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# Associative learning of host presence in non-host environments influences parasitoid foraging

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**Abstract.** 1. Parasitoids are known to utilise learning of herbivore-induced plant volatiles (HIPVs) when foraging for their herbivorous host. In natural situations these hosts share food plants with other, non-suitable herbivores (non-hosts). Simultaneous infestation of plants by hosts and non-hosts has been found to result in induction of HIPVs that differ from host-infested plants. Each non-host herbivore may have different effects on HIPVs when sharing the food plant with hosts, and thus parasitoids may learn that plants with a specific non-host herbivore also contain the host.

2. This study investigated the adaptive nature of learning by a foraging parasitoid that had acquired oviposition experience on a plant infested with both hosts and different non-hosts in the laboratory and in semi-field experiments.

3. In two-choice preference tests, the parasitoid *Cotesia glomerata* shifted its preference towards HIPVs of a plant–host–non-host complex previously associated with an oviposition experience. It could, indeed, learn that the presence of its host is associated with HIPVs induced by simultaneous feeding of its host *Pieris brassicae* and either the non-host caterpillar *Mamestra brassicae* or the non-host aphid *Myzus persicae*. However, the learned preference found in the laboratory did not translate into parasitisation preferences for hosts accompanying non-host caterpillars or aphids in a semi-field situation.

4. This paper discusses the importance of learning in parasitoid foraging, and debates why observed learned preferences for HIPVs in the laboratory may cancel out under some field experimental conditions.

**Key words.** Associative learning, *Cotesia glomerata*, dual herbivory, field-tent experiment, non-host, parasitoid behaviour.

## Introduction

The ability to learn to associate certain cues with the presence of a food reward is important for animals to optimise their foraging success (Ishii & Shimada, 2010; Hoedjes *et al.*, 2011; Mery, 2013; Dridi & Lehmann 2016). Parasitic wasps, or parasitoids, lay their eggs in or on other arthropods, their hosts. The larvae emerging from these eggs feed on this host and eventually the host dies (Godfray, 1994). To find the herbivorous hosts in the complex environment in which they forage, parasitoids

use direct cues from the host (e.g. from frass) (Van Alphen *et al.*, 2003; Colazza *et al.*, 2014) and indirect cues produced by the food plant of the host in response to herbivore feeding (herbivore-induced plant volatiles, HIPVs) (Vet & Dicke, 1992; Van Alphen *et al.*, 2003; Hare, 2011). By learning to associate such cues with the presence of hosts, parasitoids can increase their foraging efficiency (Ishii & Shimada, 2010; Hoedjes *et al.*, 2011; Mery, 2013).

In natural situations, hosts often share their food plant with non-host herbivores that are not suitable for parasitoid larval development. Mixed infestations of plants with host and non-host herbivores affect the HIPVs and herbivore-related cues that parasitoids encounter during foraging for hosts (De Rijk

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*et al.*, 2013). These cues may be more similar when host and non-host herbivores are of the same feeding guild than when herbivores differ in feeding guild. At the level of indirect cues, phloem-feeding herbivores like aphids generally induce the emission of volatile compounds regulated by the salicylic acid signal-transduction pathway of the plant, whereas leaf-chewing herbivores such as caterpillars generally induce the emission of a different group of volatiles regulated by the activation of the plant jasmonic acid pathway (Arimura *et al.*, 2011). In fact, when phloem feeders and leaf chewers attack the same plant, the plant signal-transduction pathways can interact (cross-talk), and consequently a unique volatile blend is emitted (Dicke *et al.*, 2009; Thaler *et al.*, 2012). In addition, the direct cues produced by phloem feeders and leaf chewers are dissimilar, e.g. different body odours, frass, and patterns of feeding damage. By the induction of different volatile blends and the production of different herbivore-related direct cues, the presence of non-host herbivores with various feeding styles partly determines the olfactory environment of the parasitoid (De Rijk *et al.*, 2013; Aartsma *et al.*, 2017). For a parasitoid associated with a caterpillar host, non-host caterpillars on neighbouring or host-infested plants were found to reduce host location, whereas parasitoids found hosts on plants infested with aphids more readily (Bukovinszky *et al.*, 2012; De Rijk *et al.*, 2016a). In diverse herbivore communities, individual herbivore species were found to influence parasitoid host location more strongly than diversity *per se* (De Rijk *et al.*, 2016b). Learning to associate HIPVs of a specific plant–host–non-host complex with host presence may allow parasitoids to fine-tune their foraging in environments with various non-host herbivore species.

Although thorough laboratory studies have identified the potential of learning by parasitoids as well as some of the underlying mechanisms (Tumlinson *et al.*, 1993; Turlings *et al.*, 1993; Vet *et al.*, 1995; Hoedjes *et al.*, 2011; Wäschke *et al.*, 2013), the significance of learning in foraging under complex field conditions has seldom been studied (Lewis & Martin, 1990; Mery, 2013, but see Papaj & Vet, 1990; Hare *et al.*, 1997; Kruidhof *et al.*, 2015). The few field studies show that both predators and herbivores have enhanced foraging success under field conditions when they associated the presence of their food with a distinct odour (Zrelec *et al.*, 2013; Janssen *et al.*, 2014). In parasitoids, oviposition experience on a host-infested plant enhanced the number of host patches visited per time unit in a field-tent setup. The learned cue associated with host presence allowed experienced parasitoids to find host patches more efficiently than did naive parasitoids when host patches were surrounded by other vegetation (Kruidhof *et al.*, 2015). However, how learning may benefit parasitoids in foraging under complex field conditions at the level of the herbivore community has not been studied.

Here, we studied, in a field-tent setup, the adaptive nature of learning in the parasitoid *Cotesia glomerata* foraging for its preferred host *Pieris brassicae* when it had acquired oviposition experience on a plant–host–non-host complex with its associated odours in the laboratory. In laboratory settings, *C. glomerata* is able to associate volatiles produced by plants in response to host feeding with the presence of the host (Geervliet *et al.*, 1998a,b; Bleeker *et al.*, 2006; Smid *et al.*, 2007; Kruidhof

*et al.*, 2012a,b). In this study we tested if *C. glomerata* could learn to associate cues induced and produced by simultaneous feeding of host and non-host herbivores with the presence of the host. We specifically tested whether conditioning on a plant–host–non-host complex with different non-hosts, i.e. the aphid *Myzus persicae* or caterpillars of *Mamestra brassicae*: (i) resulted in a learned preference for the specific conditioned plant–host–non-host complex in two-choice tests between the aphid and caterpillar plant–host–non-host complex; and (ii) led to enhanced parasitism rates of hosts on the conditioned plant–host–non-host complex over the non-conditioned complex in a field-tent environment. We predicted that parasitoids in the two-choice test would show a preference for the plant infestation type previously experienced, or show no preference when both plant infestation types were experienced, as a consequence of associative learning (Vet *et al.*, 1990). Previously we found that after landing on a plant infested with hosts and non-hosts, *C. glomerata* was more efficient in finding its host in the presence of non-host phloem feeders than in the presence of non-host leaf chewers (De Rijk *et al.*, 2016a). Based on this finding, we additionally predicted that parasitoids that previously experienced a plant infested by hosts plus non-host aphids would prefer to land on these plants and would be able to find hosts more quickly than on plants with hosts and non-host caterpillars. This would lead to overall higher parasitisation efficiency (number of hosts parasitised per time unit) for parasitoids that learned that hosts were present on aphid-infested plants compared with parasitoids that experienced host presence on a plant infested by hosts plus non-host caterpillars. Parasitoids that previously experienced both types of plant infestation were expected to show an intermediate parasitisation efficiency.

## Materials and methods

### Plants and insects

*Brassica oleracea* var. *gemmifera* Cyrus plants were used in the experiments. The plants were grown in a greenhouse ( $20 \pm 2$  °C, RH  $60 \pm 10\%$ , LD 16:8 h) in 0.7-litre pots containing potting soil (Lentse potgrond, no. 4; Lent, the Netherlands). Two to three times per week the plants were fertilised with a liquid fertiliser (Kristalon; Yara Vlaardingingen B.V., Vlaardingingen, the Netherlands). Cultures of the host caterpillar *Pieris brassicae*, non-host caterpillar *Mamestra brassicae* and non-host aphid *Myzus persicae* were maintained on *B. oleracea* plants in a greenhouse ( $20 \pm 2$  °C, RH  $60 \pm 10\%$ , LD 16:8 h). For both experiments, first-instar caterpillars and wingless adult aphids were used. A culture of *C. glomerata* was maintained on *P. brassicae* caterpillars in a greenhouse ( $20 \pm 2$  °C, RH  $60 \pm 10\%$ , LD 16:8 h). Parasitoid cocoons were kept in a climate cabinet (21 °C, LD 16:8 h) away from plant volatiles and emerged adults were provided with water and honey *ad libitum*. The parasitoids were allowed to mate and 1- to 6-day-old inexperienced female parasitoids were conditioned and tested in the experiments. The parasitoid *C. glomerata* is specialised on caterpillars of the genus *Pieris*, but is a generalist within this genus, with *P. brassicae* as its preferred host (Geervliet *et al.*, 2000). *Pieris* caterpillars are oligophagous, feeding on plants within the

Brassicaceae family and both the parasitoid and the caterpillar are gregarious species.

#### *Preparation of plants*

Plants used for the conditioning of parasitoids were prepared 3 days in advance in order to allow plants to fully deploy their response to the inducing herbivores. To create host- plus non-host aphid-infested plants, 10 first-instar *P. brassicae* and 40 *M. persicae* individuals were transferred to the youngest fully expanded leaf of a 5-week-old plant. Of those, all aphids and five caterpillars were placed in a clip cage (diameter 60 mm) to ensure concentrated feeding damage by both herbivore species and to provide sufficient leaf area for feeding during the confinement in the clip cage. Because of limited leaf area inside the clip cage, the remaining five caterpillars were placed outside the clip cage. Host- plus non-host caterpillar-infested plants were prepared in a similar way, but instead of 40 aphids, 10 second-instar *M. brassicae* caterpillars were transferred to the plant. Of those, five were placed inside the clip cage together with the five *P. brassicae* caterpillars. To prevent the herbivores outside of the clip cages from moving to other leaves, cotton wool was wrapped around the stalk of the leaf and a fine mesh bag (35 × 27 cm) was placed around the leaf. Prepared plants were kept in a greenhouse (23 ± 2 °C, RH 60%, LD 16:8 h). Before conditioning the parasitoids, plants were brought to a laboratory environment, and mesh bags, cotton wool, clip cages and herbivores were removed. Both host and non-host herbivores were removed, but not their excretions, to prevent the possibility that the learning experiences would greatly differ among parasitoids (e.g. where one parasitoid would encounter a non-host individual and the other parasitoid would not). First-instar *P. brassicae* caterpillars were placed on the previously infested leaf, on the spot where the clip cage had been. New hosts were offered to the parasitoid, because the 3-day-old hosts would better defend themselves against oviposition that could negatively affect the conditioning, whereas younger caterpillars defend themselves less well (Gross, 1993). To encourage the parasitoids to search for their host, frass of first-instar *P. brassicae* larvae was spread on the leaf. Plants used in the wind-tunnel and field-tent experiments were prepared the same way, but without the use of clip cages. For the wind-tunnel experiment, plants were prepared 4 days in advance, for logistical reasons.

#### *Conditioning of parasitoids*

Parasitoids were inexperienced (inexperienced) or were given an experience by allowing them one oviposition in a host on a plant that was infested by: (i) the host in combination with the non-host caterpillar *M. brassicae* (caterpillar conditioning); (ii) the host and the non-host aphid *M. persicae* (aphid conditioning); or (iii) oviposition on a plant infested by the host and non-host caterpillar immediately followed by oviposition on a plant infested by the host and non-host aphid (dual conditioning) (Fig. 1). Each conditioning treatment was given twice, with 10 min in between, so-called spaced training (Smid *et al.*, 2007), to create long-term memory that would last for at least

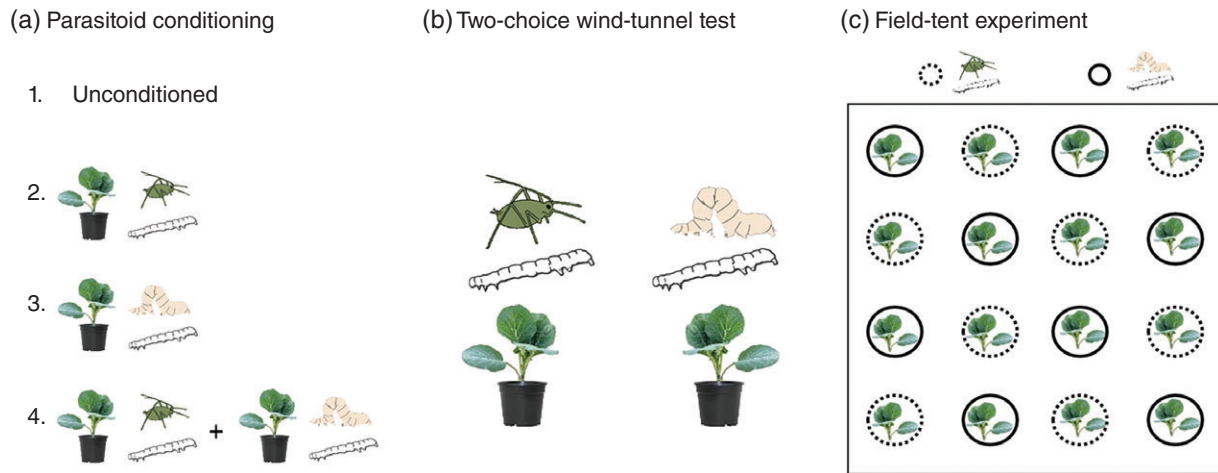
2 days. Immediately after collecting an individual parasitoid in a 1.5-ml Eppendorf tube, the parasitoid was allowed to walk on the infested part of the leaf, and after parasitizing one host, the parasitoid was again collected in the tube. After 10 min, the parasitoid underwent the second part of the conditioning, which was the same as the first part in the case of caterpillar or aphid conditioning. In the dual conditioning, the parasitoid first experienced one oviposition on a caterpillar-infested plant, and this was immediately followed by an oviposition on an aphid-infested plant. After the 10-min interval, the parasitoid experienced these two ovipositions again, but in reversed order. The two possible orders of oviposition experiences for this treatment were equally divided over the parasitoids of this treatment. Attacked hosts were replaced by new ones after removal of the parasitoid. For each set of two plants, 12 parasitoids were conditioned. Inexperienced parasitoids were kept in the collection tube for 10 min. Parasitoids of all treatments were kept in cages with honey and water *ad libitum* after conditioning. At 1 day after conditioning, the parasitoids were released individually (wind-tunnel experiment) or in groups of three (field-tent experiment). Each parasitoid was tested only once.

#### *Experimental setup*

The preference of inexperienced and conditioned *C. glomerata* parasitoids for plants infested with hosts plus non-host caterpillars and plants infested with hosts plus non-host aphids was tested in the laboratory using a wind tunnel as described by Geervliet *et al.* (1994) (Fig. 1). Plants were prepared as described earlier. Before placing one plant of each type in the wind tunnel, the cotton wool and mesh bags to confine herbivores on the leaves were removed. Herbivores were not removed. In the wind tunnel (25 ± 1 °C, RH 64–70%, wind speed 0.1 m s<sup>-1</sup>) plant pairs were placed upwind, and individual (un)conditioned parasitoids were released from a distance of 70 cm from the plants. The first plant the parasitoid landed on was considered as the preferred plant; when a parasitoid did not land on one of the plants within 5 min, this parasitoid was considered unresponsive. After every two responding parasitoids, the position of the plants was switched to control for unforeseen asymmetry in the setup. For each treatment, 13 or 14 sets of plants were used to test 12 parasitoids per plant pair in a random order spread over 7 weeks.

The parasitisation preference of inexperienced and conditioned *C. glomerata* parasitoids was tested using a field-tent experiment conducted from 1 May 2013 to 4 October 2013 (Fig. 1). Two sets of eight tents were used (length × width × height, 3 × 4 × 2 m) made out of insect screen (mesh diameter 0.6 mm) and positioned in an agricultural field in the vicinity of Wageningen, the Netherlands. The two sets of tents were used alternately, with at least 11 days between using the same set of tents to exclude the presence of previously released insects. After growing in a greenhouse for 4 weeks, *B. oleracea* plants were transferred outside to acclimatise for 1 week. Five-week-old plants were planted in the tents, with 16 plants per tent in a 4 × 4 grid and with ± 60 cm distance between plants. During the sixth week, all of the plants in the





**Fig. 1.** Experimental design of conditioning of the parasitoid *Cotesia glomerata* on different plant–host–non-host complexes and its effect on preference for a plant–host–non-host complex in two-choice wind-tunnel tests as well as parasitisation efficiency in small plant communities in field tents. (a) Parasitoids used in the experiments were inexperienced or trained on an aphid plant–host–non-host complex, a caterpillar plant–host–non-host complex, or both of the plant–host–non-host complexes. (b) They were subsequently tested in a two-choice test in a wind tunnel for preference for the aphid or caterpillar plant–host–non-host complex. (c) In a field tent, the conditioned parasitoids were released to measure their parasitism preference and efficiency for hosts on plants infested with non-host aphids or caterpillars. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

tents were infested with 10 *P. brassicae* caterpillars. Every other plant was additionally infested with 40 *M. persicae* aphids, whereas the remaining eight plants were additionally infested with 10 *M. brassicae* caterpillars using methods described earlier. Three days after herbivore introduction, three female (un)conditioned parasitoids and three male parasitoids were released after removal of the mesh bags to confine insects to plants. The parasitoid-conditioning treatments were randomly distributed over the tents (one conditioning treatment per tent, two tents per conditioning treatment for each trial). Two plastic lids (diameter 3 cm) with drops of honey were placed in the tents as a food source for the parasitoids. After 24 h the plants in the tents were harvested and kept individually in labelled plastic bags at 4 °C. All *P. brassicae* caterpillars found back on the plants were dissected under a stereomicroscope to check for parasitisation. We ran nine trials of the experiment over time, yielding 16 replicates per treatment.

#### Statistical analysis

To test for equal preference for the two plants offered to the parasitoids in the wind-tunnel experiment, initially, per treatment, a logistic regression model was fitted, with just a constant on the logit scale, and a multiplicative dispersion factor for the binomial variance of the dependent variable of parasitoid preference for one of the two odour sources, to account for extra variation between pairs of plants. An approximate *t*-test was used to check whether the constant differed from 0, i.e. whether parasitoids showed a preference. When the estimated dispersion factor was smaller than 1, the binomial test was used instead, assuming independence of choices of parasitoids within and between pairs of plants. To analyse if the preference and the response rate of the parasitoids were affected by conditioning treatment, a logistic

regression analysis was conducted comprising an extra unknown multiplicative dispersion factor for the binomial variance, an explanatory variable for the day of testing, and main effects for conditioning treatment on the logit scale. There was no significant effect of the day of testing, so it was subsequently dropped from the model. Statistical inference was based on the Wald test.

To test for equal preference for the two plant types offered to the parasitoids in the field-tent experiment, initially, per treatment, a logistic regression model was fitted, comprising just a constant as a fixed effect, in addition to random effects for tents, on the logit scale, and a multiplicative dispersion factor for the binomial variance, to account for extra variation between groups of plants within the same tent. An approximate *t*-test was used to check whether the constant differed from 0, i.e. whether parasitoids showed a preference. When the estimated variance component for tents was negligible and the estimated dispersion factor was smaller than 1, the binomial test was used instead, assuming independence of choices of parasitoids within and between groups of plants within the same tent. To analyse if the preference for the two plant types was affected by conditioning treatment, a generalised linear mixed model (GLMM) was used, comprising an extra unknown multiplicative dispersion factor for the binomial variance, a random factor for tent number, an explanatory variable for the time of testing, and main effects for conditioning treatment on the logit scale. There was no significant effect of the moment of testing, so it was subsequently dropped. To analyse the percentage of parasitised hosts out of the total number of hosts per tent, a similar GLMM was used, with the moment of testing included. In addition to an overall comparison of the four treatments, a test was performed for the contrast between the inexperienced and the average of the experienced parasitoids, all on the logit scale. One of the trials was excluded from all analyses because the

parasitoids did not fly out of the release tubes, most likely because of low temperatures, bringing the total number of replicates per treatment to 16. Inference was based on penalised quasi-likelihood (PQL) (Breslow & Clayton, 1993). Quasi-Wald tests and approximate *F*-tests (Kenward & Roger, 1997) applied to the approximate linear mixed model from the last step of the iterative reweighted REML algorithm were used. Calculations were performed using GENSTAT, 17th edition (VSN International Ltd, Hemel Hempstead, U.K.).

## Results

### Wind-tunnel experiment

The percentage of parasitoids that responded in the wind tunnel was, on average, 89% and was not affected by conditioning treatment [generalised linear model (GLM), d.f. = 3,  $F = 2.34$ ,  $P = 0.089$ ]. Inexperienced parasitoids preferred to land on plants infested with hosts plus non-host caterpillars over plants infested with hosts plus non-host aphids (approximate *t*-test,  $P = 0.007$ ). Likewise, parasitoids with an oviposition experience on plants infested with hosts plus non-host caterpillars (caterpillar conditioning) also preferred this plant type (binomial test,  $P = 0.011$ ). However, when parasitoids had experienced oviposition on plants infested with hosts plus aphids (aphid conditioning), or in combination with an oviposition on plants infested with hosts plus non-host caterpillars (dual conditioning), the distribution of preference shifted from host-plus non-host caterpillar-infested plants to a no-preference situation (approximate *t*-test,  $P = 0.561$  and  $P = 0.504$ , respectively) (Fig. 2). The percentage of parasitoids choosing the host-plus non-host caterpillar-infested plant was highest for the inexperienced parasitoids as compared with the aphid- and dual-conditioned parasitoids. In addition, the percentage of parasitoids choosing the host-plus non-host aphid-infested plant was higher for the aphid-conditioned parasitoids than for the caterpillar-conditioned parasitoids (GLM, d.f. = 3,  $F = 4.07$ ,  $P = 0.013$ ) (Fig. 2).

### Field-tent experiment

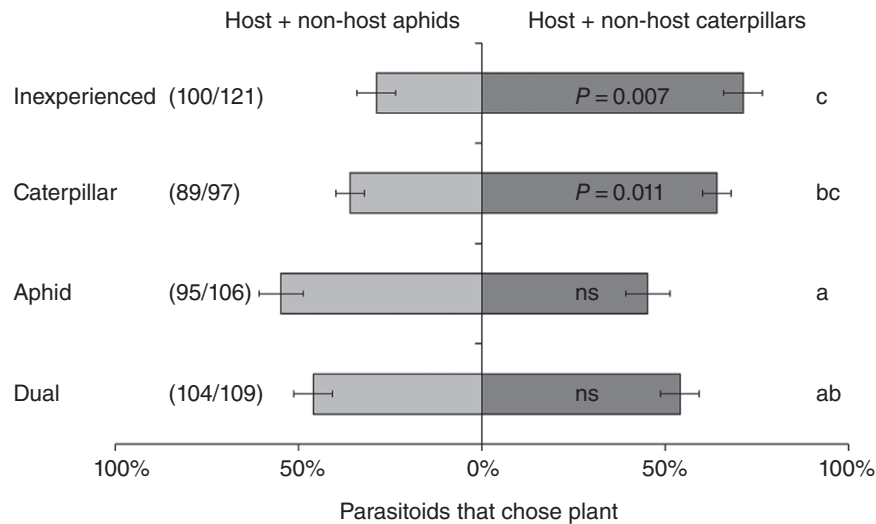
Of the 10 240 hosts transferred to the plants in the tents, 7517 hosts were re-collected (73%). Of those hosts that were re-collected, 2137 were parasitised (28%). The time at which the separate replicates of the experiment were performed influenced the percentage of hosts parasitised (GLMM, n.d.f. = 7, d.d.f. = 41.7,  $P < 0.001$ ), most likely because of variable weather conditions. Conditioning of parasitoids did not affect the number of host-plus aphid-infested or host-plus non-host caterpillar-infested plants with parasitised hosts (GLMM, n.d.f. = 3, d.d.f. = 60.0,  $P = 0.515$ ) (Fig. 3). Parasitised hosts were equally spread over host-plus non-host aphid-infested and host-plus non-host caterpillar-infested plants in all treatments (binomial test: all  $P > 0.2$ ) (Fig. 3). On average ( $\pm$  SE), 28.8% ( $\pm 1.75$ ) of the hosts in the tents were parasitised. This was not affected by conditioning in general (inexperienced versus conditioned, GLMM, n.d.f. = 1, d.d.f. = 50.6,  $P = 0.155$ ), or

by the different types of conditioning (GLMM, n.d.f. = 3, d.d.f. = 52.0,  $P = 0.320$ ).

## Discussion

In this study we showed that parasitoids learn to associate cues of non-host identity with the presence of hosts. In a laboratory setting, the landing preference of *C. glomerata* parasitoids shifted in the direction of plants infested by hosts plus non-host aphids if, previously, cues from non-host aphids were experienced in the presence of hosts. This learned association did not, however, translate into parasitisation preferences in a field-tent situation. In the field setting, *C. glomerata* parasitised hosts on similar numbers of plants infested by hosts plus non-host caterpillars as on plants infested by hosts plus non-host aphids, irrespective of their previous experience.

Based on previous findings, it was predicted that parasitoids would prefer to land on and parasitise hosts on plants with the type of herbivore infestation they had previously experienced (but see Desurmont *et al.*, 2017 for a counter-example). When both of the plant infestation types were previously experienced, no landing preference or parasitisation preference was expected. Inexperienced parasitoids were predicted to prefer host-plus non-host caterpillar-induced plants. Supporting our predictions, inexperienced parasitoids preferred host-plus non-host caterpillar-induced plants over host-plus non-host aphid-induced plants in the wind-tunnel experiment. This preference shifted in the direction of host-plus non-host aphid-induced plants when parasitoids experienced host oviposition on plants infested by hosts plus non-host aphids or were also experiencing a plant with hosts and non-host caterpillars in addition. The innate preference of inexperienced parasitoids for host-plus non-host caterpillar-damaged plants could be caused by a quantitative difference in emitted volatiles related to caterpillar feeding, as a result of unequal numbers of caterpillars on the two plant infestation types (Geervliet *et al.*, 1998a). However, previous research showed that differences in density of host and non-host caterpillars between two plants do not affect the preference of *C. glomerata* (Bukovinszky *et al.*, 2012). Quantitative differences could also have occurred because of changed emission rates of caterpillar-related volatile compounds due to co-infestation by phloem-feeding insects such as aphids (Rodriguez-Saona *et al.*, 2003; Zhang *et al.*, 2013; Ponzio *et al.*, 2016; Li *et al.*, 2017). Besides quantitative differences, qualitative differences are also hypothesised to occur between the volatile blends of plants dually attacked by aphids and caterpillars and plants attacked by only caterpillars (host and non-host) (Dicke *et al.*, 2009). An attack by aphids can affect a plant's response to an attack by caterpillars via cross-talk of the signal transduction pathways of the plant, which could lead to a qualitative difference in the volatile blend (Thaler *et al.*, 2012; Kroes *et al.*, 2015). Parasitoids conditioned on host-plus non-host aphid-infested plants might have experienced that hosts can also be found on plants emitting volatiles quantitatively and/or qualitatively different from plants infested with hosts that are innately preferred. This new experience levelled out the preference for plants induced by only host caterpillars in the wind-tunnel experiment.

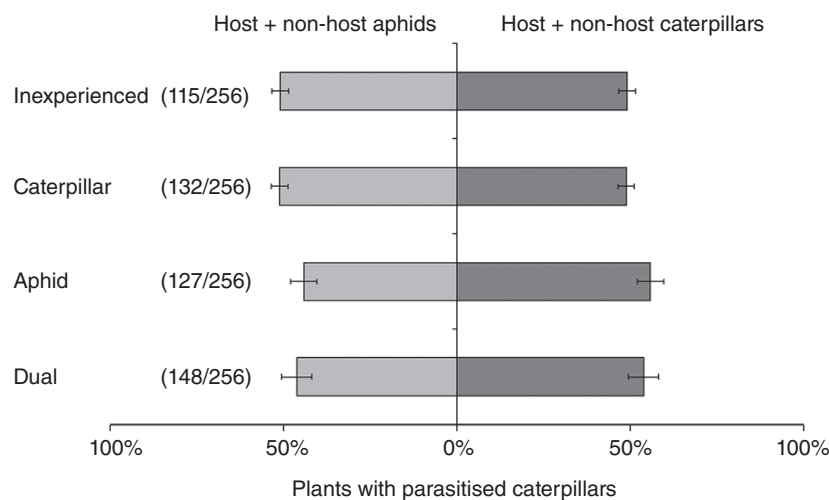


**Fig. 2.** Mean percentages of parasitoids in the wind-tunnel experiment that chose to land on a plant infested with hosts plus non-host aphids (left) and mean percentage of parasitoids that chose to land on a plant infested with hosts plus non-host caterpillars (right). Error bars indicate the standard errors of different plant pairs ( $n = 13$  or 14 plant pairs per conditioning treatment). Parasitoids were inexperienced (inexperienced), experienced with host oviposition on a plant infested with hosts plus non-host caterpillars (caterpillar), experienced with host oviposition on a plant infested with hosts plus non-host aphids (aphid), or experienced with host oviposition on a plant infested with hosts plus non-host caterpillars as well as on a plant infested with hosts plus non-host aphids (dual). Values in bars show results of approximate  $t$ -tests (for inexperienced, aphid and dual) or binomial tests (for caterpillar) for equal preference ( $\alpha = 0.05$ , ns = not significant); different letters indicate significant differences between treatments based on logistic regression ( $P = 0.013$ ) and least significant differences ( $\alpha = 0.05$ ). Values in brackets are the number of responding parasitoids/the number of parasitoids tested.

Only very few previous studies have shown that insects that can learn to associate cues with the presence of their host/food in the laboratory also accordingly show altered behaviour in (semi-)field situations (Lewis & Martin, 1990; Papaj & Vet, 1990; Hare *et al.*, 1997; Raine & Chittka, 2008; Zrelec *et al.*, 2013; Janssen *et al.*, 2014; Kruidhof *et al.*, 2015). *Cotesia glomerata* is well known for its ability to learn in the laboratory (Geervliet *et al.*, 1998a,b; Bleeker *et al.*, 2006; Smid *et al.*, 2007; Kruidhof *et al.*, 2012a,b). Although its landing preference was indeed affected by conditioning in our wind-tunnel experiment, its parasitisation preference in field-tent situations was not influenced by previous oviposition experience. An explanation for this mismatch between laboratory and field observations could be found in the incompleteness of the information that we offered to the parasitoids. In natural situations, parasitoids experience not only rewarding situations, e.g. being attracted by an odour followed by finding a host, but also unrewarding situations, e.g. being attracted by an odour followed by not finding hosts. By facing both rewarding and unrewarding situations, parasitoids get complete information about their environment (Vet *et al.*, 1998). Vet *et al.* (1998) showed that when parasitoids are provided with complete information, the response to the rewarding situation increases. In our experiment, the conditioning of the parasitoids was incomplete for the complex situation the parasitoids faced in the field tents. If we had given the parasitoids both a rewarding (e.g. oviposition on a host- plus non-host caterpillar-infested plant) and an unrewarding experience (e.g. no oviposition on a host- plus non-host aphid-infested plant), the parasitoids would have had complete information and the response to the 'rewarding plant' may have been stronger (Kruidhof *et al.*, 2015).

Additionally, our field-tent setup, in which host caterpillars were feeding on all plants, did not penalise parasitoids that landed on plants other than the plant infestation type they had learned to associate with host presence. Based on the results from our wind-tunnel experiment and on preference tests from the literature (e.g. Geervliet *et al.*, 1998a,b; Kruidhof *et al.*, 2012a,b) it is expected that the preference of *C. glomerata* would not be 100% for either of the two plant infestation types. This would mean that an individual parasitoid in the field-tent experiment would mostly fly to its preferred plant, but sometimes also to its non-preferred plant which harboured equal numbers of hosts as the preferred plant. In the field-tent experiment, the parasitoids may therefore have gained oviposition experience on the non-preferred plants in addition to the conditioned cue of host presence. After every oviposition experience on a non-preferred plant, the search image [temporary specialisation on a single host type while ignoring alternatives (Ishii & Shimada, 2010)] based on laboratory conditioning could have been gradually weakened, resulting in equal parasitism preference for both plant infestation types.

We speculate that, in our field-tent experiment, the incomplete information given to the parasitoids and the environment that was saturated with hosts played a major role in the observed absence of effects of previous oviposition experience on parasitisation preference. Because the parasitoids were provided with incomplete information, they possibly made more 'mistakes' by landing on the plant treatment they were not conditioned on. An oviposition experience on this non-conditioned plant treatment added to the experience the parasitoids acquired in the laboratory, leading to a less specific search image. As a result, parasitised hosts were found on similar numbers of plants



**Fig. 3.** Mean percentages of plants with parasitised hosts in the field-tent experiment. Plants were infested by hosts plus non-host aphids (left) or hosts plus non-host caterpillars (right). Error bars indicate the standard errors of different tents ( $n = 16$  tents per conditioning treatment). Parasitoids were inexperienced (inexperienced), experienced with host oviposition on a plant infested with hosts plus non-host caterpillars (caterpillar), experienced with host oviposition on a plant infested with hosts plus non-host aphids (aphid), or experienced with host oviposition on a plant infested with hosts plus non-host caterpillars as well as on a plant infested with hosts plus non-host aphids (dual). Based on generalised linear mixed models ( $\alpha = 0.05$ ) no significant differences were found between treatments. Based on binomial tests ( $\alpha = 0.05$ ) no significant differences were found between numbers of plants harbouring parasitised hosts of the two different plant infestation types. Values in brackets are the total number of plants with parasitised hosts/the number of plants with hosts present.

that were infested with hosts plus non-host caterpillars as plants that were infested with hosts plus non-host aphids, irrespective of the parasitoids' experience in the laboratory. In previous work, we found that parasitoids exploited aphid co-infested host patches faster than patches with non-host caterpillars by parasitising more caterpillars per unit time (De Rijk *et al.*, 2016a). Because of the absence of parasitisation preferences, we also did not observe that parasitoids that had previously experienced oviposition on host- plus non-host aphid-infested plants showed an increased parasitisation efficiency.

Our study shows that an initial associative learning experience may not translate into a long-term effect on parasitisation preferences of parasitoids in habitats with abundant hosts. It remains to be tested if this is still the case when parasitoids are given complete information about the environment in which they will forage for hosts and how a match or a mismatch of this experience with the foraging environment affects their parasitisation efficiency.

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### References

Aartsma, Y., Bianchi, F.J.J.A., van der Werf, W., Poelman, E.H. & Dicke, M. (2017) Herbivore-induced plant volatiles and tritrophic interactions across spatial scales. *New Phytologist*, **216**, 1054–1063.

- Arimura, G.I., Ozawa, R. & Maffei, M.E. (2011) Recent advances in plant early signaling in response to herbivory. *International Journal of Molecular Sciences*, **12**, 3723–3739.
- Bleeker, M.A.K., Smid, H.M., Steidle, J.L.M., Kruidhof, H.M., Van Loon, J.J.A. & Vet, L.E.M. (2006) Differences in memory dynamics between two closely related parasitoid wasp species. *Animal Behaviour*, **71**, 1343–1350.
- Breslow, N.E. & Clayton, D.G. (1993) Approximate inference in generalized linear mixed models. *Journal of the American Statistical Association*, **88**, 9–25.
- Bukovinszky, T., Poelman, E.H., Kamp, A., Hemerik, L., Prekatsakis, G. & Dicke, M. (2012) Plants under multiple herbivory: consequences for parasitoid search behaviour and foraging efficiency. *Animal Behaviour*, **83**, 501–509.
- Colazza, S., Cusumano, A., Lo Giudice, D. & Peri, E. (2014) Chemo-orientation responses in hymenopteran parasitoids induced by substrate-borne semiochemicals. *BioControl*, **59**, 1–17.
- De Rijk, M., Dicke, M. & Poelman, E.H. (2013) Foraging behaviour by parasitoids in multiherbivore communities. *Animal Behaviour*, **85**, 1517–1528.
- De Rijk, M., Yang, D., Engel, B., Dicke, M. & Poelman, E.H. (2016a) Feeding guild of non-host community members affect host-foraging efficiency of a parasitic wasp. *Ecology*, **97**, 1388–1399.
- De Rijk, M., Wang, Q., Papagiannaki, E., Dicke, M. & Poelman, E.H. (2016b) Herbivore species identity rather than diversity of the non-host community determines foraging behaviour of the parasitoid wasp *Cotesia glomerata*. *Entomologia Experimentalis et Applicata*, **161**, 20–30.
- Desurmont, G.A., Guiguet, A. & Turlings, T.C.J. (2017) Invasive insect herbivores as disrupters of chemically-mediated tritrophic interactions: effects of herbivore density and parasitoid learning. *Biological Invasions*. <https://doi.org/10.1007/s10530-017-1526-x>.
- Dicke, M., Van Loon, J.J.A. & Soler, R. (2009) Chemical complexity of volatiles from plants induced by multiple attack. *Nature Chemical Biology*, **5**, 317–324.



- Dridi, S. & Lehmann, L. (2016) Environmental complexity favors the evolution of learning. *Behavioral Ecology*, **27**, 842–850.
- Geervliet, J.B.F., Vet, L.E.M. & Dicke, M. (1994) Volatiles from damaged plants as major cues in long-range host-searching by the specialist parasitoid *Cotesia rubecula*. *Entomologia Experimentalis et Applicata*, **73**, 289–297.
- Geervliet, J.B.F., Ariëns, S., Dicke, M. & Vet, L.E.M. (1998a) Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae). *Biological Control*, **11**, 113–121.
- Geervliet, J.B.F., Vreugdenhil, A.I., Dicke, M. & Vet, L.E.M. (1998b) Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*. *Entomologia Experimentalis et Applicata*, **86**, 241–252.
- Geervliet, J.B.F., Verdel, M.S.W., Snellen, H., Schaub, J., Dicke, M. & Vet, L.E.M. (2000) Coexistence and niche segregation by field populations of the parasitoids *Cotesia glomerata* and *C. rubecula* in the Netherlands: predicting field performance from laboratory data. *Oecologia*, **124**, 55–63.
- Godfray, H.C.J. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton, New Jersey.
- Gross, P. (1993) Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology*, **38**, 251–273.
- Hare, J.D. (2011) Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annual Review of Entomology*, **56**, 161–180.
- Hare, J.D., Morgan, D.J.W. & Nguyen, T. (1997) Increased parasitization of California red scale in the field after exposing its parasitoid, *Aphytis melinus*, to a synthetic kairomone. *Entomologia Experimentalis et Applicata*, **82**, 73–81.
- Hoedjes, K.M., Kruidhof, H.M., Huigens, M.E., Dicke, M., Vet, L.E.M. & Smid, H.M. (2011) Natural variation in learning rate and memory dynamics in parasitoid wasps: opportunities for converging ecology and neuroscience. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 889–897.
- Ishii, Y. & Shimada, M. (2010) The effect of learning and search images on predator-prey interactions. *Population Ecology*, **52**, 27–35.
- Janssen, A., Fonseca, J.O., Colares, F., Silva, L., Pedrosa, A.R.P., Lima, E.R. *et al.* (2014) Time scales of associating food and odor by predator communities in the field. *Behavioral Ecology*, **25**, 1123–1130.
- Kenward, M.G. & Roger, J.H. (1997) Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, **53**, 983–997.
- Kroes, A., Van Loon, J.J.A. & Dicke, M. (2015) Density-dependent interference of aphids with caterpillar-induced defenses in *Arabidopsis*: involvement of phytohormones and transcription factors. *Plant and Cell Physiology*, **56**, 98–106.
- Kruidhof, H.M., de Rijk, M., Hoffmann, D., Harvey, J.A., Vet, L.E.M. & Soler, R. (2012a) Effect of belowground herbivory on parasitoid associative learning of plant odours. *Oikos*, **122**, 1094–1100.
- Kruidhof, H.M., Pashalidou, F.G., Fatouros, N.E., Figueroa, I.A., Vet, L.E.M., Smid, H.M. *et al.* (2012b) Reward value determines memory consolidation in parasitic wasps. *PLoS One*, **7**, e39615.
- Kruidhof, H.M., Roberts, A.L., Magdaraog, P., Muñoz, D., Gols, R., Vet, L.E.M. *et al.* (2015) Habitat complexity reduces parasitoid foraging efficiency, but does not prevent orientation towards learned host plant odours. *Oecologia*, **179**, 353–361.
- Lewis, W.J. & Martin, W.R. (1990) Semiochemicals for use with parasitoids: status and future. *Journal of Chemical Ecology*, **16**, 3067–3089.
- Li, Y., Weldegeris, B.T., Chamontri, S., Dicke, M. & Gols, R. (2017) Does aphid infestation interfere with plant indirect defence against lepidopteran caterpillars in wild cabbage? *Journal of Chemical Ecology*, **43**, 493–505.
- Mery, F. (2013) Natural variation in learning and memory. *Current Opinion in Neurobiology*, **23**, 52–56.
- Papaj, D.R. & Vet, L.E.M. (1990) Odor learning and foraging success in the parasitoid, *Leptopilina heterotoma*. *Journal of Chemical Ecology*, **16**, 3137–3150.
- Ponzio, C., Cascone, C., Cusumano, A., Weldegeris, B.T., Fatouros, N.E., Guerrieri, E. *et al.* (2016) Volatile-mediated foraging behaviour of three parasitoid species under conditions of dual insect herbivore attack. *Animal Behaviour*, **111**, 197–206.
- Raine, N.E. & Chittka, L. (2008) The correlation of learning speed and natural foraging success in bumble-bees. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 803–808.
- Rodriguez-Saona, C., Crafts-Brandner, S.J. & Cañas, L.A. (2003) Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. *Journal of Chemical Ecology*, **29**, 2539–2550.
- Smid, H.M., Wang, G., Bukovinszky, T., Steidle, J.L.M., Bleeker, M.A.K., Van Loon, J.J.A. *et al.* (2007) Species-specific acquisition and consolidation of long-term memory in parasitic wasps. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 1539–1546.
- Thaler, J.S., Humphrey, P.T. & Whiteman, N.K. (2012) Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, **17**, 260–270.
- Tumlinson, J.H., Lewis, W.J. & Vet, L.E.M. (1993) How parasitic wasps find their hosts. *Scientific American*, **268**, 100–106.
- Turlings, T.L., Wäckers, F., Vet, L.M., Lewis, W.J. & Tumlinson, J. (1993) Learning of host-finding cues by Hymenopterous parasitoids. *Insect Learning: Ecological and Evolutionary Perspectives* (ed. by D. Papaj and A. Lewis), pp. 51–78. Chapman & Hall, New York, New York.
- Van Alphen, J.J.M., Bernstein, C. & Driessen, G. (2003) Information acquisition and time allocation in insect parasitoids. *Trends in Ecology and Evolution*, **18**, 81–87.
- Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, **37**, 141–172.
- Vet, L.E.M., De Jong, A.G., Franchi, E. & Papaj, D.R. (1998) The effect of complete versus incomplete information on odour discrimination in a parasitic wasp. *Animal Behaviour*, **55**, 1271–1279.
- Vet, L.E.M., Lewis, W.J., Papaj, D.R. & van Lenteren, J.C. (1990) A variable-response model for parasitoid foraging behavior. *Journal of Insect Behavior*, **3**, 471–490.
- Vet, L.M., Lewis, W.J. & Cardé, R. (1995) Parasitoid foraging and learning. *Chemical Ecology of Insects 2* (ed. by R. Cardé and W. J. Bell), pp. 65–101. Chapman & Hall, New York, New York.
- Wäschke, N., Meiners, T. & Rostás, M. (2013) Foraging strategies of parasitoids in complex chemical environments. *Chemical Ecology of Insect Parasitoids* (ed. by E. Wajnberg and S. Colazza), pp. 37–63. John Wiley & Sons, Oxford, U.K.
- Zhang, P.J., Broekgaarden, C., Zheng, S.J., Snoeren, T.A.L., van Loon, J.J.A., Gols, R. *et al.* (2013) Jasmonate and ethylene signaling mediate whitefly-induced interference with indirect plant defense in *Arabidopsis thaliana*. *New Phytologist*, **197**, 1291–1299.
- Zrelec, V., Zini, M., Guarino, S., Mermoud, J., Oppliger, J., Valtat, A. *et al.* (2013) *Drosophila* rely on learning while foraging under semi-natural conditions. *Ecology and Evolution*, **3**, 4139–4148.

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