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Barbara L. INGEGNO, Marco G. PANSA, Luciana TAVELLA*

DIVAPRA, Entomologia e Zoologia applicate all'Ambiente "Carlo Vidano", University of Torino, via Leonardo da Vinci, 44. I-10095 Grugliasco (TO), Italy.

* Corresponding author: phone number: +39.011.670.85.33; fax number: +39.011.670.85.35; e-mail address: luciana.tavella@unito.it

Abstract

Macrolophus pygmaeus (Heteroptera: Miridae) is an omnivourus predator used to control several pests of horticultural greenhouses. With the aim to explore the relationship between *M. pygmaeus* and different host plants compared with tomato, plant preferences and bio-cycle traits were studied using: *Capsicum annuum, Calendula officinalis, Salvia officinalis, Parietaria officinalis* and *Solanum nigrum*. Species were selected among natural host crop and wild plants. Plant preference was measured by multi-choice host plant selection and olfactometric bioassays. Bio-cycle traits were assessed on reproduction and on nymphal development with and without animal diet support. Among tested plants, *P. officinalis* was the least attractive under laboratory conditions. Furthermore the availability of prey was crucial for the successful establishment of *M. pygmaeus* on tested plants, suggesting the inability of nymphs to complete development to adulthood on a strictly phytophagous diet. Nevertheless *M. pygmaeus* seemed to prefer plants where phytophagy provides a fitness benefit.

Key words

Dicyphini, olfactometer bioassay, host plant selection, oviposition preference, biological control, tomato

Introduction

Species of *Macrolophus* (Heteroptera: Miridae) belonging to the subfamily Bryocorinae, tribe Dicyphini, are generalist predators well-known for their role in the control of several pests (e.g. aphids, mites, moths, thrips, whiteflies) in horticultural crops in Europe (Avilla et al., 2004). In fact, starting from the end of the ’80s, their presence was reported in IPM vegetable crops of different regions of southern Europe, as a consequence of the reduced insecticide pressure (Tavella and Goula, 2001). In particular, unlike other predatory bugs, such as anthocorids of the genus *Orius*, that are hampered by glandular trichomes (Coll and Ridgway, 1995), tomato plants represent a very suitable host for Dicyphini (Riudavets and Castañé 1998; Tavella and Goula, 2001).

Dicyphini are characterized by zoophytophagous behaviour, thus they are strictly related to the plant besides the prey. Zoophytophagy is positive because predators can survive in the crop even when prey are scarce or totally absent (Eubanks and Denno, 1999). The plant can provide not only water essential for predation (Sinia et al., 2004), but also nutrients. In fact, some species can develop and reproduce in the absence of prey by feeding on plants, but only on some plant species or even on some parts of them (Lucas and Alomar, 2001). For example, in previous laboratory trials *Macrolophus* species proved to develop on various crop plants, i.e. leaves of tomato, eggplant, pepper, cucumber, melon and broad bean (Perdikis and Lykouressis, 1997, 1999, 2000), and French bean pods (Tavella and Arzone, 1996), whereas they are unable to reach adulthood feeding on Chinese cabbage, cabbage and Brussel sprouts (Hatherly et al., 2009). Anyway, the developmental time on a plant diet is often considerably longer, while emergence rate and adult size are smaller, as a further confirmation of their improved performance in the presence of prey.

Plants release volatile compounds varying quantitatively and qualitatively depending on plant species and attacks of specific pests, and able to attract predators (Paré and Tumlinson, 1999;

Dudareva et al., 2006). The tritrophic interactions regulating the plant-prey-predator relationships are very complicated in these zoophytophagous mirid bugs. In spring, predatory bugs, especially fertile females, migrate from winter refuges onto tomato where, if not disturbed by chemicals, they establish and contribute efficiently to control pest outbreaks (Tavella et al., 1997). An earlier colonization of tomato seems to be affected by the presence and abundance of natural host plants, rather than by their abundance in the agroecosystem (Alomar et al., 2002; Ingegno et al., 2009). Their density within crops in fact can be related to composition and abundance of the surrounding vegetation and to topographic characteristics, suggesting the importance of host plant proximity to enhance early movement of these predators into the fields (Alomar et al., 1994; Gabarra et al., 2004). Concerning host range, Dicyphini show a preference for glandular and sticky plants; in fact, most of the northwestern Italian species have been collected on hairy plant species belonging to Solanaceae, Lamiaceae and Geraniaceae (Ingegno et al., 2008; Tavella and Goula, 2001).

Among the Dicyphini species colonizing tomato crops in the Mediterranean region, the species initially identified as *M. caliginosus* Wagner seemed to be the most promising: in fact, it is now reared by several commercial producers and largely used in programmes of biological control and IPM. Recent studies on molecular taxonomy of *Macrolophus* species revealed that species marketed for several years as *M. caliginosus* is actually *M. pygmaeus* (Rambur) (Martinez-Cascales et al., 2006 a, b).

Due to their effectiveness as pest control agents, bio-ethological studies on *Macrolophus* species have been carried out to assess their predatory activity in different conditions (temperature, humidity, photoperiod) and on different prey (whiteflies, aphids, thrips, mites), and their functional response (Montserrat et al., 2000; Enkegaard et al., 2001; Perdikis 2002; Perdikis and Lykouressis 2002; Montserrat et al., 2004; Lykouressis et al., 2007). Artificial diets have also been tested and developed for rearing of predatory bugs to improve their

massive production (Castañé and Zapata, 2005; Zapata et al., 2005). Furthermore, laboratory trials were carried out to evaluate the side-effects of the most widely used pesticides in horticulture (Figuls et al., 1999; Tedeschi et al., 2001; Tedeschi et al., 2002). Recently, studies on responses of some Dicyphini species to volatile compounds produced by plants and prey were carried out above all in laboratory conditions (McGregor and Gillespie, 2004; Moayeri et al., 2006a, b; Moayeri et al., 2007a, b).

To enhance the presence and activity of Dicyphini in the crops, the factors stimulating predatory bugs to leave the natural host plants and colonize the crop should be investigated thoroughly for an economically and ecologically sustainable farming. Thus the present research has been aimed at assessing preference and bio-ethological responses of the zoophytophagous *M. pygmaeus* on different plant species in comparison with tomato, the crop plant where it is more frequently and abundantly found.

Material and methods

Insect mass rearing and plant growing

M. pygmaeus used in the experiments came from a laboratory colony derived from Bioplanet, Italy, and reared in insect cages (MegaView, Taiwan) on tobacco plants [*Nicotiana tabacum* L. (Solanaceae)], supplemented with eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). Identity of *M. pygmaeus* was further assessed by molecular analyses as described in Martinez-Cascales et al. (2006 a, b). Mass rearing was maintained at 24±1°C, RH 65±5% and L16:D8.

Among crop (vegetable and garden) and wild plants, reported as host plants for Dicyphini species in NW Italy (Ingegno et al., 2008, 2009, personal observation), the following six plant species were selected: tomato [*Lycopersicon esculentum* Miller, cv Marmande (Solanaceae)], pepper [*Capsicum annuum* L., cv Quadrato d'Asti giallo (Solanaceae)], pot marigold

[*Calendula officinalis* L. (Asteraceae)], sage [*Salvia officinalis* L. (Lamiaceae)], pellitory-of-the-wall [*Parietaria officinalis* L. (Urticaceae)], and European black nightshade [*Solanum nigrum* L. (Solanaceae)]. To obtain plants of similar age and size (approximately 20cm high), they were periodically seeded, and afterwards transplanted in plastic pots (∅ 12cm), and maintained in a heated greenhouse without any pesticide use.

Reproduction and development on different plant species

Reproduction of *M. pygmaeus* was studied on single plants of the six species, supplemented with eggs of *E. kuehniella*. Each plant was isolated in a Plexiglas cylinder (height 195mm, ∅ 110mm), wedged in the pot soil, and enclosed at the upper extremity by net. Three females and two males 1-week-old of *M. pygmaeus* were introduced in each cylinder and removed after one week. *E. kuehniella* eggs glued on a paper strip with a honey solution were periodically supplied as food source. Egg hatching and nymph emergence were monitored every 48 hours until no nymphs were seen for four days; all newly-emerged nymphs were removed and counted. Five replications (i. e. five cylinders) were performed for each plant species.

The survival rate and time of nymphal development were studied on the six plant species in presence and absence of *E. kuehniella* eggs. Freshly hatched nymphs (<1-day-old) from each plant species were placed individually on leaf discs of the same plant in cells of $2cm^2$ (24-well tissue culture plate, Sarstedt, Germany). For each plant species 24 nymphs, 12 with *E. kuehniella* eggs (directly supplied on leaf discs) and 12 without *E. kuehniella* eggs, were observed during their life span. Moulting, evident from the presence of the exuvia, or death of each nymph were daily recorded and used to determine time and survival at each nymphal instar. All the emerged adults were examined under stereo-microscope to determine their sex, and to measure their length (from vertex to the end of hemielytra).

All the assays were carried out in climatic chambers at 24±1°C, RH 65±5%, and L16:D8.

After performing tests of homogeneity of variance (Levene) and normality (Kolmogorov-Smirnov), data of reproduction and development, and of measures of the adults emerged on each plant species were analyzed with one-way ANOVA; means were then separated by Tukey's test ($P<0.05$) (SPSS version12.0; SPSS Inc., Chicago, IL, USA).

Host plant selection experiments

The host plant selection experiments were set up as multi-choice assays where whole plants or single leaves of the six plant species were simultaneously offered to *M. pygmaeus*.

Whole plant choice

To assess the adult preference for the six plants being to be tested, one potted plant of each species was placed inside a 47.5×47.5×93cm net insect cage (MegaView, Taiwan). Five cages as five replications were set up. The position of the plant species in a 2×3 matrix was assigned randomly, taking care that the plants did not touch each other. Five 1-week-old females and two males were released on each plant (for a total of 30 females and 12 males per cage). After one week, each plant was wrapped in a net bag, removed from the cage, and inspected to count the adults of *M. pygmaeus*. Then the plants were singly isolated in Plexiglas cylinders (height 195mm, ∅ 110mm), and checked to observe egg hatching and nymph emergence every 48 hours until no nymphs were seen for four days. All emergent nymphs were removed and counted.

Single leaf choice

The plant preference by ovipositing females was tested by offering a single leaf or, in the case of the plants with small leaves (i.e. *P. officinalis*), a piece of stem with some leaves, of the six plant species simultaneously in a 20×20×30cm net cage (MegaView, Taiwan). Ten cages as ten replications were set up. The single leaf or the piece of stem with some leaves were put into a 1.5mL plastic tube filled with water, and introduced through equidistant hole in a polystyrene support randomly in a 2×3 matrix. Leaf areas were about equivalent for the six

plant species. Oviposition preference was tested in presence of prey, *E. kuehniella* eggs, supplied in the centre of the matrix on paper strip glued with a honey solution. Two 1-week-old females and one male were released for each plant species (for a total of 12 females and six males per cage). The adults of *M. pygmaeus* were removed after 48 hours. The number of eggs laid on each plant species was counted under a stereo-microscope five days later. The plant preference by ovipositing females was expressed as the proportion of eggs laid on each plant species in the cage as described by Thompson (1988).

All the multi-choice assays were carried out at 24±1°C, RH 65±5%, and L16:D8.

In the whole plant multi-choice experiment the relationship between percentage of recaptured adults and number of emerged nymphs was estimated with Pearson's correlation (SPSS version 12.0). In the leaf multi-choice experiment, the percentages of eggs laid on each plant species in the cages were compared, after arcsine square-root transformation, using ANOVA, and means were separated by Tukey's test ($P<0.05$) (SPSS version 12.0).

Olfactometric bioassays

In the bioassays, 1-week-old females, kept without prey and plant in a glass tube (length 120mm, ∅ 23mm) for 18 hours, were used to assess olfactory responses of *M. pygmaeus* to the odours of tomato compared with those of the other tested plants. The bioassays were carried out in a Y-shaped Pyrex tube (internal Ø 23mm) formed by an entry arm, 250mm long, and two side arms, 200mm long (70°angle), and positioned vertically as in other studies with Dicyphini (McGregor and Gillespie, 2004; Moayeri et al., 2006a, b; Moayeri et al., 2007a, b). Each side arm was connected to a glass cylindrical chamber (height 500mm, Ø 130mm) as an odour-source container. Airflow was provided by an air pump (Air 275R, Sera, Germany). Before reaching the odour-source chambers, air passed in an activated CO_2 filter, in a flow meter (EK-2NRK, Comer, Italy) to set the airflow, and in a 1-L water bubbler half-

filled with deionized water. The odour-source chambers were held behind a black panel, so that *M. pygmaeus* females could not see the plants during the bioassays in the Y-tube.

In all experiments, the flow rate through flow meter was set at 2.5L min^{-1} and measured at the downwind end with a digital anemometer (TA-410, PCE Group, Italy) to control any flow leak. Before each trial, an air flow was established in the Y-tube by adjusting the flow rate using the air pump knob and the flow meter. After the flow was established, a single *M. pygmaeus* female was introduced into the tube. Each female was observed until she had walked at least 6cm up one of the side arms or until 20min had elapsed. Females that did not choose a side arm within 20min were considered as "no choice" and were not counted in the subsequent data analysis. Each female was tested only once. The odour sources chosen by females that responded were recorded. Twenty-five responses were recorded for each pair of odour sources.

After testing a batch of five females, the odour sources were switched between the left-hand and right-hand side arms to minimize any spatial effect on choices, whereas after testing two batches (i.e. after 10 insect responses) the Y-tube was cleaned with neutral soap and alcohol (70%$_v$). Chambers were washed after each trial with neutral soap and alcohol (70%$_v$) and sterilised in autoclave at 120°C for 20 min. The olfactometric bioassays were conducted at 24±1°C, RH 25-30%, and 540±30lux.

Two experiments were carried out to assess: i) the preference of *M. pygmaeus* to tomato and the other five plants; ii) the responses of *M. pygmaeus* to the odours of tomato plants uninfested, presently infested and previously infested by the whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) (Table 1). The odour sources consisted of one entire potted plant. The infested plants were grown separately from uninfested plants. Moreover, to evaluate an eventual systemic effect of whitefly infestation, about 50 individuals of *T. vaporariorum* were introduced into a fine mesh net covering the apex of the plant. After

15 days, the apex was cut to remove the parts that hosted the pest. Also the apex of the uninfested tomato plants used in comparison with these ones was cut to prevent any influence due to the mechanic damage.

In the olfactometric bioassays, responses of *M. pygmaeus* females were analyzed by Chi-square test with significance levels of 90% and 95% (SPSS version 12.0). The null hypothesis was that predatory females had 50:50 distribution across the two odour sources. Females that did not make a choice were excluded from the statistical analysis.

Results

Reproduction and development on different plant species

Nymphal hatching from the tested plant species supplemented with eggs of *E. kuehniella* was significantly different only between *C. officinalis* and *P. officinalis* (ANOVA: df=29, F=2.844, P=0.037), with on average 27.4±6.2 and 2.8±0.7 nymphs emerged, respectively (Figure 1).

The development times from egg-hatching to adulthood on the six plant species with prey were significantly different (ANOVA: df=53, F=4.182, P=0.003), the longest on *S. officinalis* (19.3 days) and the shortest on *C. officinalis* and *C. annuum* (14.4 and 15.5 days, respectively) (Table 2). The percentage of individuals that reached adulthood ranged from 58% on tomato and *S. officinalis* to 100% on *S. nigrum*, (Table 2). The sex ratio was variable on the tested plants, ranging from 0.30 on *P. officinalis* to 0.75 on *C. annuum* and *S. nigrum*; however we could analyze only the measures of females because we did not obtain males enough on all tested plants. The length of female adults was significantly different between *S. officinalis* (3.144±0.045 mm) and *P. officinalis* (3.367±0.035 mm) (ANOVA: df=31, F=2.673, P=0.045).

M. pygmaeus was able to complete development on all the tested plants when supplemented with *E. kuehniella* eggs, whereas no nymphs reached adulthood when fed on plant alone of the different species without prey (Table 2). Many 1st instar nymphs could reach the 2nd instar, but after only on *C. officinalis* and *S. nigrum* very few nymphs reached the 5th instar whereas on *P. officinalis* and *S. officinalis* no nymphs got over the 2nd instar (Table 2).

Host plant selection and oviposition multi-choice experiments

In the whole plant multi-choice experiment, no significant differences were found between numbers of nymphs emerged on the tested plant species (ANOVA: df=29, F=0.794, P=0.565), ranging on average from 27.0 on *L. esculentum* to 12.6 on *P. officinalis* (Table 3). As surveyed for the emerged nymphs, also the numbers of adults recaptured on each plant at end of experiment were not significantly different between the six plant species. However, the lowest percentage of *M. pygmaeus* adults was recaptured on *P. officinalis*, on which the lowest number of nymphs was also observed (Table 3). Furthermore, a positive correlation was found between the percentage of recaptured adults and the number of emerged nymphs on the same plant species (Pearson's correlation=0.507, P=0.004; n=30) (Figure 2).

In the leaf multi-choice experiment, the percentage of eggs laid per plant in each cage was significantly higher on tomato compared to sage (ANOVA: df=59, F=2.946, P=0.020), on which on average 30.8% and 2.5% of eggs were laid respectively (Table 4).

Olfactometric bioassays

In the first experiment (Table 1), no significant preference was detected between healthy tomato and the following plants (Figure 3): *C. officinalis* (χ^2=0.04, P=0.84), *C. annuum* (χ^2=0.04, P=0.84), *S. officinalis* (χ^2=0.36, P=0.55) and *S. nigrum* (χ^2=0.36, P=0.55). By contrast, *P. officinalis* resulted unattractive in comparison with tomato (χ^2=4.84, P=0.03) as well as the empty chamber (χ^2=3.24, P=0.07). The females that did not choose any odour

sources were always very few; at the most three non-responding females were observed in the comparison with *C. officinalis*, *C. annuum* and *S. officinalis*.

In the second experiment (Table 1), *M. pygmaeus* females proved to be more attracted by the whitefly infested tomato compared to the uninfested tomato (χ^2=3.24, P=0.07) and by the uninfested tomato compared to the whiteflies (χ^2=3.24, P=0.07) (Figure 4). No significant differences in responses of *M. pygmaeus* females were found comparing the healthy tomato with the previously infested one (χ^2=1.00, P=0.32), as well as comparing whitefly odours with the empty chamber (χ^2=0.36, P=0.55) (Figure 4). As in the first experiment, the females non-responding to the compared odorous source were always very few, at the maximum two females in the comparison with whitefly alone and whitefly infested tomato.

Discussion

The obtained results confirmed that host plant selection in *M. pygmaeus* has a substantial influence on survival and development, and that the benefits of particular host plants vary in the presence or absence of prey. When *M. pygmaeus* nymphs were provided with both plant and prey, they were able to complete development on all plant species showing similar survival rates between plants, whereas without prey no nymphs reached adulthood in the same experimental conditions. Nevertheless, our results showed that the absence of prey could be tolerated longer on *S. nigrum*, *C. officinalis*, *C. annuum* and tomato than on *S. officinalis* and *P. officinalis*. Development times on tested plants without prey varied greatly from the second instar, probably due to the various nutritional values of the plants or to the presence of inhibitor compounds. The availability of prey seems to be crucial for successful establishment of *M. pygmaeus* on tested plants in our experimental conditions. However, even if in other laboratory experiments (Perdikis and Lykouressis, 1999, 2000; Tavella and Arzone, 1996) *M. pygmaeus* could reach the adulthood on prey-free plants, worse biological traits (i.e. longer

development time and higher mortality) observed on plants without prey than with prey suggest the difficulty of the species to complete development to adulthood on a strictly phytophagous diet. This need was observed also in other omnivorous mirid bugs as *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae) (Urbaneja et al., 2005), *Dicyphus errans* (Wolff) (Guidone et al., 2005) and *D. hesperus* Knight (Sanchez et al., 2004) that did not complete development when feeding on tomato alone. Phytophagy mainly provides the water necessary for vital functions and although plants appear to be a much poorer food resource than prey for *M. pygmaeus*, nutrients collected from them may greatly improve the survival of individuals as prey become scarce.

Among the host plants tested in laboratory experiments, *P. officinalis* was the least attractive, even if during field surveys in NW Italy individuals of *M. pygmaeus* were often collected on this plant (Tavella and Goula, 2001). Although in the whole plant selection experiment no significant differences were found, an obvious separation emerged in positive relationship among percentages of recaptured adults and emerged nymphs of *M. pygmaeus* between *P. officinalis* and the other tested plants. The colonization of *P. officinalis* in natural conditions by Dicyphini (Alomar et al., 1994; Gabarra et al., 2004; Tavella and Goula, 2001), in contrast with the unattractiveness in laboratory assays, is probably due both to the location of this plant, usually grown on walls in sheltered sites, and to the concurrent presence of prey, possible source of food. These facts make *P. officinalis* a suitable refuge for *M. pygmaeus*, especially during winter in absence of tomato crop. In fact, a previous study showed that abundance and vicinity of this plant to the tomato crop facilitate the early entrance of predatory bugs (Gabarra et al., 2004).

Other plant species confirming their important role as natural hosts also in laboratory experiments were *S. nigrum* and *C. officinalis*. *S. nigrum* is one of the most common weeds in the vegetable agroecosystems of NW Italy, and also the most favourite host for another

predatory bug widespread in the area, *D. errans* (Tavella and Goula, 2001; Ingegno et al., 2008). In the development experiments, *S. nigrum* supported the highest survival rate of *M. pygmaeus* nymphs with prey and, even if no nymphs could complete the development to adulthood, also without prey. This weed therefore can contribute to the conservation of predatory bugs in the field as suggested also in another recent study (Lykourressis et al., 2008). On the other hand, *C. officinalis* is a common garden plant, now widely naturalized throughout NW Italy, where it represents one the most favourite natural host of *M. pygmaeus* (Tavella and Goula, 2001). Like *S. nigrum*, *C. officinalis* supported a longer nymphal development in absence of prey; moreover, in the reproduction experiments the highest numbers of nymphs emerged on this plant species.

Natural presence of predatory bugs in vegetable agrecosystems of NW Italy seems to be strictly related to the environmental conditions; in fact, Dicyphini are found mainly in agroecosystems characterized by a high environmental complexity, i.e. patchy landscape where vegetable crops are surrounded by natural corridors wasteland and woodland (Ingegno et al., 2009). Therefore, since abundance of natural host plants acting as source affects greatly the presence of predatory bugs, plants species, like *S. nigrum* and *C. officinalis*, can carry out an important role in conserving and augmenting *M. pygmaeus* in agroecosystems. To implement natural control augmentation strategies in vegetable crops these plants should be conserved and, if scarcely present, increased. Conservation of natural host plant, such as *Dittrichia viscosa* L. (Asteraceae) for *M. melanotoma* (Costa) (Perdikis et al., 2007), or their use as banker plants in vegetable greenhouses, e.g. tobacco for *Macrolophus* sp. (Arnó et al., 2000) and mullein [*Verbascum thapsus* L. (Scrophulariaceae)] for *D. hesperus* (Sanchez et al., 2003a), have been already proposed to favour the early establishment of native predators on tomato crops in other geographic areas.

Concerning the two vegetables compared in our experiments, besides tomato also pepper appeared rather attractive for *M. pygmaeus*, especially in leaf multi-choice experiments. By contrast, in the field this mirid species was surveyed on pepper (Goula and Alomar, 1994), but only occasionally (Tavella and Goula, 2001), and not everywhere (Sanchez et al., 2003b). In fact, *M. pygmaeus* is found on various vegetable crops in southern Europe but it is primarily used in conservative and inoculative biological control strategies, sometimes in combination with parasitoids, to control whiteflies and other pests in tomato greenhouses, as documented by a rich literature (Avilla et al., 2004; Castañé et al. 2004). Therefore, some producers suggest to release the species as control agents on tomato and egg-plant. Moreover, *M. pygmaeus* also shows potential for the control of whiteflies in greenhouse melons (Alomar et al., 2006).

The differences in plant preference between field surveys and laboratory experiments are probably due to several environmental factors that may influence the zoophytophagous behaviour of these omnivorous mirid bugs. In fact, even if Dicyphini are abundant and widespread in the Mediterranean Basin, the species colonizing vegetable crops vary from region to region, probably in relation to the presence and abundance of natural host plants growing in the agroecosystem. Various species mainly belonging to the genera *Macrolophus*, *Dicyphus* and *Nesidiocoris* were reported in different areas of Europe (Alomar et al., 1994; Carnero-Hernández et al., 2000; Gabarra et al., 1988; Perdikis and Lykouressis, 1996; Sanchez et al., 2003b; Sanchez et al., 2006; Tavella et al., 1997; Tavella and Goula, 2001). Therefore, results obtained under laboratory conditions should be validated in the field in specific situation in order to suggest successful control augmentation strategies.

Besides the plant, the prey and above all the interaction plant-prey are fundamental (Dicke and Loon, 2000). In olfactometric bioassays, *M. pygmaeus* females were attracted from whitefly infested tomato as previously observed for *D. hesperus* (McGregor and Gillespie,

2004), while no differences emerged between the previously infested tomato and the healthy one, likely excluding a systemic effect of whitefly infestation on plant. The actual presence of the prey on tomato is important for attracting *M. pygmaeus*, consistent with the results of another study conducted in Y-tube olfactometer where the predatory species preferred spider mite infested green bean plants to uninfested plants (Moayeri et al., 2006b). However, the only presence of the whitefly, without host plant, was not sufficient for attracting *M. pygmaeus*, as observed with other potential prey, the aphid *Myzus persicae* (Sulzer) and the spider mite *Tetranychus urticae* Koch (Moayeri et al., 2006a, b). Although in the bioassays the infested plants after prey removal were not significantly attractive, further research on responses of *M. pygmaeus* is needed to investigate thoroughly a possible systemic effect due to prey feeding on tomato, and to identify plant volatiles that, acting as indirect defences, constitutive and/or induced, can affect adversely the herbivorous victim by attracting the predatory bug.

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Tables

Table 1. Theses in comparison in the two experiments in olfactometric bioassays.

Experiment 1			**Experiment 2**		
clean air	*vs*	tomato	clean air	*vs*	uninfested tomato
C. officinalis	*vs*	tomato	clean air	*vs*	whitefly
C. annuum	*vs*	tomato	whitefly	*vs*	uninfested tomato
P. officinalis	*vs*	tomato	infested tomato	*vs*	uninfested tomato
S. officinalis	*vs*	tomato	uninfested tomato	*vs*	previously infested tomato
S. nigrum	*vs*	tomato			

524 **Table 2.** Development times (days±SE) of *M. pygmaeus* (n=12), for each nymphal instar and
525 to reach adulthood on six plant species supplemented or not with *E. kuehniella* eggs.
526 Percentages of survivorship for each nymphal instar. In the column means followed by
527 different letters are significantly different (Tukey's test, P<0.05).

		Instar						*sex ratio*
	Plant	**First**	**Second**	**Third**	**Forth**	**Fifth**	**Total**	♀/(♀ + ♂)
with E. kuehniella eggs	*C. officinalis*	3.4 ± 0.4	2.4 ± 0.3	2.2 ± 0.2	2.6 ± 0.3	3.2 ± 0.6	14.4 ± 0.7 **b**	0.67
		100.0%	100.0%	100.0%	83.3%	90.0%	75.0%	
	C. annuum	2.8 ± 0.3	2.9 ± 0.3	2.8 ± 0.2	2.7 ± 0.3	3.8 ± 0.4	15.5 ± 0.7 **b**	0.75
		100.0%	91.7%	90.9%	80.0%	100.0%	66.7%	
	L. esculentum	3.3 ± 0.4	2.5 ± 0.2	2.6 ± 0.2	2.8 ± 0.3	5.1 ± 0.4	16.8 ± 0.8 **ab**	0.43
		100.0%	100.0%	91.7%	81.8%	77.8%	58.3%	
	P. officinalis	3.0 ± 0.4	3.0 ± 0.4	3.0 ± 0.3	2.7 ± 0.3	5.4 ± 0.5	17.0 ± 0.8 **ab**	0.30
		100.0%	83.3%	100.0%	100.0%	100.0%	83.3%	
	S. officinalis	4.8 ± 0.5	3.5 ± 0.4	2.6 ± 0.2	3.8 ± 0.7	3.7 ± 0.4	19.3 ± 0.8 **a**	0.71
		100.0%	91.7%	90.9%	70.0%	100.0%	58.3%	
	S. nigrum	2.8 ± 0.3	3.0 ± 0.3	2.9 ± 0.2	3.5 ± 0.5	4.4 ± 0.2	16.7 ± 0.7 **ab**	0.75
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	
without E. kuehniella eggs	*C. officinalis*	2.7 ± 0.4	3.0 ± 0.7	1.0	2.0	-		
		83.3%	50.0%	20.0%	100.0%	0.0%		
	C. annuum	2.0 ± 0.2	1.0	1.0	-			
		83.3%	10.0%	100.0%	0.0%			
	L. esculentum	2.0 ± 0.4	3.4 ± 0.7	8.5 ± 1.5	-			
		91.7%	45.5%	40.0%	0.0%			
	P. officinalis	2.8 ± 0.6	-					
		41.7%	0.0%					
	S. officinalis	2.8 ± 0.5	-					
		83.3%	0.0%					
	S. nigrum	2.6 ± 0.3	4.0 ± 0.6	2.7 ± 0.7	3.0	-		
		100.0%	75.0%	33.3%	33.3%	0.0%		

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Table 3. *M. pygmaeus* adults recaptured (mean % ± SE) and nymphs emerged (mean number ± SE) on each whole plant of the six species in multi-choice selection experiment. Significant differences between theses were not found (ANOVA, P>0.05).

Plant species	**Mean % of adult recaptured ± SE**	**Mean no. of nymphs ± SE**
C. officinalis	17.7 ± 4.5	24.0 ± 2.3
C. annuum	17.4 ± 4.9	21.8 ±11.1
L. esculentum	17.8 ± 4.9	27.0 ± 4.6
P. officinalis	8.0 ± 3.0	12.6 ± 3.6
S. officinalis	19.7 ± 6.1	25.4 ± 8.1
S. nigrum	19.5 ± 2.5	21.8 ±5.9

Table 4. Percentage of eggs (means ± SE) laid by *M. pygmaeus* females on leaf area of each plant species in multi-choice oviposition experiment. Mean percentages followed by different letters are significantly different (Tukey's test, $P<0.05$).

Plant species	**Mean percentage of eggs ± SE**
C. officinalis	18.53 ± 5.48 **ab**
C. annuum	26.49 ± 8.35 **ab**
L. esculentum	30.77 ± 9.16 **a**
P. officinalis	6.33 ± 4.12 **ab**
S. officinalis	2.48 ± 1.12 **b**
S. nigrum	15.40 ± 9.58 **ab**

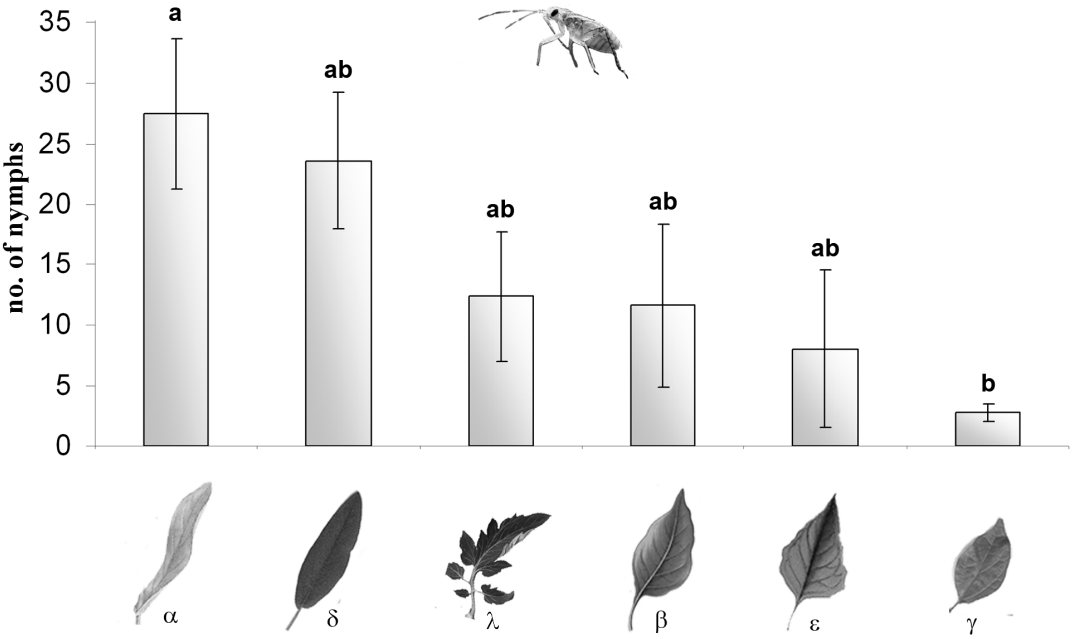


Figure 1. Nymphs of *M. pygmaeus* (mean number ± SE) emerged on the six plants in reproduction experiment: α= *C. officinalis*; δ= *S. officinalis*; λ= *L. esculentum*; β= *C. annuum*; ε= *S. nigrum*; γ= *P. officinalis*. Means characterized by different letters are significantly different (Tukey's test, $P<0.05$).

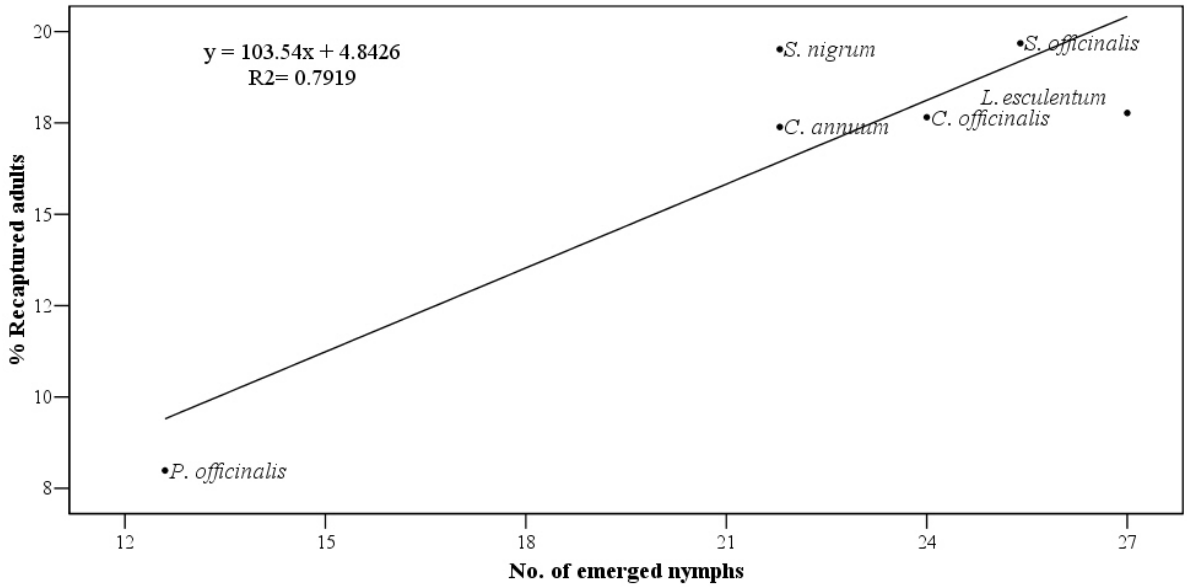


Figure 2. Relationship between percentage of recaptured adults of *M. pygmaeus* in multi-choice selection experiment and number of emerged nymphs (Pearson's correlation, $P<0.01$).

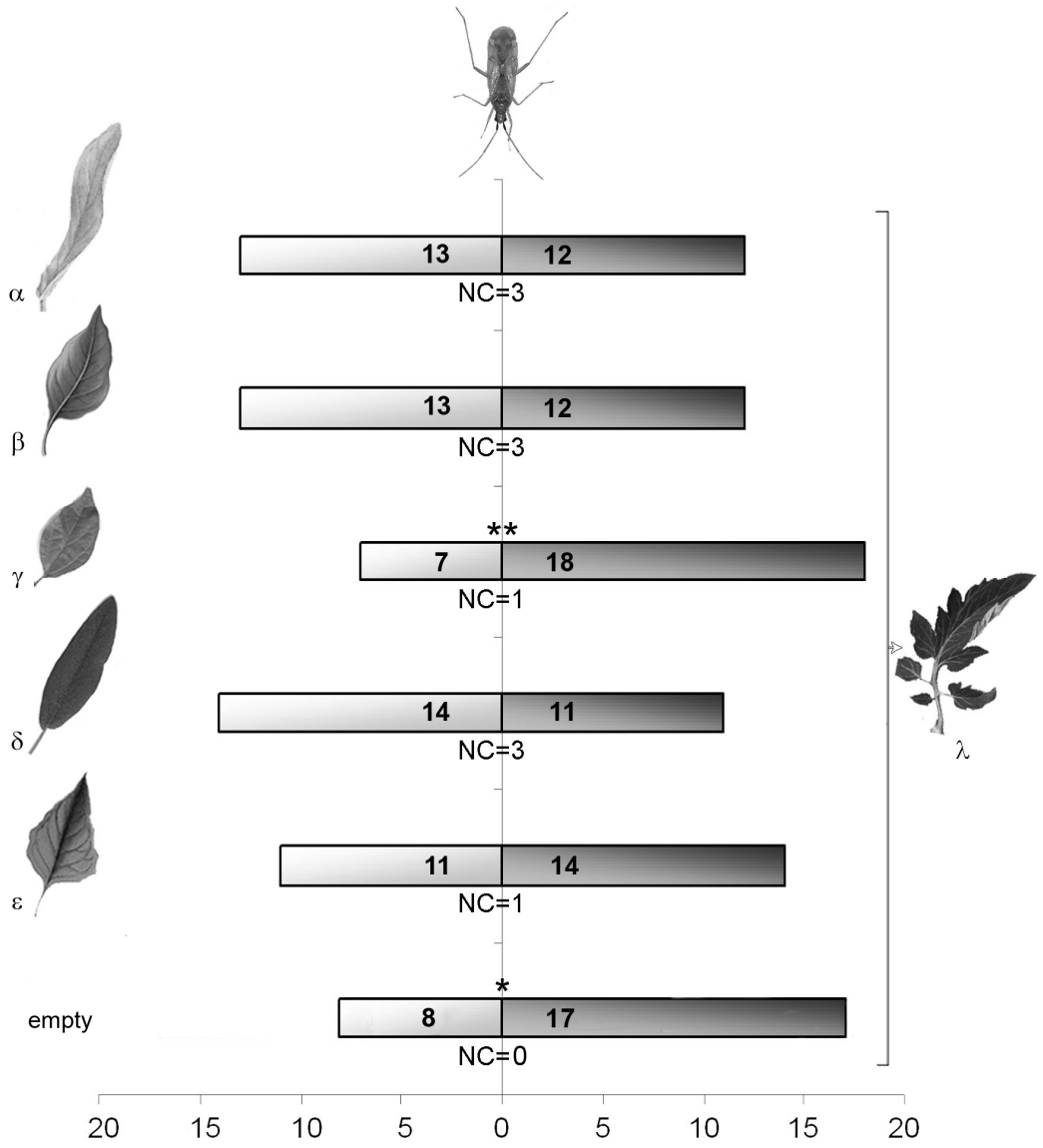
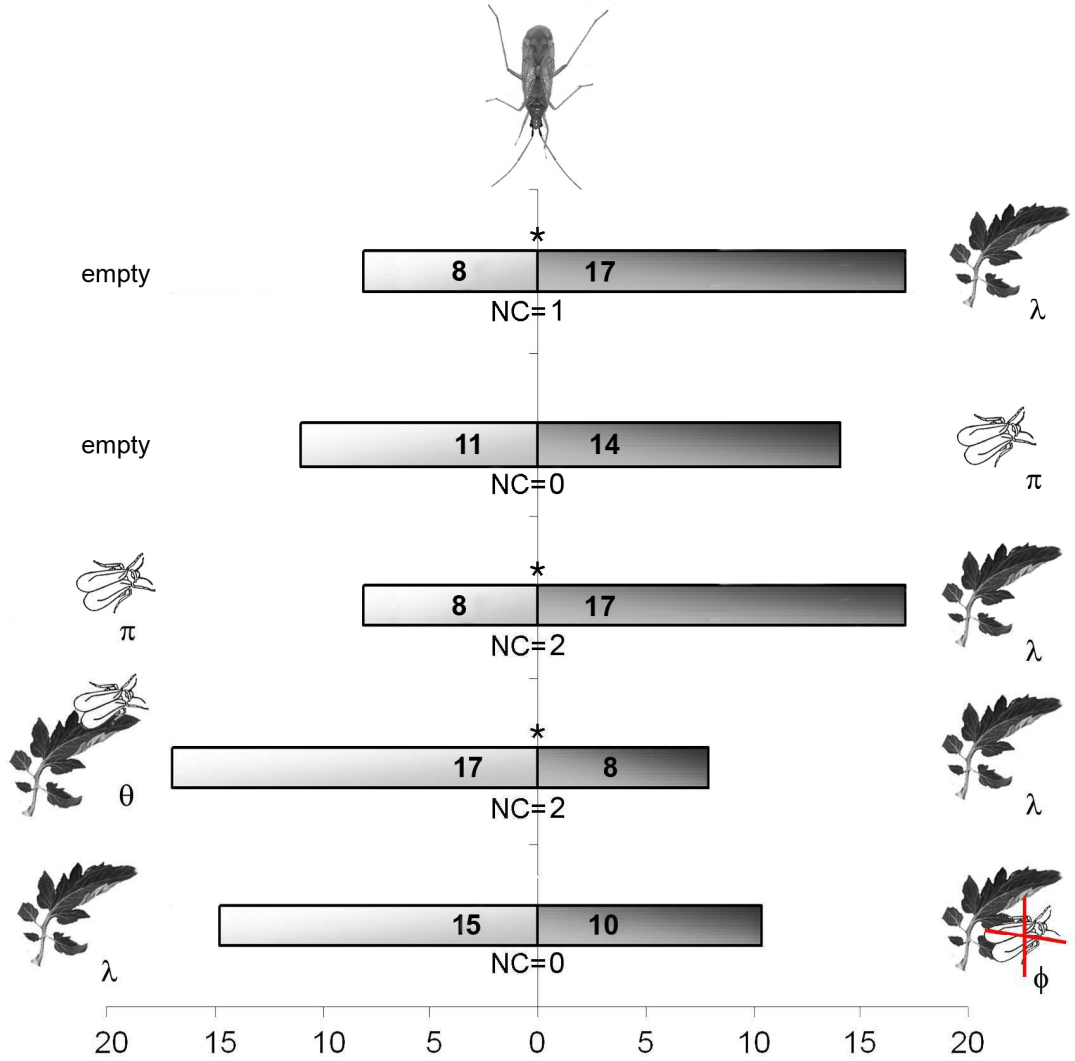


Figure 3. Responses of *M. pygmaeus* (no. of responding females in bars) in a Y-tube olfactometer and number of non-responding individuals (NC) to the odours of the plants for each compared pair. The following plants were tested against healthy tomato individuals (λ= *L. esculentum*): α= *C. officinalis*; β= *C. annuum*; γ= *P. officinalis*, δ= *S. officinalis*; ε= *S. nigrum*. Numbers in bars represent individual mirids that moved toward the volatiles. χ^2 statistics (**P<0.05, *P<0.10; df:1) tested the hypothesis that the distribution of side-arm choices deviated from a null model where odour sources were chosen with equal frequency.



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561 **Figure 4.** Responses of *M. pygmaeus* (no. of responding females in bars) in a Y-tube

562 olfactometer and number of non-responding individuals (NC) to the odours of infested (θ),

563 previously infested (ϕ) or uninfested (λ) tomato with the whitefly *T. vaporariorum* (π) for

564 each compared pair. Numbers in bars represent individual mirids that moved toward the

565 volatiles. χ^2 statistics (*P<0.10; df:1) tested the hypothesis that the distribution of side-arm

566 choices deviated from a null model where odour sources were chosen with equal frequency.