Dealing with food shortage: larval dispersal behaviour and survival on non-prey food of the hoverfly *Episyrphus balteatus*

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Abstract. 1. Predatory larvae often have to face food shortages during their development, and thus the ability to disperse and find new feeding sites is crucial for survival. However, the dispersal capacity of predatory larvae, the host finding cues employed, and their use of alternative food sources are largely unknown. These aspects of the foraging behaviour of the aphidophagous hoverfly (*Episyrphus balteatus* De Geer) larvae were investigated in the present study.

2. It was shown that these hoverfly larvae do not leave a plant as long as there are aphids available, but that dispersing larvae are able to find other aphid colonies in the field. Dispersing hoverfly larvae accumulated on large aphid colonies, but did not distinguish between different pea aphid race–plant species combinations. Large aphid colonies might be easier to detect because of intensified searching by hoverfly larvae following the encounter of aphid cues like honeydew that accumulate around large colonies.

3. It was further shown that non-prey food, such as diluted honey or pollen, was insufficient for hoverfly larvae to gain weight, but prolonged the survival of the larvae compared with unfed individuals. As soon as larvae were switched back to an aphid diet, they rapidly gained weight and some pupated after a few days. Although pupation and adult hatching rates were strongly reduced compared with hoverflies continuously fed with aphids, the consumption of non-prey food most probably increases the probability that hoverfly larvae find an aphid colony and complete their development.

Key words. Aphids, hoverflies, non-prey food, predatory larvae, searching behaviour, Syrphidae.

Introduction

The survival of insects depends on several crucial events such as finding the right food or avoiding predation. Most of the decisions an insect makes directly influence the individual itself, but ovipositing females make decisions that influence their offspring. In most insect species, females oviposit on or close to larval food sources and often select oviposition sites that maximise larval survival (Thompson & Pellmyr, 1991; Ohsaki & Sato, 1994; Singer *et al.,* 2004; Gripenberg *et al.,* 2010). But unpredictability of food sources or trade-offs between female foraging and offspring performance (Thompson, 1988; Scheirs & De Bruyn, 2002) might lead the egg-laying female to make

suboptimal decisions. In these cases it would be an advantage if developing larvae could disperse and find new feeding sites. Whereas dispersal of insect larvae has been reported (Doak, 2000), little is known about their actual dispersal capacities and how they orient if they switch feeding sites (Chew, 1977; Bernays & Chapman, 1994; Berdegué *et al.,* 1998; Soler *et al.,* 2012). Whilst herbivorous larvae are often attracted by volatiles from their host plants (e.g. Visser, 1986; Dickens, 2002; Castrejon *et al.,* 2006; Becher & Guerin, 2009; Soler *et al.,* 2012), studies considering the orientation of predatory larvae are rare (Branco *et al.,* 2006), even though predatory larvae are more likely than herbivorous larvae to encounter food shortages. This is especially true if predatory larvae are specialised on prey with an unpredictable distribution. Aphids, for example, are a highly unpredictable food source. Even though aphid colonies can survive sporadically up to 50 days, the majority of aphid colonies survive less than a week (e.g. Weisser, 2000; Weisser & Härri, 2005; Outreman *et al.,* 2010; Vosteen *et al.,* 2016a,b). The development of ladybird larvae usually takes more than 2 weeks

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while larvae of the hoverfly *Episyrphus balteatus* pupate after as little as 8 days under optimal conditions, but need much longer if temperatures are below 17 °C (Hart *et al.,* 1997; Lanzoni *et al.,* 2004). Larval development may therefore take much longer than the availability of a single aphid colony, and consequently larval development in most cases cannot be completed with one aphid colony as a food source. In the search for additional food to complete their development, it is known that aphidophagous ladybird larvae use pollen, extrafloral nectar and foliage as alternative food sources and are often able to complete their development with these alternative food sources (Lundgren, 2009). Aphidophagous hoverfly larvae are thought to feed mainly on aphids, even though consumption of other soft-bodied prey has also been reported. It is further assumed that they only have a limited dispersal capacity (e.g. Sadeghi & Gilbert, 2000; Rojo *et al.,* 2003; Almohamad *et al.,* 2009; Gomez-Polo *et al.,* 2015). Our field observations showed, however, that hoverfly larvae left plants if aphid colonies went extinct (Vosteen *et al.,* 2016a,b). As nothing is known about the dispersal behaviour of hoverfly larvae, we aimed to find out whether movement between aphid colonies is a general behaviour in *E. balteatus* larvae and under what circumstances they leave a plant.

If hoverfly larvae leave plants, they also have to find new aphid-infested plants. The decision to climb a certain plant might depend not only on the availability of aphids but also on the plant species itself, possibly due to factors like plant architecture or surface structures. For instance, it was shown by Verheggen *et al.* (2009) that trichomes hamper the movement of hoverfly larvae. To test whether different plant–aphid combinations have altered attractiveness for hoverfly larvae, we used different combinations of pea aphid races and legume species. The pea aphid (*Acyrthosiphon pisum* HARRIS) is actually a species complex consisting of at least 15 genetically distinct host races which are native to particular legume species, but can all develop very well on the universal host plant *Vicia faba* (Ferrari *et al.,* 2006; Ferrari *et al.,* 2008; Peccoud *et al.,* 2009a; Schwarzkopf *et al.,* 2013; Peccoud *et al.,* 2015). It is assumed that natural enemies contribute to the maintenance of the different host races by preferring to prey on aphids living on the general host plant *V. faba*, and therefore minimising the occurrence of mixed colonies (Vosteen *et al.,* 2016a). After we had repeatedly found that *E. balteatus* prefers to oviposit on *V. faba* and *Pisum sativum* (Vosteen *et al.,* 2016a,b), our first guess was that this preference could be due to differences in hoverfly larval performance on aphids from the different legume plants, as was found for ladybirds and lacewings (Giles *et al.,* 2000, 2001, 2002). We further wondered if dispersing hoverfly larvae would prefer certain host race–plant species combinations as this may impact aphid colony survival on the different host plant species.

To get a better understanding of the effect of hoverfly larvae on aphid colony development in the field, we investigated their food preferences and dispersal behaviour and focused on the following questions: do hoverfly larvae leave a plant only after most aphids are consumed; and is this dispersal a general phenomenon? If hoverfly larval dispersal is a common behaviour, the larvae not only have to decide when to leave a plant but also have to find a new aphid-infested plant. We tested whether hoverfly larvae were able to find aphid-infested plants

and whether aphid colony size or different pea aphid–plant combinations changed the attractiveness for the hoverfly larvae. We also explored the use and impact of alternative food during transit to new aphid colonies.

Material and methods

Organisms

Three different host races of the pea aphid complex were used for this study: the *Trifolium* race (clone T3-8V1), the *Pisum* race (clone P136) and the *Medicago* race (clone L1–22). They were originally collected from their native host plants *Trifolium pratense* L., *P. sativum* L. and *Medicago sativa* L., respectively, and genotypically assigned to the specific host race (for detailed information, see Table S1 in Peccoud *et al.*, 2009b). Stock cultures of each race were maintained for several generations in a climate chamber (20 °C, LD 16:8 h, 70% RH) on their native hosts and on the universal host plant *V. faba* L. Plants used in the experiments and for aphid rearing were 3–4 weeks old and were cultivated in soil (7:20 mixture of Klasmann Tonsubstrat and Klasmann Kultursubstrat TS1) in climate chambers under the same conditions. *P. sativum* cv. 'Baccara' and *V. faba* cv. 'The Sutton' were grown individually in pots (diameter 10 cm), while *T. pratense* cv. 'Dajana' and *M. sativa* cv. 'Giulia' were grown in groups of three to seven plants to get a similar plant biomass in each pot. All plants hosting aphids were covered with air-permeable cellophane bags (18.8 × 39 cm; Armin Zeller, Nachf. Schütz & Co, Langenthal, Switzerland) to prevent the escape of aphids.

Hoverfly eggs (*E. balteatus*) were obtained from a commercial supplier (Katz Biotech AG, Baruth, Germany) and hatching larvae were fed with aphids until they were used in the experiments (for rearing details, see the description of experiments). Rearing of insects and all laboratory experiments were done in climate chambers under the same conditions. The leaving rate and performance experiments were performed in insect-rearing tents (60 × 60 × 60 cm; Bugdorm, MegaView Science Co. Ltd, Taiwan).

Leaving rate experiment

To test if hoverfly larvae leave an aphid-infested plant if aphid-infested and non-infested plants are present in the vicinity, four plants (two aphid-infested and two uninfested plants) were placed in an insect tent. The two aphid-infested plants were placed 10 cm apart. The two non-infested plants were placed orthogonal to the infested plants such that the non-infested plants were 10 cm away from the aphid-infested plants, and the four plants were at the vertices of a rhombus (Fig. 1). To prepare the aphid-infested plants, *V. faba* were infested with 40 adult *Pisum* race aphids 1 day before the start of the experiment. One hoverfly larva was placed on one of the aphid-infested plants and the position of the larva was noted 24 h later. Due to lack of space, the 10 replicates were carried out on two consecutive days. Hoverfly larvae used in the experiment were taken from
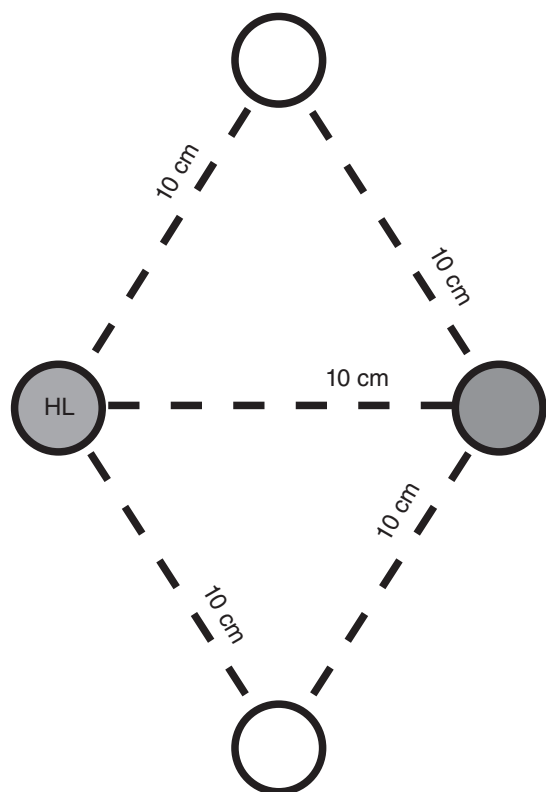
Fig. 1. Experimental setup of the leaving rate experiment. Grey circles represent aphid-infested plants; white circles represent non-infested plants. One hoverfly larva (HL) was placed on one of the aphid-infested plants.

heavily *Pisum* race aphid-infested *V. faba* plants where they could develop until they were 5–6 days old.

Larval preference – effect of aphid number

Experimental *V. faba* plants were infested with 0, 10, 20, 40 and 60 adult *Pisum* race aphids 1 day before they were used in the experiment. Plants were arranged in an insect-rearing tent randomly around a Petri dish at an equal distance from each other and 10 cm from the Petri dish. Each plant treatment was present once in each tent. Eight larvae were placed in the Petri dish and were allowed to forage for 24 h in the tent, after which their position and the number of surviving aphids on each plant were recorded. Due to spatial limitations, the 20 replicates were done on four consecutive days. Hoverfly larvae were kept on *V. faba* plants that were strongly infested with *Pisum* race aphids until they were 3–6 days old and were then used in the experiment. Every day the biggest larvae from the cohort were selected for the experiments.

Larval preference – effect of plant species

To test if hoverfly larvae prefer certain host plant–aphid race combinations, 20 adult aphids of each of the three pea

aphid host races were placed either on their native host plant or on the universal host plant 1 day before the start of the experiment. Plants were again arranged in a random order in the insect-rearing tents 10 cm away from a Petri dish which contained 10 hoverfly larvae. Each plant treatment was present once in each tent. Larvae were allowed to forage for 21 h and their position and the number of surviving aphids were recorded at the end of the experiment. Due to ageing of hoverfly larvae, the experiment was done with two larval cohorts. The first cohort was reared in a large plastic box ($19 \times 25 \times 39$ cm, covered with gauze) that contained 15–20 *Raphanus sativus* var. *sativus* infested with *Myzus persicae* aphids to avoid habituation to cues from the plant species used in the experiments. Due to the low body mass and slow growth of *M. persicae*, larvae had to be fed additionally with a mixture of pea aphids that contained all three host races, reared on either their native host plant or the universal host plant. Because of the unavailability of sufficient amounts of *M. persicae*, the second cohort was fed daily with a mixture of only pea aphids that contained the same amounts of the three host races, reared on either their native or the universal host plant. In order to avoid any effect of previous plant experience on larval preference, the second cohort was reared without plants in a plastic box ($16 \times 12 \times 6$ cm, covered with air-permeable cellophane to prevent escape of larvae). Larvae developed slower under these rearing conditions than in the previous experiment. Therefore, larvae were used at the age of 5–9 days, which was still some days before pupation. Due to spatial limitations, only five replicates could be done simultaneously. With the first larval cohort, 25 replicates were done on five consecutive days, while with the second larval cohort 20 replicates were done on four consecutive days.

Hoverfly larval distribution in the field

To test how hoverfly larvae are distributed in the field, native and universal host plants were each infested with 10 aphids (3 days old) of the *Trifolium*, *Pisum* and *Medicago* races. After 8 days of aphid colony growth, plants were placed in the field on 14 June 2013 and were subjected to the natural hoverfly population. Each plant–aphid combination was replicated 12–17 times, depending on the availability of plants (Table 1). The aphid-infested plants were distributed in three double rows which were 3 m apart from each other. The order of the different plant–aphid combinations within and between the rows was completely random. Plants within one double row were placed 35 cm apart and the leaves did not touch each other. To prevent the escape of aphids, the plants were tied to sticks to ensure an upright position. The pots (diameter 10 cm) containing the aphid-infested plants were placed in bigger pots (diameter 19 cm) which were half-filled with soil, thus all aphids dropping off the plant would fall into the big pot. Fluon (Sigma-Aldrich Chemie GmbH, Munich, Germany) covering the inner and outer sides of the big pots hindered the aphids from leaving the pot. After 7 days in the field, plants were brought into the laboratory and the numbers of aphids, hoverfly larvae, and hoverfly eggs on the different host plants were counted. The conditions of the hoverfly eggs were examined in order to estimate the age of the eggs

Table 1. Number of replications of each treatment of the field experiment.

Aphid race	Host plant	Number of replications
Pisum race	*Pisum sativum*	17
Pisum race	*Vicia faba*	12
Trifolium race	*Trifolium pratense*	15
Trifolium race	*Vicia faba*	13
Medicago race	*Medicago sativa*	17
Medicago race	*Vicia faba*	12

Table 2. Different diets that were used to test the effect of non-prey diet on hoverfly larval development.

Treatment	Phase 1 (day 0–3)	Phase 2 (day 4–9)	Phase 3 (day 10–20)
Water	Aphids	Water (no food)	— (larvae were dead)
Aphids	Aphids	Aphids	— (larvae had pupated)
Honey	Aphids	Honey	Honey
Honey/aphids	Aphids	Honey	Aphids
Pollen	Aphids	Pollen	Pollen
Pollen/aphids	Aphids	Pollen	Aphids
Honey + pollen	Aphids	Honey + pollen	Honey + pollen
Honey + pollen/aphids	Aphids	Honey + pollen	Aphids

and evaluate whether and how many hoverfly larvae hatched on the plants. Dead eggs and hatched eggs can be distinguished from newly laid hoverfly eggs by their texture, which appears dried out compared with a living egg. Therefore, the number of dry eggs is a measure of the maximal number of hoverfly larvae that could have hatched on a plant and reflects hoverfly oviposition at the beginning of the experiment when aphid number was mainly influenced by aphid reproduction rate under laboratory conditions and less so by predation. The majority of hoverfly larvae found on the plants were identified as *E. balteatus*, but some *Scaeva pyrastri* L. were also observed.

Effect of different aphid races on hoverfly larval performance

To test if larval performance differs depending on their food, freshly hatched larvae were placed individually in small Petri dishes (diameter 5.5 cm) that contained a moist piece of paper towel (2 × 2 cm) to prevent desiccation of larvae. Larvae were fed daily with 15 or 40 mg juvenile aphids of the three host races that were reared either on their native host plant or on the universal host plant. Larval survival and development stage (instar and pupal stage) were checked daily. Afterwards larvae were transferred to clean Petri dishes containing moist pieces of paper towel and the aphid prey. At day 7, larval weights were also recorded. Hoverfly development was followed until adults hatched from the pupae. Adults were sexed and their head width, as a robust measurement of the body size, was measured under a stereomicroscope. Due to limitations of space, the experiment was split into two parts. In part A, larvae were fed with *Pisum* and *Medicago* race aphids reared on their native and universal host plants, and in part B larvae were fed with *Trifolium* race aphids reared on the native and universal hosts. To check if larval performance differed between part A and B, larvae fed with *Pisum* race aphids reared on the universal host were again included in part B. Each experimental part was repeated four times, starting each time with a new set of larvae. Each treatment was replicated 32 times. Larval weight was only recorded in part A.

Effect of non-prey food on hoverfly larval performance

In order to test if hoverfly larvae are able to survive on a non-prey diet in the absence of aphids, an experiment that consisted of three phases was designed (Table 2). During the first phase, larvae were kept in groups of approximately 60

larvae in Petri dishes and were fed with *Trifolium* race aphids *ad libitum* for 3 days. In the second phase, larvae were randomly assigned to five different diet treatments, consisting of aphids, water and three different types on non-prey food (honey water, pollen, honey water + pollen). After 6 days – in the third phase of the experiment – half of the larvae that had been fed on a non-prey diet were fed again with aphids until pupation or larval death, mimicking aphid colony finding. The other half was continued on their respective non-prey diet. During the second and third phases of the experiment, larvae were kept individually in small Petri dishes (diameter 5.5 cm) and moved to new Petri dishes with fresh food every day. Honey (diluted 1:10 with water) was supplied on a piece of paper towel (2 × 2 cm). Larvae assigned to the pollen and the mixed honey/pollen treatment received 10–15 mg of mixed pollen each day. Water in the form of a moist piece of paper towel (2 × 2 cm) was provided for all larvae without food or supplied only with pollen. The amount of aphids as food was increased during the course of the experiment according to the increased food consumption of developing larvae. When one or more larvae had consumed all aphids, all larvae were fed a larger amount the following day. During the second phase, larvae were fed with 30 mg of aphids on the first, 40 mg on the second, and 50 mg on the third and fourth days. During the third phase, larvae were fed with 30 mg on the first and second days, 40 mg on the third day and 50 mg on the fourth and fifth days. After the 5th day, larvae were close to pupation and did not feed anymore. Therefore no aphids were added on day 6 or later. Larval survival was recorded every day. Body weight was recorded on days 6, 9 and 12 of the experiment and after larvae had pupated. Pupae were kept until the adults hatched and hatching rate was recorded. Each treatment was replicated 10 times.

Statistical analyses

For all larval preference tests, presence/absence data was analysed with generalised linear mixed models (GLMMs with the glmer function of the lme4 package; Bates *et al.,* 2014) to account for the block design. Experimental tents (blocks) were treated as random effects (random intercept), and treatments as

fixed effects. *P*-values for explanatory variables were obtained by deleting explanatory variables one after another and comparison of the likelihood of the more complex model with that of the simpler model with a χ^2 test (Zuur *et al.,* 2009).

Field data were analysed with generalised linear models (GLMs). Whether the presence of dry hoverfly eggs on a plant was dependent on the host plant–aphid race combination was analysed using a GLM with the Bernoulli error structure. For the analysis of the number of hoverfly larvae and aphids on the plants, a GLM with the Poisson error structure was used. Due to overdispersion, a negative binomial error structure was applied for the analysis of the number of aphids.

Survival data were analysed with Cox proportional hazard models. In the experiment, where larvae were fed with different aphid races reared on native and universal hosts, the amount of food and the plant species–aphid race combination were used as explanatory variables. In the experiment, where larvae were fed with aphids and non-prey diets, diet was used as explanatory variable. Hoverflies that survived until pupation were recorded as surviving until the day they pupated, if they died in the pupa. Those hoverflies that survived the pupal stage were recorded as surviving until the day they hatched from the pupa.

The influence of the amount of food, the kind of food (host plant–aphid race combination) and the sex of the hoverfly on larval weight, larval and total developmental time and head width were analysed using linear mixed effects models (lme function of the nlme package; Pinheiro *et al.,* 2015), to account for the different larval batches used. Larval batches were treated as random effects, and food amount, the kind of food and gender as fixed effects. As only adult hoverflies can be sexed, data were only used from larvae that successfully developed into adult hoverflies.

The influence of diet type on larval weight on day 6 and on pupal weight was analysed using a one-way ANOVA with diet as explanatory variable. The Tukey's honest significant difference test was used for *post hoc* comparison. As those larvae that were fed with aphids or water had pupated or died before day 9 of the experiment, weight changes of hoverfly larvae over time could only be analysed for those larvae that were fed with non-prey food. Weight changes over time were analysed with a linear mixed effects model (lme function of the nlme package; Pinheiro *et al.,* 2015).The identity of each individual larva was treated as a random effect, and food type and time as fixed effects.

The influence of different types of non-prey diet on the survival until pupation was analysed using a GLM with the Bernoulli error structure.

For all analyses except the ANOVA, *P*-values for explanatory variables were obtained by deleting explanatory variables one after another and comparing the likelihood of the more complex model with that of the simpler model with a χ^2 test or likelihood ratio test (Zuur *et al.,* 2009). In cases of significant differences, factor-level reductions were used to reveal differences between levels of a treatment. Therefore, the two most similar factor levels were merged and models before and after merging of factor levels were compared. This procedure was repeated until merging of factor levels led to significant differences between the simpler and more complex models (Crawley, 2013).

Model assumptions were checked by visual inspections of the residual plots. All data were analysed with R version 3.1.1 (R Development Core Team, 2014).

Results

Leaving rate

In the experiment to determine if hoverfly larvae would leave a plant if aphids were still present, nine out of 10 hoverfly larvae survived until the end of the experiment. None of the surviving larvae left the aphid-infested plant within 24 h. They consumed, on average, 17 ± 1.5 of the 40 adult aphids placed on the plant before the start of the experiment.
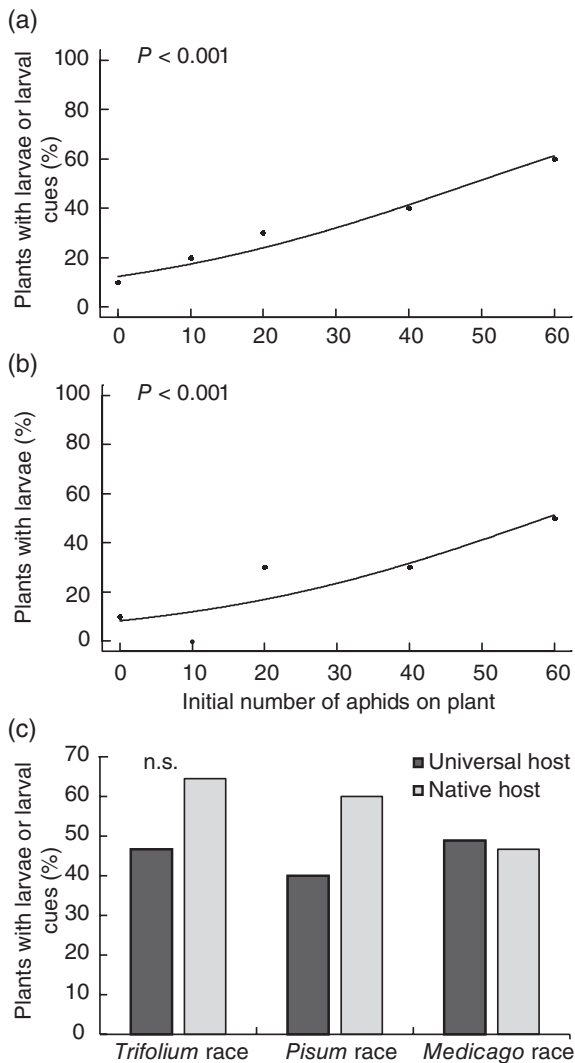
Larval preference

Hoverfly larvae preferred plants with higher numbers of aphids in experiments conducted under laboratory conditions. The number of plants with either hoverfly larvae or showing evidence of previous hoverfly presence (faeces, remains of aphid feeding) significantly increased with the number of aphids infesting the plant ($\chi_1^2 = 14.000$, $P < 0.001$; Fig. 2a). The number of plants where larvae were present at the end of the experiment also increased with the number of aphids infesting the plant ($\chi_1^2 = 13.357$, $P < 0.001$, Fig. 2b). However, the number of plants with either larvae or larval cues present at the end of the experiment was not influenced by the various host plant–aphid race combinations tested ($\chi_5^2 = 8.907$, $P = 0.113$; Fig. 2c).

Hoverfly larval distribution in the field

When aphid-infested plants were placed in the field, the distribution of hoverfly eggs that were laid at the beginning of the experiment, when the aphid population size was not yet altered by predation, was influenced by the host plant–aphid race combination (binomial GLM, likelihood ratio = 19.003, $P = 0.002$). Dried-out eggs, which had been laid during the first days of the experiment, were more frequent on the universal host plant *V. faba* than on the native host plants (Fig. 3a).
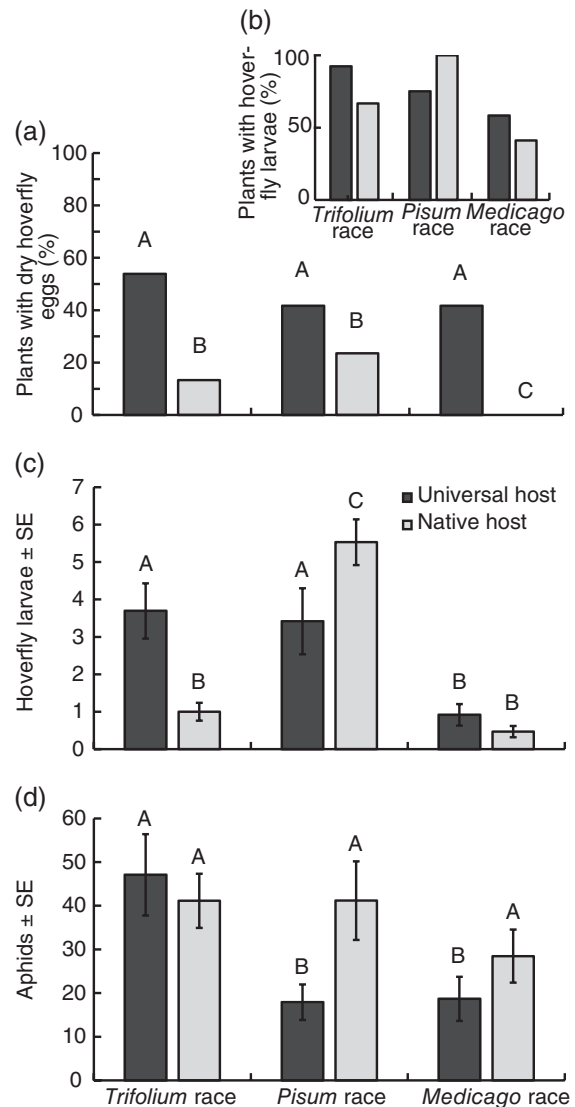
After 7 days in the field, hoverfly larvae (*E. balteatus* and *S. pyrastri*) were present on an average of 70% of all experimental plants (Fig. 3b), whereas hoverfly eggs, which were laid at the beginning of the experiment, were present on an average of only 25% of experimental plants, indicating that most hoverfly larvae had migrated to the experimental plants. The number of hoverfly larvae found on the plants after 7 days was not dependent on the number of hoverfly eggs laid at the beginning of the experiment (Poisson GLM, likelihood ratio = 1.292, $P = 0.256$), but dependent on the host plant–aphid race combination (Poisson GLM, likelihood ratio = 119.339, $P < 0.001$; Table S1). The highest number of hoverfly larvae was found on *P. sativum*, while intermediate numbers of hoverfly larvae were recorded on the universal host, *V. faba*, infested with *Trifolium* or *Pisum* race aphids. The lowest number of hoverfly larvae was found on *T. pratense*, *M. sativa* and *V. faba* infested with

Fig. 2. *Episyrphus balteatus* larval preference depending on pea aphid (*Acyrthosiphon pisum*) population size (a, b) and host plant–pea aphid race combination (c). Graphs depict the proportion of plants with evidence of hoverfly larvae presence (a, c), and the proportion of plants with larvae actually present after 24 h (b). The curves represent the regression lines obtained from the generalised linear mixed effects model. n.s., differences are non-significant.

Fig. 3. Effect of various aphid race–host plant combinations on hoverfly presence in the field. (a, b) Proportion of plants with hoverfly eggs that were laid at the beginning of the experiment (a) and with hoverfly larvae after 7 days (b). (c, d) Number of hoverfly larvae (c) and aphids (d) found on native and universal host plants after 7 days in the field (July 2013). Different letters indicate significant differences. (c, d) Bars represent means with SE.

Medicago race aphids (Fig. 3c). The number of aphids that were present at the end of the experiment had an additional influence on the number on hoverfly larvae (Poisson GLM, likelihood ratio = 8.593, $P = 0.003$; Table S1 (Online Resource 1)) with more larvae on plants with higher aphid numbers.

The number of aphids after 7 days in the field was mainly influenced by the plant species–aphid race combination (negative binomial GLM, likelihood ratio = 15.998, $P = 0.007$; Fig. 3d, Table S2). Fewer aphids were found on *V. faba* infested with *Pisum* and *Medicago* race aphids compared with all other plants. Neither the number of hoverfly eggs at the beginning of the experiment (negative binomial GLM, likelihood ratio = 3.555, $P = 0.059$) nor the number of hoverfly larvae

at the end (negative binomial GLM, likelihood ratio = 3.115, $P = 0.078$) significantly influenced the number of aphids on the plants (Table S2).

Effect of different aphid races on hoverfly larval performance

The amount of aphids offered as food strongly influenced all tested hoverfly parameters. Feeding of more aphids resulted in heavier larvae, shorter larval development and total development, and wider heads of adult hoverflies (Table 3).

Table 3. Influence of food amount (pea aphids), gender of hoverflies and type of food (host plant−aphid race combination) on hoverfly larval weight, larval development time, total development time, and adult head width. Data are means ± SE, and the likelihood ratio (LR) and *P*-values of linear mixed effects models are given. Different letters indicate significant differences between the treatments.

|	Larval weight (mg)	Larval development time (days)	Total development time (days)	Adult head width (mm)
Experiment A				
Food amount	**LR = 57.773**	**LR = 13.875**	**LR = 8.711**	**LR = 18.870**
	***P* < 0.001**	***P* < 0.001**	***P* = 0.003**	***P* < 0.001**
15 mg day^{-1}	22.12 ± 0.78	10.05 ± 0.25	17.63 ± 0.23	4.481 ± 0.047
40 mg day^{-1}	34.18 ± 1.48	9.02 ± 0.30	16.83 ± 0.31	4.761 ± 0.040
Hoverfly gender	**LR = 7.629**	LR < 0.001	LR = 0.124	**LR = 24.941**
	***P* = 0.006**	*P* = 1.000	*P* = 0.724	***P* < 0.001**
Female	26.12 ± 1.19	9.59 ± 0.24	17.29 ± 0.24	4.513 ± 0.033
Male	31.38 ± 1.99	9.41 ± 0.37	17.07 ± 0.35	4.821 ± 0.060
Type of food	LR = 5.882	LR = 1.306	LR = 0.357	LR = 5.545
	P = 0.118	*P* = 0.728	*P* = 0.949	*P* = 0.136
Pisum race from *Vicia faba*	26.15 ± 2.28	10.06 ± 0.42	17.53 ± 0.27	4.723 ± 0.101
Pisum race from *Pisum sativum*	24.93 ± 1.96	9.55 ± 0.47	17.20 ± 0.52	4.553 ± 0.051
Medicago race from *V. faba*	30.15 ± 2.24	9.21 ± 0.31	17.05 ± 0.33	4.635 ± 0.064
Medicago race from *Medicago sativa*	30.49 ± 1.95	9.35 ± 0.41	17.13 ± 0.41	4.601 ± 0.059
Interactions				
Type of food × hoverfly gender	LR = 2.241	LR = 1.591	LR = 2.964	LR = 1.524
	P = 0.524	*P* = 0.661	*P* = 0.397	*P* = 0.677
Food amount × type of food	LR = 1.507	LR = 3.167	LR = 3.847	LR = 3.124
	P = 0.681	*P* = 0.367	*P* = 0.279	*P* = 0.373
Food amount × hoverfly gender	LR = 0.047	LR = 0.328	LR = 0.674	LR = 0.733
	P = 0.828	*P* = 0.567	*P* = 0.412	*P* = 0.392
Experiment B				
Food amount		**LR = 4.026**	LR = 3.459	**LR = 22.420**
		***P* = 0.045**	*P* = 0.063	***P* < 0.001**
15 mg day^{-1}		9.67 ± 0.25	17.70 ± 0.31	2.616 ± 0.030
40 mg day^{-1}		8.93 ± 0.22	16.79 ± 0.26	2.800 ± 0.027
Gender		LR = 0.382	LR = 2.107	**LR = 9.749**
		P = 0.537	*P* = 0.147	***P* = 0.002**
Female		9.19 ± 0.18	16.89 ± 0.25	2.658 ± 0.032
Male		9.4 ± 0.32	17.17 ± 0.35	2.795 ± 0.032
Type of food		**LR = 7.124**	LR = 4.522	**LR = 6.205**
		***P* = 0.028**	*P* = 0.104	***P* = 0.045**
Pisum race from *V. faba*		9.89 ± 0.29 **A**	17.77 ± 0.42	2.623 ± 0.043 **A**
Trifolium race from *V. faba*		9.04 ± 0.26 **B**	17.07 ± 0.34	2.744 ± 0.031 **B**
Trifolium race from *T. pratense*		8.94 ± 0.30 **B**	16.875 ± 0.31	2.767 ± 0.036 **B**
Interactions				
Type of food × hoverfly gender		LR = 0.013	LR = 0.201	LR = 0.608
		P = 0.994	*P* = 0.904	*P* = 0.738
Food amount × type of food		LR = 0.073	LR = 0.214	LR = 0.130
		P = 0.964	*P* = 0.900	*P* = 0.937
Food amount × hoverfly gender		LR = 0.364	LR = 0.039	LR = 0.341
		P = 0.546	*P* = 0.844	*P* = 0.559

Bold text indicates significant differences (*P* < 0.05).

Whilst males and females differed in size (as larvae and as adults), with males being the bigger individuals, they needed similar times for their development (Table 3).

The influence of the kind of aphid food (host plant−aphid race combination) was different for the two experimental parts. It did not have an influence on any of the measured parameters (larval weight, larval and total development time, head width) in Experiment A (*Pisum* race and *Medicago* race from native and universal hosts), while it influenced larval development time and adult head width in Experiment B (*Pisum* race from universal host, *Trifolium* race from native and universal host; Table 2). Larvae fed with *Trifolium* race aphids had a shorter development time than those fed with *Pisum* race aphids. The resulting adult hoverflies also had wider heads when fed with *Trifolium* race aphids (Table 3).

The survival of hoverfly larvae was not influenced by either the amount or the kind of food (host plant−aphid race combination) offered (Experiment A − *Medicago* and *Pisum* race: kind of food, likelihood ratio = 5.159, *P* = 0.161; amount of food, likelihood ratio = 0.352, *P* = 0.553; interaction, likelihood
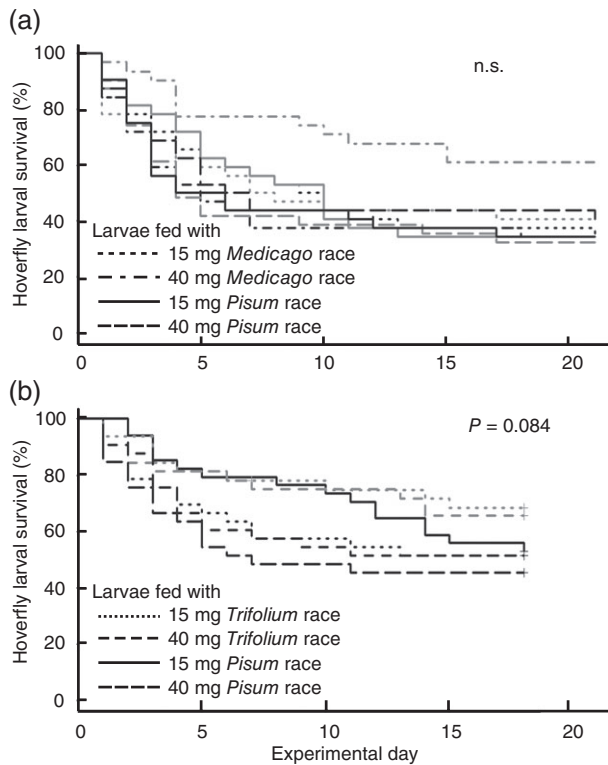
Fig. 4. Kaplan–Meier survival curves of hoverfly larvae fed with different amounts and types of aphids. (a, b) Larvae were fed with different amounts of *Medicago* and *Pisum* race aphids reared on their native and universal host plants (a) or different amounts of *Trifolium* race aphids reared on their native and universal hosts and with *Pisum* race aphids reared on the universal host (b). Dark colours, survival curves of hoverfly larvae fed with aphids originating from the universal host *Vicia faba*; grey colours, survival curves of hoverfly larvae fed with aphids reared on their native host plants. n.s., differences are non-significant.

ratio = 4.070, $P = 0.254$; Experiment B – *Trifolium* and *Pisum* race: kind of food, likelihood ratio = 4.965, $P = 0.084$; amount of food, likelihood ratio = 0.811, $P = 0.368$; interaction, likelihood ratio = 0.667, $P = 0.716$; Fig. 4).

Effect of non-prey food on hoverfly larval performance

To test if hoverfly larvae are able to survive and develop on non-prey diets, larvae that had been fed with aphids for 3 days were kept without food, with no-prey diet (honey, pollen, honey and pollen) or were fed with an excess of pea aphids. Survival differed significantly between the different diets (likelihood ratio = 37.690, $P < 0.001$; Fig. 5a). Non-fed larvae died after 3–5 days without food, while larvae that were kept on a non-prey diet survived up to 17 days without aphids, but were not able to pupate. Survival did not differ between the different types of non-prey food and was not significantly increased if larvae were switched back to an aphid diet on day 9 due to a high mortality in the pupal stage. Hoverflies that were fed with aphids continuously had a high survival (nine out of 10 larvae survived) and pupated when they were 7–9 days old.

Three days after the first diet change (to non-prey diet or water), the weight of hoverfly larvae that were fed with non-prey diet was not significantly different from that of non-fed larvae, but much lower than the weight of aphid-fed larvae ($F_{4, 69} = 406.000$, $P < 0.001$; Fig. 5b). The weight of larvae that were kept on non-prey diet and survived for at least 12 days differed from that of larvae that were supplied again with aphids after day 9 (likelihood ratio = 25.092, $P < 0.001$) and changed over time (likelihood ratio = 44.521, $P < 0.001$; Fig. 5c). Weight development over time depended significantly on food type, because only those larvae that were switched back to an aphid diet could gain weight (likelihood ratio = 97.751, $P < 0.001$). Weight of hoverfly larvae that were kept on non-prey food without additional aphids did not change over time and did not differ between the different non-prey food types (honey, pollen, honey + pollen). After switching back to aphid prey, larvae rapidly gained weight and at the age of 12 days they were significantly heavier than larvae that were kept on non-prey food throughout the experiment.

There is a strong tendency for pupation rate to be reduced when larvae have to overcome some time without aphid prey ($\chi^2 = 7.766$, $P = 0.051$; Fig. 5d). About 40% of the larvae that were switched back to an aphid diet were able to pupate, independent of the type of former non-prey diet, while 90% of larvae that were continuously fed with aphids pupated. Pupal weight was significantly influenced by the food type ($F_{3, 17} = 4.519$, $P = 0.017$; Fig. 5e). Pupae of larvae that were fed with aphids throughout the experiment were significantly heavier than those of larvae fed with honey in the second phase of the experiment and with aphids in the third phase. Larvae with pollen in their diet developed into medium-weight pupae. Of the 13 pupae originating from hoverfly larvae fed with non-prey food (and aphids in the third phase of the experiment), only one (fed with honey and pollen/aphids) hatched, while all pupae arising from larvae fed solely with aphids developed into adult hoverflies.

Discussion

It is generally assumed that hoverfly larvae are sedentary and, therefore, that hoverfly oviposition choice determines the fate of the developing larvae (e.g. Sadeghi & Gilbert, 2000; Almohamad *et al.*, 2009). Oviposition choice was detected in adult hoverflies: they prefer certain aphid species and host races and distinguish between aphids feeding on different plant species. It was shown that they generally prefer aphids that result in a high performance of their offspring and plants where aphids have a high reproductive rate (Almohamad *et al.*, 2007, 2009; Vosteen *et al.*, 2016a,b). However, there are also clear hints from the literature that hoverfly larvae move between aphid colonies. Banks (1968) found several hoverfly larvae in field experiments that must have moved to experimental aphid colonies. Similarly, Kan (1988a,b) observed older hoverfly larvae that moved between aphid colonies in the field, and Chandler (1969) reported that even 'unfed first instar larvae were able to travel considerable distances, certainly well in excess of 1 m'. We found that *E. balteatus* larvae do not leave a plant as
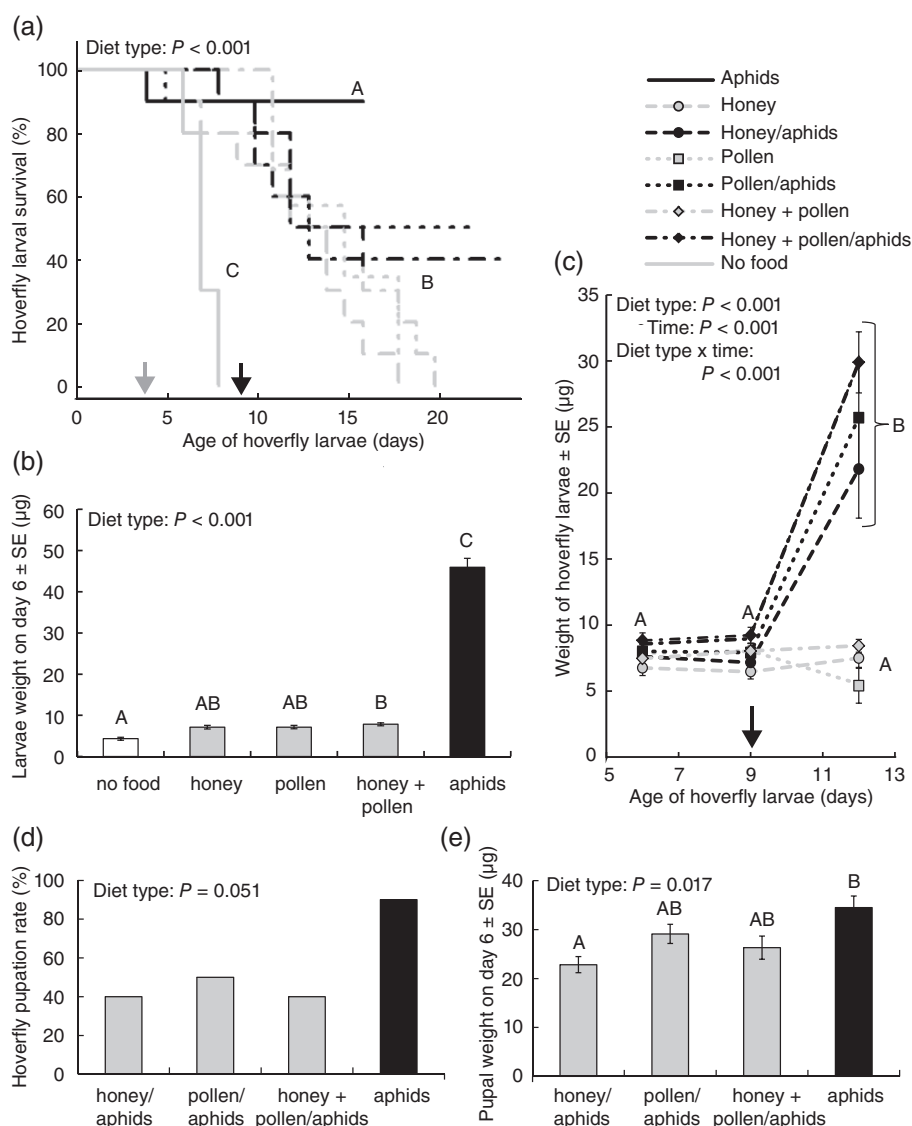
Fig. 5. Survival and performance of hoverfly larvae fed with prey and non-prey food (honey, pollen, honey + pollen). (a) Kaplan−Meier survival curves of hoverflies fed with different types of food; (b) weight of hoverfly larvae after 6 days; (c) weight development of hoverfly larvae fed with non-prey food; (d) pupation rate of hoverfly larvae; (e) weight of hoverfly pupae. Different letters show significant differences among diet treatments obtained by factor-level reductions or Tukey's *post hoc* test. n.s., differences are non-significant. The grey arrow (a) points towards the day when larvae were switched from aphid feeding (first phase of experiment) to different food types (second phase). The black arrow (a, c) indicates the day when half of the larvae were switched from non-prey food (second phase of experiment) to aphid prey (third phase of experiment).

long as there are aphids available as food. However, when most aphids on a plant are consumed, larvae leave the plant to search for additional food to complete their development (Vosteen *et al.,* 2016a,b). Arrival of migrating larvae was observed in our field experiment, where we discovered new hoverfly larvae in aphid colonies. More than half of the observed hoverfly larvae must have moved to the experimental plants because the number of dried-out hoverfly eggs (equivalent to the maximum number of larvae that could have hatched on the plant) was often much lower than the number of larvae actually present. Most likely, the number of dispersing larvae was even higher, as some young

larvae on the plant may have been killed by intraguild predators. Most hoverfly larvae were found on those host plant–aphid race combinations where the aphids have high reproductive rates, such as *V. faba* infested with *Trifolium* or *Pisum* race aphids and *P. sativum* infested with *Pisum* race aphids, but differences between host plants seemed to be less important (Vosteen *et al.,* 2016a). But how do hoverfly larvae find aphid colonies?

We showed that the probability that a dispersing *E. balteatus* larva will visit a certain plant increases with increasing aphid number. As it is known that hoverfly larvae intensify their search activity in areas where honeydew is present (Leroy *et al.,* 2014),

high amounts of honeydew that accumulate on aphid-infested plants and in the vicinity might be an important search cue for *E. balteatus* larvae. Larger aphid colonies produce more honeydew, which increases the probability that larvae will climb plants that contain high numbers of aphids. Once a hoverfly larva has encountered an aphid, it will increase its turning rate and search the surrounding area more thoroughly (Chandler, 1969). This would again increase the probability of encountering more aphids in large colonies and explains why more hoverfly larvae were observed to remain in larger colonies.

As already pointed out, honeydew is a likely cue that informs hoverfly larvae that they are close to an aphid colony. However, it is not known if larvae are able to perceive aphid colonies over greater distances. Honeydew and aphid volatiles can only be perceived over a few centimetres and *E. balteatus* larvae are not attracted by volatiles from pea aphid–*V. faba* complexes (Bargen *et al.*, 1998). This suggests that hoverfly larvae do not perceive aphid colonies over larger distances and that they randomly search the vegetation canopy until they encounter honeydew or aphids. During the local search on an aphid-infested plant, they may mark the areas they have already searched to avoid examining them again, as it is the case for ladybird larvae (Meisner & Ives, 2013). In close vicinity to an aphid colony under attack, they may be attracted by the aphid alarm pheromone (*E*)-*β*-farnesene (Vosteen *et al.*, 2016c).

During their search for aphid colonies, larvae might encounter various aphid–plant species combinations. However, not all aphid colonies might offer suitable prey. Hoverfly performance can depend on the aphid species the hoverfly larvae are feeding on, especially as some aphid species were reported to be toxic for hoverfly larvae (e.g. Ruzika, 1975; Sadeghi & Gilbert, 2000; Almohamad *et al.*, 2007). The suitability of aphids for larval development may also depend on which plant species or cultivar they were feeding on (Giles *et al.*, 2000; Almohamad *et al.*, 2007; e.g.; Vanhaelen *et al.*, 2002; Kos *et al.*, 2011). For example, the mortality of hoverfly larvae fed with the specialist cabbage aphid *Brevicoryne brassicae* increased drastically when aphids had been reared on a glucosinolate-rich host plant as compared with hosts that contain fewer glucosinolates (Vanhaelen *et al.*, 2002; Chaplin-Kramer *et al.*, 2011). Pea aphids reared on *M. sativa* were shown to have a higher caloric content than pea aphids reared on *V. faba*, which results in better survival and faster development of lacewings and ladybirds (Giles *et al.*, 2000, 2001, 2002). However, our experiments showed that aphids of all three pea aphid host races tested allow successful development of *E. balteatus* larvae, and that survival was not influenced by the host plant species the aphid was feeding on. This fits well with our observations that dispersing *E. balteatus* larvae did not prefer any single host plant–aphid race combination in the laboratory experiment and that larvae were found on all combinations in the field experiment.

Assuming that *E. balteatus* larvae forage for aphids randomly within the vegetation canopy, it may take them several days to find another aphid colony. The ability of hoverfly larvae to survive starvation periods increases with larval age: 3-day-old *E. balteatus* are able to survive 3 days of starvation, while 7-day-old larvae survive 6 days without food at 19–21 °C (Rojo *et al.*, 1996). The use of alternative food sources could

further prolong survival while searching for aphids. We showed that compared with starvation, feeding on a non-prey diet significantly prolonged the survival of *E. balteatus* larvae, but that they were not able to gain weight or pupate on such diets. Similar effects of a non-prey diet were observed for ladybird and lacewing development. These larvae survived well on non-prey diets, but development was prolonged or not completed (Limburg & Rosenheim, 2001; Berkvens *et al.*, 2008; Meissle *et al.*, 2014). Interestingly, *E. balteatus* larval weight and survival did not differ between the different types of non-prey diet (honey, pollen, honey + pollen), indicating that none of the non-prey diets, whether protein-rich, sugar-rich or containing ample protein and sugar, was more suitable for larval survival than any of the others. After 6 days on a non-prey diet, half of the larvae were fed with aphids again. These larvae rapidly gained weight and 3 days later weighed three to four times more than those larvae left on non-prey diets. Larvae that experienced an interim period of non-prey food weighed about 50% less and had a lower pupation rate (43%) than larvae that had been fed continuously with aphids (90% pupation rate) even though both food treatments resulted in a similar time of aphid feeding. Similarly, pupae of larvae that had been kept on non-prey diets for 6 days were lighter than pupae of larvae that were fed with aphid continuously, even though this difference was only significant for one diet type and only one hoverfly hatched from a pupa of the non-prey diet cohort. That food shortage drastically reduces larval and pupal survival as well as pupal weight of different hoverfly species was also shown in studies where hoverfly larvae were continuously fed with limited numbers of aphids (Barlow, 1979; Cornelius & Barlow, 1980; Rojo *et al.*, 1996). Interestingly, development times of *Syrphus (Eupeodes) corollae* larvae only increased slightly during food shortage, and larvae pupated after they had consumed half of the energy amount that was consumed by larvae on a non-limited diet, even though pupal weight was decreased by two-thirds and none of the pupae survived (Cornelius & Barlow, 1980). Contrary to these studies, larvae in our experiment were fed with plenty of aphid prey during the feeding phases, but were forced to feed on non-prey food for a period of 6 days during their development. Even though larvae that had to survive on non-prey food were provided with a similar amount of aphids to those that were continuously fed with aphids, they were much lighter as pupae and had lower survival rates, indicating that an interim period on non-prey food decreases the assimilation efficiency of *E. balteatus* larvae.

Our results indicate that, even though feeding on non-prey diets for several days strongly increases larval survival, it has lasting negative effects on *E. balteatus* development. However, these negative effects probably depend on the duration of feeding on non-prey diets and might be lower if larvae are able to consume some insect prey while they search for aphid colonies. Larvae of different aphidophagous hoverflies were found to prey to some extent on other soft-bodied prey as well, such as thrips, whiteflies, mealybugs and springtails (Rojo *et al.*, 2003; Gomez-Polo *et al.*, 2015). Studies on intraguild predation further show that hoverfly larvae are able to consume other non-aphid prey if these insects are small enough to be caught (Hindayana, 2001; Fréchette *et al.*, 2007). Thus, it is likely that

hoverfly larvae would prey mostly on small insects that they encounter during their search for aphids and might only consume non-prey food to avoid starvation.

In Europe *E. balteatus* is the most abundant aphid predator in many crop species (e.g. Cowgill *et al.,* 1993; Miñarro *et al.,* 2005; Pineda & Marcos-García, 2008a) and is commercially available for releases in horticulture. While most work has focused on optimising conditions for adult hoverflies (e.g. Cowgill *et al.,* 1993; Pineda & Marcos-García, 2008b; van Rijn *et al.,* 2006), our work suggests that there may be some potential to increase larval survival in agricultural crops. Provision of non-pest prey to hoverfly larvae may help to build up hoverfly populations in crops for preventive biocontrol efforts, if ambient infestation levels are too low to allow larval survival. Similarly, in curative biocontrol, provision of non-pest prey may increase the survival of highly voracious late-instar larvae during dispersal to new aphid colonies after the first aphid colony has been depleted. In horticulture, biocontrol of aphids with *E. balteatus* can be hampered by the high dispersal capacity of adult females, which often leave the greenhouse before they start ovipositing (Pineda & Marcos-García, 2008b). Thus, it may be more feasible to release eggs (Leroy *et al.,* 2010) on equally spaced aphid-infested plants and let the highly mobile larvae disperse to adjacent aphid-infested plants.

Our study shows that the fate of *E. balteatus* larvae does not depend purely on the oviposition choice of female hoverflies, but that larvae actively forage for aphid colonies and can survive several days on non-prey food. Future work should focus on identifying the cues used during foraging and determining whether hoverfly larvae are able to survive and develop on non-aphid prey. From an applied perspective, it is important, to test whether the provision of non-pest prey increases biocontrol of aphids in agricultural fields and greenhouses.

Conclusion

One aphid colony is often not sufficient to enable complete hoverfly larval development and so older hoverfly larvae sometimes migrate in search of other aphid colonies. We showed that larvae of *E. balteatus* accumulate in large aphid colonies, probably because these are easier to detect and the act of encountering aphids or honeydew stimulates larvae to stay on heavily infested plants. Hoverfly larvae are probably not able to use long-range cues to detect an aphid colony over larger distances, but instead search the vegetation canopy randomly until they encounter honeydew. Feeding on non-prey food increases the survival of *E. balteatus* larvae and thus the chances of encountering another aphid colony on which they can complete their development.

Acknowledgements

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Statistical values for the analysis of hoverfly larval number after 7 days in the field: GLM with Poisson error structure. Fixed effects: *Trifolium*, *Pisum* and *Medicago* race aphids on their native and universal host plants (aphid race−plant species combination), number of hoverfly eggs that were laid at the beginning of the experiment, and aphid number after 7 days in the field.

Table S2. Statistical values for the analysis of aphid number after 7 days in the field: GLM with negative binomial error structure. Fixed effects: *Trifolium*, *Pisum*, and *Medicago* race aphids on their native and universal host plants (aphid race−plant species combination), number of hoverfly eggs that were laid at the beginning of the experiment, and number of hoverfly larvae after 7 days in the field.

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