



Predation by *Macrolophus pygmaeus* (Hemiptera: Miridae) on *Acyrtosiphon pisum* (Hemiptera: Aphididae): Influence of prey age/size and predator's intraspecific interactions

JULIANA DURÁN PRIETO¹, VINCENZO TROTTA¹, PAOLO FANTI¹, CRISTINA CASTAÑÉ² and DONATELLA BATTAGLIA¹

¹Dipartimento di Scienze, Università degli studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy; e-mails: juliana.duran@unibas.it, vincenzo.trotta@unibas.it, paolo.fanti@unibas.it, donatella.battaglia@unibas.it

²Department de Protecció Vegetal, IRTA, Centre de Cabriels, 08348 Cabriels, Barcelona, Spain; e-mail: cristina.castane@irta.es

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Abstract. *Macrolophus pygmaeus* (Hemiptera: Miridae) is an important predator of pests of horticultural crops and here its ability as a predator of *Acyrtosiphon pisum* (Hemiptera: Aphididae) is addressed for the first time. The percentage predation of the different aphid instars and the number partially consumed were studied. Our results, obtained using choice and no-choice tests, revealed that *M. pygmaeus* caught and consumed more young than later instars of *A. pisum*, which confirms results of previous studies using other species of aphids. We also studied the interactions between predators (male/female) foraging in the same patch. When the prey/predator ratio is kept constant at 10 : 1 the average percentage of aphids completely consumed by individual females or males does not change with increase in the number of foraging predators. However, the number of partially consumed aphids decreased when females shared the same patch. In contrast, there was an increase in the number of aphids partially consumed when two males shared the same patch. The results were discussed in terms of potential predator foraging strategies since intraspecific competition is a key factor modulating the dynamics of prey-predator systems.

INTRODUCTION

Predatory mirid bugs are effective biological control agents of many of the insect pests of horticultural crops (Alomar et al., 1994; Wheeler, 2001; Perdikis et al., 2008). The mirid bug *Macrolophus pygmaeus* Rambur is a zoophytophagous predator native to the Mediterranean region, where it often colonizes tomato crops (Alomar et al., 2002; Castañé et al., 2004). This species has been successfully used in Europe mainly to protect tomato crops, but also eggplant, pepper, melon and cucumber crops, both in open fields and in glasshouses (Perdikis et al., 1999; Perdikis & Lykouressis, 2000; Lykouressis et al., 2001; Castañé et al., 2004; Gabarra et al., 2004; Alomar et al., 2006; Pérez-Hedo & Urbaneja, 2014). Due to the suitability of *M. pygmaeus* as a biocontrol agent, most of the available literature deals with its predatory activity (Perdikis et al., 1999; Lykouressis et al., 2007; Fantinou et al., 2008, 2009). Several aspects of the predatory behaviour of *M. pygmaeus* when feeding on the pest aphids *Myzus persicae* (Sulzer), *Aphis gossypii* Glover and *Macrosiphum euphorbiae* L. have been investigated (Alvarado et al., 1997; Perdikis et al., 1999; Lykouressis et al., 2007; Fantinou et al., 2008, 2009; Pérez-Hedo & Urbaneja, 2014). *M. pygmaeus*' pref-

erence for feeding on these aphids and their different instars (Lykouressis et al., 2007; Fantinou et al., 2008, 2009) and the influence of temperature (Perdikis et al., 1999; Fantinou et al., 2008) and photoperiod on prey consumption are documented (Perdikis et al., 1999).

Here we study the predatory behaviour of *M. pygmaeus* attacking the aphid *Acyrtosiphon pisum* (Harris), a species that is larger and more mobile than those mentioned above. This aphid is easy to rear and it has often been used as a model for studying the interactions between phytophagous and entomophagous insects (Battaglia et al., 1994; Pennacchio et al., 1999; Christiansen-Weniger & Hardie, 2000; Bensadia et al., 2006; Trotta et al., 2014, 2015) and tritrophic interactions, including the role of the host plant (Powell et al., 1998; Guerrieri et al., 2002; Chang et al., 2004; Hodge et al., 2011). *A. pisum* is the main pest of forage crops such as pea, broad bean, clover and alfalfa (Ellsbury & Nielson, 1981) and although it is not a common species of prey of *M. pygmaeus*, it is attacked and eaten by this polyphagous predator in the laboratory (Trotta et al., 2015). Although *Vicia faba* L. (Fabaceae, common name broad bean) is not a host plant of *M. pygmaeus*, under laboratory conditions, this zoophytophagous mirid bug will

Table 1. Combinations of *M. pygmaeus* females and/or males used in experiment 3 (Intraspecific interactions).

Combination treatment	<i>M. pygmaeus</i> females (inds.)	<i>M. pygmaeus</i> males (inds.)	Replicates
1F	1	–	62
2F	2	–	31
5F	5	–	47
FM	1	1	54
1M	–	1	30
2M	–	2	30

lay eggs on it and its offspring successfully complete their development feeding on *A. pisum* infesting this plant (pers. observ.).

Our research aimed to assess the consumption and feeding preferences of *M. pygmaeus* for each nymphal instar of the pea aphid in no-choice and choice tests. According to Optimal Foraging Theory, prey size/age can potentially affect the number of each prey item consumed, depending on the intrinsic value of the prey in terms of its nutrient and energy content and handling time (the time a predator requires to extract the food from a prey item) (Pulliam, 1974; Pyke et al., 1977; Pyke, 1984). The handling time is influenced by the size, mobility and defensive ability of the prey (Sih & Christensen, 2001; Jeschke et al., 2002). However, the number of prey consumed is also determined by competition between individuals of the same species (Andersen, 1960; Klomp, 1964). In this sense, intraspecific competition is a key factor, which also modulates the dynamics of prey-predator systems (Andersen, 1960; Klomp, 1964; Lucas & Alomar, 2002; DeLong & Vasseur, 2011).

Diverse types of potential interactions may occur between two or more individuals of the same species when competing for food, space and/or oviposition sites (Klomp, 1964; Alley, 1982; Symondson et al., 2002; DeLong & Vasseur, 2011). When predators compete for prey, one often observes non-interactive as well as interactive individuals, which by mutual interference or synergic interactions (density dependent predation), influence in different ways prey population dynamics (Klomp, 1964; Alley, 1982; Losey & Denno, 1998; DeLong & Vasseur, 2011). Until now, the effect of intraspecific competition between individuals of *M. pygmaeus* has only been recorded in two studies in which whitefly was the prey (Lucas & Alomar, 2002; Moreno-Ripoll et al., 2012). In the present study we report the effect of intraspecific interactions between individuals of *M. pygmaeus* on its consumption of *A. pisum* as these interactions could influence prey population dynamics.

Studying how prey size/stage and intraspecific interactions between predators can influence the numbers of *A. pisum* consumed by *M. pygmaeus* is important in determining whether it is likely to be an effective biological control agent of aphids and relevant to the current tendency to integrate *M. pygmaeus* into biological control programmes against aphids (Perdikis et al., 2008; Pérez-Hedo & Urbaneja, 2014).

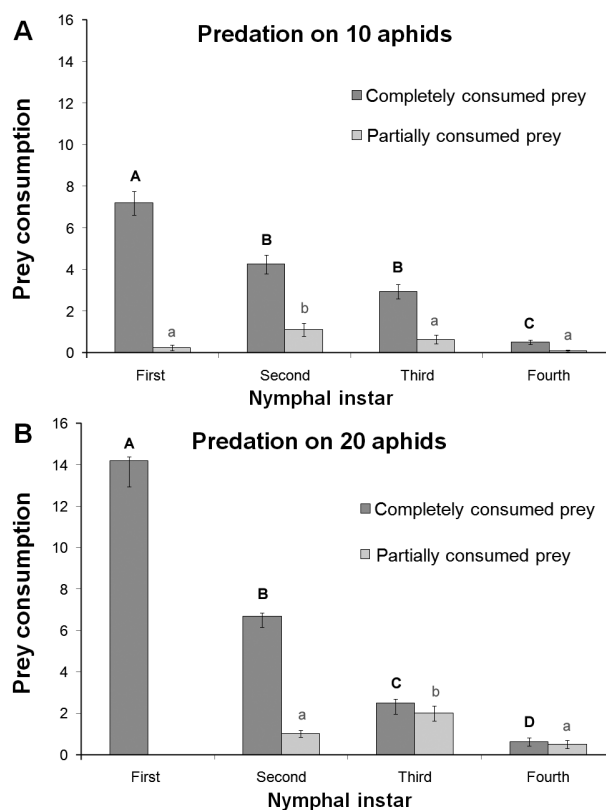


Fig. 1. Number of *A. pisum* nymphs (mean \pm SE) completely or partially consumed by one *M. pygmaeus* female in 24 h when provided with 10 (A) and 20 (B) aphids. Significant differences among means based on Tukey post-hoc tests for multiple comparisons are indicated by different letters. Upper-case letters on bars indicate highly significant differences ($P < 0.001$, Tukey's test) for the number of entirely consumed aphids and lower-case letters indicate significant differences for the number of partially consumed aphids ($P < 0.05$, Tukey's test).

MATERIAL AND METHODS

Insect cultures

M. pygmaeus came from a colony established in a laboratory in Potenza, Italy on potted tomato plants kept at 21°C (18L : 6D; 80–90% RH). This colony was established and renewed with individuals purchased from Koppert Italia srl., which were reared under similar conditions to the original culture. Sterilized *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, purchased from the same company, were used to supplement their diet. For this study we chose the adults of *M. pygmaeus* because they are more mobile and therefore more likely to move to a non-host plant attracted by the presence of prey.

Acyrtosiphon pisum was reared and maintained on broad bean plants (*Vicia faba* c.v. Agua dulce) in a climatic chamber at 21°C, 80–90% RH and a 18L : 6D photoperiod. The colony was started in 1985 with a few hundred specimens collected during spring from *Medicago sativa* L. (Fabales: Fabaceae) near Salerno, Italy (40°37'01"N, 15°03'23"E) and reared in the laboratory on broad bean plants. Broad bean plants were grown in pots (10 cm diameter) containing commercial soil (COMPO SANA® Universal Potting Soil) in a greenhouse.

For experiments that required same-aged aphids of each nymphal instar, approximately 100 adult virginoparae females were isolated from the aphid culture and put on a fresh potted broad bean plant kept in a plastic box (22 \times 15 \times 40 cm high) for 24 h at 21°C (18L : 6D; 80–90% RH). Females were then removed and

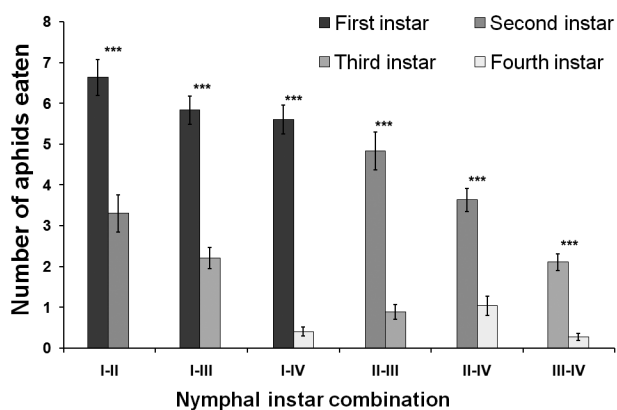


Fig. 2. Number of aphids eaten (mean ± SE) by a *M. pygmaeus* female in 24 h when provided with different combinations of instars of *A. pisum* (***) $P < 0.001$, pairwise *t* tests).

discarded. The neonate nymphs were maintained as a synchronous colony on a broad bean plant at 21°C for specific periods of time: 24, 48, 72 and 120 h; at the end of which the nymphs were, respectively, in L1, L2, L3 and L4 nymphal instars. Nevertheless, before their use in the experiments, aphids were examined under a stereo-microscope and all nymphs that were not in the appropriate stage, based on the morphological features described in Digilio (1995), were discarded.

Number of aphids killed by *Macrolophus pygmaeus* in no-choice tests

The predatory activity of females of *M. pygmaeus* based on the number of each of the four nymphal stages of *A. pisum* consumed was first assessed in a no-choice experiment. A cut broad bean leaf, with the stalk inside an Eppendorf tube filled with water and sealed with parafilm to prevent desiccation, was placed in a plastic cylinder (diameter: 5.5 cm, height: 7.5 cm, volume: 150 ml) with a mesh covered ventilation hole in the screw top (4.5 cm in diameter). Aphids of the same instar were gently placed on a leaf and allowed to settle. Preliminary experiments showed that careful manipulation caused negligible aphid mortality. Two prey densities, ten and twenty aphids per leaf, were tested. One *M. pygmaeus* adult female, taken from the rearing culture, which had emerged one week before the start of the experiment, was introduced into the cylinder and allowed to forage for 24 h. These females were not starved before the experiment, indeed they were taken directly from the rearing cage, where *E. kuehniella* eggs were available ad libitum. The predator was allowed to forage for 24 h and subsequently removed. The number of dead aphids were recorded, making a distinction between those completely consumed, those partially consumed and those unconsumed. An aphid was regarded as completely consumed when the predator had extracted its body fluids and only its exoskeleton remained. Killed but unconsumed aphids were those found dead with a droplet on their siphunculi, as described in Fantinou et al. (2009). In addition, a small melanized area on the abdomen was generally evident. Partially consumed aphids were those in which the body contents were not completely removed by sucking. Different levels of partially consumed aphids were observed: with a third, half or more than half of their body contents removed. For each aphid instar, there were 25 and 17 replicates of the experiments with 10 and 20 aphids, respectively.

Feeding preferences of *Macrolophus pygmaeus* in choice tests

This predator’s preference for different aphid instars was assessed in choice tests where two aphid instars were provided,

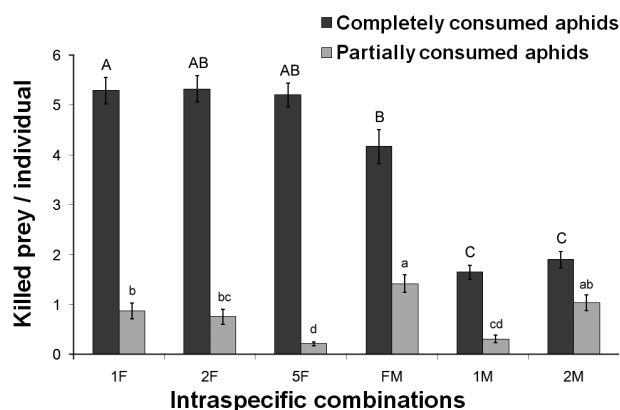


Fig. 3. Numbers of completely and partially consumed aphids (mean ± SE) per predator recorded in the different combination of females and/or males of *M. pygmaeus* over two days. Upper-case letters on bars indicate highly significant differences (at least $P < 0.05$, Tukey’s test) for the number of completely consumed aphids and lower-case letters indicate significant differences in the number of partially consumed aphids (at least $P < 0.05$, Tukey’s test). 1F – one female; 2F – two females; 5F – five females; FM – one female and one male; 1M – one male; 2M – 2 males.

which involved a total of six sets of replicated choice tests: 1st vs 2nd, 1st vs 3rd, 1st vs 4th, 2nd vs 3rd, 2nd vs 4th and 3rd vs 4th. There were thirty replicates of each aphid size/stage combination with the exception of the 2nd vs 4th test, for which there were forty replicates. All trials were carried out as described above for the no-choice experiment, but the predator was provided with a total of twenty aphids made up of ten of each instar/choice.

Intraspecific interactions

Predatory activity of both *M. pygmaeus* males and females was assessed at different predator densities, keeping the *A. pisum* nymphs/predator ratio constant, at 10 : 1. To assess the density and/or effect of sex on the numbers of aphids killed by the interacting predators, the effect of prey age/size was kept constant by always providing them with only 2nd instar aphids. Prey consumption in each of six different male and/or female *M. pygmaeus* combinations, was recorded after 24 h (Table 1). Partially consumed prey was also recorded.

Statistical analyses

Numbers of completely and partially consumed prey were analyzed using two-way ANOVAs with the “prey instar” (four levels) and “prey density” (two levels, ten and twenty aphids) as fixed effects. Tukey post-hoc tests for multiple comparisons of means were also performed.

Predator preferences in terms of the number of aphids killed in the different aphid instar pairings (dichotomous choice tests) were analyzed using pairwise *t*-tests. Data on predatory activity were always calculated in terms of the number of aphids consumed per predator, and were analyzed using one-way ANOVA with female/male combination as the main factor, followed by a Tukey post-hoc comparison of means. Statistical analyses were performed using R 3.0.1 for windows (www.r-project.org). The numbers of completely consumed aphids, those killed but not consumed and those partially consumed were recorded. Given the overall low incidence of aphids killed but not consumed during this assay, we grouped them together with the number of partially consumed prey in the statistical analysis. In the text and graphs the aphids killed but not consumed are recorded as partially consumed.

RESULTS

Number of aphids killed by *Macrolophus pygmaeus* in no-choice tests

In the no-choice assay, there were significant differences in the number of aphids of the different aphid instars completely and partially consumed ($F_{3,155} = 88.2$, $P < 0.001$ and $F_{3,145} = 14.1$, $P < 0.001$, respectively, Fig. 1A and B) and at the different aphid densities ($F_{1,155} = 33.4$, $P < 0.001$ and $F_{1,145} = 3.95$, $P < 0.05$, respectively, Fig. 1A and B). In general, at both prey densities, more of the young than of the older aphids were completely consumed (Tukey post-hoc tests: $P < 0.001$), although more aphids were consumed when twenty aphids were provided rather than ten. More interestingly, there were significant interactions between “aphid instar” and “aphid density” in terms of both completely and partially consumed aphids ($F_{3,155} = 13.2$, $P < 0.001$ and $F_{3,145} = 5.2$, $P < 0.001$, respectively). When twenty aphids were provided *M. pygmaeus* completely consumed more first and second instar aphids than they did when only 10 aphids were provided, whereas for the other two aphid stages the number completely consumed was similar at both densities. When provided with ten aphids, significantly more second instar than the other instars of aphids were consumed (Tukey post-hoc test: $P < 0.05$, Fig. 1A), whereas when twenty aphids were provided the number of partially consumed aphids of the third instar was significantly higher than for the other three aphid stages (Tukey post-hoc test: $P < 0.05$, Fig. 1B).

Feeding preferences of *Macrolophus pygmaeus* in choice tests

The numbers of aphids killed by each female in the pairwise tests (Choice assay) is shown in Fig. 2. For each instar combination, the number of younger aphids killed was greater than the number of older aphids (1st vs 2nd: $t_{29} = 5.76$; 1st vs 3rd: $t_{29} = 9.09$; 1st vs 4th: $t_{29} = 14.79$; 2nd vs 3rd: $t_{39} = 7.6$; 2nd vs 4th: $t_{27} = 9.09$; 3rd vs 4th: $t_{29} = 7.73$; $P < 0.001$ in all cases).

Intraspecific interactions

Fig. 3 shows the number of aphids partially and completely consumed by a single *M. pygmaeus* when on its own and in the presence of other conspecifics. There were significant differences in the total number of aphids killed per predator in the different female/male *M. pygmaeus* combinations ($F_{5,248} = 50.9$, $P < 0.001$). These differences are mainly due to the low number of aphids killed by males. The average number of aphids killed by each female was the same in the treatments with different numbers of females (F1, F2 and F5 treatments). The number of partially consumed aphids differed statistically in the different female and/or male combinations ($F_{5,208} = 12.2$, $P < 0.001$). For females, the lowest number of partially consumed aphids was recorded at the highest female density (Tukey post-hoc test: $P < 0.001$ for F5 vs F1 and $P < 0.05$ for F5 vs F2, Fig. 3). In contrast, when two males of *M. pygmaeus* were put together, the number of aphids consumed per individual did not change, but the number of aphids partially consumed increased (Tukey post-hoc test: $P < 0.01$,

Fig. 3). Also, the number of aphids partially consumed per predator increased when a male was present together with a female in comparison to that recorded when only one female (Tukey post-hoc test: $P < 0.05$, Fig. 3) or one male was present (Tukey post-hoc test: $P < 0.01$, Fig. 3).

DISCUSSION

In the present study, although the ability of *M. pygmaeus* to complete its development feeding only on *A. pisum* was not checked, we showed that at least in the short term, *A. pisum* is a suitable prey for *M. pygmaeus* females and males. The predatory activity of adults was evaluated because they colonize and make use of new hosts and prey resources. In this case, *Vicia faba* infested with *A. pisum* was the new host-plant system for *M. pygmaeus*.

The expectation was that adult *M. pygmaeus* would be more voracious than the fifth instar nymph. For this reason we cannot compare the number of *A. pisum* consumed by *M. pygmaeus* with previously reported numbers of *M. persicae* and *M. euphorbiae* consumed, as fifth instar nymphs were used in those studies (Lykouressis et al., 2007; Fantinou et al., 2008, 2009). The difference in voracity of these two stages could explain why, for example, when ten first instar aphid nymphs of *A. pisum* were provided, *M. pygmaeus* in this study consumed seven, whereas it consumes 3.54 *M. persicae* and 3.24 *M. euphorbiae* (Lykouressis et al., 2007), despite the fact that first instar nymph of *A. pisum* weighs twice as much as *M. euphorbiae* and four times as much as *M. persicae*.

On the other hand, *M. pygmaeus* consumed a greater number of the younger *A. pisum* instars in both choice and no-choice tests, which is in accordance with the reports in the literature for *M. euphorbiae* and *M. persicae* (Lykouressis et al., 2007; Fantinou et al., 2008, 2009; Pérez-Hedo & Urbaneja, 2014). Although older *A. pisum* instars are larger than young ones and potentially contain more nutritional resources, they are more difficult to kill since they can defend themselves better than young ones by kicking, dropping, running or walking away from predators (Dixon, 1958; Evans, 1978; Roitberg & Myers, 1978, 1979; Gerling et al., 1990; Wyckhuys et al., 2008). This defensive behaviour probably leads to an increase in the average handling time (and energy) spent by *M. pygmaeus* when attacking an older aphid, which reduces the time available to catch and consume further prey. Optimal Foraging Theory states that the fitness of a foraging organism is a function of the amount of energy gained while foraging and that natural selection favours those organisms that, while foraging, are more efficient in acquiring energy (Krebs, 1977; Pyke et al., 1977). *M. pygmaeus* preference for feeding on young pea aphids could be because the net gain in energy when young aphids are the prey is greater than when older aphids are the prey.

A greater number of pea aphids were consumed (completely plus partially) when more were provided, which is similar to the results previously reported for *M. pygmaeus* feeding on *M. persicae* (Fantinou et al., 2008, 2009), as is the behaviour of killing aphids but not consuming them

(Alvarado et al., 1997; Fantinou et al., 2008, 2009). However, in studies on *M. persicae*, *M. euphorbiae* and *A. gossypii* (Alvarado et al., 1997; Fantinou et al., 2008, 2009), the presence of partially consumed aphids in addition to unconsumed ones was not recorded.

In the present study the presence of partially consumed aphids might be related to the greater size of *A. pisum*. Large prey usually take longer to kill and completely consume (Flinn et al., 1985; Baily, 1986; Foglar et al., 1990; Milonas et al., 2011). A disadvantage of preying on large prey item is they take longer to kill and consume, which increases the risk of the predator being attacked (Sih, 1980b; Abrams, 1982). Attacking and feeding on large prey also reduces the time available to the predator for other fitness-enhancing activities (e.g. ovipositing) (Abrams, 1982). Thus, in the case of large prey, to only partially consume it could be adaptive, because Optimal Foraging Theory (Krebs, 1977; Pyke et al., 1977) predicts that predators should optimize the food intake per unit of feeding time by selectively feeding on the most easily digestible or most nutritionally valuable parts of its prey (Sih et al., 1980a). Consequently, this might account for why pea aphids were abandoned before they were completely consumed.

Partial consumption of prey is an important factor to be considered when studying the predatory behavior of a biocontrol agent because it influences the number of prey kill when attacking different prey population densities of p , which is referred to as the functional response and includes attack rate, time taken to handle prey and predator/prey size ratios (Cohen, 1995).

In the last part of this study we addressed for the first time how intraspecific interactions among different numbers of mirid predators influences the number of prey killed when *M. pygmaeus* feeds on aphid prey. In this bioassay, we did not record cannibalism between conspecifics when feeding on aphids, which confirms results of previous studies on different species of prey (Lucas & Alomar, 2002; Moreno-Ripoll et al., 2012). Cannibalism is only recorded for adult females of *M. pygmaeus* kept at high densities and only fed leaflets of tomato (Moreno-Ripoll et al., 2012). Here, we maintained a constant predator-aphid ratio of 1 : 10 when the female/male density was increased. Under these conditions, in the treatments without males, the average number of aphids killed by each female did not change with increase in the number of females. Foraging theory predicts that the number killed per predator should increase with increase in prey abundance because the prey encounter rate increases, reducing the time spent by a predator searching for prey (Sih, 1980b; Abrams, 1982). This is only true if predators foraging in the same patch do not disturb each other. In the absence of interactions between predators, a higher individual consumption of aphids is expected in patches with higher numbers of predators at higher prey densities. For both males and females the number of aphids killed did not change when the number provided was increased. This could be a consequence of the females interacting with one another and in the case of males by their low voracity.

The number of aphids eaten by males is clearly less than by females. The greater consumption of prey by females is associated with the need to support reproduction, which is a major factor determining their fitness (Pyke et al., 1977; Reznick, 1985; Zera & Harshman, 2001; Harshman & Zera, 2007). Since *M. pygmaeus* females have telotrophic ovaries, egg production requires a continuous supply of energy throughout adult life with egg development independent of mating (Castañé et al., 2007; Franco et al., 2011). Arnó et al. (2003) and Urbaneja et al. (2009) report that *M. pygmaeus* females consume more prey than males when fed on *Lyriomyza trifolii* (Burgess) (Diptera: Agromyzidae) larvae and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) eggs, which is similar to our results.

Regarding partial prey consumption, it is likely that a predator leaves already killed but unconsumed or partially consumed prey if disturbed by a conspecific while feeding. This should occur more frequently as the number of predators in the same patch increases. Therefore, at the highest predator density a higher level of partial prey consumption was expected. However, we found that partial aphid consumption decreased or increased with increase in predator density, depending on the sex of the interacting individuals. In the case of females, the number of partially consumed aphids decreased when more than one female shared the same patch; the opposite pattern was recorded for males.

The interaction between *M. pygmaeus* females seems to result in them maximizing their exploitation of individual prey items when there is competition for a limited resource. In this way, it is possible that reproductive females optimize their food intake by avoiding prey waste as female density increases.

In contrast, the interaction between males results in an increase in the number of partially consumed aphids probably due to mutual interference when foraging, or alternatively, to an increase in the aphid encounter rate due to their higher relative abundance. Studies on other predators have also documented an increase in partially consumed prey with increase in prey abundance (Sih et al., 1980a; Samu & Bíró, 1993; Lang & Gsodl, 2003). Given that males consumed relatively few aphids, then when a male and female interacted, the presence of the male may not have significantly affected the number of aphids entirely consumed by a single female, but the number of partially consumed aphids increased.

In conclusion, females and males influence in different ways the number of pea aphids consumed depending on the way in which they interacted. It is important, however, to highlight that plant architecture and plant surface may affect these interactions. Moreover, we cannot totally exclude that aphid behaviour could have played a role in the observed interactions between predators. Therefore, these results cannot be generalized without further study.

Commercially available *M. pygmaeus* are primarily sold to control whiteflies and *Tuta absoluta* in tomato crops, although prospects for its use as a biocontrol agent of aphids in other crops was recently considered (Perez-Hedo & Urbaneja, 2014). In this context, our results are relevant and

highlight the importance of considering aphid size/age in developing models that describe the effect of mirid predation on the age structure of aphid colonies. We also demonstrate that intraspecific competition is a key component in predator-aphid dynamics and predicting the efficiency of *M. pygmaeus* for controlling pest aphids, specially when it is used in augmentative biological control.

M. pygmaeus predation on *A. pisum* is described here for the first time. This novel system can be used to test predictions of theoretical models of inter- and intraspecific interactions, as done here and in another recently published work (Trotta et al., 2015). In the field *M. pygmaeus* has never been reported feeding on the pea aphid probably because this predator mainly forages on plants belonging to families other than legumes (Alomar et al., 1994). However, the observation that *M. pygmaeus* feeds on *A. pisum* in the laboratory indicates some interesting lines of research. For example, it would be interesting to study whether the intercropping of legumes and Solanaceae plants results in an increase in the number of *M. pygmaeus* on legumes. If this were the case then this mirid bug could positively contribute to the biological control of pea aphid. Moreover, *A. pisum* and *M. pygmaeus* might encounter each other in tomato greenhouses when the aphid parasitoid *Aphidius ervi* is introduced on banker plants (Huang et al., 2011). In this case, interference by *M. pygmaeus* is possible, so it would be interesting to study the extent of this phenomenon and the conditions affecting it.

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REFERENCES

- ABRAMS P.A. 1982: Functional response of optimal foragers. — *Am. Nat.* **120**: 382–390.
- ALLEY T.R. 1982: Competition theory, evolution, and the concept of an ecological niche. — *Acta Biotheor.* **31**: 165–179.
- ALOMAR O., GOULA M. & ALBAJES R. 1994: Mirid bugs for biological control: identification, survey in non-cultivated winter plants, and colonization of tomato fields. — *IOBC/WPRS Bull.* **17**: 217–223.
- ALOMAR O., GOULA M. & ALBAJES R. 2002: Colonization of tomato fields by predatory mirid bugs (Hemiptera: Heteroptera) in northern Spain. — *Agric. Ecosyst. Environ.* **89**: 105–115.
- ALOMAR O., RIUDAVETS J. & CASTAÑE C. 2006: *Macrolophus caliginosus* in the biological control of *Bemisia tabaci* on greenhouse melons. — *Biol. Contr.* **36**: 154–162.
- ALVARADO P., BALTÀ O. & ALOMAR O. 1997: Efficiency of four heteroptera as predators of *Aphis gossypii* and *Macrosiphum euphorbiae* (Hom.: Aphididae). — *Entomophaga* **42**: 215–226.
- ANDERSEN F.S. 1960: Competition in populations consisting of one age group. — *Biometrics* **16**: 19–27.
- 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**: 79–84.
- BAILY P.C.E. 1986: The feeding behavior of a sit and wait predator, *Ranatra dispar*, (Heteroptera: Nepidae) description of behavioral component of prey capture, and the effect of food deprivation on predator arousal and capture dynamics. — *Behavior* **97**: 66–93.
- BATTAGLIA D., PENNACCHIO F., ROMANO A. & TRANFAGLIA A. 1994: The role of physical cues in the regulation of host recognition and acceptance behaviour of *Aphidius ervi* Haliday (Hymenoptera: Braconidae). — *J. Insect Behav.* **8**: 739–749.
- BENSADIA F., BOUDREAU S., GUAYA J.-F., MICHAUD D. & CLOUTIERA C. 2006: Aphid clonal resistance to a parasitoid fails under heat stress. — *J. Insect Physiol.* **52**: 146–157.
- CASTAÑE C., ALOMAR O., GOULA M. & GABARRA R. 2004: Colonization of tomato greenhouses by the predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*. — *Biol. Contr.* **30**: 591–597.
- CASTAÑE C., ALOMAR O., RIUDAVETS J. & GEMENO C. 2007: Reproductive biology of the predator *Macrolophus caliginosus*: Effect of age in sexual maturity and mating. — *Biol. Contr.* **43**: 278–286.
- CHANG G.C., NEUFELD J., DURR D., DUETTING P.S. & EIGENBRODE S.D. 2004: Waxy bloom in peas influences the performance and behavior of *Aphidius ervi*, a parasitoid of the pea aphid. — *Entomol. Exp. Appl.* **110**: 257–265.
- CHRISTIANSEN-WENIGER P. & HARDIE J. 2000: The influence of parasitism on wing development in male and female pea aphids. — *J. Insect Physiol.* **46**: 861–867.
- COHEN A.C. 1995: Extra-oral digestion in predaceous terrestrial arthropoda. — *Annu. Rev. Entomol.* **40**: 85–103.
- DELONG J.P. & VASSEUR D.A. 2011: Mutual interference is common and mostly intermediate in magnitude. — *BMC Ecol.* **11**: 1–8.
- DIGILIO M.C. 1995: Identificazione rapida degli stadi giovanili di *Acyrtosiphon pisum* (Harris). — *Boll. Zool. Agr. Bachicolt.* **27**: 111–116.
- DIXON A.F.G. 1958: Escape responses shown by certain aphids to the presence of the coccinellid, *Adalia decempunctata* (L.). — *Trans. R. Entomol. Soc. Lond.* **10**: 319–334.
- ELLSBURY M.M. & NIELSON M.W. 1981: Comparative host plant range studies of the blue alfalfa aphid, *Acyrtosiphon kondoi* Shinji, and the pea aphid, *A. pisum* (Harris) (Homoptera: Aphididae). — *USDA Tech. Bull.* **1639**: 1–14.
- EVANS H.F. 1978: The role of predator-prey size ratio in determining the efficiency of capture by *Anthocoris nemorum* and the escape reactions of its prey, *Acyrtosiphon pisum*. — *Ecol. Entomol.* **1**: 85–90.
- FANTINOU A.A., PERDIKIS D.C., MASELOU D.A. & LAMBROPOULOS P.D. 2008: Prey killing without consumption: Does *Macrolophus pygmaeus* show adaptive foraging behaviour? — *Biol. Contr.* **47**: 187–193.
- FANTINOU A.A., PERDIKIS D.C., LABROPOULOS P.D. & MASELOU D.A. 2009: Preference and consumption of *Macrolophus pygmaeus* preying on mixed instar assemblages of *Myzus persicae*. — *Biol. Contr.* **51**: 76–80.
- FLINN P.W., HOWER A.A. & TAYLOR R.A.J. 1985: Preference of *Reduviolus americanoferus* (Hemiptera: Nabidae) for potato leafhopper nymphs and pea aphid. — *Can. Entomol.* **117**: 1503–1508.
- FOGLAR H., MALAUSA J.C. & WAINBERG E. 1990: The functional response and preference of *Macrolophus caliginosus* (Heteroptera: Miridae) for two of its prey: *Myzus persicae* and *Tetranychus urticae*. — *Entomophaga* **35**: 465–474.
- FRANCO K., JAUSET A. & CASTAÑE C. 2011: Monogamy and polygamy in two species of mirids bugs: A functional-based approach. — *J. Insect Physiol.* **57**: 307–315.
- GABARRA R., ALOMAR O., CASTAÑE C., GOULA M. & ALBAJES R. 2004: Movement of the greenhouse whitefly and its predators between in- and outside of Mediterranean greenhouses. — *Agric. Ecosyst. Environ.* **102**: 341–348.

- GERLING D., ROITBERG B.D. & MACKAUER M. 1990: Instar-specific defense of the pea aphid *Acyrtosiphon pisum*: Influence on oviposition success of the parasite *Aphelinus asychis* (Hymenoptera: Aphelinidae). — *J. Insect Behav.* **3**: 501–514.
- GUERRIERI E., POPPY G.M., POWELL W., RAO R. & PENNACCHIO F. 2002: Plant-to-plant communication mediating in-flight orientation of *Aphidius ervi*. — *J. Chem. Ecol.* **28**: 1703–1715.
- HARSHMAN L.G. & ZERA A.J. 2007: The cost of reproduction: the devil in the details. — *Trends Ecol. Evol.* **22**: 80–86.
- HODGE S., WARD J.L., GALSTER A.M., BEALE M.H. & POWELL G. 2011: The effects of a plant defence priming compound, β -aminobutyric acid, on multitrophic interactions with an insect herbivore and a hymenopterous parasitoid. — *BioControl* **56**: 699–711.
- HUANG N., ENKEGAARD A., OSBORNE L.S., RAMAKERS P.M.J., MESSELINK G.J., PIJNAKKER J. & MURPHY G. 2011: The banker plant method in biological control. — *Crit. Rev. Plant. Sci.* **30**: 259–278.
- JESCHKE J.N., KOPP M. & TOLLRIAN R. 2002: Predator functional responses: Discriminating between handling and digesting prey. — *Ecol. Monogr.* **72**: 94–112.
- KLOMP H. 1964: Intraspecific competition and the regulation of insect numbers. — *Annu. Rev. Entomol.* **9**: 17–40.
- KREBS J. 1977: Optimal foraging: Theory and experiment. — *Nature* **268**: 583–584.
- LANG A. & GSODL S. 2003: Superfluous killing of aphids: a potentially beneficial behaviour of the predator *Poecilus cupreus* (L.) (Coleoptera: Carabidae)? — *J. Plant Dis. Protect.* **100**: 583–590.
- LOSEY J. & DENNO R. 1998: The escape response of pea aphids to foliar-foraging predators: actors affecting dropping behavior. — *Ecol. Entomol.* **23**: 53–61.
- LUCAS E. & ALOMAR O. 2002: Impact of the presence of *Dicyphus tamaninii* Wagner (Heteroptera: Miridae) on whitefly (Homoptera: Aleyrodidae) predation by *Macrolophus caliginosus* (Wagner) (Heteroptera: Miridae). — *Biol. Contr.* **25**: 123–128.
- LYKOURESSIS D., PERDIKIS D. & MICHALAKI M. 2001: Nymphal development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) on two eggplant varieties as affected by temperature and presence/absence of prey. — *Biol. Contr.* **20**: 222–227.
- LYKOURESSIS D.P., PERDIKIS D.C. & GASPARI M.D. 2007: Prey preference and biomass consumption of *Macrolophus pygmaeus* (Hemiptera: Miridae) fed *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae). — *Eur. J. Entomol.* **104**: 199–204.
- MILONAS P.G., KONTODIMAS D.C. & MARTINOU A.F. 2011: A predators functional response: Influence of prey species and size. *Biol. Contr.* **59**: 141–146.
- MORENO-RIPOLL R., AGUSTÍ N., BERRUEZO R. & GABARRA R. 2012: Conspecific and heterospecific interactions between two omnivorous predators on tomato. — *Biol. Contr.* **62**: 189–196.
- PENNACCHIO F., FANTI P., FALABELLA P., DIGILIO M.C., BISACCIA F. & TREMBLAY E. 1999: Development and nutrition of the braconid wasp, *Aphidius ervi* in aposymbiotic host aphids. — *Arch. Insect Biochem. Physiol.* **40**: 53–63.
- 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. Contr.* **17**: 55–60.
- PERDIKIS D., LYKOURESSIS D. & ECONOMOU L.P. 1999: The influence of temperature, photoperiod and plant type on the predation rate of *Macrolophus pygmaeus* Rambur on *Myzus persicae* (Sulzer). — *BioControl* **44**: 281–289.
- PERDIKIS D.C., KAPAXIDI E. & PAPADOULIS G. 2008: Biological control of insect and mite pests in greenhouse solanaceous crops. — *Eur. J. Plant. Sci. Biotech.* **2**: 125–144.
- PÉREZ-HEDO M. & URBANEJA U. 2014: Prospects for predatory mirid bugs as biocontrol agents of aphids in sweet peppers. — *J. Pest Sci.* **88**: 65–73.
- POWELL W., PENNACCHIO F., POPPY G.M. & TREMBLAY E. 1998: Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). — *Biol. Contr.* **11**: 104–112.
- PULLIAM H.R. 1974: On the theory of optimal diets. — *Am. Nat.* **108**: 59–75.
- PYKE G.H. 1984: Optimal foraging theory: A critical review. — *Annu. Rev. Ecol. Syst.* **15**: 523–575.
- PYKE G.H., PULLIAN H.R. & CHARNOV E.L. 1977: Optimal foraging: A selective review of theory and test. — *Quart. Rev. Biol.* **52**: 137–153.
- R CORE TEAM 2013: *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. URL <http://www.R-project.org/>.
- REZNICK D. 1985: Cost of reproduction: An evaluation of the empirical evidence. — *Oikos* **44**: 257–267.
- ROITBERG B.D. & MYERS J.M. 1978: Adaptations of alarm pheromone responses of the pea aphid *Acyrtosiphon pisum* (Harris). — *Can. Entomol.* **56**: 103–108.
- ROITBERG B.D. & MYERS J.M. 1979: Behavioural and physiological adaptations of pea aphids (Homoptera: Aphididae) to high ground temperatures and predator disturbance. — *Can. Entomol.* **111**: 515–520.
- SAMU F. & BIRÓ Z. 1993: Functional response, multiple feeding and wasteful killing in a wolf spider (Araneae: Lycosidae). — *Eur. J. Entomol.* **90**: 471–479.
- SIH A. 1980a: Optimal foraging: Partial consumption of prey. — *Am. Nat.* **116**: 281–290.
- SIH A. 1980b: Optimal behavior can foragers balance two conflicting demands. — *Science* **210**: 1041–1042.
- SIH A. & CHRISTENSEN B. 2001: Optimal diet theory: When does it work, and when and why does it fail? — *Anim. Behav.* **61**: 379–390.
- Symondson W.O.C., Sunderland K.D. & Greenstone M.H. 2002: Can generalist predators be effective biocontrol agents? — *Annu. Rev. Entomol.* **47**: 561–594.
- TROTTA V., DURAN PRIETO J., BATTAGLIA D. & FANTI P. 2014: Plastic responses of some life history traits and cellular components of body size in *Aphidius ervi* as related to the age of its *Acyrtosiphon pisum*. — *Biol. J. Linn. Soc.* **113**: 439–454.
- TROTTA V., DURAN PRIETO J., FANTI P. & BATTAGLIA D. 2015: Prey abundance and intraguild predation between *Adalia bipunctata* (Coleoptera: Coccinellidae) and *Macrolophus pygmaeus* (Hemiptera: Miridae). — *Eur. J. Entomol.* **112**: 862–865.
- URBANEJA A., MONTÓN H. & MOLLÁ O. 2009: Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. — *J. Appl. Entomol.* **133**: 292–296.
- WHEELER A.G. JR 2001: *Biology of the Plant Bugs (Hemiptera: Miridae). Pests, Predators, Opportunists*. Cornell University Press, Ithaca, NY, 507 pp.
- WYCKHUYS K.A.G., STONE L., DESNEUX N., HOPPER K.R. & HEIMPEL G.E. 2008: Parasitism of the soybean aphid, *Aphis glycines* by *Binodoxys communis*: The role of aphid defensive behaviour and parasitoid reproductive performance. — *Bull. Entomol. Res.* **98**: 361–370.
- ZERA A.J. & HARSHMAN L.G. 2001: The physiology of life history trade-offs in animals. — *Annu. Rev. Ecol. Syst.* **32**: 95–126.

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