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Predatory mirid *Nesidiocoris tenuis* (Heteroptera: Miridae) as biological control agent in greenhouse tomato production

Predatorska stenica *Nesidiocoris tenuis* (Heteroptera: Miridae), prirodni neprijatelj štetnih insekta u plasteničkoj proizvodnji paradajza

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ABSTRACT

Species of zoophytophagous genus *Dicyphina*, plant bugs (Hemiptera: Miridae: Bryocorinae) are among the most abundant and effective predators of tomato pests. *Nesidiocoris tenuis* Reuter, 1895 (Hemiptera: Miridae) is the predominant species of *Dicyphina* sp. and it is considered to be a major natural enemy of insect pests in tomato crops in the Mediterranean region. This predatory bug is proved to be particularly successful in suppressing *Tuta absoluta* Meadow, 1917 (Lepidoptera: Gelechiidae), a major pest of tomato in greenhouse production. Aims of the study were to 1) investigate the efficiency of the mirid predatory bug *N. tenuis* in greenhouse tomato production and 2) to estimate the possibilities of its application in commercial vegetable production in Western Balkan countries of Europe. The significance of the study results relies on the possibility of commercialisation and general promotion of biological agent *N. tenuis* in tomato production in South East part of Europe, where the usage of pesticides still dominates the field of plant protection, both in vegetables and other agricultural sectors. According to the obtained results, the biological agent *N. tenuis* represents a reliable tool for controlling the level of the tomato leaf miner population by keeping the pest below its economic and intervention threshold.

Keywords: *Tuta absoluta*, biocontrol, *Dicyphina* sp., integrated pest management

APSTRAKT

Zoofitofagne vrste iz roda *Dicyphina* (Hemiptera: Miridae: Briocorinae) spadaju u grupu najzastupljenijih i najefikasnijih predatora štetočina paradajza. U usevima paradajza, vrsta *Nesidiocoris tenuis* Reuter, 1895 (Hemiptera: Miridae) je najzastupljeniji predstavnik roda *Dicyphina* i smatra se najvažnijim prirodnim neprijateljem štetočina paradajza u Mediteranskoj regiji. Ova predatorska stenica se pokazala posebno uspešno u suzbijanju moljca paradajza *Tuta absoluta*, 1917 (Lepidoptera: Gelechiidae), glavne štetočine u proizvodnji paradajza u zatvorenom prostoru. Ciljevi sprovedenog istraživanja su 1) ispitivanje efikasnosti predatorske stenice *N. tenuis* u proizvodnji paradajza u zatvorenom prostoru, odnosno 2) procena njene praktične primene u komercijalnoj proizvodnji povrća u zemljama zapadnog Balkana. Dobijeni rezultati doprinose promovisanju biološkog agensa *N. tenuis* i ukazuju na mogućnost komercijalne upotrebe ove predatorske stenice u proizvodnji paradajza u jugoistočnom delu Evrope, gde primena pesticida i dalje dominira u oblasti zaštite bilja, kako u povrtarstvu tako i u drugim poljoprivrednim sektorima. Prema dobijenim rezultatima, biološki agens *N. tenuis* predstavlja efikasno sredstvo za suzbijanje *T. absoluta*, kontrolom brojnosti populacije moljca paradajza ispod nivoa ekonomskog praga štetnosti.

Ključne reči: *Tuta absoluta*, biokontrola, *Dicyphina*, integralna zaštita bilja

INTRODUCTION

Intensive production of *Solanum lycopersicum* L. (Solanaceae) is accompanied by various types of pests. Among them, harmful insects attract the biggest attention in the greenhouse production of vegetables, specifically their evident influence on the quantity and quality of yields. In addition to harmful insects, there are also insects in tomato crops that belong to the group of beneficial insects, as natural enemies of tomato pests. These insects belong to different orders (Hemiptera, Diptera, Hymenoptera, Thysanoptera) and can be predators, parasites or parasitoids (Ivezić and Jezerkić, 2019). According to Maceljski et al., (2002), predatory mirid bugs are among the most important natural enemies of agricultural pests, belonging to families Anthocoridae, Geocoridae, Miridae, Nabidae, Pentatomidae and Reduviidae (Hemiptera). Predatory species within these families feed on eggs and larvae of harmful insects, weakened adult individuals, and are mostly polyphagous (Kuštera, 2018). Zoophytophagous mirid bugs of the genus *Dicyphina* (Hemiptera: Miridae, Bryocorinae) are the most widespread and efficient predators of tomato pests (Alomar and Albajes, 1996; Malausa and Trottin-Caudal, 1996; Sánchez et al., 2003; Sánchez et al., 2006; Castañé et al., 2004; Gilespie et al., 2007; Perdikis et al., 2008; Sánchez, 2008; Calvo et al., 2009; Calvo et al., 2012a; Calvo et al., 2012b). *Nesidiocoris tenuis* Reuter, 1895 (Hemiptera: Miridae) is one of the most widespread members of the *Dicyphina* genus in tomato crops and is considered the most important natural enemy of tomato pests (Goula and Alomar, 1994; Sánchez et al., 2003; Perdikis et al., 2011, Esparza-Diaz et al., 2021).

Adults of this species are good fliers and very active in warm tropical conditions. Females lay eggs in plant tissue and eggs are very difficult to detect without proper magnification (Goula and Alomar, 1994). The larvae can be seen on the back of the leaves and are very active and fast. They develop through 5 larval instars, they are 1 to 4 mm long and have no wings (Goula and Alomar, 1994). The life cycle of *N. tenuis* varies from 14.9 days at 35 °C, 21.8 days at 25 °C, to 86.7 days at 15 °C (Sánchez et al., 2009). In laboratory conditions at a temperature of 25 °C, hatching of the larvae begins 7 days after oviposition, while the larval stage at the same temperature lasts for 12.9 days (Sánchez et al., 2009). In addition to the influence of the external environment, especially the temperature, the length of the developmental cycle also depends on the type of prey, so the life cycle of *N. tenuis* is shortened if the larvae feed exclusively on whiteflies (*Trialeurodes vaporariorum*, *Bemisia tabaci*), compared to feeding on thrips or mites (Calvo et al., 2012a). Adults are very voracious and eat more than 100 eggs a day in the laboratory conditions (Urbaneja et al., 2009). At temperatures between 20 and 35 °C, the fertility rate is from 60 to 80 larvae, which enables rapid population growth but decreases significantly at temperatures above 40 °C and below 15 °C (Sánchez, 2009). Reproductive potential decreases with decreasing temperature, while the temperature threshold for development is 12 - 13 °C (Jacobson, 2018).

Although it originates from the tropics, *N. tenuis* occurs regularly in Europe, especially in the countries of the Mediterranean region (Jacobson, 2018). This predatory mirid bug, common in vegetable production, feeds on almost all small arthropods present in tomato production (Sánchez et al., 2006; Peridinis et al., 2008; Sánchez, 2008, Calvo et al., 2009), but also on arthropods characteristic for several other vegetables (Urbaneja et al., 2005) and tobacco (Perdikis et al., 2011). In integrated and organic agriculture, *N. tenuis* has been particularly successful in controlling the tomato leaf miner *Tuta absoluta* Meyrick, 1917 (Lepidoptera: Gelechiidae), the greenhouse whitefly *Trialeurodes vaporariorum* Westwood, 1856 (Hemiptera: Aleyrodidae) and tobacco whitefly, *Bemisia tabaci* Genademius 1889 (Hemiptera: Aleyrodidae) (Carnero-Hernández et al., 2000; Calvo et al., 2009); while used to a lesser extent to control thrips, leaf miners, lice, mites and eggs of harmful Lepidoptera (Urbaneja et al., 2005; Perdikis and Lykouressis, 2002; Calvo and Urbaneja, 2003). In addition to all the above mentioned pests that it feeds on, *N. tenuis* is most often commercially used to control tomato leaf miner, one of the most common indoor tomato pests (Mollá et al., 2011; Al-Jboory et al., 2012; El Arnaouty and Kortam, 2012; Mollá et al., 2014).

The development of integrated pest management (IPM) and usage of selective insecticides stimulated implementation of *N. tenuis* within vegetable protection programme (Arnó et al., 2009; Mollá et al., 2011; Urbaneja et al., 2012). Numerous studies indicate the application of *N. tenuis* in the control of tomato leaf miner primarily because this predatory mirid bug feeds on all developmental stages of *T. absoluta*, but also due to the fact that in tomato crops with high population of *N. tenuis*, damage from tomato leaf miner is very low (Urbaneja et al., 2009; Arnó et al., 2009).

The commercial use of *N. tenuis* was preceded by laboratory and field testing of various strategies for the successful application of this predatory mirid bug and effective control of harmful organisms (Sánchez et al., 2014). Sánchez et al., (2014) also point out that a successful strategy for the application of *N. tenuis* in the control of tomato moth involves increasing mirid population just before the colonisation of the crop by the pest itself. This strategy requires a precise definition of the optimal moment for the application of mirids in tomato crops. The fact that these insects show a greater tendency towards eggs and lower larval stages compared to higher larval stages and adults of *T. absoluta*, increasing *N. tenuis* population number before the emergence of *T. absoluta* supports this strategy (Urbaneja et al., 2009; Arnó et al., 2009).

Aims of this study were 1) to investigate the efficiency of the mirid predatory bug *Nesidiocoris tenuis* in greenhouse tomato production and 2) to estimate the possibilities of its application in commercial vegetable production in South East of Europe. Since the insecticides usage is the most common method of the pest control in the previously mentioned part of Europe, validation of the *N. tenuis* biological control effectiveness was conducted with simple comparison of results from biological control experiment with the results from chemical treatment of tomatoes, respectively.

MATERIAL AND METHODS

Trial description

During 2021, the Regional Centre of Kikinda, Department of Forecasting and Warning Service in Plant Protection (www.pissrbija.com) conducted an experiment to test application of *N. tenuis* in greenhouse tomato production. For this purpose, biological product Nesidiocoris system® (Virginia d.o.o., Belgrade, Serbia), consisting of an inert mineral substrate and *N. tenuis* adult predatory mirid bugs, was used. The experiment was conducted in controlled conditions in the greenhouse production of tomatoes at the locality of Kikinda (Latitude: 48° 48’ 36,072”; Longitude: 20°25’13,908”). For the purpose of the experiment, two tomato crops were established in 2 separate greenhouses (hereinafter: Greenhouse 1 and Greenhouse 2). Both greenhouses were of the same dimensions (8 × 26 meters) and with the same technology of tomato production (irrigation, foliar feeding, aeration). An early tomato hybrid (Cherokee®, Distributor: Agro-gru) was planted in both greenhouses, with a distance between plants of 50 × 50 cm. Sticky delta pheromone traps® (Csalomon, Hungary) were used to monitor the dynamics of the tomato leaf miner population. In each tomato crop, one pheromone trap was placed in the central part of the greenhouse following product instructions. Pheromone trap placed in Greenhouse 1 was marked as pheromone trap 1 and pheromone trap placed in Greenhouse 2 was marked as pheromone trap 2. In addition to pheromone traps, one sticky hunting trap® (Csalomon, Hungary) was set up in each tomato crop in the central part of the greenhouse to monitor the presence and activity of other tomato pests such as thrips, whiteflies, and silverleaf whiteflies. In Greenhouse 1, the population of *T. absoluta* was primarily controlled using mirid predatory bug *N. tenuis*, with only one insecticide treatment due to an increased population of thrips. In the second greenhouse, tomato leaf miner was treated with chemical agents only.

Greenhouse 1 experiment design

In Greenhouse 1, two commercial packages of Nesidiocoris system of 500 adult *N. tenuis* (a total of 1000 mirid bugs + substrate) were used for conducting the trial. Each package of Nesidiocoris system was used for individual treatment and thus the recommended population density of 0.5-3 units per m^2 was achieved. According to the distributor's recommendation (Virginia d.o.o), the treatment should be performed twice during the growing season, with the first application of *N. tenuis* immediately after transplanting tomato plants, and the second application 10 to 14 days after the first application. Since the *N. tenuis* species switches from predatory or carnivorous regime to phytophagous diet in the absence of food sources (prey), it is necessary to monitor the activity of mirid bugs on tomato plants and after registering characteristic symptoms of damage (concentric circles on plant tissue, aborted flowers), insecticidal treatment should be carried out to control the population level of predatory mirid bugs. In Greenhouse 1, only one chemical treatment was performed with the Laser® insecticide (a.m. spinosad; Distributor: Agrimatco, Serbia) on 15 June 2021 in the concentration of 0.03-0.05% due to the increased population of thrips registered by sticky hunting traps. Laser was chosen because of its extremely small or negligible negative effect on beneficial organisms. Spinosad preserved natural populations of beneficial insects, making it an ideal choice in IPM programmes (Williams et al., 2003; Miles and Eelen, 2006).

Tomato transplanting procedure and mirid bug application

Tomato transplanting was conducted on 16 April, 2021, and the first application of predatory mirid bugs was carried out on 22 April. During the application, the content of one package of Nesidiocoris system (500 adults) was divided into 6 plastics, sterile cups, which were randomly distributed over the total area of the Greenhouse 1 to ensure uniform distribution of predatory bugs. The treatment was performed in the afternoon (6 pm). Before the introduction of mirid bugs, the Greenhouse 1 was covered with insect net on both sides to prevent migration of applied individuals. The greenhouse remained closed overnight to allow the applied specimens to adapt to environmental conditions (temperature, air) and to be evenly deployed within the treated greenhouse. The day after application, a visual inspection of the tomato crop was performed and the presence of applied individuals on the examined plants was confirmed, which indicated that the individuals left the substrate and were evenly distributed within the treated greenhouse. The second application of predatory mirid bugs was performed on 4 May 2021, 12 days after the first application, following the same methodology.

Greenhouse 2 experimental design

In Greenhouse 2, insecticide Verimark® (a.m. cyanthraniliprol; Distributor: Agromaket, Serbia) was used to control tomato leaf miner. It was applied through an irrigation system at the dose of 0.5 l/ha, while for the control of thrips and aphids, insecticide Laser® (a.m. spinosad; Distributor: Agrimatco, Serbia) was used in a concentration of 0.03-0.05%. In Greenhouse 2, a total of 4 chemical treatments were carried out. Two treatments were performed to control *T. absoluta*, but only after economic threshold of 2.25 larvae per plant was achieved (Tadele and Emana, 2018). It was applied through the irrigation system on 24 June 2021 and on 2 August 2021. The other two treatments were carried out with insecticide Tepeki® (a.m. flonicamid; Distributer: Agromarket, Serbia) on 3 June 2021 after recording the increased presence of aphids on tomato plants and sticky hunting traps, with the Laser® insecticide on 15 June 2021 due to increased population of thrips. Calvo and Urbaneja (2004) recommended spraying against *N. tenuis* when the population reach 0.5-1.5 individuals per leaf. Since the level of the *N. tenuis* population in the Greenhouse 1 did not exceed the abovementioned threshold, chemical treatment to reduce the population of predatory mirid bugs was omitted.

Monitoring methodology

In both greenhouses, monitoring was based on catch of imago on pheromone traps and visual inspection of plants in order to determine the presence of characteristic symptoms caused by tomato leaf miner (leaf mines, damaged fruits). With detection of characteristic symptoms, damaged leaves and fruits were removed from the greenhouse so that previously recorded symptoms would not be taken under consideration during the next examination. In addition, 100 ripe tomato fruits were isolated in each greenhouse during the harvest period and examined for the presence and damage by *T. absoluta* larvae. Monitoring of adult *T. absoluta* caught on pheromone traps was checked 2 to 3 times per week in both experimental greenhouses (1 and 2), while visual observations of tomato crops were performed once or twice a week depending on the activity of *T. absoluta*. During the period of intensified tomato leaf miner activity (July and August), visual observations were conducted twice a week. During the visual inspections, 20 randomly selected tomato plants were examined in each greenhouse to monitor the development of *T. absoluta* and to estimate the health status of the cultivated crop. During each examination, minimum and maximum temperatures were measured using digital thermos-hygro sensor (TFA®, Product code: HO30.5035) after which a number of damaged plants with characteristic symptoms on the leaves and fruits were registered and removed.

Statistical analysis

For all statistical analyses, a programming language R was used (4.1.3). For each trap, classical (mean, standard deviation, coefficient of variation, overdispersion factor and number of zeros) and robust (median, median absolute deviation and robust coefficient of variation) parameters of descriptive statistics were calculated (Table1). The differences between two pheromone traps were statistically analysed with two types of *t*-tests for independent samples: the non-parametric Wilcoxon rank sum test for testing the difference between medians of the two samples and the Yuen two-sample test as well. This test is based on 20% trimmed means and 20% windsorised variances. If there are no extreme observations, this test becomes a Welch's test when there is no trimming.

Temperature effect on the abundance of insects

The 'pscl' (Jackman, 2010) and 'MASS' (Venables and Ripley, 2013) libraries from R statistical computing environment were used to fit several count models without and with zero inflation. The best fitting model was selected based on the Akaike Information Criterion (AIC) and confirmed by comparing the half-normal plots with simulated envelope among different models. Most suitable models for our data were negative binominal and zero-inflated negative binominal with similar AIC values between them.

RESULTS

Quantification of Tuta absoluta adults

Abundance of *T. absoluta* adults caught on pheromone traps in both greenhouses are given in Figure 1. The highest number of adult specimens caught in Greenhouse 1 was recorded at the beginning of August and it was 9 (max. temp = 39.85 °C; min. temp = 21.07 °C). The highest number of caught individuals in Greenhouse 2 was 38 and was recorded in the middle of August (Figure 1) (max. temp = 34.90 °C; min. temp=19.84 °C). In the Greenhouse 1, a total number of 78 adults was recorded on pheromone traps during 4 months of monitoring. In the Greenhouse 2, the total number of adults on pheromone traps treated only with insecticides during the same period was 499 individuals.

Quantified morphological changes of tomato plants

Visual examination of plants in the Greenhouse 1, with applied *N. tenuis* specimens, showed a total of 23 damaged plants, while a total of 75 damaged plants were registered in the Greenhouse 2 (Figure 2). Examination of ripe tomato fruits in Greenhouse 1 revealed 3 damaged fruits with the symptoms of *T. absoluta* activity, while 7 damaged fruits were registered in the Greenhouse 2.

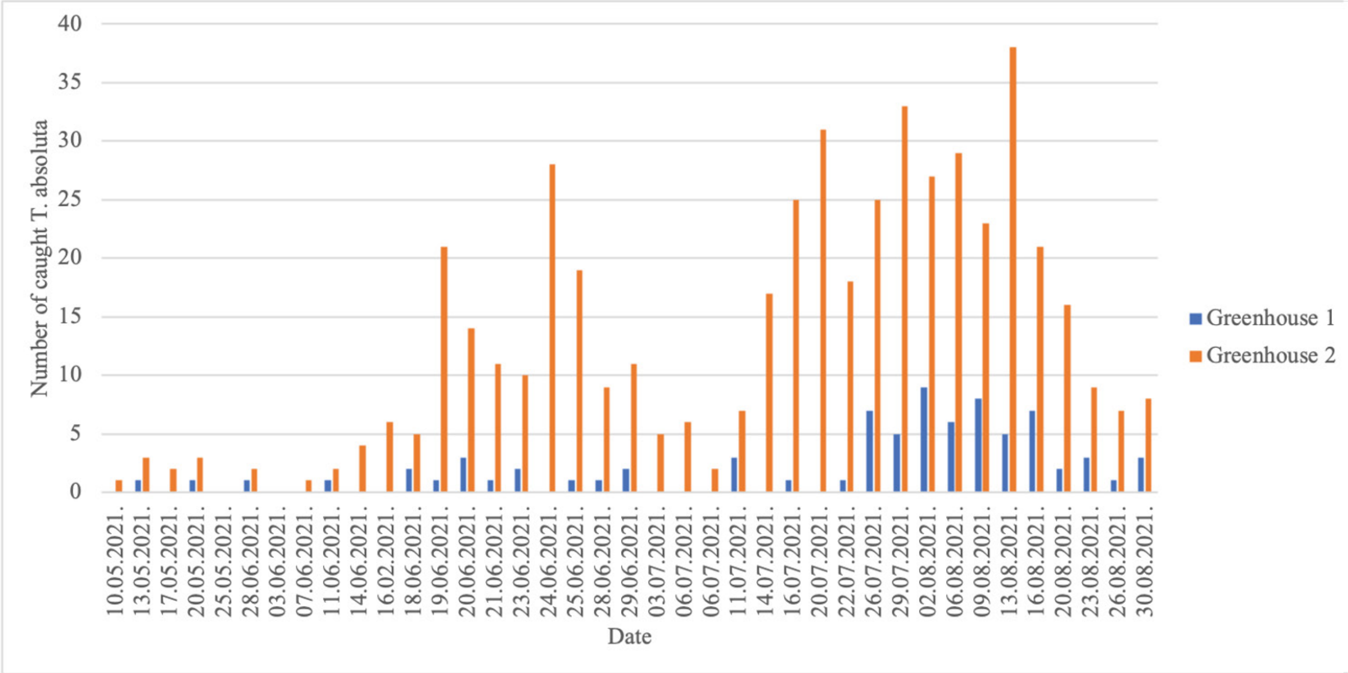

Figure 1. A total number of *T. absoluta* adults caught on pheromone traps (Y axis) by date of sampling (X axis)

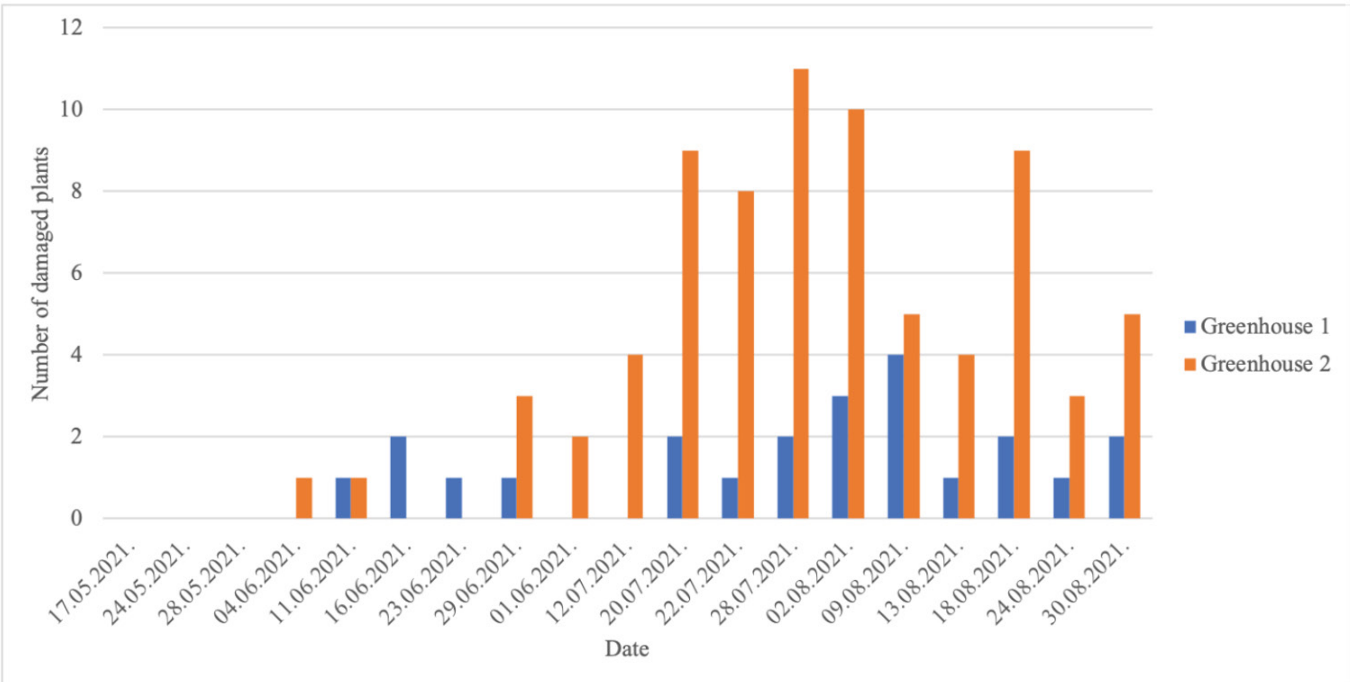

Figure 2. A total number of tomato plants damaged by *T. absoluta* (Y axis) by date of examination (X axes)

Descriptive statistics and regression coefficients

The results of descriptive statistics for trap data and statistics for difference between two pheromone traps are given in Table 1. Test statistics for Yuen two-sample test was -4.38, whereas *W* value for Wilcoxon rang sum test was 212.5. Both tests showed statistically highly significant difference between the two observed greenhouses in terms of total number of adults caught on pheromone traps and total number of damaged plants, but not in terms of registered damaged ripe fruits during harvest. Regression coefficients for negative binominal and zero-inflated negative binominal models and their statistical inference are given in Table 2. These regression coefficients are used to calculate the temperature effect on insect abundance e.g., the maximum temperature effect for pheromone trap 1 can be exponentially calculated using the following formula: $e^{\beta 1} = e^{0.174} = 1.190$, which means that the increase of max temperature of 1 °C will increase the number of insects for 19%.

Table 1. Classical and robust parameters of descriptive statistics of data gained via pheromone traps

Parameter	Pheromone trap 1	Pheromone trap 2
	Classic estimators	
Mean	2.0	12.8
Standard deviation	2.49	10.7
Coefficient of variation (%)	124.5	83.6
Overdispersion factor	3.105	9.030
Number of zeros	13 (33.3%)	2 (5.1%)
	Robust estimators	
Median	1.0	9.0
Median absolute deviation	1.48	10.4
Robust coefficient of variation (%)	148.0	115.6

Table 2. Regression coefficients from negative binomial and negative binomial zero-inflated models and their statistical inference

Predictor	Negative binomial model		Negative binomial zero-inflated model	
	Pheromone trap 1	Pheromone trap 2	Pheromone trap 1	Pheromone trap 2
Minimum temperature	0.106^{ns}	-0.004^{ns}	-	-0.023^{ns}
Maximum temperature	0.104^{ns}	0.108*	0.174*	0.107*
Temperature variation	0.028^{ns}	0.064^{ns}	0.067^{ns}	0.066^{ns}

* *P*<0.05; ns - not significant

DISCUSSION

The protected area of biological control programmes based on the controlled use of polyphagous predators such as *N. tenuis*, with the use of selective insecticides and habitat control, have enabled the successful control of economically most important tomato pests such as whitefly and tomato leaf miner (Urbaneja et al., 2009; Mollá et al., 2014; Sylla et al., 2016). Van Lenteren (2012) states that *N. tenuis* is commercially produced and used worldwide as a biological agent to control various agricultural pests. Another reason for the mass application of *N. tenuis* stems is the fact that this species is very easily produced in laboratory condition (De Puysseleyr, 2013).

To achieve desired results and determine the optimal period for application of biological agent, it is necessary to know biology of beneficial insect and biology of the targeted pest. The elementary cause that limits the application of *N. tenuis* is the fact that this mirid bug, even under favourable environmental conditions, needs 5 to 8 weeks to reach the population necessary for successful control of tomato moth, while under adverse conditions it very rarely reaches the level of a population that is competitive with the pest (Mollá et al., 2011; Urbaneja et al., 2012). As for the influence of environmental conditions, the influence of temperature is often highlighted as a key factor in the population dynamics of this mirid bug. According to Esparza-Diaz et al. (2021), *N. tenuis* adults are very susceptible to cold or high temperatures. The same authors indicate that optimal temperatures for Tmax and Tmin ranged from 23.8 °C to 25.4 °C, and from 12 °C to 15.4 °C, respectively. In a high temperature of 40 °C, *N. tenuis* is unable to develop and barely can reproduce (Sánchez et al., 2009). In addition, Sánchez (2009) reported that the optimal temperature for *N. tenuis* development oscillates between 20 °C and 30 °C; temperatures that are common in the Mediterranean region, where *N. tenuis* populations can thrive.

In our study, the first application of *N. tenuis* in accordance with the literature recommendations and manufacturer instructions was carried out immediately after tomato transplanting, which provided enough time for predatory bug to reach the population level sufficient for effective pest control. The first significant increase of *T. absoluta* population in Greenhouse 2 was recorded in the 6th week after tomato transplanting, when 21 individuals were registered. No drastic increase in pest population was registered in Greenhouse 1 during the entire monitoring period. Our results showed statistically significant difference in the number of *T. absoluta* adults caught on pheromone traps in Greenhouses 1 and 2, which indicates that the applied biological agent effectively controlled the level of pest population. Similar conclusions were obtained by statistical analysis of monitoring results and determination of the number of damaged tomato plants. A total number of damaged plants was more than three times lower in Greenhouse 1 where *N. tenuis* specimens had been applied, compared to the number of damaged tomato plants in the Greenhouse 2.

To ensure effective application of beneficial insects and introduction of biocontrol methods into regular agricultural practice, a detailed evaluation of all available strategies must be considered and most adequate moment for application must be selected. Such a systematic approach is necessary to achieve long-term effects with the least harmful consequences. Calvo et al., (2012a) state that it is necessary to raise the mirid bug population to approximately one adult per tomato leaf just before the pest emerges in the cultivated crop to successfully control *T. absoluta* by means of *N. tenuis*, while Arnó et al. (2009) recorded a low percentage of damage of tomato fruits by *T. absoluta* in greenhouses in which the mirid bug population has reached 4.5 individuals per tomato plant. Sánchez et al. (2014) point out that a good strategy for the application of *N. tenuis* is before transplanting tomato plants in the case of seedlings treatment. This technique initially gave good results in the application of *Macrolophus pigmeus* Rambur, 1839 (Hemiptera: Miridae) (Lenfrant et al., 2000), but it is also very successfully used in the application of *N. tenuis* (Calvo et al., 2012a, 2012b). Calvo et al. (2012a) stated that *N. tenuis* successfully suppresses *T. absoluta* and *B. tabaci* in seedling production of tomato, both individually and when both pests are present in the crop at the same time.

Another strategy that contributes to the growth of the *N. tenuis* population is the introduction of the eggs of the flour moth *Euphestia kuhniela* Zeller, 1879 (Lepidoptera: Pyralidae) into protected area (Sánchez et al., 2014). Flour moth eggs are alternative prey and supplementary food for mirid bugs before the emergence of pests such as *T. absoluta* or *B. tabaci* and enable the establishment of the desired level of the population of *N. tenuis* at a critical moment (Calvo et al., 2012a, 2012b).

In contrast to good results, the potential risk of the mentioned strategies is evident, because *N. tenuis* can damage the cultivated crop if the increase in mirid bug population is not correlated with the increase in the population of targeted pests (Sánchez et al., 2014). Although we did not register damaged tomato plants in our study due to the activities of applied biological agent, numerous studies have confirmed that *N. tenuis* intensifies the phytophagous diet and damages tomato plants in the absence of pray (Sánchez et al., 2006; Sánchez, 2008; Sánchez and Lacasa, 2008; Calvo et al., 2009; Arnó et al., 2010; Castañé et al., 2011). Like many other species of the genus *Dicyphina*, *N. tenuis* is an omnivorous species that can occur in the same cultivated crop both as a pest and as a beneficial insect (Trottin-Caudal, 2011). If the pest population is high, this omnivorous mirid bug has a predominantly carnivorous diet (Sánchez, 2008), while a low prey population favours herbivorous diet, which can consequently cause damage to the crop itself (Urbaneja et al., 2005).

Characteristic symptoms on plants are necrotic rings on stems and leaf petioles and damaged flowers (Urbaneja et al., 2005; Sánchez, 2008; Calvo et al., 2009; Castañé et al., 2011). In Southern France, this predatory mirid bug is even considered a pest of tomatoes in protected areas due to the overpopulation of the native population (Trottin-Caudal, 2011), while other authors (Wheeler, 2000; Sánchez, 2008; Franin and Barić, 2012) state that this *Dicyphina*, even in high numbers, does not cause economically significant damage to the stems and flowers of the tomato. In case of overpopulation, the number of *N. tenuis* is controlled by insecticides, while insecticides based on the active substances of indoxacarb and acetamiprid stand out as particularly effective, which on the other hand show poor efficiency in controlling *T. absoluta* but still very effective on *N. tenuis* (Khoshabi et al., 2016).

CONCLUSIONS

To achieve satisfactory results in biocontrol, the application of biological agents is preceded by thorough and systematic crop monitoring, accurate identification and biology of pests and beneficial insects, as well as locating the appropriate time of application. Since organic agriculture is a type of production that strives to preserve and protect the environment, it is necessary to create favourable conditions for the survival of all beneficial organisms, regardless of whether they are members of native fauna in certain agroecosystems or introduced species. For this reason, biological crop protection is an environmentally friendly alternative to the protection of indoor vegetable crops, as opposed to conventional protection based on the excessive use of toxic chemicals and the unnecessary pollution of the environment. Based on the obtained results, it can be concluded that the applied biological agent *Nesidiocorus tenuis* is an excellent environmentally friendly alternative to insecticides which can control the level of the *Tuta absoluta* population very effectively. In our experiment, *T. absoluta* did not exceed the threshold of damage initiating the need for chemical treatment. On the other hand, in the greenhouse with an integrated approach to tomato protection, despite significantly higher catch of adults on pheromone traps and a significantly higher number of damaged plants, the number of damaged fruits did not endanger production profitability, which means that applied chemicals detained population expansion and prevented a higher degree of pest attack.

This study is a great starting point for further biological control research using various host-pest combinations and interactions, especially in controlled and closed conditions. This approach will upgrade IPM. All other achieved results favouring usage of beneficial insects in organic agriculture should be promoted, especially in the South East Europe, where extensive usage of chemical pesticide is present and its toxicity in human food and nutrition.

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