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**Journal of Pest Science**

ISSN 1612-4758

J Pest Sci

DOI 10.1007/s10340-014-0587-1



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# Prospects for predatory mirid bugs as biocontrol agents of aphids in sweet peppers

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Received: 3 January 2014 / Accepted: 30 March 2014  
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**Abstract** In recent years, biological control strategies to control many major horticultural pests have been successfully implemented in the Eastern Mediterranean basin. However, the management of some pests, such as aphids in sweet pepper crops, can still be improved. The goal of this study was to examine the potential of the omnivorous predatory mirids *Nesidiocoris tenuis*, *Macrolophus pygmaeus*, and *Dicyphus maroccanus* as biocontrol agents of aphids in sweet pepper crops. First, the capacity to detect *Myzus persicae*-infested and un-infested plants was studied in a Y-tube olfactometer. Females of the three species of predatory mirids were strongly attracted to the odor of infested *M. persicae* plants. Second, the prey suitability of young and mature nymphs of *M. persicae* for these three mirid species was studied. The three species actively preyed on *M. persicae*, although *D. maroccanus* resulted the most voracious species preying both young and mature nymphs. Finally, the capacity of the three omnivorous predators to reduce *M. persicae* in heavily infested plants was determined in semi-field conditions. The three species of mirids could reproduce on aphids and establish on sweet pepper plants. Mirids significantly reduced the number of *M. persicae* per leaf, reaching levels of aphid reduction close to 100 % when compared to the untreated control. These results suggest that mirids might play a major role in aphid management in sweet peppers. The potential

implementation methods of predatory mirids for the biological control in sweet peppers are discussed.

**Keywords** *Myzus persicae* · *Nesidiocoris tenuis* · *Macrolophus pygmaeus* · *Dicyphus maroccanus* · Prey suitability · Biological control

## Introduction

During the last decade, biological control strategies for various major pests have been successfully implemented in greenhouse crops in southern Europe (Calvo et al. 2009, 2011, 2012a; van Lenteren 2012). The two most recent and remarkable successes of biological control have occurred in tomato and sweet pepper production in southeastern Spain. These successes have occurred mainly due to the selection and implementation of generalist predators native to the Mediterranean Basin. For tomatoes, the inoculation of the predatory mirid bug *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) in the nursery proved very effective in controlling key tomato pests, such as the sweet potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Calvo et al. 2012a, b; Urbaneja et al. 2012). For sweet peppers, the release of the predatory mite *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) and the minute pirate bug *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) provided effective control of the two key pests in this crop, the *B. tabaci* and the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Blom 2008; Calvo et al. 2009). Currently, all sweet pepper (approximately 9,300 ha) and tomato (approximately 8,000 ha) production operations in southeast Spain use

Communicated by L. Shipp.

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these indigenous and polyphagous biocontrol agents as the main pest control strategy.

Generalist predators are widely recognized to significantly contribute to the biological control of populations of several agricultural pests throughout the world (Symondson et al. 2002). Zoophytophagous or plant feeding predators constitute a special case of generalist predators. Generalist zoophytophagous predators can use various food resources, such as alternative prey, and can feed on plant material, which further facilitates their establishment prior to pest infestation and their maintenance in the crop during periods of prey scarcity, resulting in crop systems that are more resilient to pest invasions (Ramakers and Rabasse 1995; Messelink et al. 2008; Castañé et al. 2011; Urbaneja et al. 2012). These special features result in one of the most important challenges currently in biological control, which is the search and selection of generalist zoophytophagous predators (Bueno et al. 2013).

The biological control of aphids in sweet pepper plants needs improvement, because this strategy has required multiple releases of natural enemies (Blom 2008). These releases are not always sufficiently effective (Belliure et al. 2008, Sanchez et al. 2011) and may considerably increase the final cost of the biocontrol program in this crop (Messelink et al. 2011). The most common species of aphids in sweet peppers are *Myzus persicae* (Sulzer), *Aphis gossypii* Glover, *Macrosiphum euphorbiae* Thomas, and *Aulacorthum solani* Kaltenbach (Hemiptera: Aphididae) (Belliure et al. 2008; Blom 2008; Sanchez et al. 2011). The first two species, which are smaller in size than the last two, are regularly controlled via the release of the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae). In addition, the introduction of banker plants that were previously infested with the cereal aphid *Rhopalosiphum padi* (L) (Hemiptera: Aphididae) is a good method to increase the reservoir populations of *A. colemani* before detecting the presence of the pest preventing outbreaks of this pest (Huang et al. 2011). However, both strategies using this parasitoid (continuous augmentative releases and introduction of banker plants) can dramatically be disrupted by the action of hyperparasitoids, which are relatively abundant in southeastern Spain (Belliure et al. 2008; Sanchez et al. 2011). Therefore, the specialized aphid predatory midge *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) and other generalist predators, such as chrysopid, syrphid, or coccinellid predators, are also frequently released against these two aphid species, but these populations fail to establish when the aphids disappear (Pineda and Marcos-Garcia 2008; Messelink et al. 2011, 2013). To date, the biological control of *M. euphorbiae* and *A. solani* has not been satisfactory in most cases using the parasitoids *Aphidius ervi* Haliday and *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae).

These problems with controlling aphids in sweet pepper plants emphasize the need to continue looking for alternative natural enemies that would satisfy the aforementioned needs. Messelink et al. (2011) stated that the biological control of aphids might be significantly enhanced by using zoophytophagous predators that can establish in a sweet pepper crop prior to aphid infestations. A group of endemic natural enemies that commonly inhabit sweet pepper crops and might meet these criteria that are the predatory mirid bugs (Hemiptera: Miridae). Indeed, previous studies suggest that the positive role of the predatory mirid *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) could have in controlling aphids in sweet pepper plants (Messelink et al. 2011; Perdikis and Lykouressis 2004). Two other predatory mirids, *N. tenuis* and *Dicyphus maroccanus* Wagner, together with *M. pygmaeus*, have been detected preying on aphids and other agricultural pests, such as spider mites, thrips, and lepidopterans in protected sweet pepper crops of southeastern Spain (Jacas et al. 2008; Gonzalez-Cabrera et al. 2011; Molla et al. 2011, 2014). Therefore, this study is aiming to further confirm the previous results obtained with *M. pygmaeus* on *M. persicae* (Perdikis and Lykouressis 2004; Messelink et al. 2011) and compare this with the other two mirid species, *N. tenuis* and *D. maroccanus*. We report here on the capacity of *N. tenuis*, *M. pygmaeus*, and *D. maroccanus* to detect *M. persicae*-infested plants and the prey suitability of *M. persicae* for these three species of mirids under laboratory conditions. Finally, the capacity of the three zoophytophagous predators to reduce *M. persicae* populations in heavily infested plants was determined under semi-field conditions. Implications of these results and whether these predatory mirid bugs could be considered biocontrol agents of aphids in sweet pepper crops are discussed.

## Materials and methods

The environmental conditions in laboratory experiments were  $25 \pm 2$  °C,  $60 \pm 10$  % RH with a photoperiod of 16:8 h (L:D).

### Experimental insects

The colonies of *M. persicae* (green phenotype) on sweet pepper plants were initiated from a laboratory stock colony maintained on potted broad bean plants (*Vicia faba* L. Fabales: Fabaceae) and housed in a climatic chamber at  $25 \pm 2$  °C, 60–80 % RH, and a 16:8 h (L:D) photoperiod at IVIA (La-Spina et al. 2008). *M. persicae*-infested bean sprouts were then reared on pesticide-free sweet pepper seedlings (var. “Lipari”, Clause Spain S.A.U. Almería,

Spain). Rearing took place in screened cages (120 × 70 × 125 cm) in which groups of six sweet pepper plants (approximately 25 cm high) were weekly introduced.

*Dicyphus maroccanus* individuals were initially collected in tomato fields located in the Valencia province (Spain) and were then reared on pesticide-free tomato seedlings “Optima” (Seminis Vegetable Seeds, Inc., Almería, Spain) using frozen eggs of the factitious prey *Ephesthia kuehniella* (Zeller) as food. *N. tenuis* and *M. pygmaeus* adults were obtained from a commercial supplier (NESIBUG<sup>®</sup> and MYRICAL<sup>®</sup>; Koppert Biological Systems, S.L., Águilas, Murcia, Spain). Each bottle contained approximately 500 specimens consisting of a mixture of mature nymphs and young adults (less than 3-day-old) fed with *E. kuehniella* eggs (FJ Calvo, Koppert BS; Personal Communication). Adults from these bottles were then kept for 24 h on sweet pepper plants and fed with *E. kuehniella* eggs before used.

#### Attraction to volatiles

To investigate the olfactory response of the omnivorous predators *N. tenuis*, *M. pygmaeus*, and *D. maroccanus*, two separate series of Y-tube experiments were conducted. Four 60-cm-long fluorescent tubes (OSRAM, L18 W/765, OSRAM GmbH, Germany) were positioned 40 cm above the arms. Light intensity over the Y-tube was measured with a ceptometer (LP-80 AccuPAR, Decagon Devices, Inc. Pullman, WA) and resulted 2,516 lux. The environmental conditions in the Y-tube experiments were 23 ± 2 °C, 60 ± 10 % RH. In the first series of experiments, we compared the responses of mirids to the presence of sweet pepper plants. In a second series of Y-tube experiments, we tested whether *M. persicae*-infested sweet pepper plants were equally attractive to the mirids as uninfested ones.

The Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) consisted of a Y-shaped glass tube of 2.4 cm in diameter with a base that was 13.5-cm long and two arms that were 5.75-cm long. The base of the Y-tube was connected to an air pump that produced a unidirectional airflow of 150 mL/min from the arms to the base of the tube. The arms were connected via a plastic tube to two identical glass jars (5 L of volume), each of which contained a tested odor source.

The first experiment was conducted to test the attractiveness of an uninfested potted sweet pepper plant (25-cm high) (var. “Lipari”) odor compared with an empty jar to females of three species (aged from 3 to 5 days). The plants were healthy and growing from natural soil mixed with local peat moss in plastic pots (8 × 8 × 8 cm). Adult females of the three omnivorous predators were maintained

individually in a plastic vial (10 mm diam. and 50 mm long) without food for 24 h prior to the olfactometer bioassays. Each vial was sealed with moistened cotton.

Mirid females were individually introduced into the base arm of the Y-tube. Each female was observed, until she had walked at least 3 cm up one of the side arms or until 15 min had elapsed (McGregor and Gillespie 2004). Females who had not made a choice within 15 min were considered to be “non-responders” and discarded in the subsequent data analysis. At least 24 responses were recorded for each pair of odor sources. After five individual females had been tested, the olfactometer arms were flipped around (180°) to minimize spatial effect on choices. After 10 females had been bioassayed, the olfactometer set-up was rinsed with soap water and acetone and then air-dried.

In the second experiment, the attraction of the three species of mirids to the volatiles emitted by *M. persicae*-infested sweet pepper plants and uninfested plants was tested. Heavily infested sweet pepper plants (75–100 nymphs per leaf) from the stock colonies and height and foliar mass similar to that of control plants (uninfested) were used in Y-tube bioassays. At least 26 responses were recorded for each pair of odor sources. The same protocol explained in the first set of experiments was followed for this second experiment.

#### Prey suitability

For each species of mirid, approximately 100 adults less than 4-d-old were placed inside a 60 × 60 × 60 cm plastic cage (BugDorm-2; MegaView Science Co., Ltd.; Taichung, Taiwan) and starved of prey for 24 h before use. In each cage, four uninfested sweet pepper plants without flowers (25 cm high) were also introduced during the prey starvation period to allow mirids to adapt to sweet pepper plants. Cannibalism was not observed for any of the three species of mirids.

*Myzus persicae*-infested leaves were detached from infested plants of the stock colony. The first and fourth instar nymphs of *M. persicae* were selected under a stereoscopic binocular microscope with a small brush and placed on sweet pepper leaves inside Petri dishes (9 cm in diameter). The fourth nymphal instars of *M. persicae* are well defined, differing both in morphology and size (Horsfall 1924). The three predator species and both sexes were separately exposed to either 20 first instar nymphs or 20 fourth instar nymphs. Water was supplied on soaked cotton plugs. Twenty replicates were performed for each density and sex. After 24 h, the predators were removed from the arenas and the number of consumed nymphs was evaluated.

Since the sex ratio of these three species of mirids is quite different, to better compare and interpret the values of predation among mirid species, the consumption data were

calculated for a theoretical population, which was estimated from the sex ratio values for each predator species. The sex ratios considered were 0.44, 0.41, and 0.85 (female/total) for *N. tenuis*, *M. pygmaeus*, and *D. maroccanus*, respectively (same authors, data in preparation). To do this, for each predator species, each value of consumption for a given sex was multiplied by the sex ratio of the opposite sex. This estimation provided the number of prey consumed by a single individual irrespective of gender of a population with the sex ratio mentioned above.

#### Efficacy reducing *M. persicae*

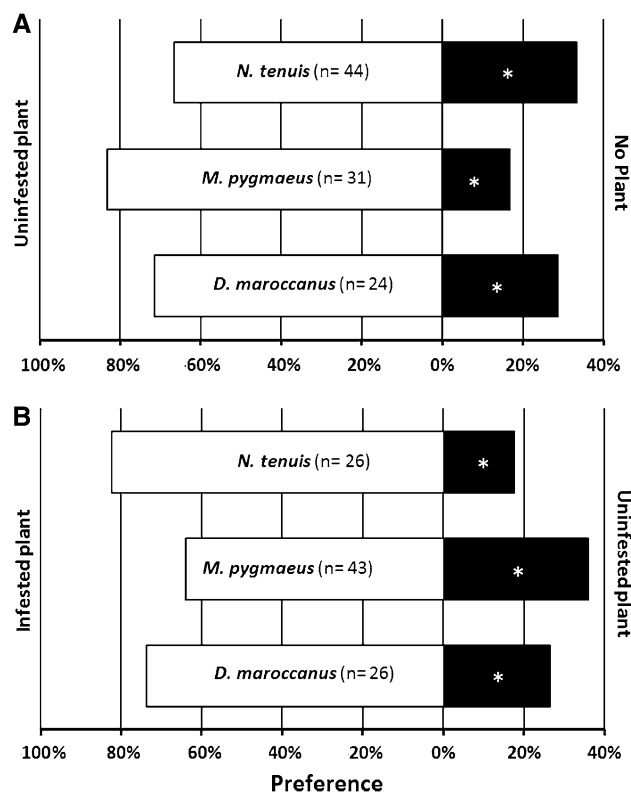
This experiment was conducted in a glasshouse located at the Instituto Valenciano de Investigaciones Agrarias IVIA (Moncada, Valencia, Spain). The climatic conditions were 23–27 °C, 65 ± 10 % RH, and a natural photoperiod (approximately 14L: 10D).

To determine the efficacy of *N. tenuis*, *M. pygmaeus*, and *D. maroccanus* in reducing *M. persicae* densities in sweet pepper plants, 16 plastic cages 60 × 60 × 60 cm (BugDorm-2; MegaView Science Co., Ltd.; Taichung, Taiwan) were used. In each cage (replicate), six heavily infested sweet pepper plants (25-cm high) and one couple of each species of mirid per plant were introduced the same day (August 20th). The control treatment did not receive any mirid release. Healthy sweet pepper plants were placed inside the stock colony of *M. persicae* during 2 weeks. A homogenous and heavy aphid infestation (around 70 nymphs per leaf) was obtained. Plants were randomly distributed in the 4 treatments, and ANOVA test confirmed that there were no statistical differences among treatments ( $F_{3,14} = 0.8241$ ,  $P = 0.5075$ ). In total, four replicates were included per treatment, with six plants per replicate.

During the following 6 weeks after the release, four randomly selected infested leaves per cage were removed weekly and introduced to a 150-mL plastic cup containing 70 % alcohol. Since flowers can serve as alternative food for predatory mirids (Messelink et al. 2011), all flower buds that were appearing in the course of the experiment were removed manually to avoid any possible interference. The collected material from each replicate and treatment was transported to the laboratory, where aphids and mirids were filtered through a sieve of 32 × 32 threads/cm<sup>2</sup> and then counted under a stereoscopic binocular microscope. These data were used to calculate the efficacy of the mirids tested using the Henderson-Tilton formula (Henderson and Tilton 1955).

#### Statistical analysis

$\chi^2$  goodness of fit tests were used to test the hypothesis that the distribution of side-arm choices between pairs of odors



**Fig. 1** Responses of *N. tenuis*, *M. pygmaeus*, and *D. maroccanus* females in a Y-tube olfactometer to the odors of **a** un-infested sweet pepper plants versus no plant and **b** *M. persicae*-infested sweet pepper plants versus un-infested sweet pepper plants. The number of individuals recorded for each odor pair is shown between brackets. (Asterisk) represents significant differences in the distribution of side-arm choices ( $\chi^2$ ;  $P < 0.05$ )

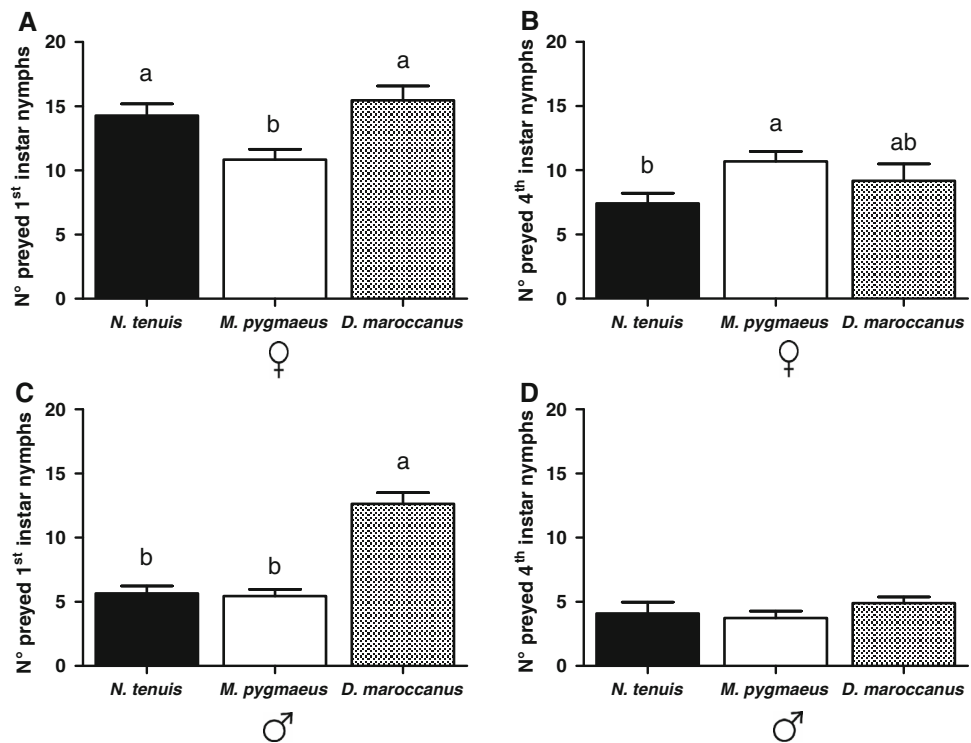
affected the olfactory responses in the experiments. In the prey suitability experiment, the data on the number of consumed *M. persicae* nymphs were subjected to a one-way analysis of variance to evaluate the effect predator species. The number of *M. persicae* and mirids per leaf in sweet pepper plants and the percentage of efficacy of the three species of mirids in reducing *M. persicae* populations were analyzed using a generalized linear mixed model with repeated measurements. Treatment was considered as a fixed factor, and time was considered random. When significant differences were found, pairwise comparisons of the fixed factor levels were performed with the least significant difference post hoc test ( $P < 0.05$ ).

## Results

### Attraction to volatiles

Females of the three species of predatory mirids were more attracted to the odor of un-infested plants compared to the plant-free control (*N. tenuis*:  $\chi^2 = 6.667$ ;  $P = 0.010$ ; *M.*

**Fig. 2** Number (mean  $\pm$  SE) of first and fourth instar nymphs of *M. persicae* consumed by females (A, B) and males (C, D) of *N. tenuis*, *M. pygmaeus*, and *D. maroccanus*. Bars topped by different letters represent means that are significantly different among species (ANOVA  $P < 0.05$ )

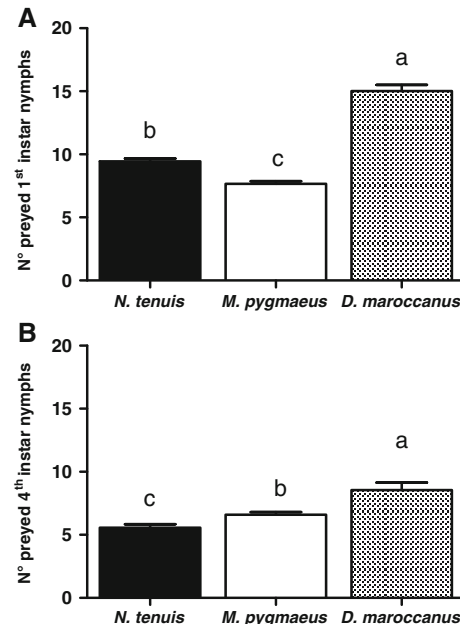


*pygmaeus*:  $\chi^2 = 10.67$ ;  $P = 0.001$  and *D. maroccanus*:  $\chi^2 = 7.714$ ;  $P = 0.005$ ) (Fig. 1A). When females were presented with a choice between the odors of *M. persicae*-infested plants and un-infested plants, they displayed a preference for the odor of *M. persicae* plants (*N. tenuis*:  $\chi^2 = 14.24$ ;  $P < 0.001$ ; *M. pygmaeus*:  $\chi^2 = 6.205$ ;  $P = 0.013$  and *D. maroccanus*:  $\chi^2 = 8.526$ ;  $P = 0.003$ ) (Fig. 1B).

#### Prey suitability

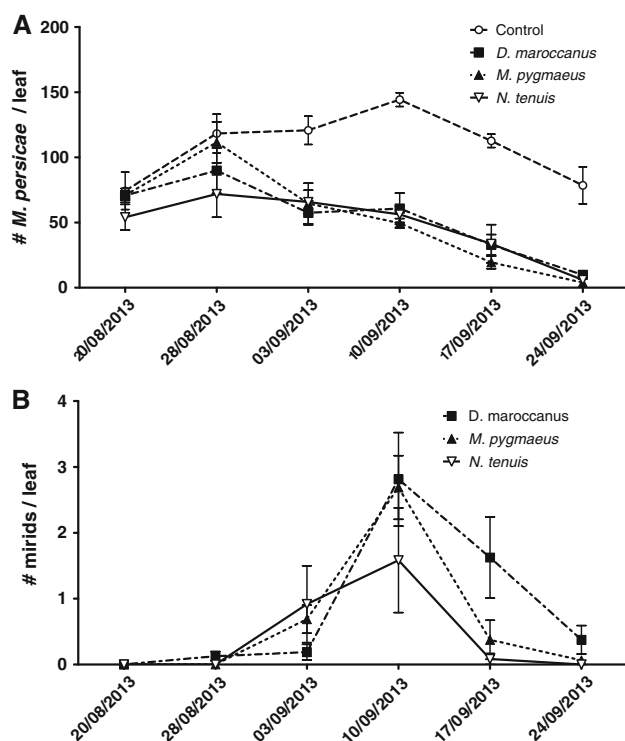
The three species of mirids preyed actively on *M. persicae* (Fig. 2). The number of first instar nymphs preyed on by *N. tenuis* and *D. maroccanus* females was significantly higher than those preyed by *M. pygmaeus* females ( $F_{2,47} = 6.720$ ,  $P = 0.0028$ ) (Fig. 2A). However, *M. pygmaeus* fed on a significantly larger number of fourth instar nymphs than *N. tenuis*, while *D. maroccanus* did not show significant differences among them ( $F_{2,56} = 4.058$ ,  $P = 0.0228$ ) (Fig. 2B). Males of *D. maroccanus* consumed significantly more first instar nymphs than males of *N. tenuis* and *M. pygmaeus* ( $F_{2,57} = 29.46$ ,  $P < 0.001$ ) (Fig. 2C). In contrast, no significant differences in fourth instar nymphs predation were found among males of the predator species tested ( $F_{2,51} = 0.4674$ ,  $P = 0.6294$ ) (Fig. 2D).

When nymphal consumption was extrapolated to theoretical population level, estimated from the sex ratio of each species (Fig. 3), *D. maroccanus* resulted the most voracious species preying first nymphal instars of *M.*



**Fig. 3** Number (mean  $\pm$  SE) of first (A) and fourth (B) instar nymphs of *M. persicae* consumed by one single individual from a reconstructed theoretical population estimated from a sex ratio of 0.44, 0.41, and 0.85 (female/total) for *N. tenuis*, *M. pygmaeus*, and *D. maroccanus*, respectively. Bars topped by different letters represent means that are significantly different among species (ANOVA  $P < 0.05$ )

*persicae*, followed by *N. tenuis* and then *M. pygmaeus* ( $F_{2,105} = 136.1$ ,  $P < 0.001$ ). Similarly, *D. maroccanus* consumed a significant greater number of fourth nymphal



**Fig. 4** Number (mean  $\pm$  SE) of **A** *M. persicae* and **B** mirids per sweet pepper leaf. One mirid couple of the corresponding species was released per plant in August 20th just after the first plant counting

instars of *M. persicae*, but on this instar was followed by *M. pygmaeus* and then by *N. tenuis* ( $F_{2,108} = 16.79$ ,  $P < 0.001$ ).

#### Efficacy reducing *M. persicae*

The initial infestation of approximately  $74.3 \pm 14.3$  individuals of *M. persicae* per sweet pepper leaf resulted in the complete collapse of control plants on September 10th, when the number of aphids per leaf reached  $144.2 \pm 5.3$ . After this date, the number of aphid per leaf in the control plants decreased to  $112.7 \pm 4.5$  at the end of the experiment. Conversely, the three species of mirids could significantly reduce the number of *M. persicae* per leaf when compared with the untreated control ( $F_{3, 84} = 28.622$ ;  $P < 0.001$ ; Pairwise comparisons: Control versus *D. maroccanus*:  $t_{84} = 7.47$ ,  $P < 0.001$ ; Control versus *M. pygmaeus*:  $t_{84} = 7.51$ ,  $P < 0.001$  and Control versus *N. tenuis*:  $t_{84} = 7.67$ ,  $P < 0.001$ ) (Fig. 4A). Indeed, plants that received a release of mirids appeared healthy at the end of the experiment in comparison with the unhealthy appearance (smaller leaves full of honeydew) of the control plants. No significant differences in the number of *M. persicae* per leaf were found among the treatments that received separately the release of three species of mirids (Pairwise comparisons: *D. maroccanus* versus *M.*

*pygmaeus*:  $t_{84} = 0.04$ ,  $P = 0.964$ ; *D. maroccanus* versus *N. tenuis*:  $t_{84} = 0.75$ ,  $P = 0.457$  and *M. pygmaeus* versus *N. tenuis*:  $t_{84} = 0.71$ ,  $P = 0.482$ ).

Independently of the species tested, releases of one couple of mirids per plant proved to be sufficient for their establishment in the experimental cages (Fig. 4B). The numbers of mirids increased regularly from their release until 4 weeks later. However, mirid population decreased in parallel to the decrease of the number of aphids present on sweet pepper plant after this time. The number of mirids per leaf was almost the same for all three treatments ( $F_{2, 63} = 1.856$ ;  $P = 0.165$ ).

The percentage of reduction of the number of aphids per plant reached numbers close to 100 % for the three species of mirids tested, with no significant differences among them ( $F_{2,52} = 0.478$ ;  $P = 0.623$ ) (Fig. 4).

#### Discussion

The three predatory mirid bugs tested in this study are native natural enemies that spontaneously appear in various crops in the Mediterranean basin (Alomar et al. 2002; Gonzalez-Cabrera et al. 2011). Two of these zoophytophagous predators, *N. tenuis* and *M. pygmaeus*, are mass-reared and have been released in augmentative biocontrol programs aimed at controlling whiteflies and *T. absoluta*, mainly in protected crops (Arnó et al. 2010; Calvo et al. 2012a, b; Urbaneja et al. 2012). However, *D. maroccanus* was recently first detected actively preying on *T. absoluta* in the Iberian Peninsula in tomato crops (Gonzalez-Cabrera et al. 2011). Even though *D. maroccanus* has been frequently observed preying on aphids and mites in tomato and egg plant crops under low pesticide-input management (Same authors, unpublished results), studies of its prey range or its possibilities to be integrated in biological control programs are lacking. In any case, little is known about the efficacy and practical use of mirids in sweet pepper agro-ecosystems. A limited number of studies have increased expectations that mirids can play a significant role in the regulation of some horticultural pest species (Perdikis and Lykouressis 2004; Messelink et al. 2011; Bueno et al. 2013) following the success of *N. tenuis* in the Mediterranean area (Calvo et al. 2012b).

The three species of predatory mirids showed a stronger response to odors from infested plants than to odors from un-infested plants. To our knowledge, this is the first olfactory study of *N. tenuis* and *D. maroccanus*, but not for *M. pygmaeus*. Our results for *M. pygmaeus* are consistent with previous results (Moayeri et al. 2006; Ingegno et al. 2011). In our work, mirids were in contact with a pepper plant during 24 h before starvation; hence, learning behavior related to plant odor could be included. It would



be interesting to clarify whether these three species of predatory mirid bugs are able to associate prey odors as honeydew with the presence of prey. In this regard, Moayeri et al. (2007) showed that *M. pygmaeus* do not seem to exploit odors emitted directly from the prey themselves, which suggests that mirids respond to herbivore-induced volatiles rather than to the prey itself.

We found that the three species of mirids successfully preyed on *M. persicae* nymphs with significant differences among them, which depended on the predator gender or the instar preyed. To our knowledge, this is the first study reporting the capacity of *D. maroccanus* for prey on *M. persicae*. However, this study was not the first of its kind for *N. tenuis* and *M. pygmaeus*. Previous studies have demonstrated that both *M. pygmaeus* and *N. tenuis* feed on *M. persicae* under laboratory conditions (Perdikis and Lykouressis 2002a, b, 2004; Valderrama et al. 2007; Fantinou et al. 2008, 2009; Vandekerckhove and De Clercq 2010). The three species tested in this study showed a higher predation rate and preference for smaller prey instars, which was also observed by Valderrama et al. (2007) for *N. tenuis* and by Fantinou et al. (2009) for *M. pygmaeus*. The number of nymphs consumed in our assays by both predators revealed that mirid females could prey on approximately 12 and 6 first and fourth nymphal instars, respectively, over 24 h. These numbers are in agreement with the data for *M. pygmaeus* and for *N. tenuis* obtained in previous works when offered the same amount of prey densities (20 nymphs). However, both predators may consume a higher number of aphid nymphs, because their predation rate depends on the amount of prey offered (Valderrama et al. 2007; Fantinou et al. 2009, 2008). *M. pygmaeus*, which shows a Type II functional response, maximizes its prey consumption in 24 h at approximately 17 and 11 first and fourth nymphal instars, respectively, densities at which the predator is satiated (Fantinou et al. 2008, 2009). Surprisingly, *N. tenuis*, which shows a Type III functional response, can prey on almost 70 third-fourth instar nymphs when a prey density of 90 nymphs is offered (Valderrama et al. 2007). Based on our personal observations, we hypothesize that *N. tenuis* would not be able to completely consume 70 nymphs of *M. persicae*. Therefore, an over-killing behavior was likely given, but Valderrama et al. (2007) did not report it in their paper. Supporting this hypothesis, Fantinou et al. (2008) demonstrated that *M. pygmaeus* exhibits an over-killing behavior that might include partial prey consumption and/or killing without consumption. Unfortunately, we could not detect this behavior in our work.

Further studies would be needed to determine the predatory behavior and performance of these mirids on the other three species of aphids that may appear in sweet pepper crops, *A. gossypii*, *A. solani*, and *M. euphorbiae*. To

date, *M. pygmaeus* is only known to actively prey on *A. gossypii* and *M. euphorbiae* (Lykouressis et al. 2007; Alvarado et al. 1997; Perdakis and Lykouressis 2000). Lykouressis et al. (2007) observed that the predation rate of *M. pygmaeus* was higher on *M. persicae* than on *M. euphorbiae*, but this difference could be related to a major amount of biomass obtained from *M. euphorbiae*. Therefore, determining the suitability of these three other aphid species as prey as well as efficacy studies that compare the control capacity of these mirid species would be interesting. In our experiment, the three species of mirids could at minimum reduce the densities of *M. persicae* on sweet pepper plants in the semi-field assay. Indeed, plants that received mirid releases appeared healthy at the end of the experiment, and some mirids still remained active on these plants. Messelink et al. (2011) evaluated the efficacy of inoculative releases of *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae), *O. laevigatus*, and *M. pygmaeus* on *M. persicae* in a sweet pepper greenhouse. They found that compared to the two *Orius* species, *M. pygmaeus* was by far the best predator for controlling *M. persicae*. Thus, they suggested that the use of mirids instead or in addition to *O. laevigatus* may be preferable in sweet pepper crops, although this approach might need additional releases of effective predatory thrips, such as *A. swirskii* (Messelink et al. 2008).

The performance of the three species of mirids reducing *M. persicae* resulted similar in our experiments. Other biological traits that are important for selection and in which these species probably differ will help us to select any of these mirid species, e.g., (1) compatibility with other natural enemies, (2) developmental rate on sweet pepper plants without prey, (3) developmental rate on alternative food and mixtures of prey, and (4) climatic preferences. In this regard, unpublished results lead us to think that *D. maroccanus* has its optimal temperature range below than *M. pygmaeus* and *N. tenuis*, which may indicate that this new biocontrol agent could be considered for use in crops with cooler temperatures.

Another further criterion of selection will be the efficacy of these predators when released prior to aphid outbreaks. In our experiment, mirids were released when aphid population densities were relatively high and even so aphid control was satisfactory but logically slow. It would, therefore, be very interesting to establish first the mirids in the crop (before the appearance of aphids) and determine their effectiveness on both, exploiting alternative food resources (e.g., pollen on flowers or other alternative prey) and the aphids that subsequently appear in the crop.

Overall, the data presented here reveal that mirids are highly efficient predators of *M. persicae*; they can detect *M. persicae*-infested sweet pepper plants and reduce heavy *M. persicae* infestations. These findings indicate that

mirids may play an important role in the control of the green peach aphids in sweet peppers. The incorporation of any of these three species of mirids in inoculative biological control strategies in sweet pepper crops will be a challenge for future studies.

**Acknowledgments** We want to thank V. Pedroche and M. Alonso-Valiente (IVIA) for their technical assistance and J. Calvo and J.E. Belda (Koppert BS) for providing the *N. tenuis* and *M. pygmaeus* individuals. The research leading to these results has received funding from the Conselleria d'Agricultura, Pesca i Alimentació de la Generalitat Valenciana.

**Conflict of interest** The authors declare that they have no conflict of interest.

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