Plant preference in the zoophytophagous generalist predator Macrolophus pygmaeus (Heteroptera: Miridae)

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UNIVERSITÀ DEGLI STUDI DI TORINO

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

2 **(Heteroptera: Miridae)**

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9   **Abstract**

10   *Macrolophus pygmaeus* (Heteroptera: Miridae) is an omnivourus 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

26

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;

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; Perdikis 2002;

75   Perdikis 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±1°C, RH 65±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 ($\varnothing$ 12cm), and

106   maintained in a heated greenhouse without any pesticide use.

107   <u>Reproduction and development on different plant species</u>

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, $\varnothing$

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^2 (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 version12.0; SPSS Inc., Chicago, IL, USA).

131 <u>Host plant selection experiments</u>

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, ∅ 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±1°C, RH 65±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°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 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 2.5L 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%$_v$). Chambers were washed after each trial with neutral soap and alcohol (70%$_v$) and

192   sterilised in autoclave at 120°C for 20 min. The olfactometric bioassays were conducted at

193   24±1°C, RH 25-30%, and 540±30lux.

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   <u>Reproduction and development on different plant species</u>

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 1st instar nymphs could reach the 2nd instar,

228 but after only on *C. officinalis* and *S. nigrum* very few nymphs reached the 5th instar whereas

229 on *P. officinalis* and *S. officinalis* no nymphs got over the 2nd instar (Table 2).

230 <u>Host plant selection and oviposition multi-choice experiments</u>

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 <u>Olfactometric bioassays</u>

244 In the first experiment (Table 1), no significant preference was detected between healthy

245 tomato and the following plants (Figure 3): *C. officinalis* (χ^2=0.04, P=0.84), *C. annuum*

246 (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

247 contrast, *P. officinalis* resulted unattractive in comparison with tomato (χ^2=4.84, P=0.03) as

248 well as the empty chamber (χ^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 (χ^2=3.24, P=0.07) and by the

253   uninfested tomato compared to the whiteflies (χ^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 (χ^2=1.00, P=0.32), as well as comparing whitefly odours with

256   the empty chamber (χ^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 agrecosystems 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; Perdikis 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.

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520 **Tables**

521 **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			

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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** First	Second	Third	Forth	Fifth	Total	*sex ratio* ♀/(♀ + ♂)
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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531　**Table 3.** *M. pygmaeus* adults recaptured (mean % ± SE) and nymphs emerged (mean number

532　± SE) on each whole plant of the six species in multi-choice selection experiment. Significant

533　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

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536   **Table 4.** Percentage of eggs (means ± 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).

| 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**

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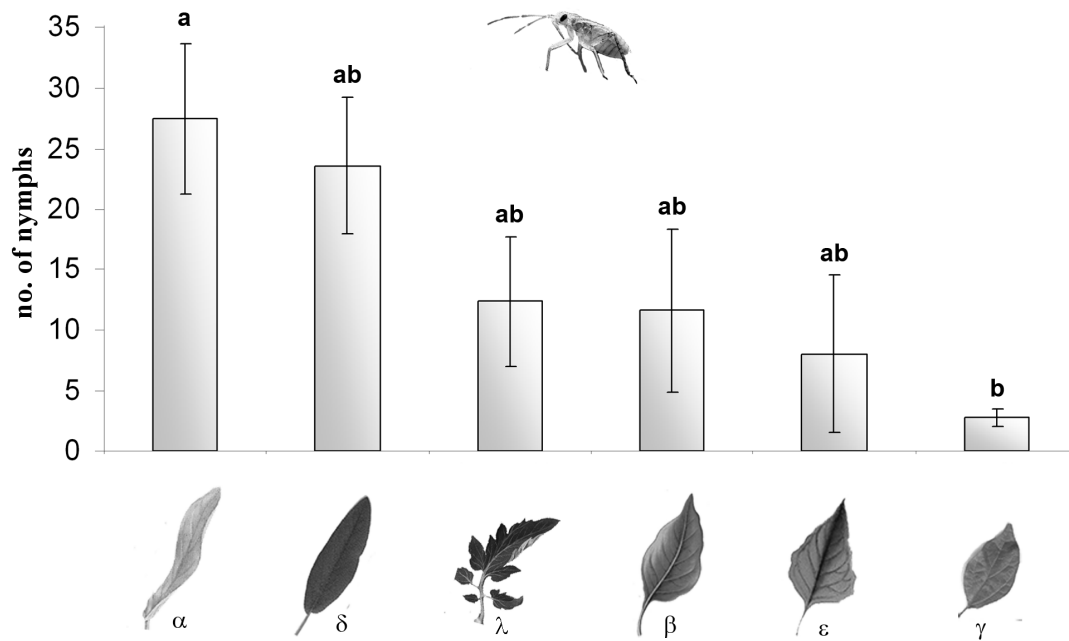
Figure 1. Nymphs of *M. pygmaeus* (mean number ± SE) emerged on the six plants in

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
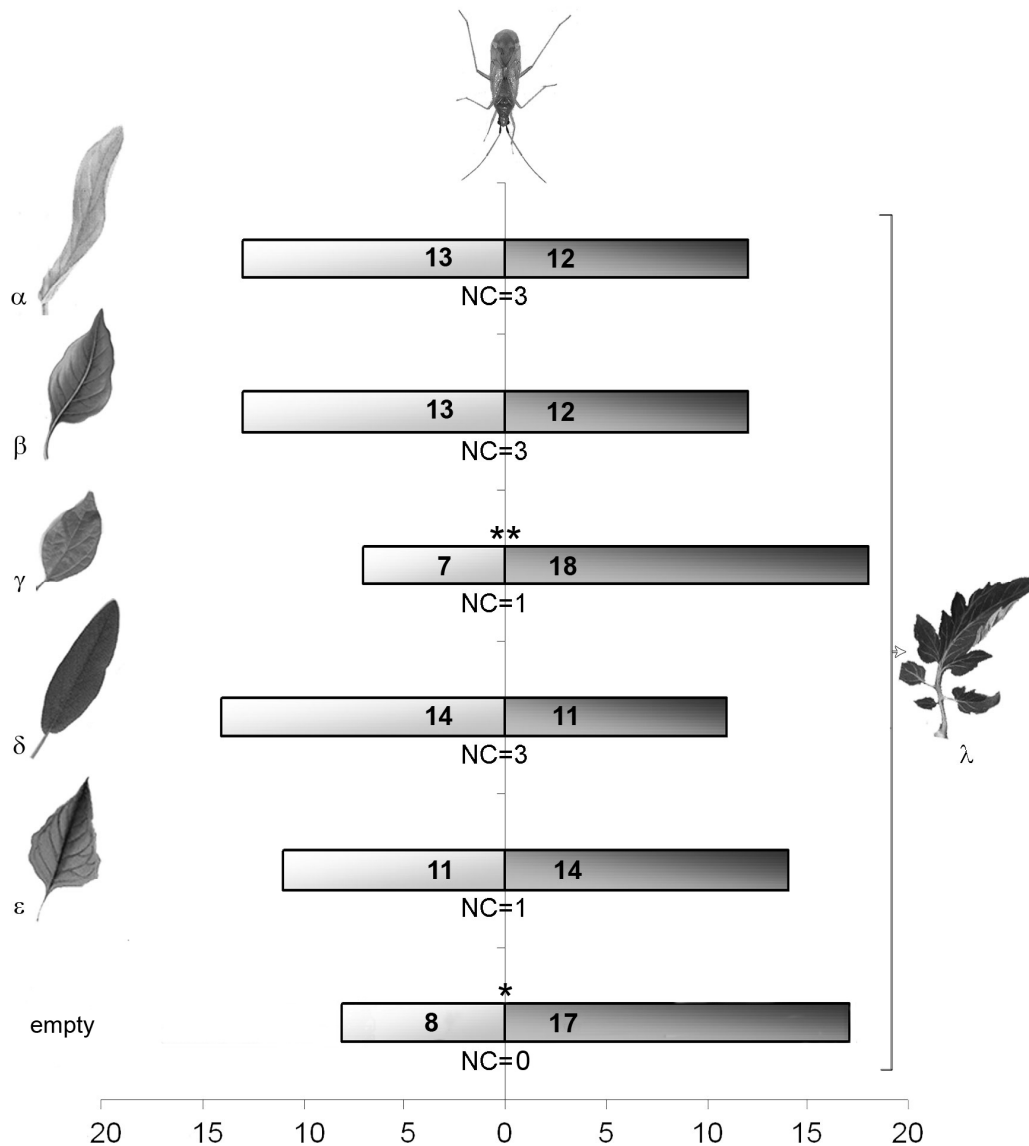
significantly different (Tukey's test, P<0.05).

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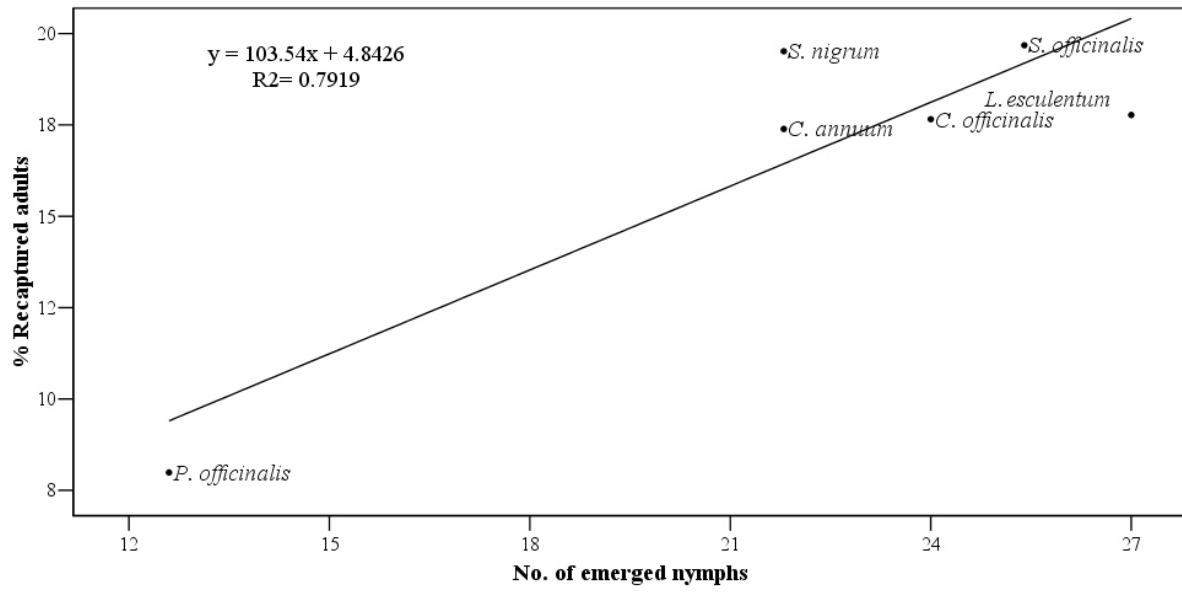
**Figure 2.** Relationship between percentage of recaptured adults of *M. pygmaeus* in multi-choice selection experiment and number of emerged nymphs (Pearson's correlation, P<0.01).

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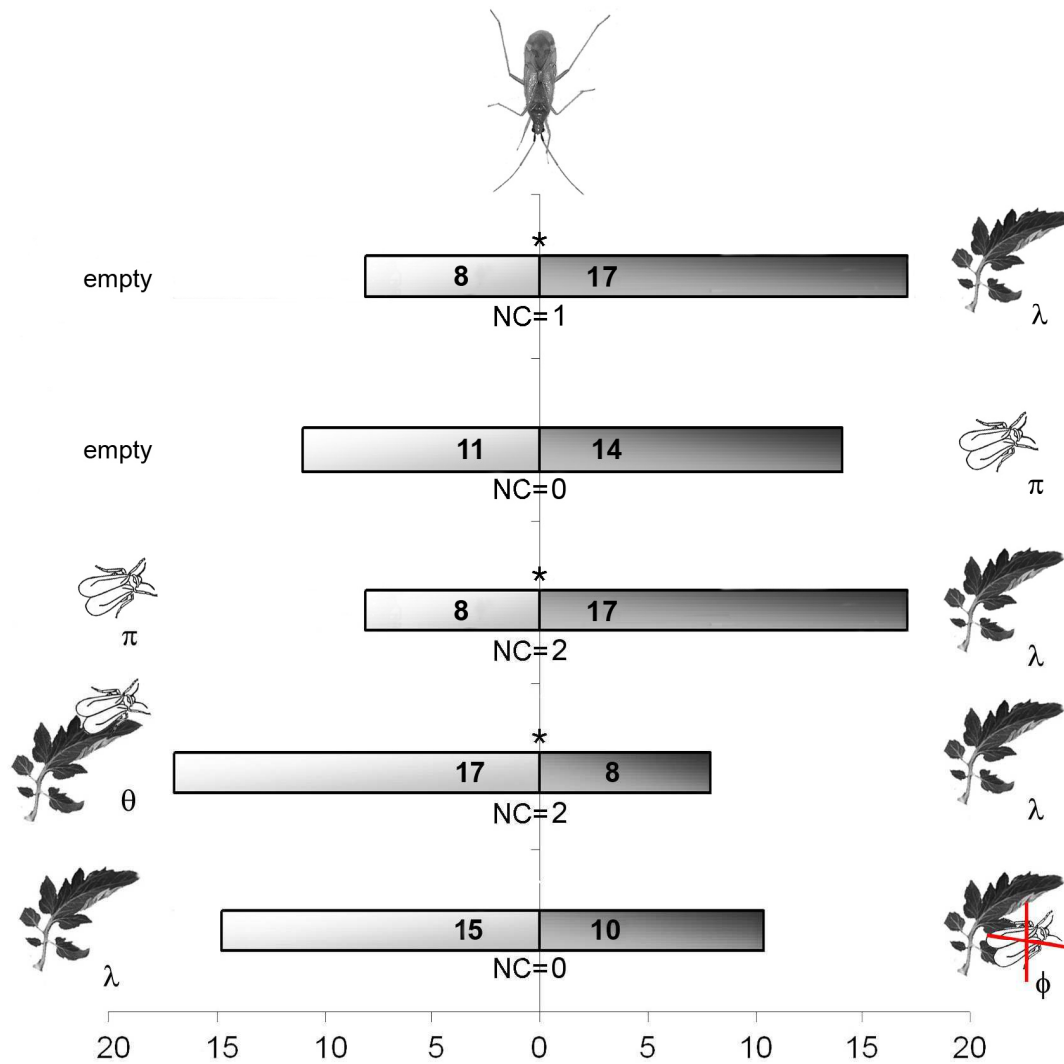
Figure 4. Responses of *M. pygmaeus* (no. of responding females in bars) in a Y-tube

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

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

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

volatiles. χ^2 statistics (*$P<0.10$; 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.