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ORIGINAL ARTICLE

The role of herbivore- and plant-related experiences in intraspecific host preference of a relatively specialized parasitoid

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Abstract Parasitoids use odor cues from infested plants and herbivore hosts to locate their hosts. Specialist parasitoids of generalist herbivores are predicted to rely more on herbivore-derived cues than plant-derived cues. *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) is a relatively specialized larval endoparasitoid of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), which is a generalist herbivore on several crops including cotton and soybean. Using *M. croceipes/H. virescens* as a model system, we tested the following predictions about specialist parasitoids of generalist herbivores: (i) naive parasitoids will show innate responses to herbivore-emitted kairomones, regardless of host plant identity and (ii) herbivore-related experience will have a greater influence on intraspecific oviposition preference than plant-related experience. Inexperienced (naive) female *M. croceipes* did not discriminate between cotton-fed and soybean-fed *H. virescens* in oviposition choice tests, supporting our first prediction. Oviposition experience alone with either host group influenced subsequent oviposition preference while experience with infested plants alone did not elicit preference in *M. croceipes*, supporting our second prediction. Furthermore, associative learning of oviposition with host-damaged plants facilitated host location. Interestingly, naive parasitoids attacked more soybean-fed than cotton-fed host larvae in two-choice tests when a background of host-infested cotton odor was supplied, and vice versa. This suggests that plant volatiles may have created an olfactory contrast effect. We discussed ecological significance of the results and concluded that both plant- and herbivore-related experiences play important role in parasitoid host foraging.

Key words *Heliothis virescens*; *Microplitis croceipes*; olfactory contrast; oviposition choice; plant volatiles

Introduction

Parasitoids are important agents of biological control against pest insects. They rely on olfactory cues to locate their herbivore hosts in a complex chemical environment. In general, successful parasitization requires at least three different but continuous stages of selection: host habitat location, host location and host acceptance (Vinson, 1976; Steiner *et al.*, 2007). The host location process is largely dependent on use of olfactory cues from plants, herbivore hosts or the interaction between plants and herbivore hosts (Elzen *et al.*, 1984; De Moraes *et al.*, 1998; Steidle & van Loon, 2003; Turlings & Wäckers, 2004; Chuche *et al.*, 2006; Fürstenberg-Hägg *et al.*, 2013; de Rijk *et al.*, 2016; Morawo & Fadamiro, 2016). The role of plant-emitted volatiles has been the main focus of several previous studies, with less attention devoted to herbivore-emitted volatiles.

Plant volatiles are more detectable and considered important long range cues for foraging parasitoids to locate host plant patch (Vet & Dicke, 1992; Röse *et al.*,

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1997; Steidle & van Loon, 2003). However, at short range, herbivore host cues are considered more reliable for host location in parasitoids (Vet & Dicke, 1992; Alborn *et al.*, 1995; De Rijk *et al.*, 2013; Colazza *et al.*, 2014; Morawo & Fadamiro, 2016). Parasitoids likely resolve the detectability-reliability problem by using plant volatiles as long range cues and herbivore odors as short range cues (Colazza *et al.*, 2014). Host acceptance is marked by oviposition, and is largely mediated by odor cues. Parasitoid species that are not egg-limited often seek out more hosts after their first oviposition experience (Mills & Wajnberg, 2008; Hopper *et al.*, 2013). Thus, parasitoids are exposed to both plant- and herbivore-related experiences in the course of foraging.

Based on their host range, parasitoids can be broadly categorized as specialist (restricted host range) and generalist (broad host range). Furthermore, their herbivore hosts can be specialists (monophagous) or generalists (polyphagous). These dietary specializations strongly influence the life strategy and foraging behavior of parasitoids. However, the degree of host specialization in parasitoids is a continuum, with only few species at the two extreme ends of the spectrum. According to the concept of dietary specialization and infochemical use proposed by Vet and Dicke (1992), the degree of polyphagy in a generalist herbivore should drive its specialist parasitoid to rely more on herbivore cues than plant cues. The foraging behavior of specialist parasitoids is likely complemented by a narrowly tuned olfactory mechanism, compared with generalist species (Cortesero, 1997; Meiners *et al.*, 2002; Chen & Fadamiro, 2007; Ngumbi *et al.*, 2009; Najar-Rodriguez *et al.*, 2015). This strategy greatly reduces the number of different chemical patterns that a specialist parasitoid would have to recognize if it only depended on plant-emitted volatiles (Wajnberg *et al.*, 2008).

Regardless of the source of olfactory cues used, several parasitoid species are able to modify their behavior by learning from various foraging experiences (Vet & Groenewold, 1990; Olson *et al.*, 2003; Hoedjes *et al.*, 2011; Ngumbi *et al.*, 2012; De Rijk *et al.*, 2013). As such, studies that seek to model host selection in parasitoids should consider the effect of experience on their foraging decisions (Vet, 1999). Parasitoids can associate an activity with a reward. Such reward may reinforce learning of new or routine stimuli. In female parasitoids, host-related odors (conditioned stimulus) may be associated with a reward (unconditioned stimulus) such as food or oviposition. In particular, associative learning with an unconditioned stimulus (e.g., oviposition) and conditioned stimulus (e.g., plant odor) has been shown to enhance attraction of the parasitoid, *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) to host-induced plant volatiles (Lewis & Takasu, 1990; Olson *et al.*, 2003; Takasu & Lewis, 2003; Ngumbi *et al.*, 2012). Therefore, it is also important to investigate the combined effect of both plant- and herbivore-related experiences on the foraging behavior of parasitoids.

Parasitoids share an intimate life history with their hosts. According to the optimal foraging theory, female parasitoids are expected to select hosts that best support the development of their progeny (Pyke, 1984; Goubault *et al.*, 2003; Steiner *et al.*, 2007; Chesnais *et al.*, 2015). Parasitoid species that are not time-limited are expected to discriminate hosts based on suitability and quality (Hopper *et al.*, 2013). In turn, the quality of herbivore host depends on plant nutrients and phytotoxins, which have direct impact on the development of immature parasitoids (Harvey *et al.*, 2013). Each plant species expresses a distinct chemical profile, even when infested by the same herbivore species (van Den Boom *et al.*, 2004). Furthermore, herbivores may retain and emit different plant-associated compounds, depending on their diet (Morawo & Fadamiro, 2016). Therefore, intraspecific host preference may be expected in a specialist parasitoid that utilizes a generalist herbivore. However, such discriminatory ability is probably influenced by the experience of a female parasitoid (Hopper *et al.*, 2013). Also, parasitoid experience likely compensates for the challenge of incomplete information in a complex chemical environment (Mills & Wajnberg, 2008).

Microplitis croceipes is a relatively specialized larval endoparasitoid of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), which is a generalist herbivore on several crops including cotton and soybean (Fitt, 1989). *Microplitis croceipes* is described as relatively specialized because it is restricted to very few host species in the *Heliothis*/*Helicoverpa* complex (Tillman & Laster, 1995). In this study, we investigated the role of certain plant- and herbivore-related experiences on short-range intraspecific host preference in *M. croceipes*. Parasitoids are generally considered good models for studying insect olfaction, and *M. croceipes* is especially considered an excellent model for cognition studies (Meiners *et al.*, 2002; Olson *et al.*, 2003; Rains *et al.*, 2006; Ngumbi *et al.*, 2012). Based on the concept of dietary specialization and infochemical use (Vet & Dicke, 1992), the following predictions were tested about specialist parasitoids of generalist herbivores using *M. criceipes/H. virescens* as model system: (i) naive parasitoids will show innate responses to herbivore-emitted kairomones, regardless of host plant identity and (ii) herbivore-related experience will have greater influence on intraspecific oviposition preference than plant-related experience. The effect of various experiences/conditioning, as well as plant backgrounds on intraspecific host preference was tested in behavioral bioassays using cotton-fed and soybean-fed *H. virescens* larvae. We discussed the ecological significance of the results.

Materials and methods

Plants

Cotton (*Gossypium hirsutum* L., var. max 9) and Soybean (*Glycine max* (L.) Merrill, var. Pioneer P49T97R-SA2P) plants were grown according to Morawo and Fadamiro (2014) in growth chambers (Auburn University) at 26.6 °C day, 24.6 °C night, and 60% RH. Illumination was provided using daylight fluorescent tubes (270 PAR) with a 16 : 8 h (L:D) photoperiod. Seeds were planted in a top soil/vermiculite/peat moss mixture in plastic pots. Plants used in bioassays were 4- to 5-week-old. In order to ensure that "plant-fed" *H. virescens* larvae used in each bioassay fed on a substantial amount of leaf tissues, plants were laid down horizontally in clear aerated plastic bowls (28 × 16 × 8 cm). Cotton and soybean were selected for this study partly because they are economically important crops in the southern United States, and partly because they are expected to have relatively different chemical profiles. For instance, herbivory significantly induces gossypol in cotton (Zebelo *et al.*, 2017) while methyl salicylate is induced in soybean (Zhu & Park, 2005).

Insects

Heliothis virescens larvae were initially reared on pinto bean artificial diet (Shorey & Hale, 1965) and adult moths fed 10% sugar water *ad libitum*. Larvae were removed from artificial diet and starved for 6 h in a plastic bowl. Afterwards, the larvae were cleaned of frass and artificial diet crumbs with moistened paper towel. Then, they were separated into two groups: one group was allowed to feed on cotton plant (cotton-fed) while the other group fed on soybean plant (soybean-fed). Before use in oviposition choice bioassays, third-instar larvae of *H. virescens* were removed from plant materials and cleaned with moistened paper towel. *Microplitis croceipes* were reared on second- and third-instar larvae of *H. virescens* and adult wasps were supplied 10% sugar water *ad libitum* upon emergence. The rearing procedures for *M. croceipes* were similar to those described by Lewis and Burton (1970). Parasitoids used in behavioral bioassays were 3- to 4-d-old females that were presumed mated after interaction with males for at least 48 h. The general rearing conditions for all insects were 25 ± 1 °C, 75% ± 5% RH and 14 : 10 h (L : D) photoperiod.

Feeding choice tests for host larvae

Feeding bioassays were conducted in no-choice and two-choice experiments to test the preference of *H. virescens* larvae for cotton or soybean. Larvae were initially fed artificial diet and cleaned as previously described. For these experiments, the leaf (cotton) or trifoliate leaf (soybean) of an infested plant was clipped at the petiole, close to the leaf blade. In no-choice tests, a leaf from either plant was placed centrally in an aerated plastic bowl (22 × 16 × 6 cm) inlayed with moistened paper towel. Ten third-instar larvae were placed on the leaf to feed for 24 h. In two-choice tests, one cotton leaf and one soybean trifoliate leaf were placed at opposite ends of a similar bowl, separated by at least 5 cm. Ten third-instar larvae were randomly placed in the space between the leaves of both plants to feed for 24 h. Leaf pictures were taken before and after infestation for leaf area analyses in ImageJ software. Experiments were performed in a randomized complete block design using each of four plant replicates on separate days.

In initial feeding tests, *H. virescens* larvae showed preference for cotton over soybean, and consumed about twofold cotton foliage compared with soybean foliage within 24 h (Fig. S1). The feeding preference of *H. virescens* larvae informed the duration or number of larvae that should infest cotton or soybean to avoid possible bias in subsequent oviposition choice tests with parasitoids. Thus, for oviposition choice experiments with parasitoids, a group of 40 *H. virescens* larvae was allowed to feed on cotton for 24 h while a similar group was allowed to feed on soybean for 48 h.

Treatments for oviposition choice tests

In two-choice oviposition tests with cotton-fed and soybean-fed hosts, female parasitoids received one of seven treatments: (i) no experience with oviposition/conditioning with host-damaged plant/plant background (Naive), (ii) experience with oviposition in cotton-fed hosts only (OV-Cotton), (iii) experience with oviposition in soybean-fed hosts only (OV-Soybean), (iv) conditioned with host-damaged cotton only (DG-Cotton), (v) conditioned with host-damaged soybean only (DG-Soybean), (vi) inexperienced parasitoids tested with a background of host-damaged cotton odors (BG-Cotton), and (vii) inexperienced parasitoids tested with a background of host-damaged soybean odors (BG-Soybean). Parasitoids with treatments BG-Cotton and BG-Soybean had no previous oviposition experience. See Figure 1 for illustration.

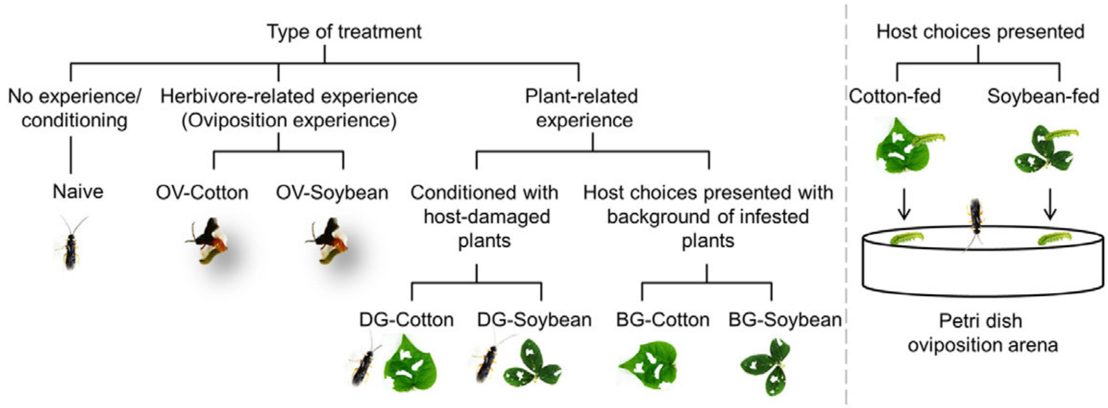

Fig. 1 An illustration showing different plant- and herbivore-related experiences received by female *Microplitis croceipes* tested in oviposition choice experiments: no experience with oviposition, no conditioning with host-damaged plant and no plant background (Naive); experience with oviposition in cotton-fed hosts only (OV-Cotton); experience with oviposition in soybean-fed hosts only (OV-Soybean); conditioned with host-damaged cotton only (DG-Cotton); conditioned with host-damaged soybean only (DG-Soybean); inexperienced parasitoids tested with a background of host-damaged cotton odors (BG-Cotton); and inexperienced parasitoids tested with a background of host-damaged soybean odors (BG-Soybean). Parasitoids with treatments BG-Cotton and BG-Soybean had no previous oviposition experience. Individual parasitoids were presented with a choice of one cotton-fed and one soybean-fed *Heliothis virescens* larva in Petri dish arena.

For OV-Cotton and OV-Soybean treatments, a female parasitoid was presented with a cotton-fed or soybean-fed host in a training Petri dish (25 × 10 mm) and allowed a single oviposition. The host larva was immediately removed while the wasp remained in isolation for about 5 min. Then, the procedure was repeated a second time with a new host larva of the same type. A previous study (Eller *et al.*, 1992) showed that a single experience with plant–host complex was insufficient to elicit subsequent preference in *M. croceipes*. Thus, parasitoids were trained two times. For DG-Cotton and DG-Soybean treatments, a plant was infested with 20 (for cotton) or 40 (for soybean) third-instar *H. virescens* larvae for 24 h to compensate for host feeding preference. Each infested plant was placed in a 5 L glass jar (Analytical Research Systems, Inc., Gainesville, FL, USA). Host larvae and frass were removed and a female parasitoid was introduced into the jar. The parasitoid was observed to antennate on the leaves (contact leaf with antenna in a probing manner) — four to five times before it was removed. The parasitoid was left in isolation for about 5 min and the procedure was repeated a second time using the same plant. After conditioning treatments, female parasitoids were left to acclimatize in arena conditions for 5 min before use in a bioassay.

For BG-Cotton and BG-Soybean treatments, plants were infested and placed in a glass jar as previously described. The Petri dish oviposition arena was perforated with 10 holes (2 mm diameter each) uniformly spread on the base. The perforated base was then placed above a second base that was connected to the glass jar through Teflon® tube. A purified (charcoal filtered) and humidified air stream of 200 mL/min was passed through the jar at room temperature into the Petri dish arena so that plant volatiles can serve as background odors.

Treatments for oviposition no-choice tests

In no-choice tests with cotton-fed or soybean-fed hosts, parasitoids received one of two treatments: (i) associative conditioning with oviposition in cotton-fed hosts in the presence of host-infested cotton odors (AC-Cotton) and (ii) associative conditioning with oviposition in soybean-fed hosts in the presence of host-infested soybean odors (AC-Soybean). Treatment controls (parasitoids with no conditioning) were also included for comparison. For associative conditioning, volatiles emitted by infested plants were passed into a training Petri dish as previously described. A parasitoid was then allowed to associate oviposition experience (unconditioned stimulus) with host-infested plant odors (conditioned stimulus). The procedure was repeated two times, similar to conditioning treatments.

Oviposition bioassays

Two-choice oviposition tests were conducted in medium-size Petri dish (100 × 15 mm) arena to investigate intraspecific host preference in *M. croceipes*. One cotton-fed and one soybean-fed third-instar host larvae of similar size were randomly positioned in the middle of the arena before introducing each wasp. A female parasitoid was released at one of 12, 3, 6 or 9 o'clock positions in the arena to avoid bias. Once introduced into the Petri dish, the parasitoid was allowed a maximum of 10 min to parasitize one host larva. A parasitoid that did not make a choice within 10 min was scored as "no-choice," removed from the arena and replaced with another parasitoid that had received the same treatment. Parasitoids, host larvae and Petri dishes were used once and discarded. Oviposition choice experiments were arranged in a randomized complete block design with equal number of replicates tested for each treatment group (Naive, OV-Cotton, OV-Soybean, DG-Cotton, DG-Soybean, BG-Cotton and BG-Soybean) on each day between 0900 and 1700 h. Oviposition choice tests were repeated two times with 30 responding parasitoids per test. Experiments testing effect of plant backgrounds were repeated three times to confirm consistency of the results. In oviposition choice bioassays, the number of parasitoids scored as "no-choice" was relatively low (range 0–3) and thus excluded from data analyses.

No-choice oviposition tests were conducted with parasitoids that received treatments AC-Cotton and AC-Soybean to investigate the effect of associative learning on host location in *M. croceipes*. The experimental design and procedure was similar to the one previously described for oviposition choice tests, with some exceptions. A relatively larger Petri dish (140 × 20 mm) arena was used to ensure considerable distance between host larva and parasitoid at the time of wasp release into the arena. The Petri dish was perforated with 20 holes (2 mm diameter each) and connected to a jar containing host-infested plant. One host larva (cotton-fed or soybean-fed) was positioned at the center of the arena before a parasitoid was released into the Petri dish at one of 12, 3, 6 or 9 o'clock positions. Time taken for a conditioned parasitoid to locate and parasitize a cotton-fed host in an arena supplied with host-infested cotton odors was recorded. A similar experiment was performed with soybean-fed host/infested soybean combination. For control experiments, time taken for unconditioned parasitoids to locate and parasitize hosts in similar arenas was also recorded. Only parasitoids that responded within 10 min were included in the analyses but the number of individuals that did not respond within 10 min (range 0–5) was also recorded. No-choice oviposition tests were repeated two times with 30 replicates per test.

Data analyses

Feeding preference of *H. virescens* larvae between cotton and soybean was analyzed by comparing leaf areas consumed using ImageJ software (v. 1.50i). Data on *H. virescens* feeding preference satisfied normality and equal variance requirements, thus, there was no need for transformation. In two-choice feeding tests for *H. virescens*, the infested leaf areas were compared using paired *T*-test. In no-choice feeding tests for *H. virescens*, the infested leaf areas were compared using two independent samples *T*-test. For *M. croceipes* two-choice oviposition tests, the deviation of parasitized hosts from a 50% : 50% distribution between cotton-fed and soybean-fed host groups was analyzed using a χ^2 test. In no-choice oviposition tests with *M. croceipes*, significant differences between time taken by conditioned and unconditioned parasitoids to parasitize hosts were analyzed using Two independent samples *T*-test. Time data satisfied normality and equal variance requirements, thus, there was no need for transformation. All analyses were conducted using SAS 9.2 with 0.05 level of significance.

Results

Two-choice oviposition tests: intraspecific host preference in M. croceipes

Intraspecific oviposition preference in female *M. croceipes* was influenced by experience, conditioning or plant background provided. Results of oviposition choice tests are shown in Figure 2. Naive parasitoids did not discriminate between cotton-fed and soybean-fed hosts ($\chi^2 = 0.53$, $P = 0.47$). However, parasitoids that only had oviposition experience with cotton-fed hosts subsequently showed preference for cotton-fed over soybean-fed hosts ($\chi^2 = 8.53$, $P = 0.004$). Similarly, parasitoids that only had oviposition experience with soybean-fed hosts subsequently showed preference for soybean-fed over cotton-fed hosts ($\chi^2 = 6.53$, $P = 0.01$). Conditioning with host-infested plant (cotton or soybean) only did not significantly elicit oviposition preference in *M. croceipes*. Interestingly, inexperienced parasitoids showed preference for soybean-fed over cotton-fed hosts when host choices were presented with background of host-infested cotton odors ($\chi^2 = 4.80$, $P = 0.03$). Similarly, inexperienced parasitoids showed preference for cotton-fed over soybean-fed hosts when host choices were presented with background of host-infested soybean odors ($\chi^2 = 6.53$, $P = 0.01$).

No-choice oviposition tests: effect of associative learning on host location in M. croceipes

Oviposition no-choice tests were conducted to investigate the effect of associative learning on host location in *M. croceipes*. Conditioned parasitoids took significantly

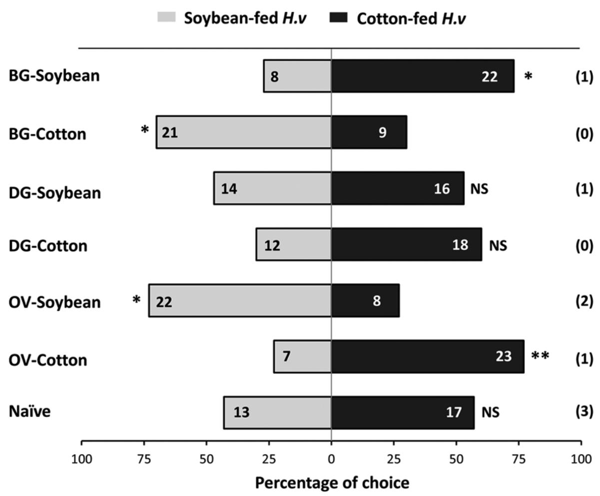

Fig. 2 Oviposition preference of female *Microplitis croceipes* for cotton-fed and soybean-fed *Heliothis virescens* (*H.v*) larvae in a Petri dish arena. Parasitoids received one of the following treatments: no experience with oviposition, no conditioning with host-damaged plant and no plant background (Naive); experience with oviposition in cotton-fed hosts only (OV-Cotton); experience with oviposition in soybean-fed hosts only (OV-Soybean); conditioned with host-damaged cotton only (DG-Cotton); conditioned with host-damaged soybean only (DG-Soybean); inexperienced parasitoids tested with a background of host-damaged cotton odors (BG-Cotton); and inexperienced parasitoids tested with a background of host-damaged soybean odors (BG-Soybean). Individual parasitoids were presented with a choice of one cotton-fed and one soybean-fed *Heliothis virescens* larva in Petri dish arena. $N = 30$ responding wasps per test. Numbers inside bars indicate actual number of parasitized hosts. Numbers in parenthesis indicate number of parasitoids that made no choice within 10 min. Asterisks (*) indicate significant deviation from a 50% : 50% distribution for total number of ovipositions made into the two types of host ($P < 0.05$; χ^2 test).

($t = -6.63$, $P < 0.0001$) less time to locate and parasitize cotton-fed hosts, compared to control (unconditioned parasitoids) (Fig. 3A). Similarly, conditioned parasitoids took significantly ($t = -6.10$, $P < 0.0001$) less time to locate and parasitize soybean-fed hosts, compared to control (Fig. 3B). Moreover, most of the parasitoids that received training took less than 5 min to locate host larvae while untrained parasitoids took between 5 and 10 min to locate host larvae (Fig. 3).

Discussion

Microplitis croceipes is a relatively specialized parasitoid of *H. virescens*, which is a generalist herbivore. According to the concept of dietary specialization and infochemical use proposed by Vet and Dicke (1992), parasitoids with such diet restrictions are predicted to rely more on herbivore-derived cues than plant-derived cues. Although widely accepted, only few empirical studies have tested this concept (Steidle & van Loon, 2003), especially in dynamics with the role of parasitoid experience. In this study, naive (inexperienced) female *M. croceipes* did not discriminate between cotton-fed and soybean-fed *H. virescens* in oviposition choice tests, supporting our first prediction that this category of parasitoids shows innate responses to herbivore-emitted kairomones, regardless of host plant identity. Furthermore, oviposition experience alone strongly influenced subsequent oviposition preference, whereas conditioning with host-infested plants alone did not elicit host preference in parasitoids. This supports our second prediction that herbivore-related experience has greater influence on intraspecific host preference than plant-related experience.

Plant chemicals acquired by herbivores can be used for metabolism and defense (Despres *et al.*, 2007). When such chemicals are emitted by their herbivore hosts, they may also serve as kairomones for parasitoids (Morawo & Fadamiro, 2016). In a previous study, we reported that plant-fed *H. virescens* larvae retained and emitted plant-associated compounds that attracted *M. croceipes* (Morawo & Fadamiro, 2016). In this study, *H. virescens* larvae showed a clear feeding preference for cotton over soybean, both in no-choice and two-choice experiments (Fig. S1). However, naive *M. croceipes* did not discriminate between *H. virescens* larvae that fed on different plants but oviposition experience influenced subsequent intraspecific host preference.

Although oviposition in soybean-fed hosts appears to be the optimal choice due to higher survival rates of host larvae (Nadgauda & Pitre, 1983), herbivore-related experience (with cotton-fed hosts) may override such considerations. This contradicts the "mother knows best" hypothesis (Henry *et al.*, 2005; Chesnais *et al.*, 2015). In fact, preference-performance hypothesis may not adequately explain host selection; possible conflicts between the needs of progeny and behavioral dynamics of mother insects should also be considered (Scheirs & De Bruyn, 2002; McCormick *et al.*, 2017). When hosts are abundant, optimal host use in parasitoids tend to be incremental, such that oviposition increases the probability of staying in a host patch to seek more hosts (Mills & Wajnberg, 2008; Kugimiya *et al.*, 2010). In general, these results suggest that herbivore-related experiences such as oviposition play important role in host discrimination in specialist parasitoids.

Nevertheless, our results also showed that plant-related experience play an important role in shaping the

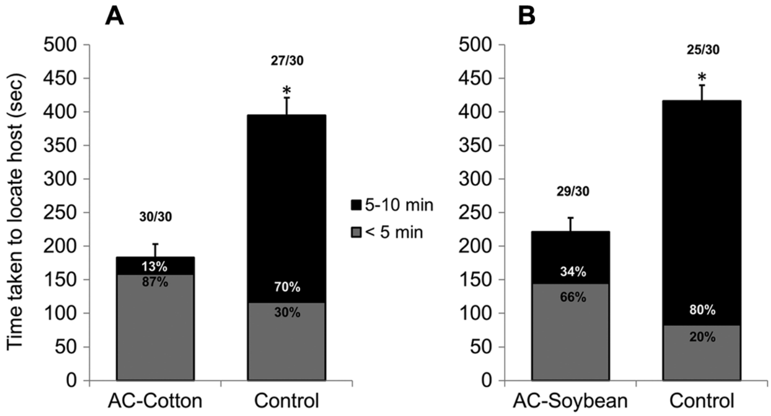

Fig. 3 Effect of associative learning on host location in *Microplitis croceipes*. Parasitoids tested in a Petri dish oviposition arena received two treatments: (A) associative conditioning with oviposition in cotton-fed hosts in the presence of host-infested cotton (AC-Cotton) and (B) associative conditioning with oviposition in soybean-fed hosts in the presence of host-infested soybean (AC-Soybean). A parasitoid was subsequently presented with a *Heliothis virescens* larva that fed on the conditioning plant. Asterisks (*) indicate significant differences between time taken (sec) by trained parasitoids and unconditioned parasitoids (Control) to locate and parasitize hosts ($P < 0.05$; two independent samples T-test). Proportions outside bars indicate number of responders that located hosts within 10 min/total number of replicates per test. Percentages inside bars represent proportions of responders that located hosts under 5 min and between 5 and 10 min durations.

foraging decisions made by parasitoids. Besides serving as highly detectable long range cues for locating host habitat (Vet & Dicke, 1992), plant volatiles may play other unique roles in short range host location. In this study, associative learning of infested plant odors with oviposition facilitated short-range host location in parasitoids. Such associative learning is a classic example of plant- and herbivore-related experiences combining to produce a synergistic effect. Although present results from laboratory experiments showed that oviposition experience alone influenced subsequent host preference in *M. croceipes*, herbivores are less likely to be encountered outside the context of their host plants. Comparing the volatiles emitted by cotton-fed *H. virecens* larvae (Morawo & Fadamiro, 2016) and those emitted by *H. virecens*-infested cotton (Morawo & Fadamiro, 2014), it can be deduced that host-emitted volatiles are mostly plant-derived but the profile differs from that of the host plant. Therefore, in addition to the effect of associative learning, it is also possible that plant odors served as background odors to facilitate recognition of herbivore host odors (Hilker & McNeil, 2008).

To further investigate the effect of background odors, naive parasitoids were tested in oviposition choice bioassays with a background of host-infested cotton or soybean odors. Parasitoids that did not receive prior conditioning or training showed preference for soybean-fed over cotton-fed hosts when host choices were presented with background of host-infested cotton odors, and vice versa. The result is noteworthy because naive parasitoid did not discriminate host groups when a plant background was not supplied in previous tests. The result is also interesting because parasitoids showed preference for the host group that did not feed on the plant background presented. A plausible explanation is that a background of soybean odors probably created more contrast with cotton-fed hosts than with soybean-fed hosts, and vice versa. The result further lends support to the olfactory contrast hypothesis proposed by Hilker and McNeil (2008). The more difference in the chemical profiles of an herbivore host and its plant background, the more olfactory contrast generated, and the more detectable a host larva becomes (Hilker & McNeil, 2008). Similar observations have been reported for male moths (Light *et al.*, 1993; Deng *et al.*, 2004; Yang *et al.*, 2004; von Arx *et al.*, 2012) and parasitoids (Xu *et al.*, 2017) in which plant volatiles synergistically enhanced the detection of female sex pheromones. The relevance of plant volatiles as background odors in parasitoid host location has been discussed in previous studies (Mumm & Hilker, 2005; Schröder & Hilker, 2008; Beyaert *et al.*, 2010).

The enemies hypothesis predicts that pest population is reduced in mixed stands (polycultures) because natural enemies thrive in complex vegetation (Root, 1973). Here, we also suggest that the olfactory contrast effect may have serious fitness consequences for generalist herbivore species such as *H. virescens* larvae in polycultures. This may also apply to more mobile generalist herbivores such as beetles that are capable of switching host plants across monoculture systems. Thus, among other factors, host switching may render a generalist herbivore more detectable to natural enemies because of the olfactory contrast generated with odors of the newly infested plant species. This leads to an opportunistic fitness benefit for specialist parasitoids of generalist herbivore species.

In summary, this study investigated the role of plant- and herbivore-related experiences in intraspecific host preference in a relatively specialized parasitoid, *M. croceipes*. Evolution should favor an olfactory mechanism that is highly sensitive and tuned to herbivore host cues in specialist parasitoids (Vet & Dicke, 1992; Steidle & van Loon, 2003; Wajnberg *et al.*, 2008). While our results partly confirm the prediction that herbivore-related experience has greater influence than plant-related experience on intraspecific host preference in *M. croceipes*, the results also indicate a possible synergistic effect of the two types of experiences during associative learning. Further, plant odors probably serve as a contrasting background with herbivore odors (Hilker & McNeil, 2008), thus facilitating host location and leading to intraspecific host discrimination in *M. croceipes*. To the best of our knowledge, this is one of the few studies to confirm the olfactory contrast hypothesis in a similar tritrophic system. Overall, these results suggest that both plant- and herbivore-related experiences play important roles that affect foraging decisions in parasitoids, especially at short range (Röse *et al.*, 1997). While this study focused on odor cues, other inputs such as tactile and visual information may contribute to host discrimination in parasitoids (Turlings *et al.*, 1993; Heipel & Casas, 2008). Future studies should compare the role of experience and other physiological factors such as nutrition and mating status affecting host discrimination in specialist versus generalist parasitoid species.

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Disclosure

The authors declare that they have no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 Feeding preference of *Heliothis virescens* larvae for cotton and soybean in (A) no-choice feeding experiments and (B) two-choice feeding experiments. Leaf area (cm^2) consumed by 10 third-instar larvae was visualized using ImageJ (v. 1.50i) software. Difference between mean (± SEM) leaf area of cotton and soybean consumed in no-choice feeding tests was analyzed using two independent samples T-test ($N = 4$). Difference between leaf area consumed in two-choice feeding tests was analyzed using paired T-test ($N = 4$). Asterisks (*) indicate significant differences ($P < 0.05$; T-tests).