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Host Suitability Affects Odor Association in *Cotesia marginiventris*: Implications in Generalist Parasitoid Host-Finding

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Abstract Insect herbivores often induce plant volatile compounds that can attract natural enemies. *Cotesia marginiventris* (Hymenoptera: Braconidae) is a generalist parasitoid wasp of noctuid caterpillars and is highly attracted to *Spodoptera exigua*-induced plant volatiles. The plasticity of *C. marginiventris* associative learning to volatile blends of various stimuli, such as host presence, also has been shown, but little is known about how this generalist parasitoid distinguishes between host species of varying suitability. *Spodoptera exigua* is an excellent host that yields high parasitoid emergence, while *Trichoplusia ni* serves as a sub-optimal host species due to high pre-imaginal wasp mortality. We have found that *S. exigua* and *T. ni* induce different volatile blends while feeding on cotton. Here, wind tunnel flight assays were used to determine the importance of differentially induced volatiles in host-finding by *C. marginiventris*. We found that, while this generalist parasitoid wasp can distinguish between the two discrete volatile blends when presented concurrently, a positive oviposition experience on the preferred host species (*S. exigua*) is more important than host-specific volatile cues in eliciting flight behavior towards plants damaged by either host species. Furthermore, wasps with oviposition experience on both host species did not exhibit a deterioration in positive flight behavior, suggesting that oviposition in the sub-optimal host species (*T. ni*) does not cause aversive odor association.

Keywords Tri-trophic interactions · Sub-optimal host · Wind tunnel · *Spodoptera exigua* · *Trichoplusia ni* · *Cotesia marginiventris*

Introduction

When damaged by insect feeding, plants release volatile organic compounds that can attract natural enemies of the herbivores (Turlings et al., 1990b). These herbivore-induced plant volatiles (HIPVs) can vary by herbivore and plant species due to differences in insect oral elicitors (Alborn et al., 1997; Schmelz, 2006) and plant physiological responses (Schnee et al., 2006; Schmelz et al., 2009), and have been shown to selectively attract parasitoids of herbivores (Turlings et al., 1995; De Moraes et al., 1998). Parasitoid wasps have the capacity to associatively learn specific odors (Lewis and Tumlinson, 1988; Lewis and Takasu, 1990) or contact cues (Jones et al., 1971) related to host and/or food presence, which may predispose them for more efficient foraging for host and food resources.

Associative learning occurs when an unconditioned stimulus, such as parasitoid oviposition in a host, is combined with a conditioned stimulus, like an odor source, to alter the subsequent behavioral response of an organism (Papaj and Lewis, 1993). The use of sequentially spaced experiences can strongly reinforce this behavior and cause long-term memory formation by synthesizing proteins in the insect

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brain (Smid et al., 2007), and can stimulate searching behavior in the wasp (Bleeker et al., 2006). This behavioral plasticity is useful for parasitic wasps, especially generalists (i.e., wasps that parasitize multiple host species) because the plant and host cues that they learn can vary greatly both spatially and temporally.

Negative associative learning can occur in the absence of reward conditioning or if a low quality reward is provided following the conditioned stimulus. When this occurs, a wasp may learn avoidance behavior in response to an odor source, or switch its odor preference (Takasu and Lewis, 1996). Few studies have explored this form of conditioning, which is probably a widespread occurrence for naturally foraging generalist wasps. Those studies that have examined negative associations have used either hosts unsuitable for oviposition (Takasu and Lewis, 2003; Costa et al., 2010) or a lack of oviposition reward (e.g., no host present) (Papaj et al., 1994). No study thus far has attempted to explain how associative learning occurs during sub-optimal host experiences, which may play a role in shaping realized host range where multiple hosts of varying quality are present in the foraging landscape.

Another area of parasitoid behavior that warrants research is the additive or diminished capacity of associative learning to form memory traces for parasitoids when multiple species are presented in sequence (e.g., a preferred and non-preferred host species). The marginal value theorem predicts that a predator or parasitoid should stay in a patch longer after a positive host encounter (Wajnberg et al., 2008), but no research has determined the effects of the presence of a less desirable host in the patch. The aphid parasitoid *Monoctonus paulensis* (Ashmead) shows a strong preference for the pea aphid [*Acyrthosiphon pisum* (Harris)] over alfalfa aphid [*Macrosiphum creelii* (Davis)] corresponding to increased fitness in the former (Chau and Mackauer, 2001). It can adjust its oviposition rate to maximize parasitization of the preferred host when either host species is presented in a sequential patch (Michaud, 1996). It has yet to be determined if residence time of a parasitoid in a patch that contains both preferred and sub-optimal hosts is different from the length of time spent in a patch with preferred hosts alone. Elucidation of these basic behaviors is necessary to predict how generalist wasps optimally forage when both hosts occur together in nature.

Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae) is a parasitoid wasp that is considered a "generalist" due to its wide host range (Turlings et al., 1989), which encompasses numerous noctuid species including *Spodoptera exigua* (Hübner) and *Trichoplusia ni* (Hübner), the beet armyworm (BAW) and cabbage looper (CL), respectively (Krombein et al., 1979). The breadth of volatile plant compounds to which *C. marginiventris* is physiologically responsive was established by Gouinguene et al. (2005), and includes as least 38 compounds. The ability of *C. marginiventris* to associate learned volatile cues to specific hosts (Dmoch et al., 1985; Tamo et al., 2006) suggests that there should be flexibility in its host-finding strategy, which has been observed in other parasitoid systems with both aggregated and regularly spaced hosts (Burger, 2006). Here, we used the sub-optimal host CL (Boling and Pitre, 1971), and a preferred host, BAW to evaluate the importance of associative learning related to sub-optimal host experience and its effect on subsequent odor preference by *C. marginiventris.* Specifically, we tested the hypothesis that *C. marginiventris* will associate volatile odors with BAW but not CL.

Methods and Materials

Insects and Plant Rearing BAW and CL were received as eggs from Benzon Research Inc. (Carlisle, PA, USA), and reared on soybean flour-wheat germ diet (Southland Products Inc., Lake Village, AR, USA) at 27±2°C and 40-70 % relative humidity with 16:8 hL:D cycle until late first or early second instar. *Cotesia marginiventris* is a solitary koinobiont endoparasitoid of host larvae. Our data suggest that *C. marginiventris* emergence success from stung caterpillars nears 80 % for BAW hosts, but only 18 % for CL hosts (Harris and Tumlinson, unpublished). A second colony of CL was obtained from Dr. Tom Baker at The Pennsylvania State University (original eggs obtained from K. Haynes, ca. 1995, Kentucky, U.S.A.) to confirm the limited suitability as a host species. Two separate colonies of *C. marginiventris* from different host histories were compared in behavioral bioassays. Colony 1 were reared on late 2nd and early 3rd instar BAW on pinto bean diet (Burton, 1969) and established in 2004 with at least 50 % replacement with field-collected wasps (trapped with sentinel caterpillars) annually. Adults were supplied a 5 % honey/water solution for food and held at 25±4°C and L:D 12:12 h to 14:10 h. Colony 2 originated from individuals collected from fall armyworm [*Spodoptera frugiperda* (J.E. Smith), FAW] hosts on bermudagrass [*Cynodon dactylon* (L.) Persoon], and reared in the laboratory on FAW on corn (*Zea mays* L.). Wasps used in wind tunnel assays were 2–7 d post-pupal emergence.

Cotton (*Gossypium hirsutum* L. var. DPL90) was grown in 10 cm diam plastic pots using Sun Gro® MetroMix 200 Series soil at 27±2°C and 40-70 % relative humidity with a 14:10 hL:D cycle and 200–500 LUX. Autoclaved soil and sterile pots/trays were used to prevent soil bacteria or insect contamination. Plants used for both volatile collection and wind tunnel assays were 28–40 d-old with 5–7 true leaves.

Volatile Collection Plants were grown in an insect-free sealed glasshouse. They were set up at 6:00 p.m. the night before collections with steel guillotines and cotton surrounding the stem, dividing the pot/soil from leaf tissue. At 5:30 a.m. the following day, twenty second instar BAW or CL were applied to each treatment plant (5 individuals on each of the top four fully expanded leaves, 20 larvae total), and glass bell jars were placed over plants. Volatiles were collected for 3 d from 6:00–10:00 a.m., 10:00 a.m.-2:00 p.m., 2:00–6:00 p.m., and 8:00 p.m.-12:00 a.m. On days 1 and 2 at 5:30 a.m. or 6:15 p.m., caterpillars were removed or added to equalize damage between plant treatments. Plants were base watered with 40 ml at 6:00 p.m. on the first and second day of collections. Following the final volatile collection period, total leaf area for each plant was quantified using UTHSCSA Image Tool® and ranged from 250–500 cm^2. Percent damage was quantified and ranged from 1-5 % of total leaf area.

A push-pull Automated Volatile Collection System (ARS, Inc. Gainesville, FL, USA) was used to collect volatile organic compounds (VOCs) emitted from intact control plants, BAW-damaged, and CL-damaged plants. Charcoal-filtered air was pushed at 1–1.5 l/min into a 3 L bell-glass chamber enclosing a plant, and was vacuumed out through polymeric adsorbent filters (Super-Q®, 30 mg, Alltech Associates) at 1 L/min (Loughrin et al., 1994). Super-Q® filters were subsequently eluted with 100 μl of 1:1 dichloromethane: hexane (J.T. Baker, 95 % purity and Burdick and Jackson High Purity, respectively). For quantification, 25 μl of an internal standard mix (8 ng/μlof nonyl acetate and 5 ng/μl *n*-octane) were added to each sample. Samples were analyzed using a non-polar methyl silicone Equity-1 column (Supelco®) via gas chromatography (GC) with flame ionization detection (FID) and identified using a non-polar HP-1 MS column (Agilent®) on GC-MS (gas chromatography coupled with mass spectrometry) in electron impact (EI) mode. Spectra were compared to known libraries in the National Institute of Standards and Technology (2002 version) for identification, and synthetic compounds were compared for retention times.

Wind Tunnel Bioassays Wind tunnel bioassays were conducted from May-September from 2008–2010. Plants used in behavioral assays were infested with 20–30 1st instar BAW or CL that were allowed to feed freely for 2 d, until they reached the wasp's preferred 2nd instar stage and had similar defoliation amounts as plants used in volatile collections. Two plants (caterpillar-damaged and undamaged control) were placed equidistant from the side walls and from each other, in a 0.61 x 0.61 x 1.83 m acrylic glass wind tunnel, 1 m upwind from a wasp release platform, with a light intensity of 630 LUX and a wind velocity of 0.5 m/s. Damaged plants used in the tunnel contained actively feeding larvae. Air entering the tunnel was passed through charcoal filters, and room and tunnel temperature was maintained at 26-30°C and 50-75 %RH (Steinberg et al., 1992). Plants were used for two wasp subject flights and then replaced with new treatment plants. All flight assays were conducted between 9 a.m. and 4 p.m. Specific details of the parasitoid treatments and assessments are described below.

Parasitoid Oviposition Training Mated 2–7 d-old wasps were given oviposition experiences on three BAW or three CL feeding on cotton in the presence of conspecific-damaged or opposite-species damaged plant tissue, with a 5 min break between each oviposition. Subsequently, they were permitted to fly upwind and to land on a cotton plant damaged by the same or opposite caterpillar species on which they had oviposition experience, and an undamaged cotton plant as a control. To test wasp association of non-plant odors, wasps were given three oviposition experiences on wheatgerm-fed BAW or CL in the presence of host frass and 3 μl diluted vanilla extract (McCormicks®, 1:4 vanilla: distilled water; stored in air-tight flask to prevent evaporation) and permitted to fly upwind to 10 μl diluted vanilla extract on Whatman #1 filter paper or a blank filter paper disc control. One μl of vanilla extract was added to the filter paper every 10 min to compensate for evaporation of the odor stimulus.

Wind tunnel assays were conducted for up to 40 min per wasp, with 25–71 female wasps tested per treatment. A "wall" choice was recorded if the wasp did not land on the odor source or control after three flight platform departures. Positions of odor source and control were alternated between wasps. Each wasp was used only once, and within 30 min of oviposition training.

For sequential experience flight studies, wasps from the fall armyworm-reared colony were experienced as above, and the first flight choice was recorded. Wasps then were removed from the wind tunnel and given an oviposition experience on the opposite host species from which they were initially trained, in the presence of the same odor source they were originally presented with. Within 30 min of the first flight, they were placed back in the tunnel and permitted to fly to damaged plants as before. Because wasps have been shown to remember visual location of hosts (Sheehan et al., 1993), position of damaged plants in tunnel was switched.

Statistical Analyses Plant compounds were analyzed individually using ANOVA for Day 3 2:00–6:00 p.m. (JMP®) when induced volatiles are detectable, and multiple comparisons were analyzed via Tukey HSD. In wind tunnel bioassays, wall-landing (no choice) was affected by treatment type (i.e., host species experience) and was included in statistical analysis. Multinomial Logistic Regression was utilized to analyze flight landing choices (SPSS®), with significance indicated between the two host species treatments. Significance within treatments was analyzed using likelihood ratio χ^2.

Results

Cotton Volatile Analysis Percent damage calculated for BAW- and CL-damaged plants ranged from 1-5 % and did not vary significantly between treatments (N=9, F=0.020; P=0.887). Cotton volatile induction varied quantitatively for all treatments, and qualitatively for undamaged vs. herbivore-damaged plants, most notably in the absence of sesquiterpenes, indole, and *cis*-jasmone in undamaged control plants (Fig. 1).

Standard Least Squares ANOVA was used to determine treatment effect for BAW, CL, and undamaged (Intact) cotton. Tukey HSD shows BAW and CL (*df* - 2, 29) treatments were significantly different for β-caryophyllene: F - 9.331, P=0.001, (*E,E*)-α-farnesene: F - 6.606, P=0.005, *trans*-bergamotene: F - 5.172, P=0.014, α-humulene: F - 12.846, P<0.001, α-pinene: F - 9.035, P=0.001, β-pinene: F - 8.840, P=0.001, β-myrcene: F - 11.969, P<0.001, limonene: F - 8.468, P=0.002, linalool: F - 9.116, P=0.001, hexanal: F - 6.378, P=0.006, (*E*)-2-hexanal: F - 5.069, P=0.015, (*Z*)-3-hexenol: F - 12.590, P<0.001, (*E*)-2-hexenol: F - 5.586, P=0.011, (*Z*)-2-pentenyl acetate F - 7.468, P=0.003, (*Z*)-3-hexenyl acetate: F - 9.343, P=0.001, and indole: F - 7.536, P=0.003 (Refer to Fig. S1, supplemental material for more detail on how individual volatile compounds varied by type of damage).

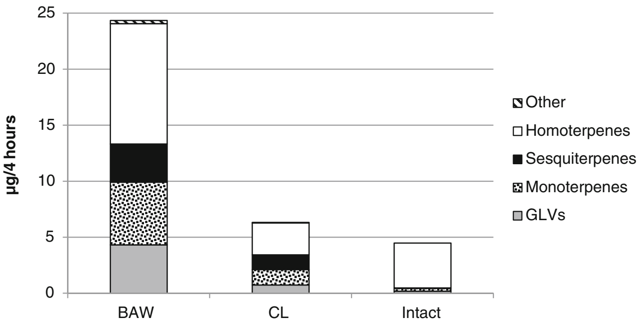

Fig. 1 Ratios of Cotton VOCs Vary Among Damage Treatments. Summed averages for each compound group on Day 2, 2–6 p.m. show different ratios between beet armyworm (BAW)-, cabbage looper (CL)- and undamaged (Intact) cotton plants. Ratios for BAW : CL : Intact are 282:47:0 (Other Compounds), 2:1:1 (Homoterpenes), 23:4:1 (Sesquiterpenes), 23:5:1 (Monoterpenes), and 81:31:1 (GLVs). Standard Least Squares ANOVA: Other Compounds ($F_{2,\ 29}$ - 9.841; P=0.001), Homoterpenes ($F_{2,\ 29}$ -10.071; P=0.001), Sesquiterpenes ($F_{2,\ 29}$ -9.969; P=0.001), Monoterpenes ($F_{2,\ 29}$ -7.850; P=0.003), GLVs ($F_{2,\ 29}$ -9.097; P=0.001). Tukey multiple comparison tests show BAW significantly different from CL and Intact for all compound groups, but CL and Intact not different from each other for any compound group

Wind Tunnel Bioassays Wasps were given oviposition experience on three BAW or three CL in the presence of conspecific-damaged cotton tissue (Fig. 2a). Wasps experienced on BAW flew to (i.e., landed on) BAW-damaged cotton 70 % (colony 1) and 92 % (colony 2) of the time, while wasps experienced on CL flew to the wall of the wind tunnel 55 % (colony 1; N=37 (BAW); 31 (CL); χ^2=14.428; P=0.001) and 54 % (colony 2; N=25 (BAW); 28 (CL); χ^2=13.425; P=0.004) of the time. The effect between colony 1 (*C. marginiventris* reared on BAW on wheatgerm diet) and colony 2 (*C. marginiventris* reared on FAW on corn) was not significant; ANOVA-GLM: Choice=Experience, Colony; P=0.285. Likelihood ratio tests for each treatment was as follows: colony 1 (BAW) χ^2=21.106; P<0.001; colony 1 (CL) χ^2=13.946; P=0.001; colony 2 (BAW) χ^2=20.720; P<0.001; colony 2 (CL) χ^2=15.798; P<0.001.

Wasps were given oviposition experience on three BAW or three CL in the presence of cotton tissue damaged by the opposite species (Fig. 2b). Wasps experienced on BAW flew to CL-damaged cotton 82 % of the time, while wasps experienced on CL flew to the wall of the wind tunnel 55 % of the time; N=28 (BAW); 29 (CL); χ^2=11.093; P=0.004. Likelihood ratio tests for each treatment as follows: BAW χ^2=30.242; P<0.001; CL χ^2=16.777; P=0.002. When given a choice between BAW- or CL- damaged cotton, wasps more often flew to plants damaged by the host species on which they were trained (Fig. 2c). BAW-experienced wasps flew to BAW-damaged cotton 61 % of the time, and CL-damaged cotton 37 % of the time (Fig. 3c; N=41; χ^2=28.966; P<0.001); of wasps experienced on CL, 36 % flew to CL-damaged cotton and 20 % flew to BAW-damaged cotton. Likelihood ratio tests for each treatment as follows: BAW χ^2=27.759; P<0.001; CL χ^2=4.139; P=0.126.

Untrained (naïve) *C. marginiventris* tested for attraction toward BAW-damaged cotton in the wind tunnel flew to the wall of the tunnel 96 % of the time (N=25; χ^2=61.438; P<0.001).

Vanilla-experienced wasps were given oviposition experience on three wheat germ-fed BAW or CL in the presence of diluted vanilla extract and host frass (Fig. 3). Wasps experienced on BAW flew to filter discs containing vanilla odor 61 % of the time, while wasps experienced on CL flew to the wall of the wind tunnel 73 % of the time; N=71 (BAW) and 67 (CL); χ^2=17.739; P<0.001. Likelihood ratio tests for each treatment as follows: BAW χ^2=52.141; P<0.001; CL χ^2=14.343; P<0.001.

Sequential Multi-species Oviposition Training Wasps from the FAW-reared colony used in 2a were removed from the tunnel after their first flight choice and given a fourth oviposition experience on the opposite host species from which they were trained (e.g., Experience 1, 2 and 3 on BAW in presence of BAW-damaged plants; Experience 4 on CL in presence of BAW-damaged plants). Wasps experienced

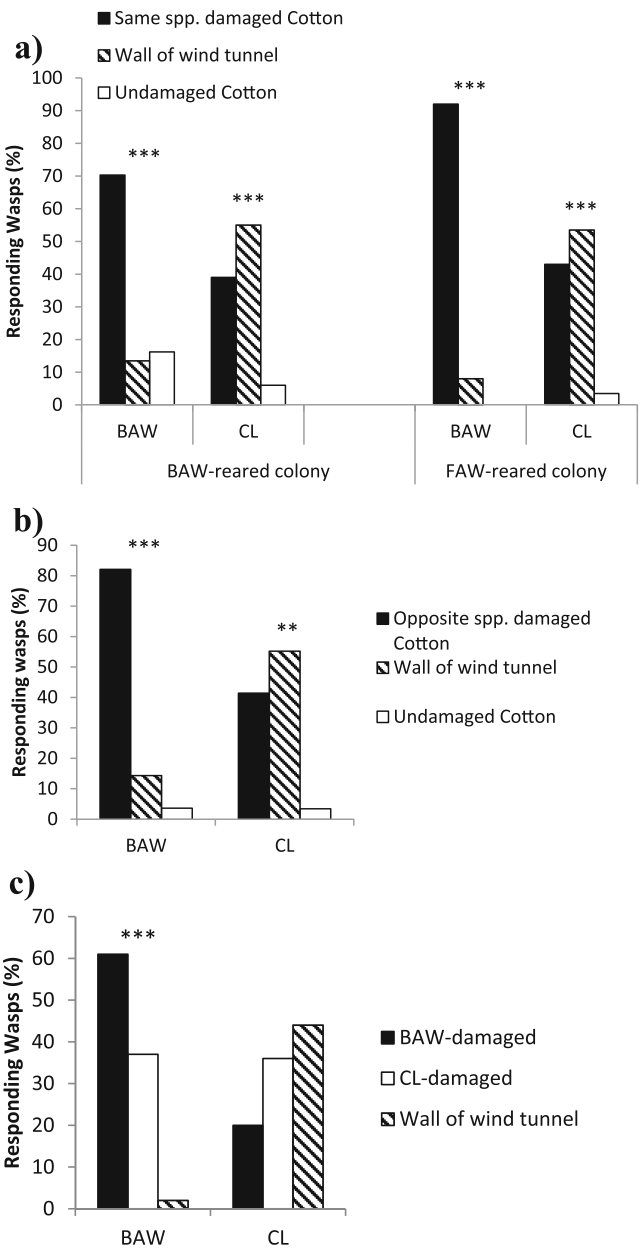

Fig. 2 Effects of host experience on flight response of Cotesia marginiventris to host-associated odors. a) Wasps experienced on beet armyworm (BAW) or cabbage looper (CL) in the presence of conspecific plant tissue, flying to cotton plants damaged by the same host species vs. undamaged plants. Multinomial Logistic Regression comparing choice of BAW- v. CL-experienced wasps as follows: BAW-reared colony: N=37 (BAW); 31 (CL); χ^2=14.428; P=0.001; fall armyworm (FAW)-reared colony: N=25 (BAW); 28 (CL); χ^2=13.425; P=0.004. Test for colony effect (BAW-reared vs. FAW-reared) using GLM: Choice=Experience, Colony; P=0.285. Likelihood ratio tests for each treatment as follows: colony 1 (BAW) χ^2=21.106; P<0.001; colony 1 (CL) χ^2=13.946; P=0.001; colony 2 (BAW) χ^2=20.720; P<0.001; colony 2 (CL) χ^2=15.798; P<0.001. Asterisks denote significance within a host species choice ratio at ***=0.001. **b)** Wasps experienced on BAW or CL in the presence of plants damaged by the opposite host species, flying to cotton plants damaged by the opposite host species vs. undamaged plants: N=28 (BAW); 29 (CL); χ^2=11.093; P=0.004. Likelihood ratio tests for each treatment as follows: BAW χ^2=30.242; P<0.001; CL χ^2=16.777; P=0.002. Asterisks denote significance at **=0.01 and ***=0.001. **c)** Wasps experienced on BAW or CL in the presence of conspecific-damaged plant tissue, flying to BAW- vs. CL-damaged cotton: N=41; χ^2=28.966; P<0.001. Likelihood ratio tests for each treatment as follows: BAW χ^2=27.759; P<0.001; CL χ^2= 4.139; P=0.126. Asterisks denote significance at ***=0.001

on BAW that flew to BAW-damaged cotton on the first flight did not have a deterioration of the positive odor stimulus and continued to land on BAW-damaged cotton after a CL-experience on BAW-damaged cotton (Fig. 4). Wasps experienced on CL that flew to the wall on the first flight had a 60 % improvement of flying to CL-damaged cotton after just one BAW experience on CL-damaged cotton.

Discussion

There is a plethora of literature suggesting the importance of differentially induced HIPVs in natural enemy host-finding. Rose et al. (1998) found that BAW and *Helicoverpa zea* (Boddie) induce almost identical volatile blends in cotton plants, and that the specialist parasitoid, *Microplitis croceipes* (Cresson), could not distinguish between the two volatile profiles. Turlings et al. (1990a) found that *C. marginiventris* innately prefers FAW over CL-damaged corn and cotton in olfactometer tests, and that this preference could be modified through prior exposure to CL-damaged plants, but it is unknown if this was due to differentially induced HIPVs. While the role of plant volatiles in the attraction of *C. marginiventris* to BAW has been widely demonstrated (Turlings et al., 1990b; Turlings and Tumlinson, 1992; Rose et al., 1998; Cardoza et al., 2003), its attraction to CL-induced plant volatiles is not as well understood. Furthermore, few of these studies have examined the actual associative learning aspect of volatile cues related to hosts of differing suitability.

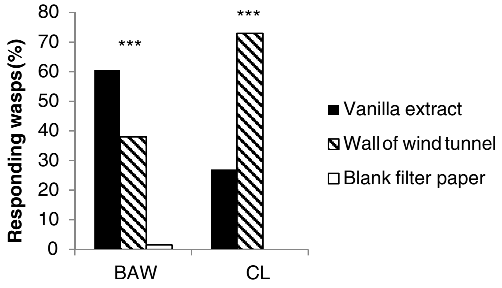

Fig. 3 Wasps flying to vanilla extract following oviposition experience on BAW or CL in the presence of diluted vanilla extract. N=71 (BAW); 67 (CL); χ^2=17.739; P<0.001. Likelihood ratio tests for each treatment as follows: BAW χ^2=52.141; P<0.001; CL χ^2=14.343; P< 0.001. Asterisks denote significance at ***=0.001

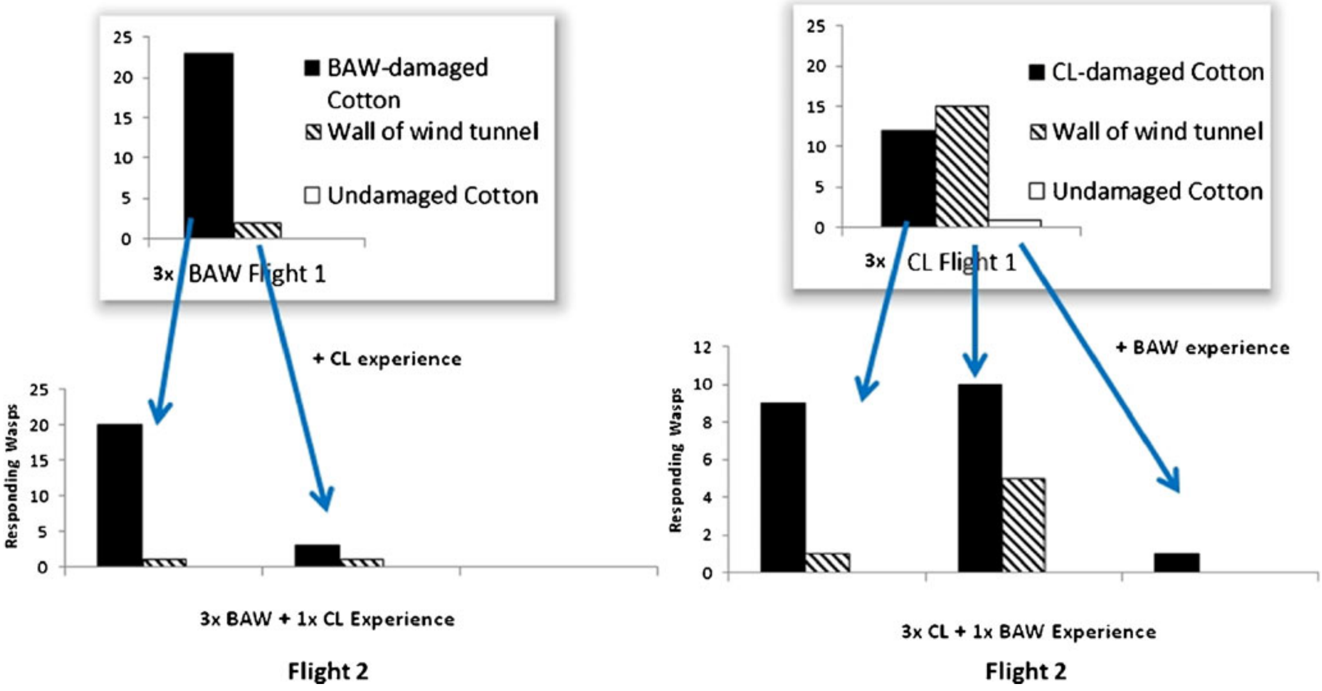

Fig. 4 Second flight of wasps flying to cotton plants after oviposition experiences on both host species. Inset boxes show first flight landing choice after 3 oviposition experiences on beet armyworm (BAW) or cabbage looper (CL) in the presence of conspecific-damaged cotton plants. The bars below show flight choice after an additional experience on the opposite host species in the presence of the original odor source. Wasps from fall armyworm (FAW)-reared colony in 2a, *N*=41

Costa et al. (2010) explored unrewarding experiences in *C. marginiventris* using the non-host *Pieris rapae* (L.) and found that wasps did not change their response to HIPVs following experience with a non-host. However, their naïve (untrained) wasp control showed an 81 % attraction to plant odors, whereas our untrained wasps flew to a damaged plant only 4 % of the time, suggesting that their control was not as effective as ours at measuring learned responses. Furthermore, by releasing multiple females at the same time, their data often showed a high tendency of positive control wasps to not move toward an odor stimulus. This is not surprising as wasps tend to disperse randomly when foraging in what they perceive to be crowded or highly competitive environments. Preliminary experiments indicated that one or both female wasps would immediately depart the release platform in the wind tunnel after contact with a conspecific. By flying *C. marginiventris* singly, we demonstrated the effects of oviposition training free of foraging pressure by conspecifics.

Our data show that BAW and CL differentially induce cotton volatiles. Differences in BAW- and CL-damaged blends were evidenced by disparities in ratios of GLV, monoterpene, sesquiterpene, and homoterpene emission. Wasps with oviposition experiences on BAW flew to BAW-damaged cotton the majority of the time, but wasps with experience on the semi-permissive host CL flew to the wall of the wind tunnel more often than to CL-damaged plants. By switching these two odor sources, wasps flew to CL-damaged cotton following BAW oviposition experience, but less frequently oriented toward BAW-damaged cotton following CL oviposition experience. This suggests that a positive oviposition experience on the preferred host (BAW) increases associative learning of HIPVs in *C. marginiventris*, which has been previously documented (Turlings et al., 1991). Choice flights where both BAW- and CL-damaged cotton were presented indicate that BAW-experienced wasps fly more often to plants damaged by the host on which they were experienced. This indicates that discrimination of the two odor blends is occurring; however, the relatively lower response of CL-experienced individuals is probably due to the sub-optimal experience. While we considered the possibility that sensitization (i.e., increased responsiveness to an unpaired stimulus when presented repeatedly) could be occurring (Papaj and Prokopy, 1989), associative learning is a more likely explanation. Sensitization, by definition, would have elicited similar flight responses of wasps regardless of host species because it is caused by mere exposure to plant volatiles, which we did not observe.

The ability of braconid wasps to associate a non-plant cue (vanilla extract) to host-finding behavior has been demonstrated previously (Lewis and Tumlinson, 1988). By using vanilla extract in oviposition training and removing all natural plant compounds, we demonstrated that odor association more frequently occurs when wasps have positive oviposition experiences on BAW. By adding an oviposition experience on the opposite host species from which wasps were originally experienced, we determined that *C. marginiventris* change post-oviposition flight behavior when presented with the preferred BAW hosts, but not with CL. In other words, CL experience does not cause deterioration of the odor-responding individuals trained on BAW, suggesting that the sub-optimal host experience is not causing negative associative learning, rather it is a sub-optimal event. However, no-choice individuals from the CL trained group need a reinforcing positive oviposition experience on BAW to continue foraging.

We tested the hypothesis that *C. marginiventris* would associate volatile odors with BAW but not CL. This was not true. Instead, behavioral analyses indicate that wasp associative learning occurs strongly in BAW, and only moderately in CL, and that the response of CL-experienced wasps can be altered by a single oviposition experience on BAW. This observed preference learning for BAW-associated volatiles may be robust in nature, as wasps reared on both BAW and FAW show the same behaviors. Our studies show that *C. marginiventris* readily sting and oviposit in CL larvae when present, but only 18 % of parasitized hosts yield wasp progeny when hosts are feeding on cotton (Harris and Tumlinson, unpublished data), suggesting that it is a sub-optimal host.

This study demonstrates that the interactive role of HIPVs in parasitoid foraging in nature may be dependent upon host species encounters. The observation of *C. marginiventris* flying to CL-damaged plants following positive BAW oviposition experiences may explain the occurrence of increased parasitism of the semi-permissive host when in the presence of abundant BAW. Conversely, abundant presence of semi-permissive hosts may limit parasitism of more suitable hosts through sub-optimal contact experiences that slow parasitoid foraging behavior or encourage patch abandonment.

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