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1 **Evaluation of mirid predatory bugs and release strategy for aphid control in sweet pepper**

2 G. J. Messelink<sup>1</sup>, C. M. J. Bloemhard<sup>1</sup>, H. Hoogerbrugge<sup>2</sup>, J. van Schelt<sup>2</sup>, B. L. Ingegno<sup>3</sup> & L.  
3 Tavella<sup>3</sup>

4 <sup>1</sup> Wageningen UR Greenhouse Horticulture, Bleiswijk, The Netherlands

5 <sup>2</sup> Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands

6 <sup>3</sup> Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino  
7 Grugliasco, Italy

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13 **Correspondence:** Gerben J. Messelink (corresponding author), Wageningen UR Greenhouse  
14 Horticulture, PO Box 20, 2265 ZG Bleiswijk, The Netherlands. E-mail: gerben.messelink@wur.nl

15

16

17 **Abstract**

18 Zoophytophagous predators of the family Miridae (Heteroptera), which feed both on plant and prey,  
19 often maintain a close relationship with certain host plants. In this study, we aimed to select a  
20 suitable mirid predatory bug for aphid control in sweet pepper. Four species were compared:  
21 *Macrolophus pygmaeus* (Rambur), *Dicyphus errans* (Wolff), *Dicyphus tamaninii* Wagner and  
22 *Deraeocoris pallens* (Reuter). They were assessed on their establishment on sweet pepper plants  
23 with and without supplemental food (eggs of the flour moth *Ephestia kuehniella* Zeller and  
24 decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg) and on their effects on aphids  
25 with releases before and after aphid infestations. None of the predator species was able to control an  
26 established population of aphids on sweet pepper plants; however, the predators *M. pygmaeus* and  
27 *D. tamaninii* could successfully reduce aphid populations when released prior to an artificially  
28 introduced aphid infestation. The best results were achieved with *M. pygmaeus* in combination with  
29 a weekly application of supplemental food. Hence, our results demonstrate that the order and level  
30 of plant colonization by mirid predators and aphids determines how successful biological control is.  
31 Further studies are needed to evaluate the performance of mirid predatory bugs in sweet pepper  
32 crops in commercial greenhouses with multiple pests and natural enemies, in particular to  
33 understand how increased variation in food sources affects their feeding behaviour and preferences.

34

## 35 **Introduction**

36 Zoophytophagous predators of the family Miridae (Heteroptera), which feed both on plant and prey,  
37 are increasingly used for pest control in greenhouse vegetable crops either through augmentative or  
38 conservation biological control (Castañé et al. 2004; Perdikis et al. 2011; van Lenteren 2012). The  
39 most commonly used and commercially mass-produced species are currently *Macrolophus*  
40 *pygmaeus* (Rambur) (formerly identified as *Macrolophus caliginosus* Wagner, Castañé et al. 2013)  
41 in northern Europe and *Nesidiocoris tenuis* (Reuter) in southern Europe. Typically these predators  
42 feed on multiple pest species such as whiteflies (Montserrat et al. 2000), aphids (Alvarado et al.  
43 1997), thrips (Riudavets and Castañé 1998), spider mites (Hansen et al. 1999), leaf miners (Arnó et  
44 al. 2003) and Lepidoptera species, including *Tuta absoluta* (Urbaneja et al. 2009). Because of their  
45 plant-feeding behaviour and oviposition in plant tissue, mirids maintain a close relationship with  
46 certain host plants (Perdikis and Lykouressis 2000; Wheeler 2001). Somehow, mirid predators of  
47 the tribe Dicyphini (belonging to *Nesidiocoris*, *Dicyphus* or *Macrolophus* genus) seem to be  
48 adapted to live on hairy plants (Wheeler 2001; Voigt et al. 2007; Ingegno et al. 2008). Their typical  
49 long and slender legs facilitate them, in contrast with many other arthropods, to walk on leaf and  
50 stem surfaces with high trichome densities (Voigt et al. 2007). The fact that some hairy plants have  
51 glandular trichomes which entrap insects might be one of the reasons why mirids became  
52 specialized in hairy plants, as the carrion of entrapped insects provides them with protein-rich food  
53 (Krimmel and Pearse 2013).

54 When selecting mirid predatory bugs for pest control, it is important to consider their strong  
55 association with certain plant traits, particularly when they are used in a preventive way and their  
56 establishment in crops is aimed before pests occur. Such crop ‘inoculation’ strategies with mirid  
57 predatory bugs at the start of a cropping cycle are commonly applied in greenhouse tomatoes and  
58 eggplants, mainly as a cost-effective method to reach high predator densities (Calvo et al. 2012).  
59 Hence, the performance of mirid predatory bugs on plants without pests is an important selection  
60 criterion in addition to their efficacy in controlling the target pest species. In this study, we aimed to  
61 select a suitable mirid predatory bug for aphid control in sweet pepper. Mirids are so far hardly used  
62 in sweet pepper crops, probably because these plants are usually not colonized by naturally  
63 occurring mirids, but more commonly by anthocorid bugs (Tavella and Goula 2001; Sanchez et al.  
64 2003; Bosco et al. 2008; Ingegno et al. 2011). However, when released in monocultures of sweet  
65 pepper, the predator *M. pygmaeus* is able to establish and reproduce in sweet pepper for several  
66 months and high densities are able to prevent outbreaks of aphids (Messelink et al. 2011; Messelink  
67 and Janssen 2014; Pérez-Hedo and Urbaneja 2014). Yet, it is not clear how effectively *M.*  
68 *pygmaeus* controls aphids in sweet pepper and how well it establishes in this crop compared with

69 other species of mirid predatory bugs. Finding other mirid predatory bugs that are even more  
70 effective in controlling aphids would be very useful, as aphids are extremely hard to control,  
71 particularly in organic sweet pepper cropping systems (Messelink et al. 2011).

72 In this study, we compared *M. pygmaeus* with three other mirid species: *Dicyphus errans* (Wolff),  
73 *Dicyphus tamaninii* Wagner and *Deraeocoris pallens* (Reuter), by assessing their establishment on  
74 sweet pepper plants and their efficacy as predators of the peach aphid *Myzus persicae* (Sulzer).

75 Laboratory studies show that all these predators can consume large numbers of aphids (Alvarado et  
76 al. 1997; Ghavami et al. 1998; Voigt 2005). *Dicyphus errans* is widespread throughout Europe on a  
77 wide range of host plants (Ingegno et al. 2008) and observed to colonize tomato crops in northern  
78 Italy where it can prey on important pests as *Tuta absoluta* (Meyrick) (Ingegno et al. 2013).

79 *Dicyphus tamaninii* has been tested for pest control in tomato and cucumber crops in Spain, but this  
80 species was never recommended because of the observed damage to the tomato fruit (Castañé et al.  
81 2011). *Deraeocoris pallens* is considered as a key predator of soft-bodied pests in cotton in the East  
82 Mediterranean region (Ghavami et al. 1998), but the predator was also observed on several other  
83 host plants (Atakan and Tunç 2010) and was able to reduce whitefly populations on eggplant  
84 (Ulubilir et al. 1997). However, the performance of *D. errans*, *D. tamaninii* and *D. pallens* on sweet  
85 pepper is unknown. Sweet pepper plants have smooth and waxy leaf surfaces with trichomes  
86 clustered in domatia only on the abaxial side of the leaves (Ferreira et al. 2008), and this lack of  
87 trichomes could be a disadvantage for hairy plant associated bugs like Dicyphini. However, even if  
88 sweet pepper is not the preferred host plant because of these morphological traits, the predators may  
89 still establish in this crop when no alternative plant species are present, as has been observed for *M.*  
90 *pygmaeus* (Messelink and Janssen 2014). Moreover, the lack of trichomes might be compensated by  
91 the pollen and nectar provided by the continuously flowering sweet pepper plants (Portillo et al.  
92 2012). Establishment might also be enhanced by supplemental food (Messelink et al. 2014). Hence,  
93 we additionally evaluated the population increase of predatory bugs on flowering sweet pepper  
94 plants provided with weekly food supplements of eggs of the flour moth *Ephesia kuehniella* Zeller  
95 and decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg, which are both suitable  
96 food sources for generalist predatory bugs (Arijs and De Clercq 2001; De Clercq et al. 2014).

97 Effects on aphids were evaluated with predator releases both before and after establishment of  
98 aphids. As mirid predatory bugs have a relatively long generation time and mix their diet with prey  
99 and plant feeding (Gillespie and McGregor 2000), we hypothesized that high initial predator–pest  
100 ratios are needed to achieve a sufficient control of aphids. This might be obtained through prior to  
101 pest releases of mirids combined with supplemental food to increase predator densities. The results

102 of this study may help to develop practical guidelines for enhancing aphid control with generalist  
103 predators in commercial sweet pepper crops.

104

## 105 **Materials and Methods**

### 106 Plants and insects

107 The origin of the four selected predatory bugs is presented in table 1. Collected predatory bugs were  
108 maintained as laboratory rearings on bean pods (*Phaseolus vulgaris* L.) and eggs (killed by  
109 freezing) of the flour moth *E. kuehniella* as food, following the methods described by Van den  
110 Meiracker and Ramakers (1991) for rearing anthocorid bugs. The culture of *D. pallens* was  
111 additionally provided with sweet pepper leaves infested by *M. persicae*, as this prey enhanced the  
112 reproduction rate. Tobacco leaves (*Nicotiana tabacum* L.) were added to the culture of *D. errans* to  
113 enhance oviposition. Sweet pepper plants (*Capsicum annuum* L.), cv Spider (Enza Zaden), were  
114 grown in peat by a commercial plant propagator without application of pesticides. Peach aphids (*M.*  
115 *persicae*) of the red phenotype were reared on sweet pepper plants of the same cultivar in a separate  
116 greenhouse compartment.

### 117 Greenhouse experiment with predator releases after aphid establishment

118 Aphid control with releases of mirid predatory bugs after establishment of aphids was evaluated in  
119 16 walk-in cages of 1\*2\*2 m made of fine insect gauze (mesh size 0.22 9 0.31 mm), which were  
120 placed in a greenhouse compartment of 144 m<sup>2</sup> at the Institute of Wageningen UR Greenhouse  
121 Horticulture. The experiment had a completely randomized design with four replicates of the  
122 following treatments: (i) no predators (only *M. persicae*), (ii) *M. pygmaeus* (iii) *D. errans* and (iv)  
123 *D. pallens*. *Dicyphus tamaninii* was not included in this experiment, because of the limited available  
124 numbers when starting the trials. Each cage was provided with four potted sweet pepper plants, cv  
125 Spider, which were planted in peat in 10 l pots. The plants were grown according to a three-stems-  
126 per-plant system, resulting in 12 sweet pepper stems per cage. Plants were provided with a standard  
127 nutrient solution through drip irrigation. The plants were infested in September with aphids when  
128 the crop was about 1 m high at densities of 20 aphids of mixed age per plant, thus 80 aphids per  
129 cage. This resulted after 2 weeks in an average density of 1325 ( $\pm$  SE 67) aphids per cage. Predators  
130 were released 1 day after this assessment of aphid establishment. Each cage was provided with 40  
131 one-week-old adults (20 males and 20 females) and 40 third instar nymphs. Thus the average  
132 predator: aphid ratio at the start of this experiment was 1 : 16.5. Densities of aphids and mirid  
133 predatory bugs were assessed weekly during four consecutive weeks by counting the total number  
134 of aphids and predators on 24 randomly selected leaves per cage: 12 leaves in the upper part of the  
135 plants and 12 leaves in the lower part. All observations on the plants were non-destructively

136 performed inside the cages between 8:00 and 12:00 AM. Temperature and relative humidity were  
137 registered every 5 min throughout the experiment with a climate recorder (Hoogendoorn Growth  
138 Management) inside one of the cages. The experiment was conducted in September– October, and  
139 during that period, the average measured temperature and relative humidity in the cages were  
140 20.7°C (range 17.5–27°C) and 75% (range 42–90%), respectively.

#### 141 Greenhouse experiment with predator releases prior to aphid releases

142 A second greenhouse experiment was set-up similar to the first one, but with releases of predatory  
143 bugs prior to aphid releases. The establishment of the predators on flowering sweet pepper plants  
144 was compared with the establishment on plants that received a weekly supply of additional food  
145 consisting of 0.6 g eggs of *E. kuehniella* (killed by freezing) and 0.3 g freeze dried decapsulated  
146 cysts of *A. franciscana* per cage, both obtained from Koppert Biological systems (non-commercial  
147 R&D materials). This food was added weekly by manually sprinkling it equally distributed over the  
148 leaves. The following eight treatments were tested in four replicates: (i) no predators, (ii) *M.*  
149 *pygmaeus*, (iii) *D. errans*, (iv) *D. pallens* (v) *M. pygmaeus* + food, (vi) *D. errans* + food, (vii) *D.*  
150 *pallens* + food and (viii) *D. tamaninii* + food. Plants were inoculated with predatory bugs by twice  
151 releasing 16 one-week-old adults (eight females and eight males) per cage. The second introduction  
152 was 2 weeks after the first, to establish populations of mixed developmental stages. Aphids were  
153 introduced 5, 6 and 7 weeks (week number 18, 19 and 20 respectively) after the last predator  
154 releases, each time 120 aphids of mixed age were released per cage (30 aphids per plant). Densities  
155 of aphids and predators were followed in the same way as in the first experiment. In addition, 10  
156 fresh flowers per cage were randomly selected to count the number of mirid predators in the  
157 flowers. The experiment was conducted in March–May, and during that period, the average  
158 measured temperature and relative humidity in the cages were 22.3°C (range 15.2– 31.8°C) and  
159 71% (range 32 – 88%), respectively. Data were analysed with the same methods as for the first  
160 greenhouse experiment.

#### 161 Predation rates in the laboratory

162 In addition to the greenhouse experiments, we measured predation rates of the two best performing  
163 predatory bugs. From each selected species 20 one-week old females were individually starved for  
164 24 h in a glass vial with only some water-soaked cotton, to ensure they were motivated to feed. The  
165 experiment was conducted in plastic boxes (Ø 6 9 5 cm) with a sweet pepper leaf disc that was  
166 embedded upside down in water agar (1% agar), making the abaxial side of the discs available to  
167 the aphids and predators. Ventilation was possible through a hole in the lid covered with insect  
168 gauze (mesh size 80 µm). Each box was provided with 40 first instar nymphs of *M. persicae*, which  
169 were directly isolated from the greenhouse colony with a small brush. Preliminary experiments

170 showed that 40 nymphs are ample prey for one female predator per day. After adding the aphids,  
171 each box was provided with one of the starved females. All 40 boxes were placed upside down  
172 (natural position of aphids) in a climate room under 16 h of artificial illumination per day, at 25°C  
173 and 70% RH. Predation of nymphs by the adult predators, recognized by the presence of bodies that  
174 were sucked partly or completely empty, was assessed after 24 h.

#### 175 Statistical analyses

176 Population dynamics of aphids and predators in the two greenhouse trials were analysed with  
177 repeated measures ANOVA, and differences among treatments were tested with Fisher's least  
178 significance difference (LSD) test ( $P < 0.05$ ). In addition, it was analysed whether the application of  
179 food (only for *M. pygmaeus*) or the presence of aphids caused a shift in the fraction of predators  
180 occupying the flowers. A two-way ANOVA was performed with arcsine square root transformed  
181 average fractions of predators in the flowers (densities in 10 flowers/densities on 24 leaves) with  
182 treatment and the presence of aphids (week 4–8 vs. week 9–14) as factors. Differences among  
183 treatments were tested with Fisher's LSD test ( $P < 0.05$ ). Predation rates of the laboratory trial were  
184 analysed using a generalized linear model (GLM) with a Poisson distribution of the data. All  
185 statistical analyses were performed using the statistical package Gen-Stat Release 16.1 (Payne et al.  
186 2010).

187

## 188 **Results**

### 189 Greenhouse experiment with predator releases after aphid establishment

190 Aphid densities increased strongly in all treatments (fig. 1a), and none of the predator species was  
191 able to significantly reduce the increase of aphids ( $F_{3,9} = 3.17$ ,  $P = 0.24$ ). Densities of predators  
192 were significantly different among treatments ( $F_{2,6} = 5.2$ ,  $P = 0.049$ ), with the highest densities  
193 observed for *M. pygmaeus* and the lowest for *D. pallens* (fig. 1b).

### 194 Greenhouse experiment with predator releases prior to aphid releases

195 The predators *D. errans* and *D. pallens* were not able to establish a population in the sweet pepper  
196 plants without application of food. *Deraeocoris pallens* was not observed anymore from 4 weeks  
197 after the second release of predators and only a few adults of *D. errans* remained alive during the  
198 trial, without producing a new generation. These treatments were therefore excluded from further  
199 analyses. The population dynamics of aphids were significantly different among the remaining  
200 treatments ( $F_{5,18} = 42.33$ ,  $P < 0.01$ ). The best control of aphids was achieved in the treatment of *M.*  
201 *pygmaeus* combined with the weekly food application, followed by *D. tamaninii* + food and *M.*  
202 *pygmaeus* without food (fig. 2). The predator + food treatments of *D. errans* and *D. pallens* were  
203 not able to prevent a strong increase of aphids (fig. 2). Densities of predators were significantly

204 different among treatments both on the leaves ( $F_{4,15} = 83.45$ ,  $P < 0.01$ ) and in the flowers ( $F_{4,15} =$   
205  $280.48$ ,  $P < 0.01$ ). The highest predator densities were achieved in the treatments with food  
206 application for the species *M. pygmaeus* and *D. tamaninii* (fig. 3). The weekly application of food  
207 was beneficial for the predator *M. pygmaeus*; aphid densities were significantly lower and predator  
208 densities significantly higher when food was added compared to the treatment without food (figs 2  
209 and 3). The fractions of predators in the flowers were significantly different among predator  
210 treatments ( $F_{4,30} = 22.04$ ,  $P < 0.01$ ) and also affected by the releases of aphids ( $F_{1,30} = 10.99$ ,  $P =$   
211  $0.003$ ) (fig. 4). Weekly food supplements significantly reduced the fraction of *M. pygmaeus*  
212 predators in the flowers compared to the treatment without food application (fig. 4). A strong shift  
213 was observed for the predator *D. errans* from the flowers to the leaves when aphids were present on  
214 the plants, whereas such a shift did not occur with the other predators species (fig. 4).

#### 215 Predation rates in the laboratory

216 The daily predation rates of first instar aphids by females of the two best performing predatory bugs  
217 *M. pygmaeus* and *D. tamaninii* were on average  $21.7$  ( $\pm$  SE  $2.7$ ) and  $22.8$  ( $\pm$  SE  $2.0$ ) individuals,  
218 respectively. These rates were not significantly different ( $F_{1,37} = 1.03$ ,  $P = 0.317$ ).

219

#### 220 **Discussion**

221 The results of this study demonstrate that the order and level of plant colonization by the predator  
222 and pest determines how successful biological control with generalist predators is. None of the  
223 predator species was able to control an established population of aphids, even at the relatively high  
224 predator: prey ratio of 1 : 16.5. The predation rate experiment in the laboratory showed that female  
225 predators can consume about 20 young aphids/day, which might potentially have been enough to  
226 consume all young aphids on the plants within a few days. One of the possible reasons for this gap  
227 between the laboratory and field might be that the predation rates are reduced by the presence of  
228 alternative food sources provided by the plant (pollen and nectar). Another laboratory study with *M.*  
229 *pygmaeus* showed such effects: consumption rates of aphids were reduced with 44% when flowers  
230 of sweet pepper were added to sweet pepper leaves (Lykouressis et al. 2014). In our study, it was  
231 also observed that the predators feed on the secreted honeydew of the aphids, which could  
232 additionally distract them from feeding on the aphids themselves. Furthermore, the age structure of  
233 the predator and aphid populations could have affected predation rates. Also, the released male  
234 predators and third instar nymphs probably had lower predation rates than the female predators,  
235 which were assessed in the laboratory. Our results are in contrast with the study of Pérez-Hedo and  
236 Urbaneja (2014), which showed a good control of aphids when mirid bugs were released after

237 infestation of aphids. However, the predator–prey ratios are not clear in this study and also the  
238 provision of alternative food was prevented by removing the flowers.

239 Much better results were achieved in our study in the second experiment with preventive releases of  
240 predators. Both the predators *M. pygmaeus* and *D. tamaninii* strongly reduced the increase of aphids  
241 compared to the plants without predators. The best results were achieved with *M. pygmaeus* in  
242 combination with a weekly application of supplemental food. This additional food increased the  
243 population of predators to very high densities which favoured the control of aphids. These results  
244 suggest that a high predator: prey ratio is needed to achieve sufficient control. Aphids multiply  
245 extremely fast by viviparous parthenogenetic reproduction, resulting in relative growth rates on  
246 sweet pepper of 0.36 females per female per day (Dewhurst et al. 2012). Aphids will soon  
247 outnumber mirid predatory bugs which have a relatively low reproduction rate: for example  
248 0.0615–0.0958 females per female per day for *M. pygmaeus* on aphids (Margaritopoulos et al. 2003;  
249 Perdikis and Lykouressis 2004). Thus, the numerical response of the predators is by far not fast  
250 enough to control aphids. Preventive releases of predators can solve this problem, as this prior to  
251 pest establishment can result in relatively high initial predator: pest ratios which are enough to  
252 control the aphids. Moreover, predators might aggregate in patches of aphids in the field.

253 Olfactometric studies showed that mirids strongly respond to odours of plants infested by pests  
254 (Ingegno et al. 2011, 2013; Pérez-Hedo and Urbaneja 2014). This suggests that the predators can  
255 have an aggregative numerical response, also referred to as short-term apparent competition (Holt  
256 and Kotler 1987). Such aggregation was also suggested by our study, because a much lower fraction  
257 of *M. pygmaeus* was found in the flowers when food was added to the leaves. Our results also  
258 showed that *D. errans* seems to shift from flowers to leaves when aphids were added. This shift was  
259 not observed for *M. pygmaeus* and *D. tamaninii*, as their fraction of predators in the flowers  
260 remained similar on plants with or without aphids. However, these two predatory bugs controlled  
261 aphids much better than the other predator species, thus a part of the predator population may have  
262 gone back to the flowers when most aphids were consumed.

263 The predators *D. errans* and *D. pallens* were not able to establish in flowering sweet pepper plants  
264 without supplemental food. The nectar and pollen provided by the flowers are apparently not  
265 suitable for adult reproduction or to complete the development of these predators. The presence of  
266 *D. errans* in the flowers suggests that they do consume the pollen and nectar, but these food sources  
267 might lack essential nutrients for the juvenile development. *Deraeocoris pallens* was only found  
268 occasionally in the flowers of sweet pepper, indicating the unsuitability of sweet pepper pollen for  
269 this predator. This species might, more than the other tested mirids, need living prey for its  
270 development. Even the supplemental food of *Ephestia* eggs and *Artemia* cysts did not result in a

271 successful establishment of this predator species. The first generation offspring of released adults of  
272 *D. pallens* was not able to complete development and died out slowly.

273 The reason why *M. pygmaeus* and *D. tamaninii* were more successful in establishing a population  
274 on sweet pepper than the other predators remains unclear. Field surveys indicate that sweet pepper  
275 is not a preferred host plant, as pepper plants are only occasionally colonized by these predators  
276 (Tavella and Goula 2001; Sanchez et al. 2003). However, these predators might be better adapted to  
277 feed on pollen and nectar than the other tested mirids (Vandekerkhove and De Clercq 2010). Their  
278 ability to reproduce on food provided by the plant is a major advantage for establishing and  
279 augmenting predator populations in sweet pepper prior to pest infestations, which makes them  
280 suitable candidates for ‘seasonal inoculative’ biological control (van Lenteren and Woets 1988).

281 Biological control of aphids is, so far, usually based on releases of specialist natural enemies such  
282 as parasitoids (mainly Aphididae), or the predatory midge *Aphidoletes aphidimyza* (Rondani)  
283 (Blümel 2004). However, preventive releases of generalist predators such as *Orius majusculus*  
284 (Reuter) or *M. pygmaeus* can, in addition to these natural enemies, enhance aphid control  
285 (Messelink et al. 2013; Messelink and Janssen 2014). This will probably also result in lower costs,  
286 as inoculative releases of generalist predators may reduce the need for repeated releases of  
287 expensive specialist aphid natural enemies. The results of this study confirm that releases of *M.*  
288 *pygmaeus* in flowering sweet pepper plants before pest arrival can be a successful strategy to  
289 prevent outbreaks of aphids. A possible drawback is the intraguild predation by these generalist  
290 predators on specialist aphid predators, such as larvae of the predatory midge *A. aphidimyza* or  
291 syrphids (Fréchette et al. 2007; Messelink et al. 2013). However, the strong contribution of  
292 generalist predators to the control of aphids may outweigh the negative effects of intraguild  
293 predation (Messelink et al. 2013). An additional benefit of using generalist predatory bugs is their  
294 contribution to the control of other pests in sweet pepper such as caterpillars, spider mites or  
295 whiteflies (Hansen et al. 1999; Montserrat et al. 2000). Hence, biological control of aphids could be  
296 enhanced with prior to pest releases of *M. pygmaeus* or *D. tamaninii* combined with application of  
297 supplemental food for ‘boosting’ their densities. However, their performance in sweet pepper crops  
298 with multiple pests and natural enemies needs to be further evaluated, in particular how increased  
299 variation in food sources affects their feeding behaviour and preferences.

300

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307

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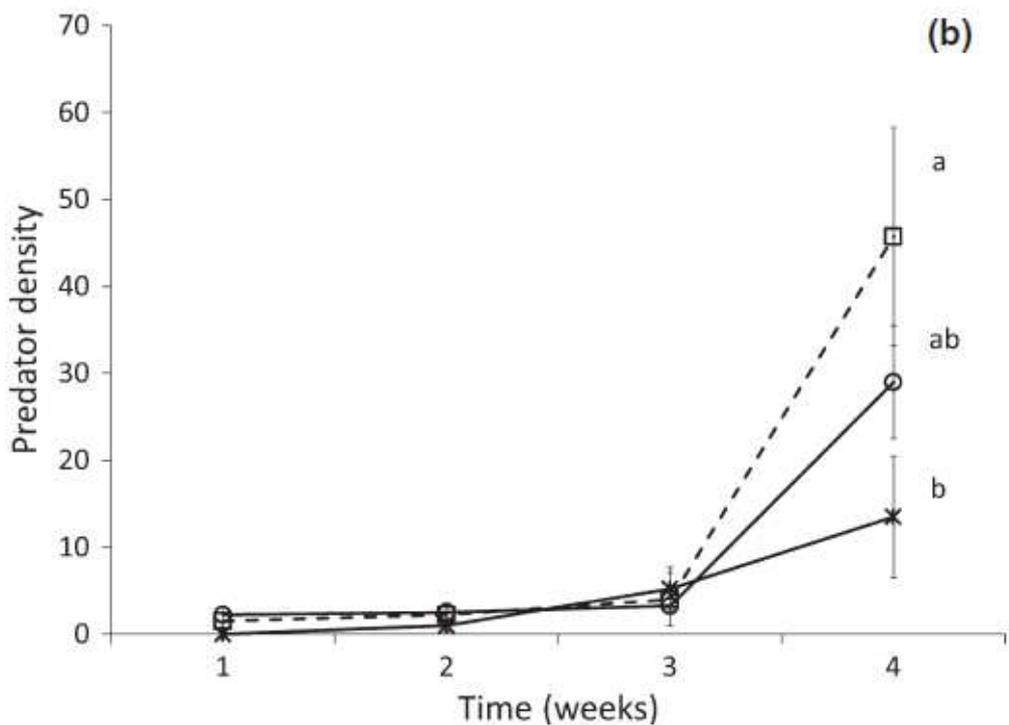
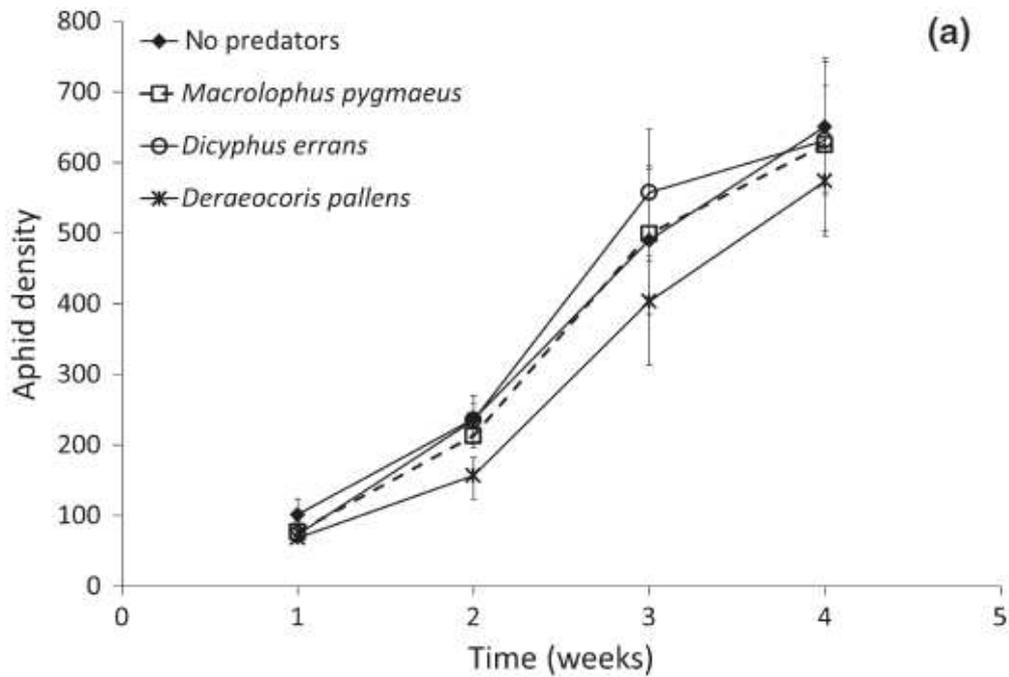
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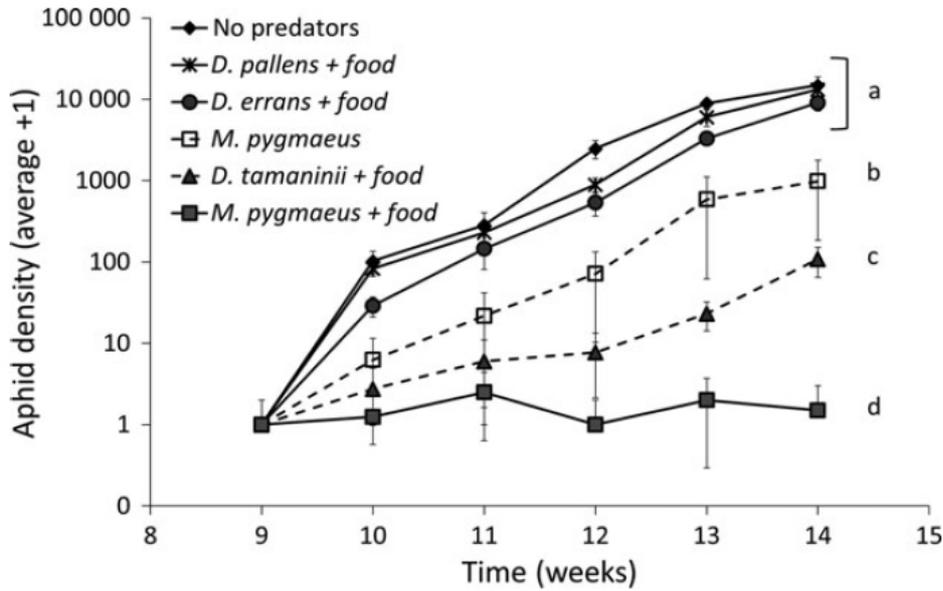
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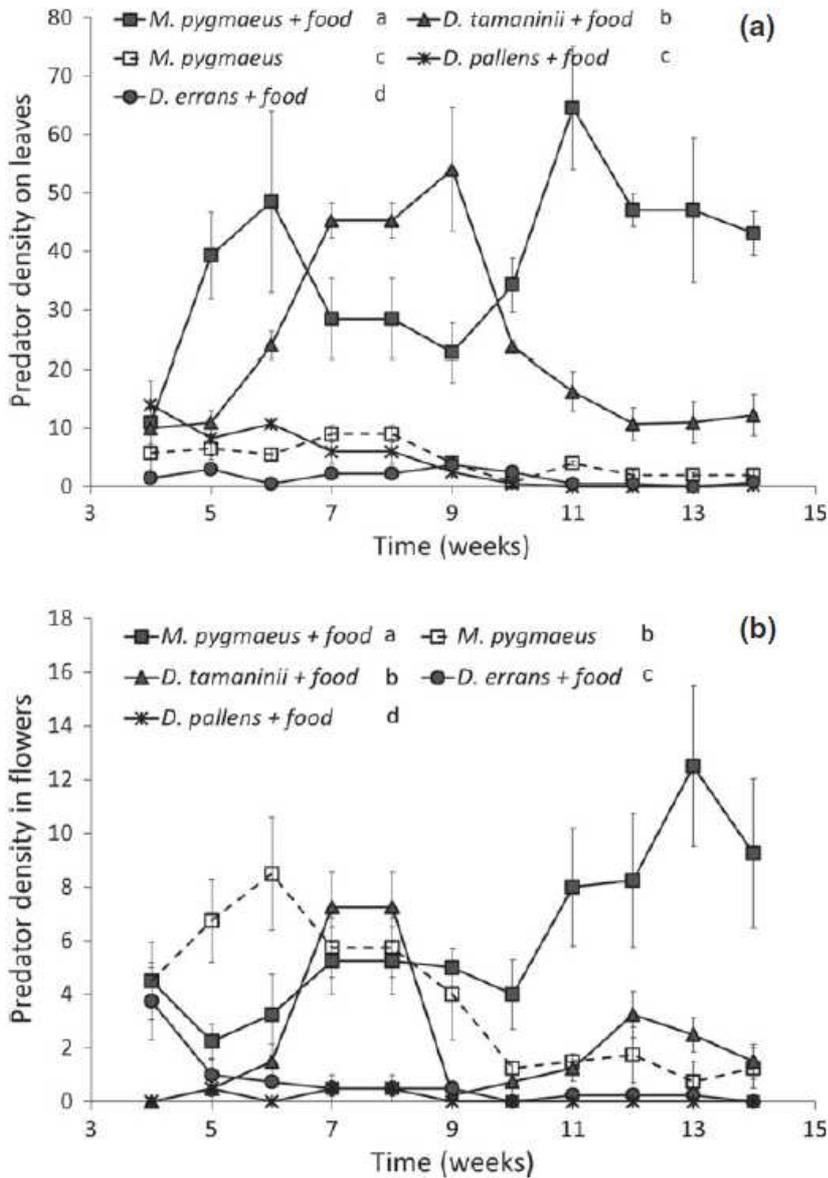
**Figure 1.** Population increase of the peach aphid *M. persicae* (A) and its predators (B) on sweet pepper plants. Aphids were released 3 weeks and predators 1 week before the first assessment. Average ( $\pm$  SE) densities per 24 leaves through time are shown. Different letters indicate significant differences among treatments through time (Fisher's LSD test,  $P < 0.05$ ).



428

429 **Figure 2.** Population dynamics of the peach aphid *M. persicae* on sweet pepper plants inoculated  
 430 with different species of predatory bugs with or without supplemental food (*Ephestia* eggs and  
 431 *Artemia* cysts) compared with plants without predators. Predators were released in week 1 and 3  
 432 and aphids in week 8, 9 and 10. Shown are average ( $\pm$  SE) densities + 1 of aphids per 24 leaves  
 433 through time. Different letters indicate significant differences among treatments through time  
 434 (Fisher's LSD test,  $P < 0.05$ ).

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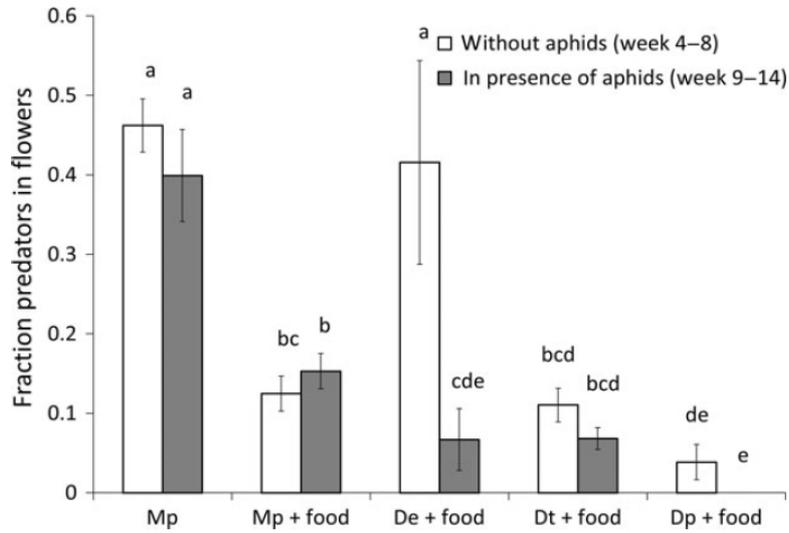


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438 **Figure 3.** Population dynamics of mirid predatory bugs on sweet pepper plants. Four treatments  
 439 were provided weekly with supplemental food (*Ephestia* eggs and *Artemia* cysts). Predators were  
 440 released in week 1 and 3 and aphids in week 8, 9 and 10. Shown are average ( $\pm$  SE) densities per 24  
 441 leaves (a) or per 10 flowers (b) through time. Different letters indicate significant differences  
 442 among treatments through time (Fisher's LSD test,  $P < 0.05$ ).

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445

446 **Figure 4.** Relative abundance of mirid predatory bugs in the flowers of sweet pepper plants in  
 447 presence or absence of aphids and with or without additional food (*Ephestia* eggs and *Artemia*  
 448 cysts). Shown are average ( $\pm$  SE) fractions of predators in the flowers, Mp = *Macrolophus*  
 449 *pygmaeus*, De = *Dicyphus errans*, Dt = *Dicyphus tamaninii* and Dp = *Deraeocoris pallens*.  
 450 Different letters indicate significant differences among treatments (Fisher's LSD test,  $P < 0.05$ ).

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453 **Table 1.** Origin and production method of selected predator species.

Predator species	Host plant from which collected	Year and area of collection
<i>Macrolophus pygmaeus</i> (Rambur)	<i>Solanum lycopersicum</i> (L.)	1994, southern France
<i>Dicyphus errans</i> (Wolff)	<i>Geranium</i> sp.	2012, Northern Italy
<i>Dicyphus tamaninii</i> Wagner	<i>Solanum luteum</i> Mill.	2012, Northern Spain
<i>Deraeocoris pallens</i> (Reuter)	<i>Inula viscosa</i> (L.)	2011, Southern Turkey

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