Bulletin of Insectology **71** *(1): 113-120, 2018*
ISSN 1721-8861

Intraguild predation between *Macrolophus pygmaeus* and *Aphidius ervi*

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Abstract

This work investigates the intraguild predation (IGP) between the predator *Macrolophus pygmaeus* Rambur (Hemiptera Miridae) and the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera Braconidae). In particular, predation on parasitized *Acyrthosiphon pisum* (Harris) (Hemiptera Aphididae) aphids was studied at two different stages of parasitism. It is known that *A. pisum* parasitized by *A. ervi* exhibits a 'suicidal' behaviour in presence of a coccinellid. This behaviour may be linked to an increased risk of being preyed, since it has been observed that parasitized *A. pisum* suffers greater predation rate by *Harmonia axyridis* (Pallas). The escape response in *A. pisum* is affected by predator species and hemipterans cause a small disturbance of aphid colony. We hypothesize that parasitized *A. pisum* at an early stage of parasitization would suffer higher predation risk also if the predator is a hemipteran. We used the generalist predator *M. pygmaeus* to test this hypothesis. Predation on newly parasitized aphids was tested by offering groups of twenty 2nd instar aphids composed of parasitized and unparasitized individuals in different proportions to the predator. The relative proportion of the healthy and parasitized aphids did not change the total prey consumption. Increased susceptibility to predation for parasitized aphids at an early stage of parasitism was observed. This result is discussed on the basis of the kin selection theory. Predation on pre-mummified and mummified *A. pisum* aphids was also studied. Our results show that *M. pygmaeus* preys, on average, about two *A. pisum* mummies in 24 h, which is fully in line with the consumption of fourth instar aphids. Pre-mummies killed by *M. pygmaeus* always showed visible damage while mummies did not always show visible signs of the predator feeding activity. The possible consequences of the mass release of generalist predators on parasitoid natural populations are briefly discussed.

Key words: IGP, risk of predation, Miridae, Braconidae, Aphididae, biological control.

Introduction

The interaction among predators foraging on the same prey is an important mortality factor, shaping natural communities of species that occupy the same trophic level (Müller and Brodeur, 2002). This phenomenon, known as intraguild predation (IGP), consists in the behaviour of killing and eating potential competitors that exploit the same, often limiting, resources (Polis *et al.*, 1989). The term "guild" is used here in the broadest sense, including parasitoids and pathogens (Brodeur and Rosenheim, 2000).

IGP between parasitoids and predators is always unidirectional. Adult parasitoids may be preyed by generalist predators (Brodeur and Rosenheim, 2000; Navarrete *et al.*, 2014). However, a more elusive form of IGP is observed when predators eat parasitized insects. Whether parasitized insects are more or less vulnerable to predation than unparasitized ones is an interesting matter both from a theoretical and a practical point of view.

One of the issues of biological control is the opportunity to release predators and parasitoids together (Cardinale *et al.*, 2003). Experimental studies and field observations seem to indicate that, at some release ratios, predators and parasitoids may have a complementary action in the control of the pest (Cardinale *et al.*, 2003, Bilu and Coll, 2007; Gontijo *et al.*, 2015). However, if predators have a strong preference for parasitized versus unparasitized prey, IGP may have a disruptive effect on parasitoid populations (Snyder and Ives, 2003).

The behaviour change in the subjects involved in IGP is a matter of evolutionary ecology.

From a theoretical point of view, IGP may be advantageous to the predator that eliminates a competitor, while it is always disadvantageous to the parasitoid that constitutes the intraguild prey. The selective pressure of IGP on parasitoids is strong and the evolution of behaviours, which involve the avoidance of plants visited by predators, has been demonstrated for several species (Nakashima *et al.*, 2006; Martinou *et al.*, 2009). Conversely, we may expect a weak selective pressure on the predator behaviour. The effect of the parasitoid on the prey population is not immediate and parasitized insects continue to be available as prey for a rather long time. Also, most predators are generalists. The reduction in the prey population can be offset by the presence of different suitable species. The preference for preys that host parasitoid juvenile stages is conditioned by their suitability, their defensive behaviour and, ultimately, by the predator ability to distinguish parasitized from unparasitized prey. During the early development stages of the parasitoid, its host appears indistinguishable from unparasitized individuals. Subsequently, the change of some physical characteristics such as opacity or sclerotization of the body surface becomes apparent. In the case of *Encarsia formosa* Gahan and *Eretmocerus emiratus* Zolnerowich et Rose (Hymenoptera Aphelinidae) parasitizing *Bemisia tabaci* (Gennadius) (Hemiptera Aleyrodidae), parasitoid development causes the host to become opaque and swell slightly (Gelman *et al.*, 2002). This change makes the parasitized early fourth instar nymphs more apparent on the leaf surface compared with the unparasitized ones that are translucent and flat. Some predator species, namely *Orius majusculus* (Reuter), *Orius insidiosus* (Say) (Hemiptera Anthocoridae), *Geocoris punctipes* (Say) (Hemiptera Geocoridae), *Hippodamia convergens* Guerin-Meneville (Coleoptera Coccinellidae), show a strong preference for parasitized hosts when offered a choice between unparasitized early fourth instar whitefly nymphs and whiteflies hosting a late larval or pupal stage of the parasitoid (Naranjo, 2007; Sohrabi *et al.*, 2013). On the contrary, different species of whitefly predators, such as *Macrolophus pygmaeus* (Rambur) (Hemiptera Miridae) and *Delphastus catalinae* (Horn) (Coleoptera Coccinellidae), tend to discriminate against whitefly nymphs containing parasitoid larvae (Zang and Liu, 2007; Malo *et al.*, 2012).

Last larval stages and pupae of the parasitoids are avoided by several predator species (Heinz *et al.*, 1994; Hoelmer *et al.*, 1994; Al-Zyoud and Sengonca, 2004; Fazal and Xiang, 2004; Kutuka *et al.*, 2011; Chailleux *et al.*, 2013; Gkounti *et al.*, 2014) perhaps because the changes associated with parasitoid development make the prey less suitable (Takizawa *et al.*, 2000). In other cases, preys harbouring older parasitoid stages are preferred due to the reduction (Paull *et al.*, 2012) or loss (Snyder and Ives, 2001) of their defensive abilities.

Based on the above, we expect that discrimination for or against parasitized prey does not occur immediately after parasitization but after some time, when the prey reduces its defensive ability as a consequence of parasitism, or it becomes easier to detect, or it changes its physical and / or nutritional characteristics.

The possibility that IGP may select the behaviour of the parasitized insect has been taken into consideration only in the case of aphids (McAllister and Roitberg, 1987; McAllister *et al.*, 1990). A ‘suicidal’ behaviour was observed in parasitized pea aphids, *Acyrthosiphon pisum* (Harris) (Hemiptera Aphididae), elicited either by the exposure to the alarm pheromone or by the presence of a coccinellid. This behaviour consists in a greater tendency to run away from the feeding site and to drop from the plant. Greater reactivity of parasitized aphids has been interpreted as an altruistic behaviour on the basis of the inclusive fitness theory stating that “a gene may receive positive selection, even though disadvantageous to its bearers, if it causes them to confer sufficiently large advantages on relatives” (Hamilton, 1964). Consistently with this theory, the behaviour of a post-reproductive animal is expected to be entirely altruistic. In fact, pea aphids parasitized at the second instar, which will not have any chance to produce offspring before mummification, perform the most dangerous escape behaviour in the presence of the predator. On the contrary, aphids parasitized at the fourth instar, which are expected to produce a few offspring, behave no differently from unparasitized ones (McAllister *et al.*, 1990).

The suicidal behaviour in *A. pisum* has been linked to an increased risk of death, for example by desiccation, but not directly to greater risk of predation (McAllister *et al.*, 1990). However, Meisner *et al.* (2011) showed that, at an early stage of parasitization, parasitized pea aphids suffer higher predation by the coccinellid predator *Harmonia axyridis* (Pallas) (Coleoptera Coccinellidae) than unparasitized aphids. Probably, for parasitized aphids, the behavioural alteration resulting from parasitization is associated with a greater risk of being preyed. If the behaviour of parasitized aphids is the cause of their more intense predation, and especially if their behaviour has an adaptive value, we should expect that parasitized aphids will suffer a greater predation also by predators other than coccinellids. The escape response in *A. pisum* is affected by predator species (Brodsky and Barlow, 1985; Losey and Denno, 1998). In particular, hemipterans cause a small disturbance of aphid colony, resulting in significantly reduced escape behaviour compared with that elicited by coccinellids (Losey and Denno, 1998). In our study, we hypothesize that parasitized *A. pisum* at an early stage of parasitization would suffer higher predation risk also if the predator is a hemipteran. We used the generalist predator *Macrolophus pygmaeus* Rambur (Hemiptera Miridae) and the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera Braconidae Aphidiinae) to test this hypothesis. Although the pea aphid is not commonly preyed upon by *M. pygmaeus* in the field, this mirid bug displays, in the laboratory, a predatory behaviour against *A. pisum* very similar to that shown against its common preys *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) (Lykouressis *et al.*, 2007; Fantinou *et al.*, 2008, 2009; Durán *et al.*, 2016). Also, this aphid has already been used as a model in the study of IGP between *M. pygmaeus* and *Adalia bipunctata* (L.) (Coleoptera Coccinellidae) (Trotta *et al.*, 2015).

This study also tested the acceptance of aphid mummies as prey by *M. pygmaeus*. Aphid mummies are reported to be preyed by a large number of predator species, including hemipteran predatory bugs such as Miridae, Nabidae and Anthocoridae (Brodeur and Rosenheim, 2000 and references therein). Under choice condition, predators often prefer to feed on living aphids than mummies (Colfer and Rosenheim, 2001; Meyhöfer and Klug, 2002), although the former can exhibit a defensive behaviour. Sometimes, predation on mummies is low even under no choice condition (Meisner *et al.*, 2011). We presume that mummies are not to *M. pygmaeus* liking, as this species tends to discriminate against whitefly at a late stage of parasitization (Malo *et al.*, 2012). Therefore, we expect a low predation rate of mummies or a high degree of partially consumed prey.

Materials and methods

Insect rearing

M. pygmaeus, initially provided by Koppert Biological Systems, was reared on potted tomato plants placed on a table without cages, at room temperature (20-25 °C and 50-70% RH) and under an 18L:6D photoperiod. Sterilized eggs of *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) provided by Koppert Biological Systems

Table 1. Schematic representation of the experimental treatments.

Treatments	Presence of *M. pygmaeus*	Parasitized aphids	Unparasitized aphids	N. of replicates
T-0%	Yes	0	20	30
T-25%	Yes	5	15	30
T-50%	Yes	10	10	30
T-75%	Yes	15	5	14
T-100%	Yes	20	0	15
T-100% - no *Mp*	No	20	0	18

were supplied as food. *M. pygmaeus* adult females used in the bioassays had emerged one week before the start of the experiment. These females were not starved before the experiment since they were taken directly from the rearing cage, where *E. kuehniella* eggs were available ad libitum.

A. ervi was also obtained from Koppert Biological Systems and reared on *A. pisum* in an environmental chamber at 21 °C, 80-90% RH and a 18L:6D photoperiod. Parasitoids used in the experiments were removed every day from the culture at the mummy stage and individually kept until adult emergence. After emergence, adult females were fed with honey and allowed to mate. All mated females used in bioassays were 2-3 days old.

A. pisum culture was started in 1985 from a few hundred specimens collected in the field and maintained on broad bean (*Vicia fabae* c.v. *Agua dulce*) in a separate environmental chamber at 21 °C, 80-90% RH and a 18L:6D photoperiod (Battaglia *et al.*, 1994).

Predation on parasitized versus unparasitized aphids

The experimental arena consisted of a fresh cut broad bean leaf inside a Polypropylene Sample Collection Container (volume: 150 ml) with a mesh covered ventilation hole (4.5 cm in diameter) in the screw-top (Trotta *et al.*, 2015; Durán *et al.*, 2016). To avoid leaf desiccation, the leaf stalk was held inside an Eppendorf tube filled with tap water and sealed with Parafilm®. Groups of twenty 2nd instar aphids, at different proportions of parasitized and unparasitized aphids (table 1), were allowed to settle on a leaf, and each group was exposed to a predator adult female for 24 hours. Twenty aphids were used since this number of preys exceeded the maximum number of 2nd instar aphids *M. pygmaeus* is able to consume during 24 h (Durán *et al.*, 2016). Parasitized aphids were offered to the predator 1-2 hours after the parasitization event. On the basis of our previous experiments on *M. pygmaeus* predation (Trotta *et al.*, 2015; Durán *et al.*, 2016), all the experiments were performed at 21 °C, under an 18L:6D photoperiod and 80-90% RH.

After removal of the predator, consumed aphids were counted. Survived aphids of each trial were reared on a single broad bean leaf for four days and then dissected under a stereo microscope. This made it possible to evaluate the number of aphids containing a larva of *A. ervi* and the number of unparasitized aphids. The mortality occurring one day after the removal of the predator was verified, resulting in less than 1% in all the experimental groups. Aphid mortality four days after the removal of the predator was also recorded. In order to discriminate the mortality occurring during this period possibly caused by previous exposure to the predator from the mortality due to parasitism side-effects, a group of 20 unparasitized aphids exposed to a predator for 24 h (T-0% control treatment) was maintained as previously described.

For the parasitization experimental procedure, an aphid was individually placed at the bottom of a glass vial (1 cm in diameter, 4 ml volume) with a parasitoid female inside and allowed to be stung. In general, a parasitoid female was used for a maximum of one hour since she is able to parasitize about 30-50 aphids in the first hour; however, in our experiments, the parasitoid and the aphid were replaced with fresh ones if no probing was observed during a time interval of 5 minutes. Parasitized aphids were transferred to the experimental arena with a fresh cut broad bean leaf inside immediately after the observed second sting by a parasitoid female.

In *A. ervi* the decision to accept or reject a host may follow the insertion of the ovipositor (Pennacchio *et al.*, 1994) that bears chemosensilla on the tip for host recognition (Larocca *et al.*, 2007). Parasitized aphids used in these experiments were observed to be stung twice by parasitoid females. Since in our previous observations we had often found that double stinging gives a higher parasitization rate, we allowed the parasitized aphids used in this experiment to be stung twice by parasitoid females. However, even by double stinging, in a small number of instances there could be no insertion of egg(s) in the host, and there is no way of knowing for sure if an aphid hosts a parasitoid egg without dissecting it. For this reason, we estimated the Actual Parasitization Rate (APR) in aphids before their exposure to predation, using a control group (“T-100% no predator”) with 20 parasitized aphids (that is, stung twice by *A. ervi*) that were dissected after five days under a stereomicroscope. This estimated parasitization rate before predation was used as a theoretical threshold to be compared with the observed parasitization rate after predation. In each experimental group, the assessment of the parasitization rate was made on the fifth day after parasitization, when parasitoid eggs were already hatched, because before hatching, *A. ervi* eggs can easily escape observation.

Predation test on pre-mummified and mummified pea aphids

This set of experiments was carried out under the same conditions described above, by offering a group of four mummies or pre-mummies for 24 hours to a single *M. pygmaeus* female in each replicate. The number of mummies or pre-mummies used for each replicate was decided on the basis of the maximum number of fourth instar aphids consumed in 24 h, since mummified aphids and live fourth instar aphids share similar size (Durán *et al.* 2016). After removal of the predator, the pre-mummies and the mummies were first examined to detect visible damage caused by the predator (pre-mummies could appear emptied, even partially, while mummies could be clearly pierced). All the pre-mummies and mummies were kept on single broad bean leaves and the number of emerged *A. ervi* adults was checked daily for at least 13 days, which is the maximum developmental time of this parasitoid (from pupa to eclosion) at 21 °C, when egg of *A. ervi* was inserted in a 2nd instar of *A. pisum* (Trotta *et al.*, 2014).

Observations on pre-mummified and mummified aphids were replicated nineteen and twenty-two times, respectively. Side controls with pre-mummies and mummies not exposed to predators were kept in the same way.

Statistical analysis

Generalized linear models (GLMs) were used for the analysis of our data since logistic regression has greater interpretability and higher power than the analyses of transformed data (Warton and Hui, 2011). Since our data have a discrete probability distribution, the binomial, the Poisson and the negative binomial models have been considered as possible models for data analysis. Among these models, the Poisson one was selected because it has the lowest Akaike Information Criterion (AIC) and, therefore, minimizes the loss of information (Johnson and Omland, 2004). The binomial model has the lowest AIC value but comparable with the Poisson model only in the case of the data on predation on pre-mummified and mummified aphids. Consequently, a GLM with a Poisson error distribution with a log link function has always been chosen to test for treatment differences or interactions in our data on predation, on aphid mortality and on parasitoid emergence in pre-mummified and mummified aphids.

For the treatments with different initial proportion of parasitized aphids, the observed mean values of parasitism (OPR) after predation ± 95% confidence intervals were compared with the Expected Parasitization Rates (EPR). The equality of OPR and EPR is in accordance with the null hypothesis that there is no difference in the predation rate on parasitized *vs* unparasitized hosts. The EPRs were estimated on the basis of the Actual Parasitization Rate (APR) of a control group of aphids parasitized but not exposed to the predator. Then EPR is equal to APR for T-100%, to APR × ¾ for T-75%, to APR / 2 for T-50% and to APR / 4 for T-25%.

The 95% confidence intervals for each of the EPR were calculated with a Bootstrapping method consisting in the generation of 1800 random "*APR X-% permutation trials*", with *X*-% = 25, 50, 75 and 100%. An "*EPR X-% permutation trial*" is composed of *X*-% of parasitized aphids randomly chosen from the "T-100% no predator" group and the remaining proportion of unparasitized ones.

All the analyses in this study were carried out using R.3.2.4 software (R Development Core Team, 2013), library MASS.

Results

Predation on parasitized aphids versus unparasitized aphids

The number of aphids consumed by *M. pygmaeus* did not statistically differ among the five experimental groups tested (analysis of deviance based on a Poisson GLM model: $\chi^2_{(4)} = 2.47$, $P = 0.65$, figure 1). With regard to aphid mortality recorded four days after exposure to the predator, the Poisson GLM model showed no significant differences among different combinations of parasitized / unparasitized aphids ($\chi^2_{(4)} = 4.51$, $P = 0.34$), suggesting that the overall aphid mortality was not affected by the different proportions of parasitized / unparasitized aphids (mean aphid mortality ± standard error for: T-0%: 6.7 ± 1.76; T-25%: 10.7 ± 2.1; T-50%: 9.2 ± 1.79; T-75%: 8.5 ± 2.3; T-100%: 13.1 ± 4.32).

In the four treatments containing parasitized aphids, the percentage (mean ± 95% Confidence Interval - CI) of parasitized *A. pisum* (dissected aphids hosting a parasitoid larva) over the number of surviving aphids after predation was compared with the expected parasitization rate assuming that there is no difference in the predation rate on parasitized vs unparasitized hosts (figure 2). Under our experimental conditions, the Actual Parasitization Rate (APR) before predation (assessed on the experimental group with 20 aphids stung twice by a parasitoid) was 93.7% ± 0.02 (mean ± CI). Based on this estimate we assessed the four expected parasitization rates in the experimental groups with different ratios of parasitized/unparasitized aphids, which is equal to APR for group T-100%, APR × ¾ for T-75%, APR / 2 for T-50% and APR / 4 for T-25%.

For T-75%, T-50% and T-25% the observed mean values of parasitization are significantly lower than their respective theoretical mean values, meaning that *M. pygmaeus* seems to prey at a significantly higher rate on aphids hosting a parasitoid egg.

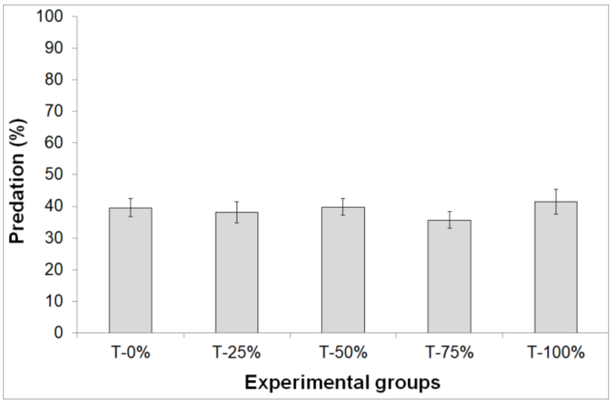

Figure 1. Predation rate (mean ± SE) of *M. pygmaeus* on *A. pisum*. Treatments: T-0%: 20 non-parasitized aphids (N = 30); T-5%: 5 parasitized aphids plus 15 non-parasitized aphids (N = 30); T-25%: 5 parasitized aphids plus 15 non-parasitized aphids (N = 30); T-50%: 10 parasitized aphids plus 10 non-parasitized aphids (N = 30); T-75%: 15 parasitized aphids plus 5 non-parasitized aphids (N = 14); T-100%: 20 parasitized aphids (N = 15).

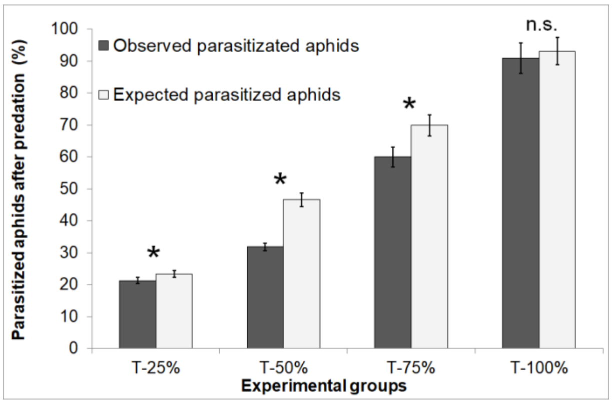

Figure 2. Mean values (± 95% CI) of the observed and expected percentage of parasitized aphids after predation (see M&M for details) in the experimental groups at different proportion of parasitized aphids: Treatments: T-25%: 5 parasitized aphids plus 15 non-parasitized aphids (N = 30); T-50%: 10 parasitized aphids plus 10 non-parasitized aphids (N = 30); T-75%: 15 parasitized aphids plus 5 non-parasitized aphids (N = 14); T-100%: 20 parasitized aphids (N = 15). * Differences statistically significant ($P < 0.05$); n.s.: not significant.

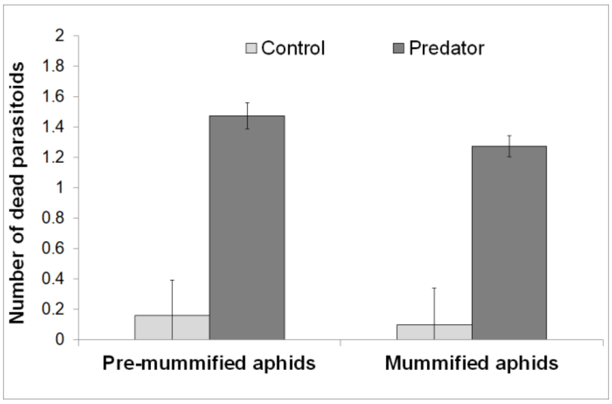

Figure 3. Number of dead parasitoids (mean ± SE) in pre-mummified (N = 19) and mummified (N = 22) pea aphids exposed or not to *M. pygmaeus*.

Predation test on pre-mummified and mummified pea aphids

M. pygmaeus preys on pre-mummified and mummified aphids of *A. ervi*, even though at a different rate. Twenty-four hours after exposure to the predator, the mean numbers (± standard errors) of pre-mummified and mummified aphids showing clear signs of predation (i.e., pre-mummies completely or partially emptied, or mummies with their cocoon pierced) were 1.16 ± 0.175 and 0.36 ± 0.124 respectively. The Poisson GLM model applied to these data showed that the values were significantly influenced by the stage (pre-mummy or mummy) offered to the predator ($\chi^2_{(1)} = 9$, $P = 0.0027$).

The overall parasitoid mortality (measured as the number of non-emerged parasitoids) in pre-mummified and mummified hosts, exposed or not to *M. pygmaeus*, is shown in figure 3. The results of the Poisson GLM model performed on the parasitoid mortality showed that significant differences were present between the groups of aphids subjected to predation and the control ones ($\chi^2_{(1)} = 47.6$, $P < 0.0001$). Parasitoid mortality did not statistically differ between the two parasitized aphid stages tested (pre-mummy and mummy, $\chi^2_{(1)} = 0.45$, $P = 0.5$) and the interaction between treatments and aphid stage was not significant ($\chi^2_{(1)} = 0.11$, $P = 0.74$).

Discussion

Meisner *et al.* (2011), specifically addressing the susceptibility to predation of aphids at an early stage of parasitism, found that *H. axyridis* has a higher predation rate on *A. pisum* parasitized by *A. ervi* compared with unparasitized aphids. We obtained a similar result using *M. pygmaeus* as predator species instead of *H. axyridis*.

The “optimal foraging theory” (Krebs, 1977) states that predator feeding preference is a function of the ratio of the energy obtained with a prey to the costs of searching, handling and consuming it (Schoener, 1971; Pyke *et al.*, 1977). This function should maximize the net rate of energy uptake to maximize predator fitness. Prey preference is then determined by prey features such as size, mobility, vulnerability and defensive abilities but also by the predator-prey encounter rates, since all of these factors may modify the cost/benefit balance of this function, influencing the amount of energy that the predator can obtain while foraging (Pyke *et al.*, 1977; Lykouressis *et al.*, 2007; Fantinou *et al.*, 2008; 2009).

In our experiment, the overall predation rate by *M. pygmaeus* has not been affected by the ratio of parasitized to unparasitized aphids; it is then possible to conclude that the energy obtained from a single prey is similar for both types of aphids (the aphids are all of the same age/size). It is also reasonable to suppose that the causes of *M. pygmaeus* preferences for parasitized aphids could be investigated by considering the aphid behaviour rather than changes in aphid physiology, and then in prey nutrient content, since the aphids were offered to the predator immediately after parasitization. We did not make accurate observations on aphid behaviour but higher mobility after parasitization was evident.

An alteration of the behavioural response of parasitized aphids to predators has already been demonstrated for *A. pisum* (McAlister and Roitberg, 1987; McAlister *et al.*, 1990). This behavioural alteration, which exposes the parasitized aphid to a higher risk of death in the presence of an approaching Coccinellidae predator, has been interpreted as ‘‘adaptive suicide”. Indeed, the death of a parasitized aphid would prevent the immature parasitoid from becoming adult and then parasitizing the offspring of its host siblings. The suicide hypothesis is based on the kin selection theory considering that the viviparous parthenogenic reproduction and the low rate of dispersal lead to aggregation of closely related individuals. In Aphids, the kin selection theory has also been called into question to explain the evolution of sociality (Abbot *et al.*, 2001).

It is plausible that the “suicidal behaviour”, already observed in *A. pisum* as a result of parasitization, by increasing the mobility of parasitized aphids affects the rate of encounter between the predator and the parasitized prey. This hypothesis seems to be confirmed by the observations of Meyhöfer and Klug (2002) concerning a different aphid - parasitoid - predator system [*Aphis fabae* Scopoli - *Lysiphlebus fabarum* (Marshall) - *Chrysoperla carnea* (Stephens)]. The above-mentioned authors found that, once a prey was consumed, "it took *C. carnea* a significantly shorter time to capture a parasitized aphid as the next victim compared to an unparasitized one". Also, *A. fabae* specimens, harbouring a parasitoid egg or young larva, are more susceptible to predation by different species of predators (Meyhöfer and Klug, 2002). In prospect, further studies could prove that greater susceptibility of parasitized aphids to predation, at an early stage of parasitism, is a common phenomenon.

Predation of the mummies is a fairly common behaviour among aphid predators, with the exception of cecidomyd flies and hoverflies (Broder and Rosenheim, 2000). Hoverflies seldom attack aphid mummies causing very low mortality (Meyhöfer and Klug, 2002). Since they are very voracious, coccinellids can destroy a large number of mummies (up to 22 in 24 hours in the case of *Hippodamia convergens* Guerin-Meneville) (Colfer and Rosenheim, 2001). In spite of that, even among Coccinellids, the propensity to consume mummies depends on the predator species. *H. axyridis* is not inclined to prey on mummies and each individual kills, in no choice condition, not more than two mummies in 24 hours (Meisner *et al*., 2011). Among predatory bugs, *Anthocoris nemorum* (L.) (Hemiptera Anthocoridae) shows no preference between mummies and unparasitized aphids (Meyhöfer and Klug, 2002; Meyling *et al*, 2004). In no choice condition, *M. pygmaeus* kills, on average, less than two mature larvae or pupae of *A. ervi* within the *A. pisum* mummies in 24 hours. The consumption of mummies by *M. pygmaeus* is, therefore, fully in line with the consumption of fourth instar aphids (Durán *et al*, 2016) that are more or less the same size as a pre-mummy or a mummy. This is the opposite of what we expected because *M. pygmaeus* tends to discriminate against whitefly nymphs hosting a parasitoid late larval or pupal stage (Zang and Liu, 2007; Malo *et al.*, 2012).

It should be noted that the pre-mummies killed by *M. pygmaeus* always show visible damage. Conversely, mummies killed by *M. pygmaeus* may not show visible signs of the predator feeding activity. For this reason, predation of aphid mummies by *M. pygmaeus*, and perhaps by other predatory bugs, might be underestimated in the field. Since there are no substantial differences between mummies of different species, except in size, we believe that this observation is easily generalizable.

In conclusion, we have demonstrated that *M. pygmaeus* more frequently preys on the aphids containing an *A. ervi* egg than unparasitized aphids. Furthermore, *M. pygmaeus* accepts mummies as prey by consuming a number equivalent to the fourth instar aphids. Our observations concerning the highest predation rate of parasitized but still alive aphids confirm those of Meisner *et al*. (2011) and of Meyhöfer and Klug (2002). We have speculated about the causes of this phenomenon attributing them to the behaviour of aphids. However, further studies are needed to show that the greatest risk of predation is due to the behaviour of parasitized aphids and that this behaviour has an adaptive value.

Greater susceptibility of aphids at an early stage of parasitization to predation could be a general phenomenon the implications of which in natural conditions are not easy to be investigated. Occurrence of IGP may lead to unstable dynamics and even to exclusion of one of the species involved (Polis and Holt, 1992; Moran *et al*., 1996; Holt and Polis, 1997; Holt and Huxel, 2007). The release of generalist predators for biological control could influence the equilibrium of communities present in the target crop and in adjacent ones. For example, the artificial increase in generalist predators' populations, repeated over years through mass releases for biological control of pests, could have consequences for parasitoids local displacement.

Acknowledgements

This study was partly funded by a grant from the PRIN MIUR "The role of root symbionts in induced indirect defence against aphids: the release of volatile organic compounds attracting predators"; Juliana Durán Prieto and Vincenzo Trotta equally contributed to the work.

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Received December 15, 2016. Accepted March 12, 2018.