



AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript		
Original Citation:		
Availability:		
This version is available http://hdl.handle.net/2318/157735	since 2020-04-06T19:52:43Z	
Published version:		
DOI:10.1111/jen.12170		
Terms of use:		
Open Access		
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.		

(Article begins on next page)

Evaluation of mirid predatory bugs and release strategy for aphid control in sweet pepper 1 G. J. Messelink¹, C. M. J. Bloemhard¹, H. Hoogerbrugge², J. van Schelt², B. L. Ingegno³ & L. 2 Tavella³ 3 ¹ Wageningen UR Greenhouse Horticulture, Bleiswijk, The Netherlands 4 ² Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands 5 6 ³ Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino 7 Grugliasco, Italy 8 9 Keywords: biological control, Deraeocoris pallens, Dicyphus errans, Dicyphus tamaninii, 10 Macrolophus pygmaeus, supplemental food 11 12 13 Correspondence: Gerben J. Messelink (corresponding author), Wageningen UR Greenhouse Horticulture, PO Box 20, 2265 ZG Bleiswijk, The Netherlands. E-mail: gerben.messelink@wur.nl 14 15 16 17 Abstract Zoophytophagous predators of the family Miridae (Heteroptera), which feed both on plant and prey, 18 often maintain a close relationship with certain host plants. In this study, we aimed to select a 19 suitable mirid predatory bug for aphid control in sweet pepper. Four species were compared: 20 Macrolophus pygmaeus (Rambur), Dicyphus errans (Wolff), Dicyphus tamaninii Wagner and 21 Deraeocoris pallens (Reuter). They were assessed on their establishment on sweet pepper plants 22 with and without supplemental food (eggs of the flour moth Ephestia kuehniella Zeller and 23 decapsulated cysts of the brine shrimp Artemia franciscana Kellogg) and on their effects on aphids 24 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 D. tamaninii could successfully reduce aphid populations when released prior to an artificially 27 introduced aphid infestation. The best results were achieved with M. pygmaeus in combination with 28 a weekly application of supplemental food. Hence, our results demonstrate that the order and level 29 of plant colonization by mirid predators and aphids determines how successful biological control is. 30 Further studies are needed to evaluate the performance of mirid predatory bugs in sweet pepper 31 crops in commercial greenhouses with multiple pests and natural enemies, in particular to 32 understand how increased variation in food sources affects their feeding behaviour and preferences. 33 34

35 Introduction

Zoophytophagous predators of the family Miridae (Heteroptera), which feed both on plant and prey, 36 are increasingly used for pest control in greenhouse vegetable crops either through augmentative or 37 conservation biological control (Castañé et al. 2004; Perdikis et al. 2011; van Lenteren 2012). The 38 most commonly used and commercially mass-produced species are currently Macrolophus 39 pygmaeus (Rambur) (formerly identified as Macrolophus caliginosus Wagner, Castañé et al. 2013) 40 in northern Europe and Nesidiocoris tenuis (Reuter) in southern Europe. Typically these predators 41 feed on multiple pest species such as whiteflies (Montserrat et al. 2000), aphids (Alvarado et al. 42 43 1997), thrips (Riudavets and Castañé 1998), spider mites (Hansen et al. 1999), leaf miners (Arnó et al. 2003) and Lepidoptera species, including Tuta absoluta (Urbaneja et al. 2009). Because of their 44 45 plant-feeding behaviour and oviposition in plant tissue, mirids maintain a close relationship with certain host plants (Perdikis and Lykouressis 2000; Wheeler 2001). Somehow, mirid predators of 46 47 the tribe Dicyphini (belonging to Nesidiocoris, Dicyphus or Macrolophus genus) seem to be adapted to live on hairy plants (Wheeler 2001; Voigt et al. 2007; Ingegno et al. 2008). Their typical 48 49 long and slender legs facilitate them, in contrast with many other arthropods, to walk on leaf and stem surfaces with high trichome densities (Voigt et al. 2007). The fact that some hairy plants have 50 glandular trichomes which entrap insects might be one of the reasons why mirids became 51 specialized in hairy plants, as the carrion of entrapped insects provides them with protein-rich food 52 (Krimmel and Pearse 2013). 53 When selecting mirid predatory bugs for pest control, it is important to consider their strong 54

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

69 other species of mirid predatory bugs. Finding other mirid predatory bugs that are even more

ro 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

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

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

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

clustered in domatia only on the abaxial side of the leaves (Ferreira et al. 2008), and this lack of

trichomes could be a disadvantage for hairy plant associated bugs like Dicyphini. However, even if

sweet pepper is not the preferred host plant because of these morphological traits, the predators may

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 *Ephestia kuehniella* Zeller

and decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg, which are both suitable

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

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

- of this study may help to develop practical guidelines for enhancing aphid control with generalistpredators 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

grown in peat by a commercial plant propagator without application of pesticides. Peach aphids (M.

persicae) of the red phenotype were reared on sweet pepper plants of the same cultivar in a separategreenhouse 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 16 walk-in cages of 1*2*2 m made of fine insect gauze (mesh size 0.22 9 0.31 mm), which were 119 placed in a greenhouse compartment of 144 m2 at the Institute of Wageningen UR Greenhouse 120 Horticulture. The experiment had a completely randomized design with four replicates of the 121 following treatments: (i) no predators (only *M. persicae*), (ii) *M. pygmaeus* (iii) *D. errans* and (iv) 122 D. pallens. Dicyphus tamaninii was not included in this experiment, because of the limited available 123 numbers when starting the trials. Each cage was provided with four potted sweet pepper plants, cv 124 Spider, which were planted in peat in 101 pots. The plants were grown according to a three-stems-125 per-plant system, resulting in 12 sweet pepper stems per cage. Plants were provided with a standard 126 nutrient solution through drip irrigation. The plants were infested in September with aphids when 127 the crop was about 1 m high at densities of 20 aphids of mixed age per plant, thus 80 aphids per 128 cage. This resulted after 2 weeks in an average density of 1325 (SE 67) aphids per cage. Predators 129 were released 1 day after this assessment of aphid establishment. Each cage was provided with 40 130 one-week-old adults (20 males and 20 females) and 40 third instar nymphs. Thus the average 131 predator: aphid ratio at the start of this experiment was 1: 16.5. Densities of aphids and mirid 132 predatory bugs were assessed weekly during four consecutive weeks by counting the total number 133 of aphids and predators on 24 randomly selected leaves per cage: 12 leaves in the upper part of the 134 plants and 12 leaves in the lower part. All observations on the plants were non-destructively 135

- performed inside the cages between 8:00 and 12:00 AM. Temperature and relative humidity were
- 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
- 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
- A second greenhouse experiment was set-up similar to the first one, but with releases of predatory 142 bugs prior to aphid releases. The establishment of the predators on flowering sweet pepper plants 143 144 was compared with the establishment on plants that received a weekly supply of additional food consisting of 0.6 g eggs of *E. kuehniella* (killed by freezing) and 0.3 g freeze dried decapsulated 145 cysts of A. franciscana per cage, both obtained from Koppert Biological systems (non-commercial 146 R&D materials). This food was added weekly by manually sprinkling it equally distributed over the 147 148 leaves. The following eight treatments were tested in four replicates: (i) no predators, (ii) M. pygmaeus, (iii) D. errans, (iv) D. pallens (v) M. pygmaeus + food, (vi) D. errans + food, (vii) D. 149 150 pallens + food and (viii) D. tamaninii + food. Plants were inoculated with predatory bugs by twice releasing 16 one-week-old adults (eight females and eight males) per cage. The second introduction 151 152 was 2 weeks after the first, to establish populations of mixed developmental stages. Aphids were introduced 5, 6 and 7 weeks (week number 18, 19 and 20 respectively) after the last predator 153 releases, each time 120 aphids of mixed age were released per cage (30 aphids per plant). Densities 154 of aphids and predators were followed in the same way as in the first experiment. In addition, 10 155 fresh flowers per cage were randomly selected to count the number of mirid predators in the 156 flowers. The experiment was conducted in March-May, and during that period, the average 157 measured temperature and relative humidity in the cages were 22.3°C (range 15.2–31.8°C) and 158 71% (range 32 - 88%), respectively. Data were analysed with the same methods as for the first 159
- 160 greenhouse experiment.
- 161 <u>Predation rates in the laboratory</u>

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

- showed that 40 nymphs are ample prey for one female predator per day. After adding the aphids,
- 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
- and 70% RH. Predation of nymphs by the adult predators, recognized by the presence of bodies that
- were sucked partly or completely empty, was assessed after 24 h.

175 <u>Statistical analyses</u>

- 176 Population dynamics of aphids and predators in the two greenhouse trials were analysed with
- 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
- average fractions of predators in the flowers (densities in 10 flowers/densities on 24 leaves) with
- treatment and the presence of aphids (week 4–8 vs. week 9–14) as factors. Differences among
- treatments were tested with Fisher's LSD test (P < 0.05). Predation rates of the laboratory trial were
- analysed using a generalized linear model (GLM) with a Poisson distribution of the data. All
- statistical analyses were performed using the statistical package Gen- Stat Release 16.1 (Payne et al.
 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
- able to significantly reduce the increase of aphids (F3,9 = 3.17, P = 0.24). Densities of predators
- were significantly different among treatments (F2,6 = 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
- after the second release of predators and only a few adults of *D. errans* remained alive during the
- trial, without producing a new generation. These treatments were therefore excluded from further
- analyses. The population dynamics of aphids were significantly different among the remaining
- treatments (F5,18 = 42.33, P < 0.01). The best control of aphids was achieved in the treatment of M.
- 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
- not able to prevent a strong increase of aphids (fig. 2). Densities of predators were significantly

- different among treatments both on the leaves (F4,15 = 83.45, P < 0.01) and in the flowers (F4,15 =
- 205 280.48, P < 0.01). The highest predator densities were achieved in the treatments with food
- 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
- and 3). The fractions of predators in the flowers were significantly different among predator
- treatments (F4,30 = 22.04, P < 0.01) and also affected by the releases of aphids (F1,30 = 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
- the plants, whereas such a shift did not occur with the other predators species (fig. 4).
- 215 <u>Predation rates in the laboratory</u>
- 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 (SE 2.7) and 22.8 (SE 2.0) individuals,
- respectively. These rates were not significantly different (F1,37 = 1.03, P = 0.317).
- 219

220 Discussion

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

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

Much better results were achieved in our study in the second experiment with preventive releases of 239 240 predators. Both the predators *M. pygmaeus* and *D. tamaninii* strongly reduced the increase of aphids compared to the plants without predators. The best results were achieved with M. pygmaeus in 241 combination with a weekly application of supplemental food. This additional food increased the 242 population of predators to very high densities which favoured the control of aphids. These results 243 suggest that a high predator: prey ratio is needed to achieve sufficient control. Aphids multiply 244 245 extremely fast by viviparous parthenogenetic reproduction, resulting in relative growth rates on sweet pepper of 0.36 females per female per day (Dewhirst et al. 2012). Aphids will soon 246 outnumber mirid predatory bugs which have a relatively low reproduction rate: for example 247 0.0615–0.0958 females per female per day for *M. pygmaeus* on aphids (Margaritopoulos et al. 2003; 248 249 Perdikis and Lykouressis 2004). Thus, the numerical response of the predators is by far not fast enough to control aphids. Preventive releases of predators can solve this problem, as this prior to 250 251 pest establishment can result in relatively high initial predator: pest ratios which are enough to control the aphids. Moreover, predators might aggregate in patches of aphids in the field. 252 253 Olfactometric studies showed that mirids strongly respond to odours of plants infested by pests (Ingegno et al. 2011, 2013; Pérez-Hedo and Urbaneja 2014). This suggests that the predators can 254 have an aggregative numerical response, also referred to as short-term apparent competition (Holt 255 and Kotler 1987). Such aggregation was also suggested by our study, because a much lower fraction 256 of *M. pygmaeus* was found in the flowers when food was added to the leaves. Our results also 257 showed that D. errans seems to shift from flowers to leaves when aphids were added. This shift was 258 259 not observed for *M. pygmaeus* and *D. tamaninii*, as their fraction of predators in the flowers remained similar on plants with or without aphids. However, these two predatory bugs controlled 260 aphids much better than the other predator species, thus a part of the predator population may have 261 262 gone back to the flowers when most aphids were consumed.

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

successful establishment of this predator species. The first generation offspring of released adults of *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 on sweet pepper than the other predators remains unclear. Field surveys indicate that sweet pepper 274 275 is not a preferred host plant, as pepper plants are only occasionally colonized by these predators (Tavella and Goula 2001; Sanchez et al. 2003). However, these predators might be better adapted to 276 feed on pollen and nectar than the other tested mirids (Vandekerkhove and De Clercq 2010). Their 277 ability to reproduce on food provided by the plant is a major advantage for establishing and 278 279 augmenting predator populations in sweet pepper prior to pest infestations, which makes them suitable candidates for 'seasonal inoculative' biological control (van Lenteren and Woets 1988). 280 Biological control of aphids is, so far, usually based on releases of specialist natural enemies such 281 as parasitoids (mainly Aphididae), or the predatory midge Aphidoletes aphidimyza (Rondani) 282 283 (Blümel 2004). However, preventive releases of generalist predators such as Orius majusculus (Reuter) or *M. pygmaeus* can, in addition to these natural enemies, enhance aphid control 284 285 (Messelink et al. 2013; Messelink and Janssen 2014). This will probably also result in lower costs, as inoculative releases of generalist predators may reduce the need for repeated releases of 286 287 expensive specialist aphid natural enemies. The results of this study confirm that releases of M. pygmaeus in flowering sweet pepper plants before pest arrival can be a successful strategy to 288 prevent outbreaks of aphids. A possible drawback is the intraguild predation by these generalist 289 predators on specialist aphid predators, such as larvae of the predatory midge A. aphidimyza or 290 syrphids (Fréchette et al. 2007; Messelink et al. 2013). However, the strong contribution of 291 generalist predators to the control of aphids may outweigh the negative effects of intraguild 292 predation (Messelink et al. 2013). An additional benefit of using generalist predatory bugs is their 293 contribution to the control of other pests in sweet pepper such as caterpillars, spider mites or 294 295 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 with multiple pests and natural enemies needs to be further evaluated, in particular how increased 298 299 variation in food sources affects their feeding behaviour and preferences.

300

301 Acknowledgements

This study was funded by the Dutch Ministry of Economic Affairs and by Koppert Biological
Systems. We are grateful to Dr. Cristina Castañé (IRTA, Spain) for collecting and sending *D*.

304 *tamaninii* and Dr. Halil Kutuk (PPRI, Turkey) for collecting and sending D. pallens. Karel

- 305 Bolckmans is acknowledged for the stimulating discussions. Laxmi Kok and Joanah Midzi are
- thanked for practical assistance in the greenhouse experiments.
- 307

308 References

- 309 Alvarado P, Balta O, Alomar Ò, 1997. Efficiency of four heteroptera as predators of Aphis gossypii
- and *Macrosiphum euphorbiae* (Hom.: Aphididae). Entomophaga 42, 215-226.
- Arijs Y, De Clercq, P, 2001. Rearing Orius laevigatus on cysts of the brine shrimp Artemia
- 312 *franciscana*. Biol. Control 21, 79-83.
- Arnó J, Alonso E, Gabarra R, 2003. Role of the parasitoid *Diglyphus isaea* (Walker) and the
- predator *Macrolophus caliginosus* Wagner in the control of leafminers. IOBC/WPRS Bull. 26, 7984.
- Atakan E, Tunç I, 2010. Seasonal abundance of hemipteran predators in relation to western flower
- 317 thrips Frankliniella occidentalis (Thysanoptera: Thripidae) on weeds in the eastern Mediterranean
- region of Turkey. Biocont. Sci. Technol. 20, 821–839.
- Blümel S, 2004. Biological control of aphids on vegetable crops. In: Biocontrol in protected culture.
- Ed. by Heinz KM, Van Driesche RG, Parrella MP, Ball Publishing, Batavia, IL, 297–312.
- Bosco L, Giacometto E, Tavella L, 2008. Colonization and predation of thrips (Thysanoptera:
- 322 Thripidae) by Orius spp. (Heteroptera: Anthocoridae) in sweet pepper greenhouses in Northwest
- 323 Italy. Biol. Control 44, 331-340.
- 324 Calvo FJ, Lorente MJ, Stansly PA, and Belda JE, 2012. Preplant release of *Nesidiocoris tenuis* and
- supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato.
- 326 Entomol. Exp. Appl. 143, 111-119.
- 327 Castañé C, Alomar Ò, Goula M, Gabarra R, 2004. Colonization of tomato greenhouses by the
- 328 predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*. Biol. Control 30, 591-597.
- 329 Castañé C, Arnó J, Gabarra R, Alomar Ò, 2011. Plant damage to vegetable crops by
- zoophytophagous mirid predators. Biol. Control 59, 22-29.
- 331 Castañé C, Agustí N, Arnó J, Gabarra R, Riudavets J, Comas J, Alomar Ò, 2013. Taxonomic
- 332 identification of *Macrolophus pygmaeus* and *Macrolophus melanotoma* based on morphometry and
- molecular markers. Bull. Entomol. Res. 103, 204-215.
- 334 De Clercq P, Coudron TA, Riddick EW, 2014. Production of heteropteran predators. In: Mass
- 335 production of beneficial organisms: invertebrates and entomopathogens. Ed. by Morales-Ramos JA,
- Rojas G, Shapiro-Ilan D, Elsevier, New York, 57-100.

- 337 Dewhirst SY, Birkett MA, Loza-Reyes E, Martin JL, Pye BJ, Smart LE, Hardie J, Pickett JA, 2012.
- Activation of defence in sweet pepper, *Capsicum annum*, by cis-jasmone, and its impact on aphid and aphid parasitoid behaviour. Pest Manag. Sci. 68, 1419-1429.
- 340 Ferreira JAM, Eshuis B, Janssen A, Sabelis MW, 2008. Domatia reduce larval cannibalism in
- 341 predatory mites. Ecol. Entomol. 33, 374-379.
- 342 Fréchette B, Rojo S, Alomar Ò, Lucas E, 2007. Intraguild predation between syrphids and mirids:
- 343 Who is the prey? Who is the predator? BioControl 52(2), 175-191.
- 344 Ghavami MD, Özgür AF, Kersting U, 1998. Prey consumption by the predator *Deraeocoris pallens*
- Reuther (Hemiptera: Miridae) on six cotton pests. J. Plant Dis. Prot. 105, 526-531.
- 346 Gillespie DR, McGregor RR, 2000. The functions of plant feeding in the omnivorous predator
- 347 *Dicyphus hesperus*: Water places limits on predation. Ecol. Entomol. 25, 380-386.
- 348 Hansen D L., Brødsgaard H F, Enkegaard A, 1999. Life table characteristics of Macrolophus
- 349 *caliginosus* preying upon *Tetranychus urticae*. Entomol. Exp. Appl. 93, 269-275.
- Holt RD, Kotler BP, 1987. Short-term apparent competition. Am. Nat. 130, 412-430.
- 351 Ingegno BL, Goula M, Navone P, Tavella L, 2008. Distribution and host plants of the genus
- *Dicyphus* (Hemiptera: Miridae) in the Alpine valleys of northwestern Italy. Bull. Insectol. 61 (1),
 139-14.
- Ingegno BL, Pansa MG, Tavella L, 2011. Plant preference in the zoophytophagous generalist
- 355 predator *Macrolophus pygmaeus* (Heteroptera: Miridae). Biol. Control 58, 174-181.
- Ingegno BL, Ferracini C, Gallinotti D, Alma A, Tavella L, 2013. Evaluation of the effectiveness of
- 357 *Dicyphus errans* (Wolff) as predator of *Tuta absoluta* (Meyrick). Biol. Control 67, 246-252.
- Krimmel BA, Pearse IS, 2013. Sticky plant traps insects to enhance indirect defence. Ecol. Lett. 16,
 219-224.
- van Lenteren JC, 2012. The state of commercial augmentative biological control: plenty of natural
- 361 enemies, but a frustrating lack of uptake. BioControl 57, 1-20.
- van Lenteren JC, Woets J, 1988. Biological and integrated pest control in greenhouses. Annu. Rev.
 Entomol. 33, 239-269.
- Lykouressis D, Perdikis D, Charalampous P, 2014. Plant food effects on prey consumption by the omnivorous predator *Macrolophus pygmaeus*. Phytoparasitica 42, 303-309.
- 366 Margaritopoulos, JT, Tsitsipis, JA, Perdikis, DC, 2003. Biological characteristics of the mirids
- 367 *Macrolophus costalis* and *Macrolophus pygmaeus* preying on the tobacco form of *Myzus persicae*
- 368 (Hemiptera: Aphididae). Bull. Entomol. Res. 93, 39–45.

- 369 Messelink GJ, Janssen A, 2014. Increased control of thrips and aphids in greenhouses with two
- 370 species of generalist predatory bugs involved in intraguild predation. Biol. Control. DOI
- 371 10.1016/j.biocontrol.2014.07.009.
- 372 Messelink GJ, Bloemhard CMJ, Kok L, Janssen A, 2011. Generalist predatory bugs control aphids
- in sweet pepper. IOBC/WPRS Bull. 68, 115-118.
- 374 Messelink GJ, Bloemhard CMJ, Sabelis MW, Janssen A, 2013. Biological control of aphids in the
- presence of thrips and their enemies. BioControl 58, 45-55.
- 376 Messelink GJ, Bennison J, Alomar Ò, Ingegno B, Tavella L, Shipp L, Palevsky E, Wäckers F,
- 2014. Approaches to conserving natural enemy populations in greenhouse crops: Current methods
- and future prospects. BioControl 59: 377–393.
- 379 Montserrat M, Albajes R, Castañé C, 2000. Functional response of four Heteropteran predators
- 380 preying on greenhouse whitefly (Homoptera: Aleyrodidae) and western flower thrips
- 381 (Thysanoptera: Thripidae). Environ. Entomol. 29, 1075-1082.
- Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM, 2010. GenStat for Windows (13th
- Edition) Introduction. VSN International, Hemel Hempstead, UK.
- Perdikis D, Lykouressis D, 2000. Effects of various items, host plants, and temperatures on the
- development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Biol. Control
 17, 55-60.
- 387 Perdikis DC, Lykouressis DP, 2004. *Myzus persicae* (Homoptera: Aphididae) as suitable prey for
- 388 *Macrolophus pygmaeus* (Hemiptera: Miridae) population increase on pepper plants. Environ.
- 389 Entomol. 33, 499-505.
- 390 Perdikis D, Fantinou A, Lykouressis D, 2011. Enhancing pest control in annual crops by
- conservation of predatory Heteroptera. Biol. Control 59, 13-21.
- Pérez-Hedo M, Urbaneja, A, 2014. Prospects for predatory mirid bugs as biocontrol agents of
 aphids in sweet peppers. J Pest Sci. (in press).
- Portillo N, Alomar Ò, Wäckers F, 2012. Nectarivory by the plant-tissue feeding predator
- 395 Macrolophus pygmaeus Rambur (Heteroptera: Miridae): Nutritional redundancy or nutritional
- 396 benefit? J. Insect Physiol. 58, 397-401.
- 397 Riudavets J, Castañé C, 1998. Identification and evaluation of native predators of *Frankliniella*
- 398 *occidentalis* (Thysanoptera: Thripidae) in the Mediterranean. Environ. Entomol. 27, 86-93.
- 399 Sanchez JA, Martinez-Cascales JI, Lacasa A, 2003. Abundance and wild host plants of predator
- 400 mirids (Heteroptera: Miridae) in horticultural crops in the Southeast of Spain. IOBC/WPRS Bull. 26
- 401 (10), 147–151.

- 402 Tavella L, Goula M, 2001. Dicyphini collected in horticultural areas of northwestern Italy
- 403 (Heteroptera Miridae). Boll. Zool. Agrar. Bachic., Ser. II. 33, 93-102.
- 404 Ulubilir A, Yigit A, Yücel S, Yabas C, 1997. Biological control of *Bemisia tabaci* Genn.
- 405 (Homoptera: Aleyrodidae) by Deraeocoris pallens Reut. (Hemiptera: Miridae) on eggplant in
- 406 plastic houses in Adana. Adv. Hortic. Sci. 11, 202-204.
- 407 Urbaneja A, Monton H, Molla O, 2009. Suitability of the tomato borer *Tuta absoluta* as prey for
- 408 *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. Appl. Entomol. 133, 292-296.
- 409 Van den Meiracker RAF, Ramakers PMJ, 1991. Biological control of the western flower thrips
- 410 *Frankliniella occidentalis*, in sweet pepper, with the anthocorid predator *Orius insidiosus*. Meded.
- 411 Fac. Landbouww. Rijksuniv. Gent. 56, 241-249.
- 412 Vandekerkhove B, De Clercq P, 2010. Pollen as an alternative or supplementary food for the mirid
- 413 predator *Macrolophus pygmaeus*. Biol. Control 53, 238-242.
- 414 Voigt D, 2005. Untersuchungen zur Morphologie, Biologie und Ökologie der räuberischen
- 415 Weichwanze Dicyphus errans Wolff (Heteroptera, Miridae, Bryocorinae). Dissertation Technischen
- 416 Universität Dresden, 154 pp.
- 417 Voigt D, Gorb E, Gorb S, 2007. Plant surface-bug interactions: *Dicyphus errans* stalking along
- 418 trichomes. Arthropod-Plant Inte. 1, 221-243.
- 419 Wheeler JAG, 2001. Biology of the plant bugs (Hemiptera: Miridae): pests, predators, opportunists.
- 420 Comstock Publishing Associates, Ithaca.
- 421
- 422









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



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





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

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

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