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1 **Plant preference in the zoophytophagous generalist predator *Macrolophus pygmaeus***

2 **(Heteroptera: Miridae)**

3

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9 **Abstract**

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

22

23 **Key words**

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

26

## 27 **Introduction**

28 Species of *Macrolophus* (Heteroptera: Miridae) belonging to the subfamily Bryocorinae, tribe  
29 Dicyphini, are generalist predators well-known for their role in the control of several pests  
30 (e.g. aphids, mites, moths, thrips, whiteflies) in horticultural crops in Europe (Avilla et al.,  
31 2004). In fact, starting from the end of the '80s, their presence was reported in IPM vegetable  
32 crops of different regions of southern Europe, as a consequence of the reduced insecticide  
33 pressure (Tavella and Goula, 2001). In particular, unlike other predatory bugs, such as  
34 anthocorids of the genus *Orius*, that are hampered by glandular trichomes (Coll and Ridgway,  
35 1995), tomato plants represent a very suitable host for Dicyphini (Riudavets and Castañé  
36 1998; Tavella and Goula, 2001).

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

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

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

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

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

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

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

89

## 90 **Material and methods**

### 91 Insect mass rearing and plant growing

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

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

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

#### 107 Reproduction and development on different plant species

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

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

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



127 After performing tests of homogeneity of variance (Levene) and normality (Kolmogorov-  
128 Smirnov), data of reproduction and development, and of measures of the adults emerged on  
129 each plant species were analyzed with one-way ANOVA; means were then separated by  
130 Tukey's test ( $P < 0.05$ ) (SPSS version 12.0; SPSS Inc., Chicago, IL, USA).

### 131 Host plant selection experiments

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

#### 134 *Whole plant choice*

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

#### 145 *Single leaf choice*

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

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

159 All the multi-choice assays were carried out at  $24\pm 1^{\circ}\text{C}$ , RH  $65\pm 5\%$ , and L16:D8.

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

#### 165 Olfactometric bioassays

166 In the bioassays, 1-week-old females, kept without prey and plant in a glass tube (length  
167 120mm,  $\varnothing$  23mm) for 18 hours, were used to assess olfactory responses of *M. pygmaeus* to  
168 the odours of tomato compared with those of the other tested plants. The bioassays were  
169 carried out in a Y-shaped Pyrex tube (internal  $\varnothing$  23mm) formed by an entry arm, 250mm  
170 long, and two side arms, 200mm long ( $70^{\circ}$  angle), and positioned vertically as in other studies  
171 with Dicyphini (McGregor and Gillespie, 2004; Moayeri et al., 2006a, b; Moayeri et al.,  
172 2007a, b). Each side arm was connected to a glass cylindrical chamber (height 500mm,  $\varnothing$   
173 130mm) as an odour-source container. Airflow was provided by an air pump (Air 275R, Sera,  
174 Germany). Before reaching the odour-source chambers, air passed in an activated  $\text{CO}_2$  filter,  
175 in a flow meter (EK-2NRK, Comer, Italy) to set the airflow, and in a 1-L water bubbler half-

176 filled with deionized water. The odour-source chambers were held behind a black panel, so  
177 that *M. pygmaeus* females could not see the plants during the bioassays in the Y-tube.  
178 In all experiments, the flow rate through flow meter was set at  $2.5\text{L min}^{-1}$  and measured at the  
179 downwind end with a digital anemometer (TA-410, PCE Group, Italy) to control any flow  
180 leak. Before each trial, an air flow was established in the Y-tube by adjusting the flow rate  
181 using the air pump knob and the flow meter. After the flow was established, a single *M.*  
182 *pygmaeus* female was introduced into the tube. Each female was observed until she had  
183 walked at least 6cm up one of the side arms or until 20min had elapsed. Females that did not  
184 choose a side arm within 20min were considered as “no choice” and were not counted in the  
185 subsequent data analysis. Each female was tested only once. The odour sources chosen by  
186 females that responded were recorded. Twenty-five responses were recorded for each pair of  
187 odour sources.

188 After testing a batch of five females, the odour sources were switched between the left-hand  
189 and right-hand side arms to minimize any spatial effect on choices, whereas after testing two  
190 batches (i.e. after 10 insect responses) the Y-tube was cleaned with neutral soap and alcohol  
191 (70%<sub>v</sub>). Chambers were washed after each trial with neutral soap and alcohol (70%<sub>v</sub>) and  
192 sterilised in autoclave at 120°C for 20 min. The olfactometric bioassays were conducted at  
193  $24\pm 1^\circ\text{C}$ , RH 25-30%, and  $540\pm 30\text{lux}$ .

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

201 15 days, the apex was cut to remove the parts that hosted the pest. Also the apex of the  
202 uninfested tomato plants used in comparison with these ones was cut to prevent any influence  
203 due to the mechanic damage.

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

208

## 209 **Results**

### 210 Reproduction and development on different plant species

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

215 The development times from egg-hatching to adulthood on the six plant species with prey  
216 were significantly different (ANOVA: df=53, F=4.182, P=0.003), the longest on *S. officinalis*  
217 (19.3 days) and the shortest on *C. officinalis* and *C. annuum* (14.4 and 15.5 days,  
218 respectively) (Table 2). The percentage of individuals that reached adulthood ranged from  
219 58% on tomato and *S. officinalis* to 100% on *S. nigrum*, (Table 2). The sex ratio was variable  
220 on the tested plants, ranging from 0.30 on *P. officinalis* to 0.75 on *C. annuum* and *S. nigrum*;  
221 however we could analyze only the measures of females because we did not obtain males  
222 enough on all tested plants. The length of female adults was significantly different between *S.*  
223 *officinalis* (3.144±0.045 mm) and *P. officinalis* (3.367±0.035 mm) (ANOVA: df=31,  
224 F=2.673, P=0.045).

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

### 230 Host plant selection and oviposition multi-choice experiments

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

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

### 243 Olfactometric bioassays

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

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

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

259

## 260 **Discussion**

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

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

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

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

299 predatory bug widespread in the area, *D. errans* (Tavella and Goula, 2001; Ingegno et al.,  
300 2008). In the development experiments, *S. nigrum* supported the highest survival rate of *M.*  
301 *pygmaeus* nymphs with prey and, even if no nymphs could complete the development to  
302 adulthood, also without prey. This weed therefore can contribute to the conservation of  
303 predatory bugs in the field as suggested also in another recent study (Lykourressis et al.,  
304 2008). On the other hand, *C. officinalis* is a common garden plant, now widely naturalized  
305 throughout NW Italy, where it represents one the most favourite natural host of *M. pygmaeus*  
306 (Tavella and Goula, 2001). Like *S. nigrum*, *C. officinalis* supported a longer nymphal  
307 development in absence of prey; moreover, in the reproduction experiments the highest  
308 numbers of nymphs emerged on this plant species.

309 Natural presence of predatory bugs in vegetable agroecosystems of NW Italy seems to be  
310 strictly related to the environmental conditions; in fact, Dicyphini are found mainly in  
311 agroecosystems characterized by a high environmental complexity, i.e. patchy landscape  
312 where vegetable crops are surrounded by natural corridors wasteland and woodland (Ingegno  
313 et al., 2009). Therefore, since abundance of natural host plants acting as source affects greatly  
314 the presence of predatory bugs, plants species, like *S. nigrum* and *C. officinalis*, can carry out  
315 an important role in conserving and augmenting *M. pygmaeus* in agroecosystems. To  
316 implement natural control augmentation strategies in vegetable crops these plants should be  
317 conserved and, if scarcely present, increased. Conservation of natural host plant, such as  
318 *Dittrichia viscosa* L. (Asteraceae) for *M. melanotoma* (Costa) (Perdikis et al., 2007), or their  
319 use as banker plants in vegetable greenhouses, e.g. tobacco for *Macrolophus* sp. (Arnó et al.,  
320 2000) and mullein [*Verbascum thapsus* L. (Scrophulariaceae)] for *D. hesperus* (Sanchez et al.,  
321 2003a), have been already proposed to favour the early establishment of native predators on  
322 tomato crops in other geographic areas.



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

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

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

348 2004), while no differences emerged between the previously infested tomato and the healthy  
349 one, likely excluding a systemic effect of whitefly infestation on plant. The actual presence of  
350 the prey on tomato is important for attracting *M. pygmaeus*, consistent with the results of  
351 another study conducted in Y-tube olfactometer where the predatory species preferred spider  
352 mite infested green bean plants to uninfested plants (Moayeri et al., 2006b). However, the  
353 only presence of the whitefly, without host plant, was not sufficient for attracting *M.*  
354 *pygmaeus*, as observed with other potential prey, the aphid *Myzus persicae* (Sulzer) and the  
355 spider mite *Tetranychus urticae* Koch (Moayeri et al., 2006a, b). Although in the bioassays  
356 the infested plants after prey removal were not significantly attractive, further research on  
357 responses of *M. pygmaeus* is needed to investigate thoroughly a possible systemic effect due  
358 to prey feeding on tomato, and to identify plant volatiles that, acting as indirect defences,  
359 constitutive and/or induced, can affect adversely the herbivorous victim by attracting the  
360 predatory bug.

361

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519

520 **Tables**

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

<b>Experiment 1</b>			<b>Experiment 2</b>		
clean air	vs	tomato	clean air	vs	uninfested tomato
<i>C. officinalis</i>	vs	tomato	clean air	vs	whitefly
<i>C. annuum</i>	vs	tomato	whitefly	vs	uninfested tomato
<i>P. officinalis</i>	vs	tomato	infested tomato	vs	uninfested tomato
<i>S. officinalis</i>	vs	tomato	uninfested tomato	vs	previously infested tomato
<i>S. nigrum</i>	vs	tomato			

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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).

Plant	Instar						sex ratio $\frac{\Omega}{(\Omega + \delta)}$
	First	Second	Third	Forth	Fifth	Total	
<i>C. officinalis</i>	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>b</b>	0.67
	100.0%	100.0%	100.0%	83.3%	90.0%	75.0%	
<i>C. annuum</i>	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>b</b>	0.75
	100.0%	91.7%	90.9%	80.0%	100.0%	66.7%	
<i>L. esculentum</i>	3.3 ± 0.4	2.5 ± 0.2	2.6 ± 0.2	2.8 ± 0.3	5.1 ± 0.4	16.8 ± 0.8 <b>ab</b>	0.43
	100.0%	100.0%	91.7%	81.8%	77.8%	58.3%	
<i>P. officinalis</i>	3.0 ± 0.4	3.0 ± 0.4	3.0 ± 0.3	2.7 ± 0.3	5.4 ± 0.5	17.0 ± 0.8 <b>ab</b>	0.30
	100.0%	83.3%	100.0%	100.0%	100.0%	83.3%	
<i>S. officinalis</i>	4.8 ± 0.5	3.5 ± 0.4	2.6 ± 0.2	3.8 ± 0.7	3.7 ± 0.4	19.3 ± 0.8 <b>a</b>	0.71
	100.0%	91.7%	90.9%	70.0%	100.0%	58.3%	
<i>S. nigrum</i>	2.8 ± 0.3	3.0 ± 0.3	2.9 ± 0.2	3.5 ± 0.5	4.4 ± 0.2	16.7 ± 0.7 <b>ab</b>	0.75
	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	
<i>C. officinalis</i>	2.7 ± 0.4	3.0 ± 0.7	1.0	2.0	-	-	-
	83.3%	50.0%	20.0%	100.0%	0.0%	0.0%	
<i>C. annuum</i>	2.0 ± 0.2	1.0	1.0	-	-	-	-
	83.3%	10.0%	100.0%	0.0%	0.0%	0.0%	
<i>L. esculentum</i>	2.0 ± 0.4	3.4 ± 0.7	8.5 ± 1.5	-	-	-	-
	91.7%	45.5%	40.0%	0.0%	0.0%	0.0%	
<i>P. officinalis</i>	2.8 ± 0.6	-	-	-	-	-	-
	41.7%	0.0%	0.0%	0.0%	0.0%	0.0%	
<i>S. officinalis</i>	2.8 ± 0.5	-	-	-	-	-	-
	83.3%	0.0%	0.0%	0.0%	0.0%	0.0%	
<i>S. nigrum</i>	2.6 ± 0.3	4.0 ± 0.6	2.7 ± 0.7	3.0	-	-	-
	100.0%	75.0%	33.3%	33.3%	0.0%	0.0%	

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530

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

<b>Plant species</b>	<b>Mean % of adult recaptured <math>\pm</math> SE</b>	<b>Mean no. of nymphs <math>\pm</math> SE</b>
<i>C. officinalis</i>	17.7 $\pm$ 4.5	24.0 $\pm$ 2.3
<i>C. annuum</i>	17.4 $\pm$ 4.9	21.8 $\pm$ 11.1
<i>L. esculentum</i>	17.8 $\pm$ 4.9	27.0 $\pm$ 4.6
<i>P. officinalis</i>	8.0 $\pm$ 3.0	12.6 $\pm$ 3.6
<i>S. officinalis</i>	19.7 $\pm$ 6.1	25.4 $\pm$ 8.1
<i>S. nigrum</i>	19.5 $\pm$ 2.5	21.8 $\pm$ 5.9

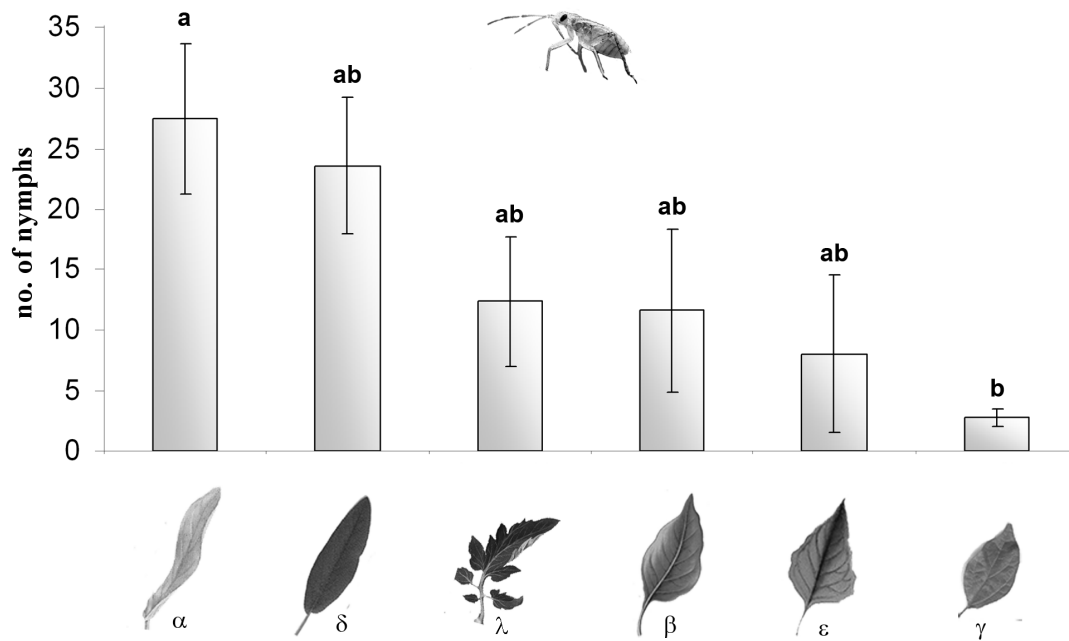
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536 **Table 4.** Percentage of eggs (means  $\pm$  SE) laid by *M. pygmaeus* females on leaf area of each  
537 plant species in multi-choice oviposition experiment. Mean percentages followed by different  
538 letters are significantly different (Tukey's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

<b>Plant species</b>	<b>Mean percentage of eggs <math>\pm</math> SE</b>
<i>C. officinalis</i>	18.53 $\pm$ 5.48 <b>ab</b>
<i>C. annuum</i>	26.49 $\pm$ 8.35 <b>ab</b>
<i>L. esculentum</i>	30.77 $\pm$ 9.16 <b>a</b>
<i>P. officinalis</i>	6.33 $\pm$ 4.12 <b>ab</b>
<i>S. officinalis</i>	2.48 $\pm$ 1.12 <b>b</b>
<i>S. nigrum</i>	15.40 $\pm$ 9.58 <b>ab</b>

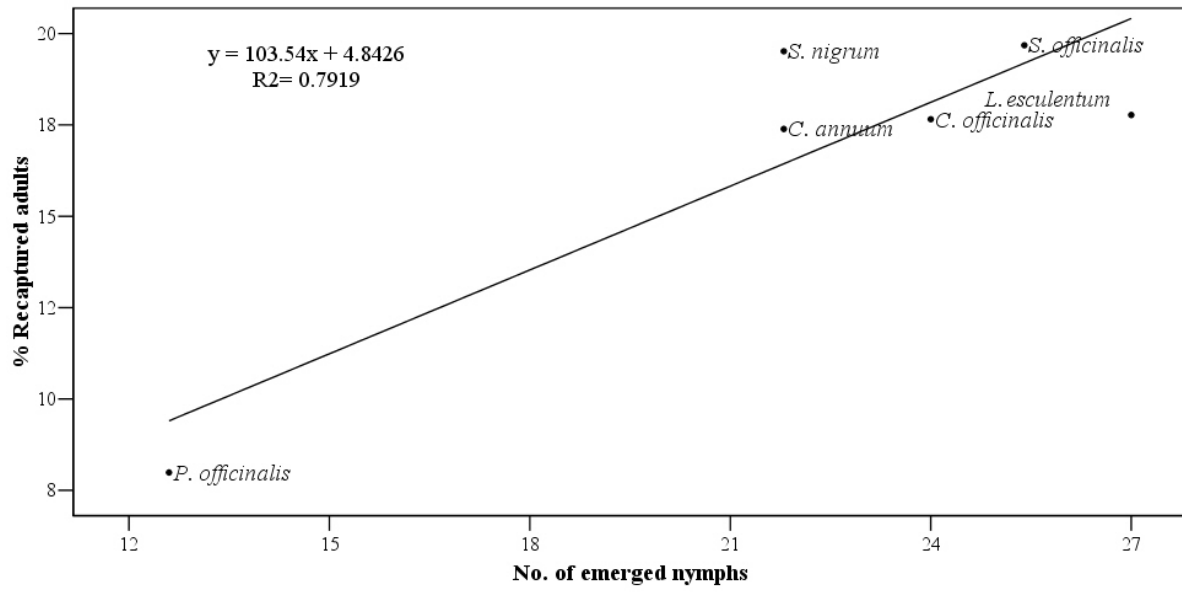
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541

542 **Figure 1.** Nymphs of *M. pygmaeus* (mean number  $\pm$  SE) emerged on the six plants in  
 543 reproduction experiment:  $\alpha$ = *C. officinalis*;  $\delta$ = *S. officinalis*;  $\lambda$ = *L. esculentum*;  $\beta$ = *C.*  
 544 *annuum*;  $\epsilon$ = *S. nigrum*;  $\gamma$ = *P. officinalis*. Means characterized by different letters are  
 545 significantly different (Tukey's test,  $P < 0.05$ ).

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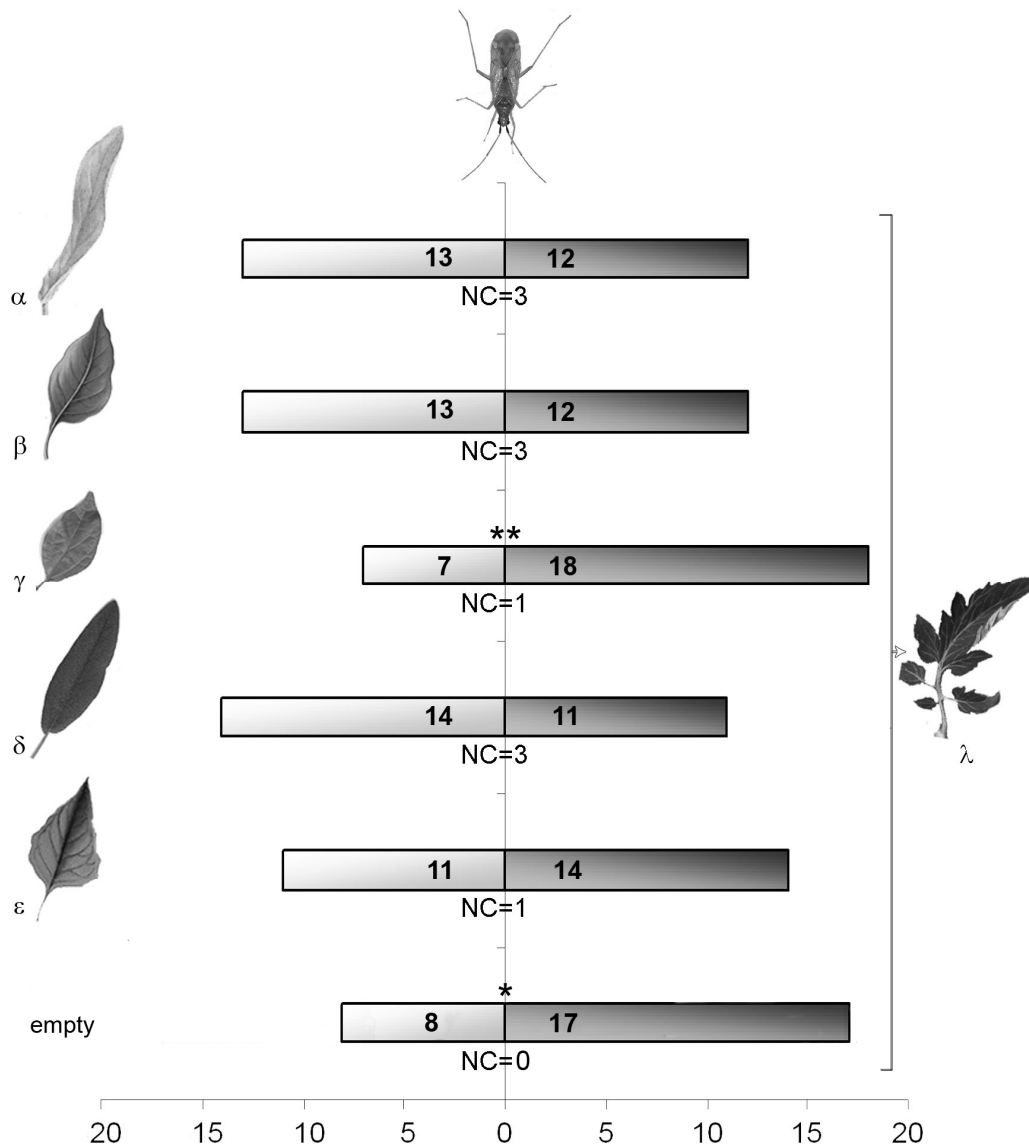
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**Figure 2.** Relationship between percentage of recaptured adults of *M. pygmaeus* in multi-

549

choice selection experiment and number of emerged nymphs (Pearson's correlation,  $P < 0.01$ ).

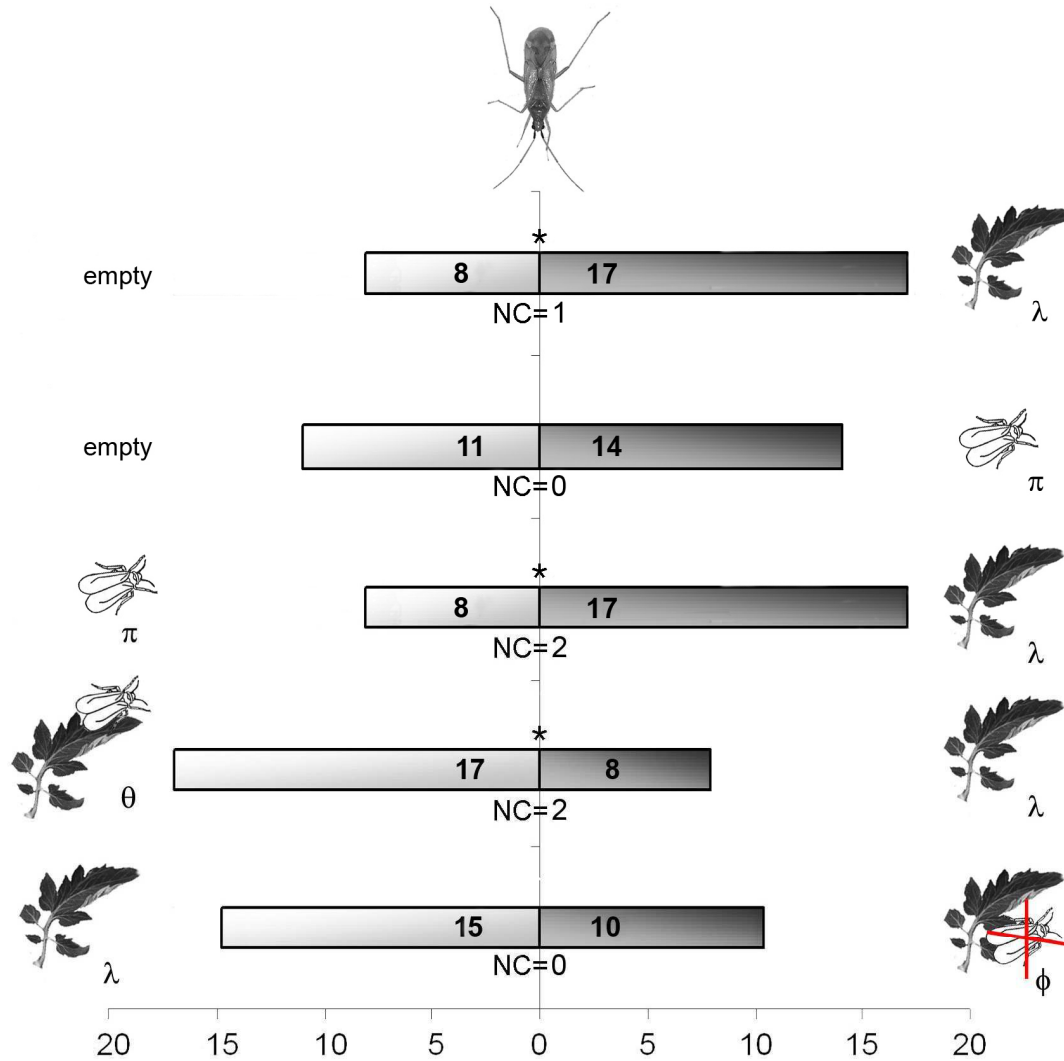
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552

553 **Figure 3.** Responses of *M. pygmaeus* (no. of responding females in bars) in a Y-tube  
 554 olfactometer and number of non-responding individuals (NC) to the odours of the plants for  
 555 each compared pair. The following plants were tested against healthy tomato individuals ( $\lambda =$   
 556 *L. esculentum*):  $\alpha = C. officinalis$ ;  $\beta = C. annuum$ ;  $\gamma = P. officinalis$ ,  $\delta = S. officinalis$ ;  $\epsilon = S.$   
 557 *nigrum*. Numbers in bars represent individual mirids that moved toward the volatiles.  $\chi^2$   
 558 statistics (\*\* $P < 0.05$ , \* $P < 0.10$ ;  $df: 1$ ) tested the hypothesis that the distribution of side-arm  
 559 choices deviated from a null model where odour sources were chosen with equal frequency.





560

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 ( $\theta$ ),  
 563 previously infested ( $\phi$ ) or uninfested ( $\lambda$ ) tomato with the whitefly *T. vaporariorum* ( $\pi$ ) for  
 564 each compared pair. Numbers in bars represent individual mirids that moved toward the  
 565 volatiles.  $\chi^2$  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.