest control, a key issue is to ensure their timely establishment on the crop, particularly in temporary agroecosystems (Ehler and Miller 1978). In areas like northwestern Italy and Greece the population of *N. tenuis* normally increases steadily only during August and September (Tavella et al. 1997, Perdikis et al. 2007, Lykouressis et al. unpublished data) and this may increase the risk for damage in cases of early development of *T. absoluta* populations. In fact, the timely establishment of *N. tenuis* has been recognized as substantial for higher efficacy against *T. absoluta* (Urbaneja et al. 2009, Calvo et al. 2012a). Thus, strategies to enhance the early establishment of the predator are required.

An alternative method for early establishment of the mirid predators represents their pre-plant release. Following this technique, adults of the predator are released in the nursery aiming to lay eggs on the seedlings. When transplanted, the plants bear eggs of the predator, enabling earlier build up of the predator's population in the crop and more uniform distribution. The strategy has been evaluated with positive impacts on biological control in greenhouses (Ridray et al. 1998, Lenfant et al. 2000, Calvo et al. 2012a, Nannini et al. 2014).

Although positive results have been obtained in greenhouses, to the best of our knowledge the potential of pre-plant release has not been evaluated for open field conditions.

The objectives of this study were to examine whether the release of *N. tenuis* in the nursery could support its earlier establishment in the open field.

Materials and Methods

The experiments were carried out in a conventional fresh market tomato (cv. Mountain Spring) field in the area of Kyparissia, western Peloponnesus in 2010. This is the second most important region for fresh market tomato production in Greece.

Effect of pre-plant release on the establishment of *Nesidiocoris tenuis*

Our previous studies showed that in western Peloponnesus, *M. pygmaeus* colonizes the crops earlier than *N. tenuis* (Perdikis et al. 2007). Therefore, it was decided to test whether the pre-plant release of *N. tenuis* in the nursery could support its earlier establishment on the crop. To investigate this potential one cage made by insect exclusion net (16:10/cm^2) was established in a greenhouse nursery. The openings of that nursery were covered by exclusion nets and the plants were inspected but no nymphs or adults of *M. pygmaeus* or *N. tenuis* or signs of *T. absoluta* damage were recorded. In the cage, 250 young tomato plants were placed from that nursery along with 500 *N. tenuis* individuals (mostly adults but also late instar nymphs) (Nesibug^{TM}, Koppert Biological Systems, The Netherlands), on May 21, 2010. Thus, the release rate was 2 *N. tenuis* per plant. On the plants sterilized eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Entofood ^{TM}, Koppert Biological Systems) were distributed twice in a quantity of 3g, until transplantation in the field. No sprayings were applied in the nursery.

On May 30, the plants from the cage in the nursery were transplanted in the field, along with the other tomato plants from the same nursery, on which no predators were present.

The field was elongated (100m long and 60m wide). The direction of the tomato plant rows was parallel to the long axis of the field (east-west direction). The tomato rows were separated by 1m and the distance between tomato plants on the row was 0.5m. The field was surrounded by olive groves.

Four plots were established along the southern long edge of the field: the first and the fourth plots were of smaller size and served as "outer" control plots to locate any possible edge effect that could affect differentially the colonization of native (spontaneously occurring) predators between the two internal (i.e. second and third) plots. The second and the third plots were the main plots assigned to compare populations of predators and pests between the second (or central control) plot which was colonized with native predators and the third plot (treatment plot) where only plants from the cage where *N. tenuis* had been released in the nursery were transplanted. Each of the east and west control plots was 10m long and 4m wide (i.e. including four rows of tomato plants). The distance of the outer border of the plots was 2m from the field edges. The two internal plots were longer (each of 20m length and 4m wide). The distance between the plots was always 12m. In each of the control plots located in the eastern and western side of the field, 8 plants were randomly selected. Within each of the two internal plots, 32 plants were randomly selected, from the 2 internal tomato rows. On each of the selected plants, one stem (replicate) was randomly selected and marked.

The effect of the pre-plant release was evaluated for a total period of two months after transplantation. Four samplings were conducted on May 31, June 17, July 7 and July 27. In each sampling, the number of nymphs of each instar and the adults of *N. tenuis* was recorded on the 8 upper leaves and the apex of each marked stem.

In parallel to the effect of pre-plant release of *N. tenuis* on its establishment and control of *T. absoluta*, the effects of native *M. pygmaeus* and *N. tenuis* populations that naturally colonize the crop were evaluated.

For this reason, at each sampling the number of each instar nymphs and adults of *M. pygmaeus* as well as the number of leaves with mines and larvae of *T. absoluta* and the numbers of pest individuals (whiteflies and thrips) were similarly recorded as described before for *N. tenuis*. At the last sampling, damage on the fruits from *T. absoluta* was also recorded by the random inspection of 20 fruits on the selected plants per plot and the stems were inspected for necrotic rings and possible flower abortion due to the plant feeding of *N. tenuis*.

During the sampling period, the numbers of *T. absoluta* males were recorded weekly in a delta trap (Russel IPM) supplied with pheromone. The trap was placed at a height of 1.2 m. The sticky plate was changed in a 2-week interval and the pheromone capsule was changed once a month.

A single insecticidal *B. thuringiensis* (Bactospeine^{TM}, Hellafarm, Greece) treatment at the recommended dose (i.e. 150gr/100lt) was conducted on July 10, when larvae of *T. absoluta* were recorded and captures were increased (see results).

Data analysis

The data collected for each predator species or *T. absoluta* in each sampling date were compared by one-way ANOVA with treatment as fixed factor. Data were square root transformed prior to the analyses to normalize the data and stabilize variances. The numbers of each instar nymphs and adults were compared among samplings using a Generalized Linear Model. Comparisons between treatments were performed with the Tukey-Kramer (HSD) test (a = 0.05). Analyses were conducted using the statistical package JMP (v. 10.0.0, SAS Institute 2012).

Results

Nesidiocoris tenuis **population levels**

The population densities (nymphs and adults) of *N. tenuis* at the different plots are shown in Table 1. In the first and the second samplings *N. tenuis* was only found in the treatment plot where the plants from the pre-plant release cage had been planted. In the third sampling, five weeks after transplantation, individuals were also recorded in the other plots, but their numbers were significantly lower than in the pre-plant release plot. However, in the last sampling, the population levels of *N. tenuis* were not different among the plots (F=0.52, df= 3,79, P=0.66).

In the first sampling, at the treatment plot, most of the *N. tenuis* individuals were young nymphs belonging mainly to the first but also to the second and third instar (Table 2). In the second sampling, the population consisted of adults only. In the two following samplings mainly nymphs were present belonging to all the instars. In the plots without pre-plant release nymphs and adults were firstly recorded in the third sampling.

Necrotic rings due to the phytophagous activity of *N. tenuis* were very rarely recorded.

***Macrolophus pygmaeus* and *Tuta absoluta* population levels**

Macrolophus pygmaeus was present from the first sampling at the two outer control plots with significantly higher numbers in the west control plot (Table 3). In the next samplings it was present in all the plots with similar numbers among them.

Alive larvae of *T. absoluta* were recorded at very low numbers in the treatment and the central control plot, during the third and the last sampling (Table 3).

TABLE 1. Mean number (±SE) of *Nesidiocoris tenuis* individuals per tomato stem, in different sampling dates after transplantation (May 31), in different plots in an open tomato field in the area of w. Peloponnesus. (Plot A: East control plot, Plot B: Central control plot, Plot C: Plot with pre-plant release of N. tenuis, Plot D: West control plot). Means followed by the same letter in a column are not statistically different.

	Sampling date	**May 31**	**June 17**	**July 7**	**July 27**
	Plot				
***Nesidiocoris tenuis* (nymphs and adults)**	**A**	0.00	0.00	0.12±0.12b	1.62±0.62a
	B	0.00	0.00	0.31±0.09b	1.28±0.28a
	C	1.06±0.21	0.16±0.09	2.31±0.42a	1.65±0.36a
	D	0.00	0.00	1.50±0.56b	0.87±0.47a

TABLE 2. Structure of *Nesidiocoris tenuis* population (mean number per stem/sampling date), in a plot where plants with pre-plant release of *N. tenuis* were planted and in the no-pre-plant release plot (central control plot), in an open tomato field in the area of w. Peloponnesus (Greece). Means followed with same letter are not significantly different among the sampling dates.

	Pre-plant release of *Nesidiocoris tenuis*, treatment plot)					
	Nymphal instar					**Adult**
Sampling date	I	II	III	IV	V	
May 31	0.39a	0.32a	0.26a	0.03a	0.00	0
June 17	0.00	0.00	0.00	0.00	0.00	0.16a
July 7	0.26b	0.23b	0.55b	0.26a	0.58a	0.26a
July 27	0.32a	0.64a	0.19a	0.16a	0.22b	0.09a
	χ^2=9.19, *P*=0.0268	χ^2=10.86, *P*=0.0125	χ^2=10.74, *P*=0.0132	χ^2=3.20, *P*=0.3608	χ^2=15.41, *P*=0.0015	χ^2=2.76, *P*=0.4289

	No pre-plant release of *Nesidiocoris tenuis*, control plot)					
	Nymphal instar					**Adult**
Sampling date	I	II	III	IV	V	
May 31	0.00	0.00	0.00	0.00	0.00	0.00
June 17	0.00	0.00	0.00	0.00	0.00	0.00
July 7	0.02a	0.10a	0.04a	0.08a	0.10a	0.12a
July 27	0.27b	0.33b	0.14b	0.12b	0.22b	0.16b
	χ^2=13.96, *P*=0.0030	χ^2=25.43, *P*<0.0001	χ^2=17.61, *P*=0.0005	χ^2=11.41, *P*=0.0097	χ^2=25.47, *P*<0.0001	χ^2=6.68, *P*=0.0825

Leaves with mines of *T. absoluta* were firstly recorded in the 3rd sampling at very low levels and increased slightly in the last sampling but were kept at low levels without differing significantly among the plots (Table 3). No damage on fruits was recorded.

TABLE 3. Mean numbers (±SE) of *Macrolophus pygmaeus* individuals, *Tuta absoluta* larvae and mines per tomato stem, in different dates after transplantation (May 31), in different plots in an open tomato field in the area of w. Peloponnesus (Greece). (Plot A: East control plot, Plot B: Central control plot, Plot C: Plot with pre-plant release of *N. tenuis*, Plot D: West control plot). Means followed with same letter are not significantly different among the plots for each sampling date.

	Plot	May 31	June 17	July 7	July 27
Macrolophus pygmaeus	**A**	0.50±0.18a	0.12±0.12a	0.50±0.40a	0.62±0.37a
	B	0.00	0.24±0.07a	1.25±0.20a	0.93±0.26a
	C	0.00	0.41±0.15a	0.59±0.20a	0.46±0.08a
	D	1.12±0.39b	0.87±0.51a	1.00±0.40a	0.62±0.18a
		$F_{3,39}$=23.32, P<0.001	$F_{3,79}$=1.81, P=0.15	$F_{3,79}$=2.05, P=0.11	$F_{3,79}$=1.03, P=0.38
***Tuta absoluta* larvae**	**A**	0.00	0.00	0.00	0.00
	B	0.00	0.00	0.00	0.09±0.05
	C	0.00	0.00	0.03±0.01	0.00
	D	0.00	0.00	0.00	0.00
***Tuta absoluta* mines**	**A**	0.00	0.00	0.25±0.16ab	1.12±0.47a
	B	0.00	0.00	1.34±0.31a	1.68±0.33a
	C	0.00	0.00	0.15±0.09b	1.28±0.22a
	D	0.00	0.00	0.00	2.12±0.58a
				$F_{3.79}$=6.35 P<0.0007	$F_{3.79}$=0.89, P=0.44

The capture rates of *T. absoluta* males in the pheromone trap are shown in Figure 1. Generally, the captures steadily increased attaining highest values from late June to middle July showing a peak on July 11 when 46 adults were trapped in a week.

At the end of July the captures were reduced to 28 males per week. Adults of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) were recorded and their numbers showed a peak of 2.12 adults (mean number per stem in all the plots) on week 2, whereas 4 adult thrips per stem were observed in the first sampling. However, their numbers were soon reduced and were not further considered.

Discussion

The release of *N. tenuis* in the nursery favoured its earlier establishment on the tomato plants in comparison to the controls. The contribution of pre-plant release in earlier establishment is indicated by the fact that only on that plot i) *N. tenuis* individuals were present in the first and the second samplings and ii) young nymphs were recorded with high numbers in the first sampling, showing that establishment was not due to adult arrival from the nearby vegetation.

Based on our earlier investigations in

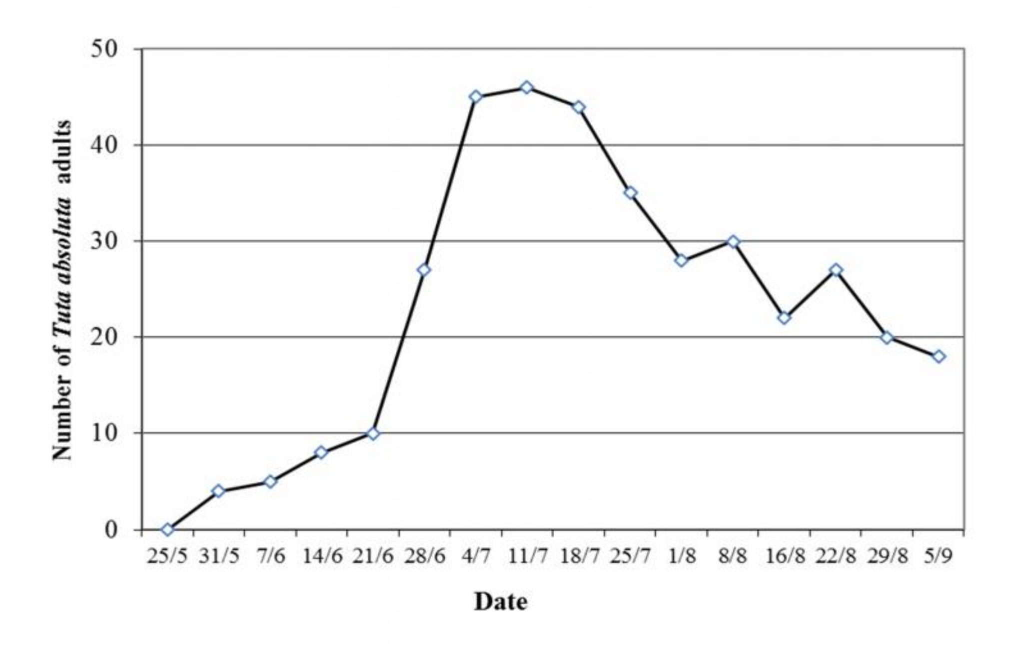

FIG. 1. Trap catches per week of *Tuta absoluta* adults in a pheromone trap in the experimental open tomato field.

the area of the study, *N. tenuis* was firstly recorded on the tomato crops in early July (Perdikis et al. 2007). Therefore, this technique favoured the earlier establishment of the predator. In greenhouse experiments, whitefly and *T. absoluta* population were significantly lower where *N. tenuis* was released, verifying that the tobacco whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), or *T. absoluta* populations can be controlled when *N. tenuis* is released before planting (Calvo et al. 2012a). In that study the pre-plant release of 0.5 adults of *N. tenuis* per plant was effective to control *T. absoluta* populations and its combination with supplementary control agents (i.e. sprays with *B. thuringiensis* and release of parasitoids) did not significantly improve the results. Similarly, Calvo et al. (2012b) showed that pre-plant release of 0.5 and 1 *N. tenuis*/plant were effective to control *B. tabaci*. At the same study, the highest release rate (2 *N. tenuis*/plant) provided more rapid suppression of *B. tabaci* was recorded than the two other rates, which also controlled the pest very effectively.

Lenfant et al. (2000) reported that *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) established earlier in the season when it was released before transplanting. The establishment of the mirid predators provided also effective control of other serious pests such as whiteflies that although appeared, their increase in numbers was prevented.

In regard to the pre-plant release, although traditionally, open field crops are considered to receive few benefits from released natural enemies, the pre-plant release method may be a valuable tool to stimulate the use of biological control in these systems. Indeed, this strategy

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enhances earlier establishment of the targeted natural enemy, in our case *N. tenuis,* that can be essential for effective control i.e. in areas where population of native natural enemies are low. However, the application of this method in open field crops has to be evaluated further under variable conditions in regard to abundance of native predators, pest pressure levels or pre-plant release rates of the predator.

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Η εξαπόλυση του αρπακτικού *Nesidiocoris tenuis* (Hemiptera: Miridae) στο φυτώριο βοήθησε στην πιο γρήγορη εγκατάστασή του σε καλλιέργεια υπαίθριας τομάτας

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ΠΕΡΙΛΗΨΗ

Ο εχθρός της τομάτας, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) που εισέβαλε στη χώρα μας, πολύ σύντομα έγινε σημαντικός εχθρός τόσο των υπαίθριων όσο και των θερμοκηπιακών καλλιεργειών. Η εξαπόλυση του αρπακτικού *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae) στο φυτώριο έχει βρεθεί ότι μπορεί να συμβάλει στην αντιμετώπιση του *T. absoluta* σε καλλιέργειες τομάτας στο θερμοκήπιο. Ο στόχος της παρούσας μελέτης είναι να αξιολογηθεί κατά πόσο σε υπαίθρια καλλιέργεια τομάτας, η εξαπόλυση του *N. tenuis* στο φυτώριο μπορεί να συμβάλει στην έγκαιρη εγκατάστασή του. Συμπερασματικά, η εξαπόλυση του *N. tenuis* συνέβαλε ουσιαστικά στην έγκαιρη αποίκηση της καλλιέργειας σε σύγκριση με τα τεμάχια του μάρτυρα. Επίσης, ο ρόλος του ιθαγενούς πληθυσμού του *M. pygmaeus* πρέπει να λαμβάνεται σοβαρά υπόψη στα προγράμματα ολοκληρωμένης αντιμετώπισης. Η μέθοδος αυτή είναι αποτελεσματική στην πιο έγκαιρη εγκατάσταση του *N. tenuis* και θα πρέπει να αξιολογηθεί περαιτέρω η συμβολή της στην αντιμετώπιση του *T. absoluta*.