Performance of a predatory hoverfly feeding on *Myzus persicae* (Hem. Aphididae) reared on two brassicaceous plants varies with ant attendance

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- The presence of ants in the colonies of aphids can reduce biological control agents efficiency.
- The presence of ants in an aphid colony significantly affected all measured performance traits of the predatory hoverfly.
- Hoverfly females showed a significant avoidance toward the presence of ants in the aphid colony.
- Overall, the indirect benefits from ant tending (reduced predation) to aphids suggest the occurrence of enemy-free space.

ABSTRACT

Food webs centered on aphids contain networks of species, some directly related to prey characteristics, others mediated via the characteristics of the host plant. These tritrophic interactions can be even more extensive and complex when ants are present in aphid colonies. This study investigated how the presence of ants (*Cataglyphis aenescens* Nylander and *Formica cunicularia* Latreille) in colonies of a generalist aphid (*Myzus persicae* Sulzer) can influence (i) the performance of an important generalist aphidophage (the common hoverfly *Episyrphus balteatus* DeGeer) feeding on aphids growing on two host plants that express different levels of the glucosinolate sinigrin (*Brassica napus* L. and *Brassica nigra* L.); and (ii), the oviposition of the aphidophage on ant-free versus ant-tended aphid colonies. Individual fitness was calculated from performance measures, and the number of eggs laid by gravid females used as a measure of preference. Ant presence significantly reduced the survival rate of predator immature stages, but there were no significant effects of host plant (sinigrin content), and host plant did not interact with ant presence. Female fecundity was significantly reduced by ant presence and varied between host plants, but these factors did not interact significantly. Syrphid females avoided ovipositing in the presence of ants, independent of plant species. Overall, the presence of ants in aphid colonies reduced predator performance and reduced oviposition by hoverfly females. Therefore the ant-aphid interaction is important in biological control as it influences the distribution of aphidophagous syrphids within the habitat.

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1. Introduction

Predicting the outcome of an aphid-aphidophage (third trophic organisms which feeding upon aphids) interaction is complicated because of multitrophic interactions that exist in these food-webs (Gilbert, 2005). Some interactions directly relate to prey characteristics, such as prey species (Hodek and Honek, 1996; Sadeghi et al., 2014) and prey availability (Sutherland et al., 2001; Almohamad et al., 2006; Amiri-Jami and Sadeghi-Namaghi, 2014), while others are mediated via traits of the host-plant (Chaplin-Kramer et al., 2011; Kos et al., 2011; Goodey et al., 2015; Amiri-Jami et al., 2016a). Usually called tritrophic, the hostplant-aphid-aphidophage system is probably much more extensive and complex (Gilbert, 2005). In this context, the presence of intraguild competitors in aphid colonies (Almohamad et al., 2010; Amiri-Jami et al., 2016b), or third parties such as the

ants associated with some aphids, could modify natural enemy efficiency (Stadler et al., 2003; Oliver et al., 2008).

Mutualism between ants and aphids is one of the most widespread and important interactions found in nature (Stadler and Dixon, 2005). About one-quarter of aphid species appear always to be tended by ants, with many more species tended facultatively (Bristow, 1991; Stadler and Dixon, 1998). Ants tend hemipterans to obtain sugar-rich honeydew (which also contains amino acids and vitamins) and in turn protect them against their natural enemies (Hölldobler and Wilson, 1990; Stadler and Dixon, 2005). In the case of aphids, ants guard them from a wide range of natural enemies including coccinellids, lacewings, midges, spiders, parasitoids and hoverflies (Gilbert, 2005; Almohamad et al., 2007). Thus ants come into conflict with aphidophages for their common food resources. Ants are usually able to defend resources effectively and often disrupt the natural enemies of aphids via increased aggression (Buckley, 1987; Dejean, 2002). This has been described as ownership behaviour (Way, 1963). Exclusion of natural enemies from aphid colonies by ants is beneficial for tended aphids in different ways, such as direct increases in development rate, adult body size, fecundity and reproductive rate (Majerus et al., 2007). However, ants do not always benefit aphids and their association with aphids can be antagonistic; for example, when ants negatively affect aphid growth and development (Stadler et al., 2002) or even prey on aphids (Stadler and Dixon, 2005; Singh et al., 2016). Moreover, it has been shown that plant genotype influences whether ant–aphid associations are mutualistic or antagonistic (Abdala-Roberts et al., 2012). Aphid performance and preference also varies across different plant genotypes or varieties (Zytynska and Weisser, 2016; Singh et al., 2016). This could further influence the effect of ants on the aphids (Sakata, 1995). Further factors such as the presence of other herbivores that alter host-plant quality could also indirectly mediate ant-aphid interactions (Walling, 2000). Understanding ant-aphid associations is crucial because they can be keystone interactions, with a major influence on the arthropod communities on plants (Styrsky and Eubanks, 2007; Zhang et al., 2012).

When feeding on toxic plants, a generalist aphid often derives some protection from its host plant, as demonstrated by various studies. For example, Mendel et al. (1992) reported that the generalist aphid, Aphis craccivora Koch, and the coccids, Icerya purchasi Maskell and Icerya aegyptiaca (Douglas), are better protected against natural enemies when feeding on the alkaloid-rich legumes, Erythrina corallodendrum L. and Spartium junceum L., than when feeding on Citrus sinensis (L.) Osbeck, Cucurbita moschata (Duschene ex Lan.) Duschesne ex Poiret, or Euphorbia tirucalli L. When preyed upon, aphids feeding on Spartium junceum induced longer developmental times in Chrysoperla carnea (Stephens), and Icerya spp. feeding on alkaloid-rich legumes induced higher mortality in Rodolia cardinalis (Mulsant) and Chilochorus bipustulatus L. However, differential aphid toxicity to ladybeetles can also be a property of the aphid lineage rather than the host plant (White et al., 2016).

Although ant tending and its subsequent effects on hemipteran performance are some of the most well-studied of interactions (e.g. Itioka and Inoue, 1996; Morales, 2000), few studies have evaluated the effects of ant presence on the performance of the aphidophages, especially hoverflies. About one third of all hoverfly species (Diptera, Syrphidae) are aphidophagous and are good models in studies of top-down forces (Dziock, 2005). Although aphidhoverfly interactions have been addressed in many studies, especially concerning oviposition patterns and biological control (Sadeghi and Gilbert, 2000a,b,c; Almohamad et al., 2008, 2009, 2010; Amiri-Jami and Sadeghi-Namaghi, 2014; Amiri-Jami et al., 2016a,b), little is known about the relationships between myrmecophilous aphids, their associated tending ants and predatory hoverflies. However, it seems that the main effect of ant-tending

on the performance of predatory hoverflies relates to their immature stages, because the adults do not feed on aphids. The negative effects of ant-tending on aphidophage performance have been shown in some studies (e.g. Schmutterer, 1972a,b, 1974; Sloggett et al., 1998; Sloggett and Majerus, 2003; Oliver et al., 2008).

Hoverflies have sedentary larvae, and newly hatched offspring have limited dispersal ability; thus the decisions of ovipositing females about where to lay their eggs are crucial. Any antagonist interaction between ants and aphidophagous hoverflies would be determined firstly by the oviposition decisions of gravid females. The oviposition response of predatory syrphids has been evaluated toward several factors, including habitat (Bell, 1990), host-plant (Cortesero et al., 2000; Sadeghi and Gilbert, 2000a; Almohamad et al., 2007; Amiri-Jami et al., 2016a), prey type (Hodek, 1993; Sadeghi and Gilbert, 2000b; Sadeghi et al., 2014), prey availability (Dixon, 1959; Chandler, 1968; Sutherland et al., 2001; Almohamad et al., 2006; Amiri-Jami and Sadeghi-Namaghi, 2014), semiochemicals (Dicke, 1999; Ninkovic et al., 2001; Francis et al., 2004; Harmel et al., 2007; Verheggen et al., 2008; Almohamad et al., 2010), the presence of intra- and interspecific competitors (Hindayana et al., 2001; Almohamad et al., 2010; Amiri-Jami et al., 2016b), female age (Sadeghi and Gilbert, 2000b; Frechette et al., 2004), and egg load and host deprivation (Sadeghi and Gilbert, 2000c).

Despite all of these studies, there is little information about the oviposition responses of gravid females toward the presence of ants. Some literature has shown avoidance responses in coccinellid females (e.g. Oliver et al., 2008) to antagonism from ants. Oviposition should reflect a preference for minimising potential sources of offspring mortality, such as the presence of antagonist ants in prey colonies. However, the evolutionary and ecological responses of aphidophagous insects towards ants tending their prey has received less attention, and it is important to find out the crucial role of third parties (ants) to improve the management of aphid pests. With this background, we investigate how the presence of ants (Cataglyphis aenescens Nylander and Formica cunicularia Latreille) in colonies of an aphid (Myzus persicae Sulzer) might influence (i) the performance of one of the most common of the aphidophagous hoverflies (Episyrphus balteatus DeGeer) feeding on aphids on two host plants with different level of defensive glucosinolates (sinigrin in Brassica napus L. and Brassica nigra L.); and (ii) the oviposition responses of syrphid females towards ants in the field.

2. Material and methods

Canola Brassica napus var. Hyola 308 (supplied from Torogh Agricultural Research Center, Razavi Khorasan Province, Iran) and black mustard Brassica nigra (supplied from Pakan-Bazr Co. Isfahan Province, Iran) were sown in plastic pots (20 cm diameter, 30 cm height, containing a 2:2:1 mixture of soil, sand and leaf compost, respectively). Plants at the stage of 4–5 fully expanded leaves were selected for the experiments, and also were sampled for HPLC analysis. To prevent ants from accessing the aphid colonies, a strip of Tanglefoot insect barrier was used at the bottom of each plant stem. For mass rearing of Myzus persicae, selected plants were infected with apterous females collected from a single colony in an oilseed field to obtain a suitable aphid population.

A stock culture of Episyrphus balteatus was established using gravid females captured at the campus of Ferdowsi University of Mashhad, NE Iran. The stock culture was maintained in a constant environment of 21–23 °C under a L14:10D photoperiod. To obtain a group of larvae of the same age, females were induced to lay eggs on cut potted plants (B. napus) infested with aphids. For experimental purposes, eggs laid over a period of 12 h were selected

and placed in a large Petri dish in an incubator (20 ± 1 °C, 60–70% RH and L16:8D photoperiod) to hatch.

Sinigrin was quantified by HPLC in plant samples. The extraction method of sinigrin was made according to the EEC Regulation N1864/90. The chromatographic analyses were carried out on an HPLC system (Knauer Corporation, Scientific Instruments, Germany) equipped with a manager 5000, pump 1000 and UV detector 2600. Sinigrin and desulfo-sinigrin were separated on a Eurospher (C 18) 100 A column (250 × 4.6 mm, 5 μm), at 30 °C. The data were processed with the software EZ Chrom Elite (Shimadzu).

2.1. Performance of Myzus persicae

Aphid population growth rate was estimated on each host plant in the presence/absence of tending ants using the equation (Chau et al., 2005): $r = \ln(N_{x+1}/N_x)/t$, where N_x is the population at time x, N_{x+1} the population at time x + 1 and t the difference in days between time x + 1 and x. The same aphid inoculum consisting of 5 adults and 5 nymphs from different instars were used at the beginning (N_x), and after two weeks the aphids were counted again (N_{x+1}) on each host plant. Sixteen replications were performed for each treatment combination.

2.2. Performance of Episyrphus balteatus

The plant-aphid-ant system was established in the field (Horticulture Garden of Ferdowsi University of Mashhad). Potted plants were placed in the vicinity (<5 m) of natural colonies of ants. The plants were infested with M. persicae, and after three weeks when the aphid population was sufficient for starting the experiments, aphid density on four treatments was manipulated to have the same number (approximately 150 aphids per plant at the beginning). All combinations of the two host-plant species (canola/mustard) and two conditions of the presence/absence of ants were used as treatments to investigate the effect of ants and sinigrin on predator performance. Ant density (per plant) was measured in each treatment by daily visual counts during the larval development of the hoverfly totally on 100 random host plant in each treatment (10 successive days from the 2-day old larva until pupation, and each day on 10 random host plants within each treatment). The ant counts were made by visual observations taken over 3 min intervals and during three different times daily on the infected host plants (10 cm of the infected main stem). To account the continuous ant movement and avoid of recount the same individual ants we divided each 3 min observation period to 10 very short periods on each plant (each time 18 successive seconds) and then calculated the average of observations as one replication for each treatment. Because the newly emerged larvae of E. balteatus are very delicate and difficult to handle, they were transferred to aphid infested plants in the field condition when they were 2 days old (45 larvae in each treatment and one larva per plant at the beginning). Larval development was monitored daily and the number of survivors recorded until pupation. Pupae were transferred to the laboratory and kept separately until adult emergence. During the experiment, great care was taken to eliminate other aphidophages (egg, larva or adult, using a camel hair brush) when observed at aphid colonies. The resulting adult females were dissected after 24 h under a stereomicroscope and their potential fecundity measured by counting the number of ovarioles (both ovaries). Development time (from egg hatch to adult emergence) was calculated from the data. All replicates in which larvae were lost for any reason during the experiment, were omitted from final analysis (7, 12, 5 and 11 replicates respectively for each treatment combination, include: canola-ant absence, canola-ant presence, black mustard-ant absence and black mustard-ant presence). Every

3 days, the location of the potted plants was rotated to minimize positional effects.

Individual fitness (r) was calculated as a performance measure (McGraw and Caswell, 1996; Sadeghi and Gilbert, 2000a) by integrating development time (D), survival (m, either 1 [survived] or 0 [died]) and potential fecundity (V) via the equation: $r = [\text{Ln} (mV)]/D$, where Ln is the natural logarithm. Fitness values were calculated for surviving females only and for all females including non-survivors. Because female larvae/pupae cannot be differentiated from males, and because the sex ratio in the emerged adults was 1:1, we assumed half the mortality to be female, rounding up when an odd number had died.

### 2.3. Oviposition preference of aphidophagous hoverflies

Oviposition preferences were determined by a field test. Plant-aphid-ant combinations were established as before. Gravid hoverfly females were offered six plants of each treatment (as a single replicate) for oviposition and left for 48 h. Treatment combinations were offered separately (at four different places). Plants were then cut and the number of eggs on each plant were counted and average among plants within treatment was calculated. These eggs were then placed in Petri dishes to hatch and the percentage hatch calculated for each treatment. The test was replicated overall six times for each treatment. During the experiments, plants were checked twice daily to remove any eggs or adults of other aphidophages.

2.4. Statistical analyses

All analyses were performed using the statistical program, R (R Core Team, 2011). The performance and preference data were analyzed by GLM with an ANOVA table built up by sequentially deleting terms from the model. The full model was composed of the two factors (host plant, presence of ant) and their interaction. Since no significant interactions were found, we focused only on the main effects. For analysis of survival (larval survival, survival to adult emergence), a glm with binomial errors was fitted to the data, checking the fit using the dispersion parameter, and testing for differences using the change in deviance (distributed as a chi-squared). A non-parametric Kruskal-Wallis test was used for individual fitness including non-survivors because none of the standard error distributions was an adequate fit. Ant densities on the two host plants, and the percentage of eggs hatched laid by hoverflies ± ants, were compared with a t-test. A simple linear regression tested the relationship between time (the development of the aphid colony) and ant density.

3. Results

The wild host plant (Brassica nigra) had high levels of sinigrin in its leaves (mean ± SE 10.83 ± 0.337 μmol g^{-1}), while the cultivated canola (Brassica napus) lacked any sinigrin. The abundance of ants on plants was not significantly different between the two host plants (t = 0.8, df = 98, P = 0.445). The number of ants per plant varied from 1.1 to 4.55 with a mean of 2.73 ± 0.32 on canola, and 3.1 ± 0.34 on black mustard (Fig. 1a). Regression analysis showed a significant relationship between the time of development of the aphid colony and ant density (Fig. 1b and c). The per capita population growth rate of aphids was significantly higher in the presence of ants (0.425 ± 0.004) than without them (0.388 ± 0.004) ($F_{1,61}$ = 41.98, P < 0.001), but host plant and interaction effects were not significant. The per capita population growth rate of aphid across ant and plant combinations are shown in Fig. 1d.
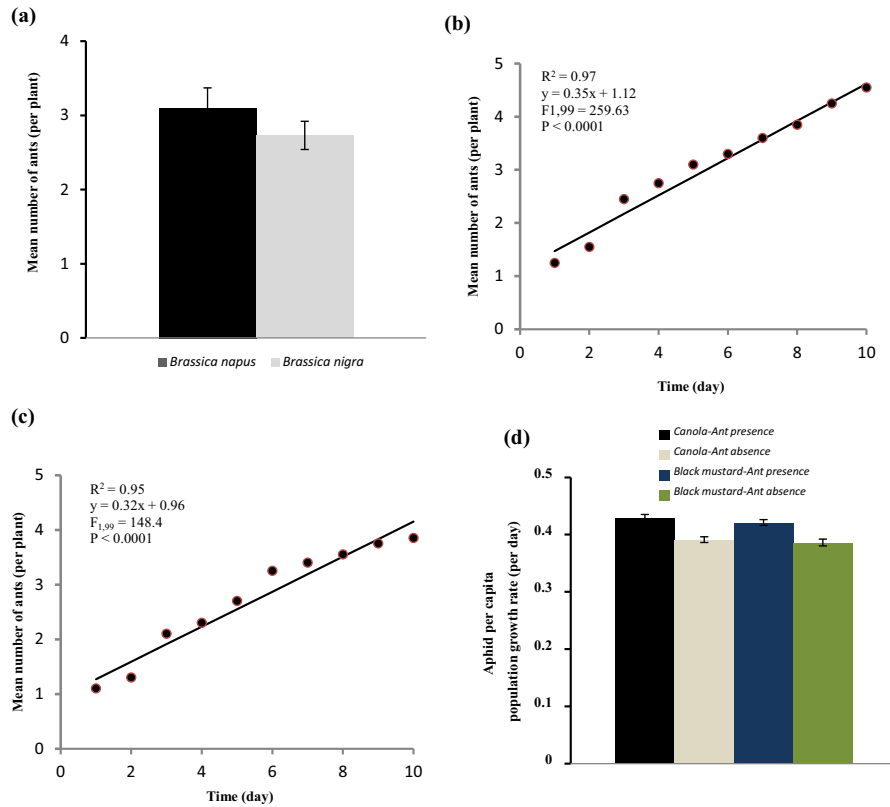
Fig. 1. Ant density at the colony of *Myzus persicae* (mean ± SE) fed on two different host plant (*Brassica napus* vs. *Brassica nigra*) (a), the relationship between time and ant density in a developing aphid colony (b) on canola (*Brassica napus*) and (c) on black mustard (*Brassica nigra*). Points and line represent the mean of observed values and predicted values, respectively. (d) Aphid population growth rate (mean ± SE) across ant and plant treatments.

The presence of ants in an aphid colony significantly affected all the performance measures of *Episyrphus balteatus*, but the host plant (i.e. sinigrin) only had a significant effect on development time of the immatures and adult fecundity, and hence on overall fitness of survivors. There was no significant interaction for any performance measure (Table 1). The values of predator performance measures are shown in Table 2 for the effect of ants and Table 3 for the effect of host plant. Almost all hoverfly larvae in the absence of ants achieved the pupal stage and most emerged as adults, whereas many of those in the presence of ants died before pupation and subsequently few emerged (Table 2; Fig. 2a and b). In the presence of ants, the fecundity of female hoverflies decreased but development time increased (Table 2). The individ-

Table 1
Summary of ANOVA results for effects of host plant and ant on performance measures of *Episyrphus balteatus* and oviposition preference of aphidophagous hoverflies.

Measure	Host plant	Ant	Host plant$_x$ Ant
Development time (day)	$F_{1,50} = 17.92$***	$F_{1,51} = 48.69$***	$F_{1,49} = 0.414$[NS]
Larval survival (%)	$X^2_1 = 0.79$[NS]	$X^2_1 = 50.37$***	$X^2_1 = 1.05$[NS]
Survival to emergence (%)	$X^2_1 = 0.32$[NS]	$X^2_1 = 39.57$***	$X^2_1 = 0.41$[NS]
Fecundity (ovariole number)	$F_{1,50} = 8.67$**	$F_{1,51} = 64.57$***	$F_{1,49} = 1.07$[NS]
Fitness, r of survivors (per day)	$F_{1,50} = 13.86$***	$F_{1,51} = 56.74$***	$F_{1,49} = 0.3$[NS]
Fitness, r of all individuals (per day)	$X^2_1 = 3.2$[NS]	$X^2_1 = 40.04$***	–
Oviposition preference (number of eggs laid)	$F_{1,141} = 3.38$[NS]	$F_{1,142} = 394.88$***	$F_{1,140} = 0.5$[NS]

[*]p < 0.05; [**]p < 0.01; [***]p < 0.001; NS means Non Significant.

Table 2
The performance measures of *Episyrphus balteatus* when fed on *Myzus persicae* with and without the presence of ant in the aphid colony.

Measure	Ant absent	Ant present
Development time (day)	19.72 ± 0.25 (32)	22.33 ± 0.26 (21)
Larval survival (%)	99 ± 1 (67)	51 ± 6 (78)
Survival to emergence (%)	91 ± 4 (67)	44 ± 6 (78)
Fecundity (ovariole number)	43.66 ± 1.03 (32)	31.14 ± 1.23 (21)
Fitness, r of survivors (per day)	0.192 ± 0.003 (32)	0.154 ± 0.003 (21)
Fitness, r of all individuals (per day)	0.176 ± 0.010 (35)	0.074 ± 0.012 (44)

Each value in the table gives the mean ± SE, with the sample size in brackets.

Table 3
The performance measures of *Episyrphus balteatus* when fed on *Myzus persicae* reared on black mustard *Brassica nigra* and canola *Brassica napus*.

Measure	Black mustard	Canola
Development time (day)	21.48 ± 0.37 (25)	20.11 ± 0.3 (28)
Larval survival (%)	70 ± 5 (74)	76 ± 5 (71)
Survival to emergence (%)	64 ± 6 (74)	68 ± 6 (71)
Fecundity (ovariole number)	36.4 ± 1.59 (25)	40.75 ± 1.59 (28)
Fitness, r of survivors (per day)	0.168 ± 0.005 (25)	0.185 ± 0.005 (28)
Fitness, r of all individuals (per day)	0.108 ± 0.013 (39)	0.13 ± 0.014 (40)

Each value in the table gives the mean ± SE, with the sample size in brackets

ual fitness of hoverfly larvae feeding on aphids on black mustard was lower than that on canola (Table 3).

Syrphid females showed a significant oviposition avoidance toward the presence of ants in the aphid colony (Table 1; Fig. 3a), while host plant and interaction effects were not significant. There was a highly significant difference in relation to the percentage of
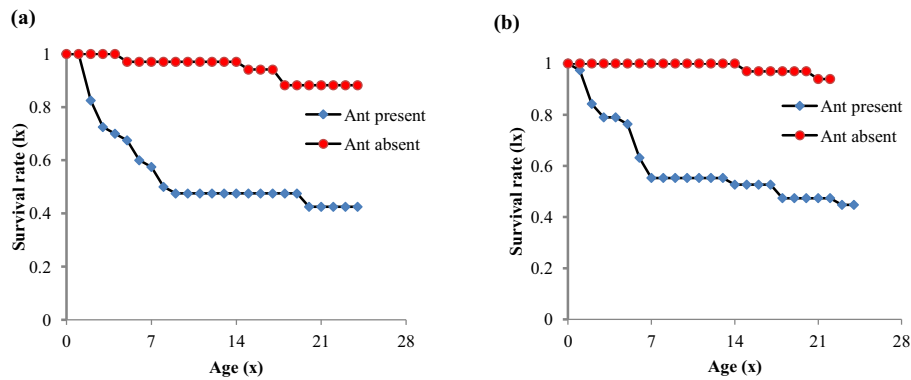
Fig. 2. Survival rate of *Episyrphus balteatus* from egg hatching until adult emergence at the presence and absence of ant at the aphid colony on two host plant: (a) *Brassica napus* and (b) *Brassica nigra.*

egg hatching in the presence or absence of ants (Fig. 3b; t = 15.48, df = 70, P < 0.0001).

4. Discussion

Many predatory hoverflies encounter aphid-tending ants because they both use resources provided by aphids. Thus conflict between ants and aphidophagous hoverflies is expected to be frequent. Here, hoverfly females avoided ant-attended aphid colonies by reducing the number of eggs laid. In addition, the performance of *Episyrphus balteatus* was obviously lower when ants were present. Similarly, Oliver et al. (2008) showed that the presence of ants in colonies of aphids had a negative impact on all ladybird developmental stages (e.g. eggs, larvae, and adults), and significantly reduced individual fitness of *Adalia bipunctata*. Ladybirds unable to tolerate ant presence are therefore likely to have adaptations to avoid patches with ants. Thus the spatial distribution of ants that share mutualisms with aphids is likely to influence the distribution of aphidophagus insects such as hoverflies. However, some studies have shown that there are adaptations that enable hoverflies and ladybirds to manage ant attacks and hence successfully to feed on the aphids of the colonies they guard (e.g. Schmutterer, 1972a,b, 1974; Mizuno et al., 1997; Sloggett et al., 1998; Sloggett and Majerus, 2003; Bachtold and Del-Claro, 2013). This conflict could be interpreted in terms of differences in the morphological, physiological or behavioural adaptations among predator species. However, adaptations by aphidophages reveal important subtleties in the relationship between aphids and aphidophages in the presence of ants.

In this experiment, the individual fitness of *Episyrphus balteatus*, as a composite performance measure of development, survival and fecundity, was obviously lower in the presence of ants (about half) as opposed to aphid colonies from which ants were excluded. Although we did not study ant behaviour directly, declines in predator performance can be attributed to the aggressive behaviour of tending ants towards predator larvae (Oliver et al., 2008). The negative impacts arising from ants can be divided into lethal (ants prey on hoverfly larvae) or sub-lethal effects (such as interference in predation and consequently insufficient nutrition). As a result, in the presence of ants, many *E. balteatus* larvae could not complete development, and survivors had longer developmental times, with relatively few emerging as adults (Fig. 2a and b). Sloggett and Majerus (2003) have shown similar results in relation to ladybirds.

If a generalist predator suffers a reduction in fitness due to interference by third parties such as mutualistic ants, we would expect adult females to avoid selecting such kinds of prey. Here, hoverfly females showed significant oviposition avoidance toward aphid colonies with ants present. Only a small percentage (ca. 25%) of eggs were laid on host plants with ants, and furthermore the percentage hatch was much lower (Fig. 3a and b). Oliver et al. (2008) showed similar results for *Adalia bipunctata* as a result of ant attack (*Lasius niger*). They found that ant semiochemicals effectively repelled predators from the homopteran resource. They also showed that there may be a threshold of semiochemical concentration determined by ant density at the prey colony, below which the oviposition strategy remains normal. Harmon and Andow (2007) showed that the oviposition strategy of ladybirds is determined
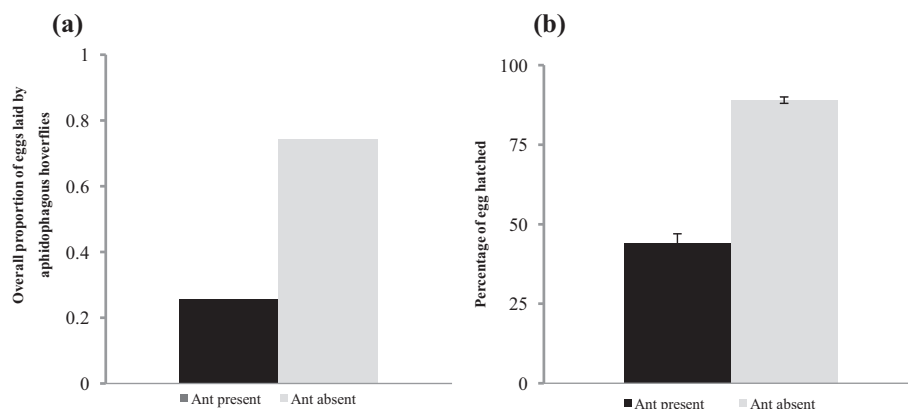
Fig. 3. Overall proportion of eggs laid by aphidophagous hoverflies (mean ± SE) in response to ant present/absence at the colony of *myzus persicae* (a) and (b) percentage of egg hatched laid by hoverfly females.

by ant density relative to aphid abundance. In our study, although host plant did not have a significant effect on the abundance of ants in the aphid colony (Fig. 1a), ant density showed a positive relationship with colony development (Fig. 1b and c). As the aphid population increased, the abundance of guarding ants increased to disrupt and deter aphidophagous predators. An alternative explanation is that the increase in ants was not to protect the aphids, but rather that they were simply attracted by the increased resource, and in turn this protected the aphids more. Our findings support these density-dependent mutualism hypotheses.

When both aphids and attendant ants are found together in a colony, their interactions should positively alter aphid population growth, if the species interaction is mutualistic. When separated, negative or neutral population change should occur. Our results support this hypothesis: aphids with ants present had a higher per capita population growth rate than those without ants.

It is well known that ants encourage aphids to produce more honeydew ('milking'). As a result, tended aphids should increase their feeding rate on their host plants and hence prey toxicity acquired from the host plant may change in the presence of ants. Black mustard had high levels of a common glucosinolate (sinigrin), and we suspect that the presence of ants effectively enhanced the toxicity of prey (*Myzus persicae*) for the predator (*E. balteatus*). However, there was no significant interaction effect between the presence of ants and of sinigrin (Table 1). It could be that when feeding on brassicaceous plants, the generalist aphid (*M. persicae*) excretes most of the glucosinolates in its honeydew, whilst specialist herbivores such as *Brevicoryne brassicae* sequester these compounds (Kos et al., 2011; Amiri-Jami et al., 2016a). Consequently, the generalist aphid may receive little or no benefit from host-plant secondary metabolites against its predators.

We found that *E. balteatus* fed on *M. persicae* on the high-sinigrin host plant (black mustard) had a significantly lower fecundity and longer development time (and hence lower individual fitness) than when fed on the same prey on the zero-sinigrin host plant (Tables 1 and 3). These two brassicaceous plants may differ in many respects other than sinigrin, such as amino-acid content and glucosinolate profile (e.g., Cole, 1997; Francis et al., 2004). For predators there is presumably little difference between their nutritional requirements and what prey provide, and additional factors such as prey toxicity are probably relatively more important (e.g. Chaplin-Kramer et al., 2011; Kos et al., 2011; Goodey et al., 2015; Amiri-Jami et al., 2016a,b). However, just as there was no significant interaction between the presence of ants and sinigrin, similarly which host-plant was involved had only a weak flow-through effect on the performance of the predatory hoverfly (*E. balteatus*).

In conclusion, our data show that the presence of mutualistic ants can completely alter the aphid-aphidophage interaction, at least for a generalist hoverfly. We did not find any interaction between ant presence and glucosinolate levels, perhaps because our experiments involved a generalist rather than a specialist aphid. Overall, the indirect benefits from ant-tending (reduced predation) to aphids suggest the occurrence of enemy-free space. Because of poor performance in the presence of ants, at least one generalist aphidophagous hoverfly has an avoidance response that benefits it by reducing the costs of predation. Thus, the spatial distribution of ants that share mutualisms with aphids is likely to influence the spatial distribution of the predators. With respect to biocontrol measure, our findings imply that the efficiency of one of the most important of the aphidophagous hoverflies in controlling aphids might be lowered in the presence of ants in aphid colonies. Myrmecophilous predators probably performa better when they feed on ant-tended aphids, and subsequently they may effectively suppress such aphids while other predators are often forced to evade ants. Improved knowledge about myrme-cophily in relation to biocontrol agents (e.g. predators and parasitoids) may help to manage aphidophages better and hence improve biological control strategies. It would therefore be interesting to study hoverfly adaptations to predation on myrmecophilous aphids.

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