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EAG-Active Herbivore-Induced Plant Volatiles Modify Behavioral Responses and Host Attack by An Egg Parasitoid

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Abstract Volatiles emitted by plants in response to feeding by *Lygus* species were tested in neurophysiological, behavioral, and parasitism trials with *Anaphes iole*, an egg parasitoid of *Lygus*. Electroantennogram analyses indicated that *A. iole* antennae responded to most herbivore-induced plant volatiles (HIPVs) tested and that females were usually more responsive than males. Antennal responses to (*Z*)-3-hexenyl acetate and methyl salicylate were among the strongest. Behavioral assays in a four-arm olfactometer demonstrated that response of female wasps to (*Z*)-3-hexenyl acetate varied greatly depending on preconditioning regime. Preconditioning wasps to complex host-plant odors led to stronger preference than did a single preconditioning stimulus, i.e., (*Z*)-3-hexenyl acetate. In a horizontal wind tunnel, female wasps were attracted by methyl salicylate and α-farnesene. Parasitism of *Lygus lineolaris* eggs by *A. iole* in a cotton field was greater when the eggs were associated with (*Z*)-3-hexenyl acetate or α-farnesene than with controls. Overall, the results of this study show that *A. iole* can perceive a variety of plant volatiles released after its host damages plants, that the degree of associative learning in *A. iole* can be manipulated based on preconditioning regime, and that single synthetic HIPVs are attractive to *A. iole* and can be used to increase attack rates on host eggs. Therefore, it appears that HIPVs have potential for use in suppression of *Lygus* population densities.

Keywords Electroantennography · Olfactometer · Herbivore-induced plant volatiles · Parasitism · *Anaphes iole* · Hymenoptera · Mymaridae · Heteroptera · Miridae · *Lygus lineolaris*

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Introduction

Herbivory induces a variety of biochemical changes in plants (Karban and Baldwin 1997). These changes include indirect defense responses such as emission of volatiles attractive to the natural enemies of herbivores inflicting the damage. The role of feeding-induced plant volatiles in host-habitat location by natural enemies is well documented (Turlings et al. 1991; Dicke et al. 1993; Tumlinson et al. 1993; Birkett et al. 2003; Wei et al. 2007). Recent studies also have demonstrated the importance of oviposition-induced volatiles in the host-searching behavior of egg parasitoids (Meiners and Hilker 2000; Colazza et al. 2004; Manrique et al. 2005; Mumm et al. 2005; Hilker and Meiners 2006). Exploitation of chemical signaling by plants that attract natural enemies has potential for enhancing biological control in agroecosystems (Hunter 2002). For example, synthetic herbivore-induced plant volatiles

(HIPVs) have been used to attract and retain beneficial insects into vineyards and hopyards (James 2003a,b, 2005), which has led to a reduction in pest densities (James and Price 2004; James and Grasswitz 2005). To date, however, little is known about practical application of HIPVs in cotton agroecosystems.

Lygus lineolaris and *Lygus hesperus* (Heteroptera: Miridae) are important pests of cotton and other crops in North America (Jackson and Graham 1983; Leigh et al. 1988; Wheeler 2001). The feeding mechanics of *L. hesperus* can be categorized as 'mechanical cell rupture-enhanced maceration' that involves stylet maceration of plant cells, injection of saliva, and ingestion of the saliva-cell content slurry (Backus et al. 2007). Selection of oviposition sites by mirids typically is preceded by probing with the mouthparts (Cobben 1978; Wheeler 2001), and we have observed this behavior by *L. hesperus* (Williams, unpublished data). Rodriguez-Saona et al. (2002) showed that feeding and oviposition by *L. hesperus* females induced emission of significant amounts of volatiles from cotton, and the level of emission was positively associated with the number of eggs laid on a plant. Williams et al. (2005) reported that feeding by virgin females of *L. hesperus* induced 2.6-fold higher emission of cotton volatiles than feeding by mated females, and treatment of maize plants with *L. hesperus* salivary gland extracts and artificial mechanical injury resulted in 2.7-fold higher emission of volatiles than artificial mechanical injury alone.

Anaphes iole (Hymenoptera: Mymaridae) is an egg parasitoid that attacks *Lygus* and other mirids in North America (Huber and Rajakulendran 1988). Parasitism rates can exceed 90%, and thus, this wasp is an important natural enemy of *Lygus* species that has potential for pest suppression (Ruberson and Williams 2000). Conti et al. (1996, 1997) demonstrated that chemical and physical cues were important for host acceptance (i.e., recognition and oviposition) by *A. iole* of *L. hesperus* eggs. Wasps on plants harboring host eggs utilized chemical cues from the host and injured host plant, as well as physical cues associated with eggs and oviposition sites for host acceptance. Oviposition behavior of *A. iole* was also influenced by the experience of female wasps (Conti et al. 1997). Naïve females spent more time examining host eggs than did females preconditioned by exposure to host eggs embedded in plant tissue. Subsequent behavioral studies demonstrated that *A. iole* females were attracted to odors derived from plants infested with *L. hesperus* in a four-arm olfactometer (Manrique et al. 2005). However, wasp perception of individual synthetic plant volatiles and their role in attraction to and parasitism of host eggs by *A. iole* have not been previously investigated.

The goal of the present study was to evaluate the responses of *A. iole* wasps to individual HIPVs by using a combination of electroantennogram (EAG), behavioral, and field studies. Because associative learning often increases the response of parasitoids to host-related volatiles (Vet and Groenewold 1990; Turlings et al. 1991; Steinberg et al. 1992; Conti et al. 1997; Drukker et al. 2000; Santolamazza-Carbone et al. 2004), we investigated the effects of experience on *A. iole* response to HIPVs. Specifically, we asked: (1) Do female and male *A. iole* antennae respond differentially to HIPVs? (2) Does experience influence *A. iole* response to (*Z*)-3-hexenyl acetate, a common green leaf volatile? (3) Does *A. iole* respond to individual HIPVs in a wind tunnel? and (4) Do HIPVs increase field parasitism by *A. iole*?

Methods and Materials

Insects *A. iole* used in this study were originally obtained from a laboratory colony maintained on *L. hesperus* Knight eggs at the US Department of Agriculture-Agricultural Research Service (USDA-ARS) Biological Control and Mass Rearing Research Unit, Mississippi State, MS, USA. After emergence, wasps were provided with distilled water and 1 M sucrose ad libitum. *A. iole* were reared in Plexiglass cages (26×26×20 cm) at 25±1°C, 60–85% relative humidity (RH), and 14:10 L/D photoperiod until experimentation (3-day-old adults).

Electroantennography Concentration Responses The compounds included in this study (Table 1) were tested individually as olfactory stimuli. For the EAG study, serial dilutions (0.1, 1.0, 10, and 100 μM) of each compound were made with paraffin oil (E. Merck, Darmstadt, Germany). Stimulus applicators were prepared by pipetting 25 μl of a test solution onto a 6×0.5 cm strip of Whatman no. 1 filter paper (Whatman International Ltd., Maidstone, Kent, UK), after which the filter paper was placed inside a 14.5-cm long glass Pasteur pipette. Fresh stimulus applicators were prepared after 2 h of use. Three controls were used: (1) an empty pipette, (2) a pipette containing 25 μl paraffin oil only on filter paper, and (3) a pipette containing 25 μl 100 μM octanal in paraffin oil on filter paper (octanal standard). Differences in volatilities (see Kovats indices, Table 1) of the test compounds permitted only relative comparisons between test chemicals, except for closely related compounds.

The EAG apparatus (Syntech Ltd., Hilversum, The Netherlands) was linked to a desktop computer (with IDAC-02 data acquisition interface board) on which recording, storing, and quantifying EAG responses were performed. The recording and indifferent electrodes were silver wires enclosed in drawn glass capillary tubes filled with phosphate-buffered saline (NaCl, 4 g; Na_2HPO_4,

Table 1 Chemicals used in EAG and olfactometer trials with *A. iole*, their purity, commercial sources, and Kovats' retention indices

Chemical	Purity (%)	Commercial source[a]	Kovats' index[b]
Green leaf volatiles			
1-Hexanol	98	Aldrich	851
(*Z*)-3-Hexen-1-ol[c]	98	Aldrich	858
(*E*)-3-Hexen-1-ol[c]	98	Aldrich	1,038
(*E*)-2-Hexenyl acetate	98	Aldrich	NA
(*Z*)-3-Hexenyl acetate[c]	≥98	Bedoukian	1,009
Monoterpenes			
β-Myrcene[c]	≥99	Aldrich	992
(±)-Linalool[c]	95–97	Sigma	1,100
β-Caryophyllene[c]	90	Sigma	1,467
(*E*,*E*)-α-Farnesene[c,d]	NA	Bedoukian	1,500
(+)-Limonene[c]	97	Aldrich	1,030
(*E*)-β-Ocimene[c]	97	Fluka	1,038
(−)-α-Pinene[c]	98	Aldrich	937
Carboxylate ester			
Methyl salicylate[c]	≥99	Sigma	1,234
Aliphatic aldehyde			
Octanal (positive control)	99	Aldrich	1,006

[a] Aldrich Chemical Co., Milwaukee, WI, USA; Bedoukian Research, Inc., Danbury, CT, USA; Fluka, Buchs, Switzerland; Sigma Corp., St. Louis, MO, USA
[b] Kovats (1958). DB-5 column
[c] Included in preconditioning regime with four-arm olfactometer
[d] A mixture of α-farnesene and isomers

0.57 g; KH_2PO_4, 0.1 g; KCl, 0.1 g in 500 ml distilled water; pH 7.4). Antennal preparations were made by cutting transversely through the mesonotum just anterior to the tegulae with a scalpel and mounting the thorax on the indifferent electrode. Both antennae remained intact, and the recording electrode was placed on the tip of one randomly chosen antenna. The antennal preparation was bathed continuously by a stream of charcoal-filtered and humidified air at a flow rate of 1 l/min. Air temperature and relative humidity was measured approximately 15 cm from the antennal preparation (overall ranges for all trials: 19.2–26.3°C, 28–52% RH).

EAG recording began 6 min after the antennal preparation was mounted. At this time, the following test protocol was used for each recording trial. The controls were tested in the following order: 1, 2, 3, 2, after which six randomly chosen chemical treatments were tested. For each chemical, order of delivery of the four concentrations was random. Delivery of controls (2, 3, 2) was made after each four-concentration series of a chemical treatment. After the final chemical treatment for each recording, controls were presented in the following order: 2, 3, 2, 1. Presentation of controls throughout the recording session permitted standardization of antennal responses. Test compounds and controls were applied (0.5-s pulse) at 30-s intervals separated by a purge of filtered–humidified air via an aluminum tube approximately 5 mm from the antenna. EAGs were measured as maximum amplitude of depolarization (mV). Each chemical was tested on 10 to 35 individuals of each gender.

Short-Range Olfactory Response to HIPVs Short-range walking response of parasitoids to HIPVs was measured with a four-arm olfactometer (Vet et al. 1983). The base of the arena was precision-machined aluminum with a groove for a rubber O-ring, and the top consisted of a circular piece of Plexiglass (30 cm diameter). The Plexiglass top had a hole (5 mm diameter) in the center to facilitate placement of a wasp into the arena. The hole was plugged with a Teflon-wrapped cork. The top of the arena was held securely to the base by spring clamps, and the O-ring seal ensured that the arena did not leak. Each arm of the olfactometer was divided into three regions: release, visit, and selection regions (Manrique et al. 2005). The airflow (25 ml/min per arm) inside the arena was equalized by using one flowmeter (Aalborg, Orangeburg, NY, USA) at each arm and a terminal flowmeter between the olfactometer and the pump (Model 400-3910, Barrant Co., Carrington, IL, USA). Charcoal-filtered and humidified air was passed through four 250 ml Erlenmeyer flasks that contained odor sources. A white 19-l plastic bucket was inverted over the olfactometer. The bottom of the bucket had been removed and covered with a light diffuser upon which a circular light (22 cm diameter) was placed. The outside of the bucket was covered with aluminum foil to ensure that it was opaque.

An observation hole (6×6 cm) was cut in the side of the bucket to facilitate visual recording of data. This hole remained covered except when observations were being made. The use of a white bucket over the olfactometer provided uniform light inside the arena and reduced bias from external visual cues. Smoke tests ensured that the system was working properly (Vet et al. 1983).

In all trials, a single HIPV treatment was compared against an untreated control. (*Z*)-3-hexenyl acetate was chosen because of the high antennal response recorded in the EAG study (Fig. 1). The HIPV stimulus was prepared in the same way that EAG stimuli were prepared (see above). After pipetting 25 μl of the test solution or paraffin oil only (untreated control) onto the strip of filter paper, it was placed inside an Erlenmeyer flask. Two treatment arms were assigned opposite one another, and the other two arms were untreated controls. Individual *A. iole* females were transferred into the center of the arena with a dissecting needle, and the hole in the top of the arena was plugged. Thereafter, the vacuum pump was turned on, and the bucket-light assembly was replaced onto the arena. Wasps not leaving the release site within 2 min of initiating a trial were replaced. Otherwise, each wasp was observed continuously for 5 min, and the time spent in each region of the arena was recorded with a multichannel timer. Individual *A. iole* females were used only once. After each trial, the inside of the arena was wiped with 70% ethanol, the arena was rotated 90° clockwise, and the treatments were randomly reassigned. The HIPV stimulus was replaced after 30 min use. Air temperature was measured inside the chamber (overall range for all trials, 20.8–25.0°C) during the bioassays (April–June; 1000 to 1700 hours CDT).

Trials were conducted to determine the effect of different preconditioning regimes on response of *A. iole* female wasps to a single HIPV. The goal was to partition the preconditioning effects of a single synthetic HIPV, a blend of 11 synthetic HIPVs, a host plant on which *L. lineolaris* had fed and oviposited, and an artificial egg pack of *L. lineolaris* eggs on the response by *A. iole*. Compounds were chosen because their levels increased after *Lygus* feeding (Rodriguez-Saona et al. 2002) and because of the strong concentration response we observed in the EAG trials (see below). *Erigeron annuus* was used because it is an important host of *Lygus* species. *E. annuus* was collected from the vicinity of Stoneville, MS, USA, and stems (6 cm-long) with flowers were caged with mated *L. lineolaris* females for approximately 12 h, thus permitting feeding and oviposition. Artificial egg packs into which *L. lineolaris* had oviposited (<24-h old) were also used as a treatment. For these last two treatments, insects were removed from plants or egg packs prior to

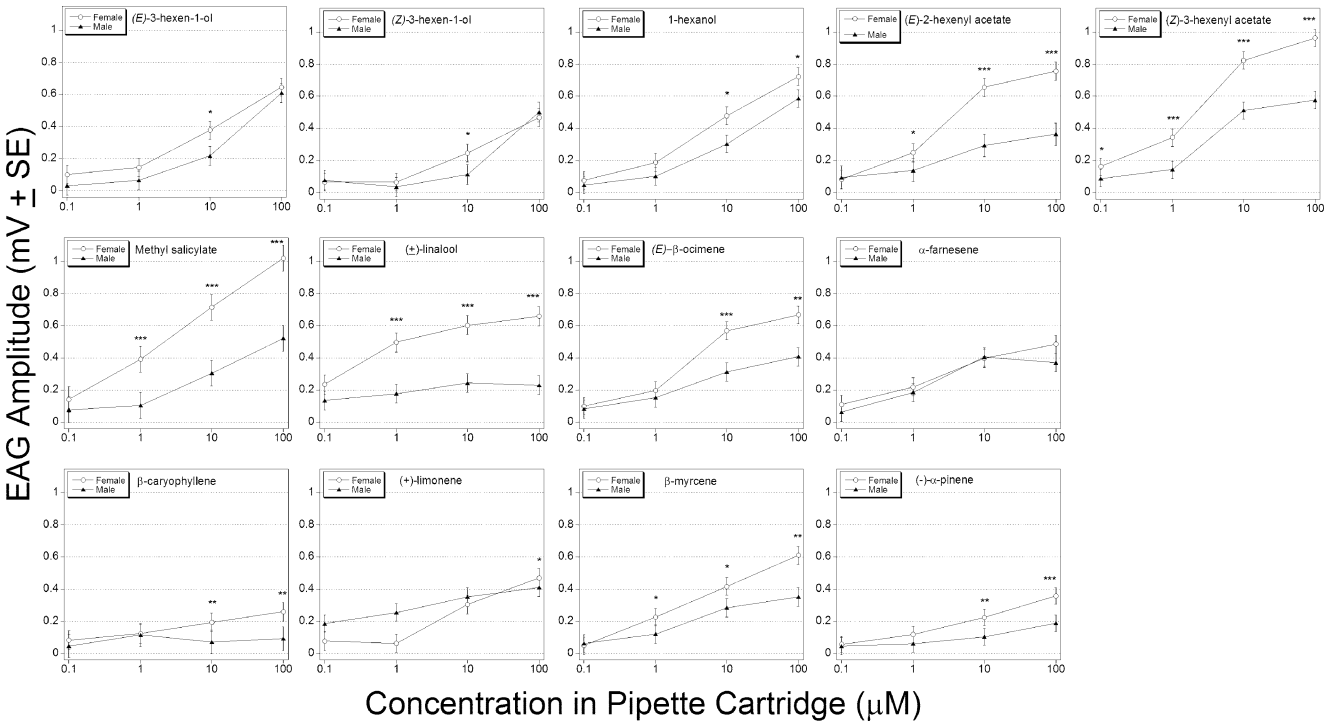

Fig. 1 EAG concentration–response curves of *A. iole* to 13 herbivore-induced plant volatiles. EAG amplitudes are control-adjusted and presented as proportional responses (mean±SE) to the standard, 100 μM octanal. Each compound was tested on 10 to 35 individuals of each gender. Significant differences between genders are noted by asterisks (*single asterisk* P=0.05–0.01, *double asterisk* P=0.01–0.001, *triple asterisk* P<0.001). P values correspond to ANOVA

experiments. Five preconditioning regimes were chosen to represent a range of signals, from simple to complex: (1) 10 μM (*Z*)-3-hexenyl acetate, (2) 1 μM each of 11 HIPVs (refer to Table 1), (3) *E. annuus* with *L. lineolaris* eggs, (4) *E. annuus* with *L. lineolaris* eggs + 10 μM (*Z*)-3-hexenyl acetate, and (5) *E. annuus* with *L. lineolaris* eggs + 1 μM each of 11 HIPVs. The exposure time for all preconditioning regimes was 45 min. For all these trials, wasps were tested with 100 μM (*Z*)-3-hexenyl acetate in the four-arm olfactometer. Different combinations of these four treatments allowed partitioning the effects of different odors on the behavior of *A. iole* wasps.

Long-Range Olfactory Response to HIPVs A horizontal wind tunnel was used to assess the behavioral response of *A. iole* at a larger scale than possible in a four-arm olfactometer. The wind tunnel was constructed of Plexiglass and measured 65 cm long×35 cm high×40 cm wide. It was subdivided into two compartments by a 34-cm-long partition at the upwind end. Each compartment received odors from either a treatment stimulus or untreated control. Charcoal filtered and humidified air was first pushed via pump (Model 400-3910, Barrant Co., Carrington, IL, USA) through 250 ml Erlenmeyer flasks containing either an odor source or blank control, and then into the two upwind wind tunnel compartments. HIPV odor sources were prepared as described for the four-arm olfactometer. Each compartment had four holes (each 5 mm diameter) to allow air flowing from the treatment chambers to enter the cage, and a flowmeter (Aalborg, Orangeburg, NY, USA) was used to regulate the overall airflow at 100 ml/min per hole. At the downwind end of the tunnel, there was a door with a rectangular window (19×15.25 cm) covered with nylon organdy that allowed introduction of wasps. At the downwind end of the tunnel, a piece of Plexiglass (20.3×35 cm) was glued from each side of the window to the side wall of the box, thus helping to funnel air toward the window. A computer muffin fan at the downwind end vented airflow from the tunnel. Smoke tests confirmed that air flowed through the chamber without mixing between treatment compartments. All outside walls of the box were covered with opaque white cardstock to provide uniform lighting and avoid external visual cues. The paper on the top of the wind tunnel had two holes (22 cm diameter) to facilitate illumination provided by two circular lights. The lights were placed parallel to the long axis of the chamber, with the outside edge of each light 10 cm from the edge of the chamber. The wind tunnel was rinsed with 70% ethanol after each bioassay. Temperature and humidity inside the room were measured (overall ranges for all trials, 20.8–24.0°C, 18–49% RH) during the bioassays (1100 to 1700 hours CDT).

In all trials with the horizontal wind tunnel, a single HIPV treatment was compared against an untreated control. (*Z*)-3-hexenyl acetate, methyl salicylate, and α-farnesene were chosen because of the high antennal responses observed in the EAG study (Fig. 1) and the responses in the four-arm olfactometer study. The HIPV stimulus was prepared in the same manner as EAG stimuli (see above); after adding 25 μl test solution or paraffin oil only (untreated control) onto a 6×0.5 cm strip of filter paper, it was placed inside an Erlenmeyer flask. The air pump, muffin fan, and lights were turned on 5 min before each release. Thereafter, 50 naïve *A. iole* females were released from each of four 20-ml glass scintillation vials (Kimble Glass, Inc., Vineland, NJ, USA) on the tunnel floor at the downwind end of the tunnel. The assay was terminated after 2 h, and the number of wasps on all walls of both compartments at the upwind end of the wind tunnel was counted. This procedure was repeated six times for each HIPV treatment, and the stimulus and blank controls were randomly reassigned to the two upwind chamber compartments after each repetition.

Field Parasitism A field investigation was conducted to determine the effect of synthetic HIPVs on parasitism of *L. lineolaris* eggs by *A. iole*. Based on our olfactometry studies and the work of Manrique et al. (2005), we hypothesized that host eggs in proximity to HIPVs would suffer greater parasitism than hosts associated with an untreated control. A cotton field (25 ha, *Gossypium hirsutum* L. var. DPL 215 BG/RR) was selected in Elizabeth, Washington Co., Mississippi (2.8 km E Stoneville; 33°25.4″ N, 90°52.8″ W). Agronomic practices (e.g., pesticide and fertilizer use) were consistent with those used in commercial cotton production, except no foliar insecticides were applied. This field was bordered on two sides by soybean, *Glycine max* (L.) Merr., one side by corn, *Zea mays* L., and on one side by a 12-m wide grass, *Cynodon dactylon* (L.) Pers., alley adjacent to natural vegetation (e.g., *Vitis* spp., *Ampelopsis arborea* (L.) Koehne, *Ambrosia trifida* L., *Quercus nigra* L., *Lonicera japonica* Thunberg, *Sambucus canadensis* L., and *Carya illinoensis* (Wangenheim) K. Koch.

A 6-ha portion of the cotton field was chosen for the study, and the experiment was arranged in a randomized complete block design with ten replicates of each of the following four treatments: (*Z*)-3-hexenyl acetate, α-farnesene, methyl salicylate, and an untreated control. These compounds were chosen because of our previous olfactometry results and previous studies on HIPVs with another mymarid (James 2005; James and Grasswitz 2005). Each plot was approximately 0.04 ha (20×20 m). HIPV bait-host egg stations were used to test the effect of synthetic HIPVs on parasitism of *L. lineolaris* eggs by *A. iole*. Each station consisted of a 45-ml

transparent plastic vial (12 dram crystal, Thornton Plastics, Salt Lake City, UT, USA) with four 1-cm diameter equidistant holes 5 mm from the top of the vial. Nested inside the plastic vial was a 20-ml glass scintillation vial (Kimble Glass, Inc., Vineland, NJ, USA) containing 2 ml of undiluted synthetic HIPV (see above) and a 1×5 cm piece of filter paper (Whatman no. 1, Whatman International, Ltd., Maidstone, England) that acted as a wick and enhanced volatilization of the HIPV. The plastic vial was closed with a white plastic cap to exclude rain and debris, and aluminum foil was wrapped around the vial, taking care not to cover the holes, to reduce ultraviolet light effects on the HIPV. In untreated controls, the scintillation vial contained only the strip of filter paper. The vial assembly was attached to a bamboo garden stake (65 cm long) so that the top of the dispenser was 45 cm above the soil surface. A *L. lineolaris* egg pack (9×9 cm) <2 days old was clipped to the bamboo stake 3 cm above the top of the dispenser.

One HIPV bait-host egg station was established in the center of each plot from 1530 to 1600 hours CST on 9 July 2004. Stations were placed in the furrow such that the top of the HIPV dispenser was 45 cm above the soil surface, and the egg pack was parallel to the row. Plants were removed such that there was no vegetation within 30 cm of the station; the destroyed plants were moved at least 10 m from the station to avoid bias of HIPV production from these plants. Approximately 1,000 naïve mixed gender wasps held in a glass scintillation vial were released 2 h later at each bait-egg station by placing the open vial on the soil beneath the host egg pack. Egg packs were recovered after 2 days and were held in an environmental chamber for 10 days at 25±1°C, 60–85% RH, and 14:10 L/D photoperiod. Egg packs were then observed under a dissecting scope at ×50, and parasitized and unparasitized eggs were counted. Parasitism data were expressed as the proportion of parasitized eggs in each egg pack. There was no difference in total host egg density per pack between HIPV treatments (mean=2,120, SE=101; F=1.73; df=3, 36; P=0.178). Voucher specimens of the wasps that were released are deposited in the National Entomological Collection, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.

Data Analysis Maximum EAG responses were control-adjusted with the paraffin oil only control and expressed as proportional responses relative to the octanal standard. These data were then square root-transformed $(0.5(\sqrt{x}+\sqrt{x}+1))$ (Zar 1996), and analysis of variance, PROC MIXED (SAS Institute 2003), was used to compare maximum EAG deflection between gender and HIPV treatment–concentration combinations. Regression analysis was also used to assess the influence of gender and HIPV treatment on EAG amplitude (PROC REG and PROC MIXED; SAS Institute 2003). Due to heteroscedasticity over concentrations, a weighted regression (reciprocal of the variance) was calculated. Data from olfactometry studies were arcsine square root-transformed $(\arcsin(\sqrt{x}+\sqrt{x}+1))$ prior to two-tailed paired t test (selection region in the four-arm olfactometer) or G test for goodness of fit (flight tunnel; SAS Institute 2003). Data on parasitism were arcsine square root-transformed $(\arcsin(\sqrt{x}+\sqrt{x}+1))$ prior to single-factor analysis of variance (ANOVA) followed by mean comparison by using a one-tailed Dunnett's test of each HIPV treatment to the control (SAS Institute 2003). Untransformed values are presented for EAG, behavioral, and parasitism results.

Results

EAG Concentration–Response Curves Overall responses of female *A. iole* were significantly greater than male responses (F=76.52; df=1, 69.7; P<0.001). Female *A. iole* responses to (*E*)-2-hexenyl acetate at 1, 10, and 100 μM (F=2.47, 4.29 and 5.08, respectively; df=1, 1,205; P=0.014, <0.001, and <0.001, respectively) and to (*Z*)-3-hexenyl acetate at 0.1, 1, 10, and 100 μM (F=2.19, 3.41, 3.98, and 4.64, respectively; df=1, 1,200; P=0.029, <0.001, <0.001, and <0.001, respectively) were significantly greater than male responses (Fig. 1). For (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, and 1-hexanol, female responses were greater than those of males at 10 μM (F=2.56, 2.32, and 2.46, respectively; df=1, 1,203, 1,203, and 1,202, respectively; P=0.011, 0.021, and 0.014, respectively) and for 1-hexanol at 100 μM (F=2.00; df=1, 1,202; P=0.046). Female responses were greater than those of males at 1, 10, and 100 μM for methyl salicylate (F=3.34, 3.72, and 3.75, respectively; df=1, 1,204; P=<0.001, <0.001, and <0.001, respectively) for (±)-linalool (F=5.11, 5.19, and 6.29, respectively; df=1, 1,204; P<0.001), and for β-ocimene at 10 and 100 μM (F=3.53 and 2.93, respectively; df=1, 1,185 and 1,223, respectively; P=<0.001 and 0.004, respectively; Fig. 1). For β-myrcene, female responses were greater than those of males at 1, 10, and 100 μM (F=2.37, 1.97, and 3.25, respectively; df=1, 1,202; P=0.018, 0.049, and 0.001, respectively). For β-caryophyllene and α-pinene, female responses were greater than those of males at 10 μM (F=2.65 and 2.79, respectively; df=1, 1,205 and 1,199, respectively; P=0.008 and 0.005, respectively) and 100 μM (F=3.01 and 3.92, respectively; df=1, 1,205 and 1,199, respectively; P=0.003 and <0.001, respectively). For (+)-limonene, female response was greater than that of males at 100 μM (F=2.26; df=1, 1,202; P=0.024). For α-farnesene, there was no difference between female and male response at any concentration (Fig. 1).

Regression analysis of the impact of gender and HIPVs on antennal response indicated a linear response between antennal receptiveness (y) and HIPV concentration (log10 x; Table 2). Contrasts of slopes between the genders revealed greater ($P<0.05$) slopes for females for seven of the 13 HIPVs (Table 2). Ranked in order of P values (greatest to least significant), the seven significant compounds were: (*E*)-2-hexenyl acetate < β-myrcene = (±)-linalool < α-pinene = methyl salicylate < β-caryophyllene < β-ocimene (Table 2).

Comparison of slopes across herbivore-induced plant volatiles by gender showed that 42 comparisons were significant ($P<0.05$) for females, and 35 were significant for males (Table 3). For example, in females, responses to (*E*)-2-hexenyl acetate and (*Z*)-3-hexenyl acetate were not significantly different from one another, but each was different from β-caryophyllene, (*Z*)-3-hexen-1-ol, α-farnesene, (±)-linalool, (+)-limonene, and α-pinene. Female response to (*Z*)-3-hexenyl acetate was different from the response to (*E*)-3-hexen-1-ol and β-ocimene. The response of females to β-caryophyllene was significantly different from responses to all HIPVs except (±)-linalool. The matrix layout of Table 3 facilitates additional comparison of slopes for each gender.

EAG analyses clearly revealed that *A. iole* responded to the compounds tested. Although female antennae generally were more responsive than male antennae, both genders responded to most HIPVs. For females, methyl salicylate and (*Z*)-3-hexenyl acetate elicited significantly higher EAG responses than the other compounds tested. Wasps were least responsive to the monoterpenes β-caryophyllene and α-pinene. The two C_6 acetates, (*E*)-2-hexenyl acetate and (*Z*)-3-hexenyl acetate, had similar shapes of concentration–response curves, although wasps were more responsive to (*Z*)-3-hexenyl acetate. Two alcohols, (*E*)-3-hexen-1-ol and (*Z*)-3-hexen-1-ol, had similar-shaped concentration–response curves, but wasps were more responsive to the *E* isomer. At the two lowest concentrations tested, female wasps seemed to be most responsive to linalool and methyl salicylate.

Short-Range Olfactory Response to HIPVs A. iole females spent significantly more time in odor fields (selection regions) containing (*Z*)-3-hexenyl acetate compared to untreated control odor fields for trials using preconditioning regimes 3 (3.08 vs. 1.92 min; $t=2.31$; $df=28$; $P=0.028$), 4 (3.09 vs. 1.91 min; $t=2.24$; $df=25$; $P=0.034$), and 5 (3.29 vs. 1.71 min; $t=3.42$; $df=29$; $P=0.002$; Fig. 2). In contrast, significant responses to (*Z*)-3-hexenyl acetate were not detected for preconditioning regimes 1 (2.40 vs. 2.60 min; $t=-0.47$; $df=46$; $P=0.644$) and 2 (2.87 vs. 2.13 min; $t=1.73$; $df=51$; $P=0.090$).

Generally, responsiveness toward (*Z*)-3-hexenyl acetate increased as complexity of the preconditioning regime increased. For example, preconditioning regimes that included host plant (*E. annuus*) that had been subjected to feeding and oviposition by *L. lineolaris* led to the greatest attraction to (*Z*)-3-hexenyl acetate. The regime with the most complex odor mixture (*E. annuus* with *L. lineolaris* eggs + 1 μM each of 11 HIPVs) led to significantly greater attraction than did *Erigeron* and host eggs alone or 1 μM of

Table 2 Regression equations, F values, and significance levels for contrasts of slopes (female versus male) describing the effects of herbivore-induced plant volatiles on *A. iole* antennal response

	Female		Male		Den. df [b]	F	P
Herbivore-induced plant volatile	Equation[a]	R^2	Equation[a]	R^2			
Green leaf volatiles							
1-Hexanol	1.53+0.31(x)	0.722	1.39+0.26(x)	0.516	168	1.07	0.301
(*Z*)-3-Hexen-1-ol	1.35+0.22(x)	0.446	1.21+0.19(x)	0.231	148	0.02	0.883
(*E*)-3-Hexen-1-ol	1.50+0.27(x)	0.543	1.33+0.24(x)	0.543	156	0.39	0.535
(*E*)-2-Hexenyl acetate	1.61+0.34(x)	0.713	1.40+0.17(x)	0.260	128	17.75	<0.001
(*Z*)-3-Hexenyl acetate	1.78+0.33(x)	0.672	1.49+0.27(x)	0.444	180	2.74	0.100
Monoterpenes							
β-Myrcene	1.51+0.28(x)	0.753	1.39+0.19(x)	0.535	164	9.17	0.003
(±)-Linalool	1.84+0.14(x)	0.353	1.42+0.05(x)	0.031	144	9.20	0.003
β-Caryophyllene	1.37+0.11(x)	0.181	1.20+0.03(x)	0.022	120	4.11	0.045
α-Farnesene	1.56+0.19(x)	0.511	1.45+0.18(x)	0.418	156	0.17	0.678
(+)-Limonene	1.36+0.26(x)	0.565	1.42+0.14(x)	0.060	160	2.16	0.143
β-Ocimene	1.58+0.30(x)	0.510	1.43+0.19(x)	0.355	164	3.95	0.049
α-Pinene	1.38+0.18(x)	0.572	1.22+0.09(x)	0.135	192	8.28	0.005
Carboxylate ester							
Methyl salicylate	1.78+0.33(x)	0.842	1.39+0.25(x)	0.714	76	8.54	0.005

[a] Antennal response=$a+b$ (log10 concentration)
[b] Numerator $df=1$

Table 3 Matrix of *P* values for comparison of slopes for concentration response of *A. iole* antennae to individual herbivore-induced plant volatiles

HIPV	1-Hexanol	(*Z*)-3-Hexen-1-ol	(*E*)-3-Hexen-1-ol	(*E*)-2-Hexenyl acetate	(*Z*)-3-Hexenyl acetate	β-Myrcene	(±)-Linalool	β-Caryophyllene	α-Farnesene	(+)-Limonene	(*E*)-β-Ocimene	α-Pinene	Methyl salicylate
1-Hexanol	XXX	0.008	0.150	0.648	0.501	0.272	<0.001	<0.001	<0.001	0.037	0.197	<0.001	0.533
(*Z*)-3-Hexen-1-ol	*0.247*	XXX	0.231	0.002	<0.001	0.116	0.150	<0.001	0.268	0.631	0.171	0.142	0.006
(*E*)-3-Hexen-1-ol	*0.784*	*0.169*	XXX	0.060	0.032	0.075	0.010	<0.001	0.022	0.493	0.870	0.007	0.075
(*E*)-2-Hexenyl acetate	*0.017*	*0.212*	*0.011*	XXX	0.843	0.122	<0.001	<0.001	<0.001	0.012	0.083	<0.001	0.802
(*Z*)-3-Hexenyl acetate	*0.879*	*0.304*	*0.672*	*0.023*	XXX	0.071	<0.001	<0.001	<0.001	0.005	0.046	<0.001	0.923
β-Myrcene	*0.076*	*0.598*	*0.048*	*0.423*	*0.100*	XXX	0.003	<0.001	0.008	0.295	0.842	0.002	0.132
(±)-Linalool	*<0.001*	*0.007*	*<0.001*	*0.062*	*<0.001*	*0.003*	XXX	0.055	0.720	0.062	0.006	0.918	0.001
β-Caryophyllene	*<0.001*	*<0.001*	*<0.001*	*0.039*	*<0.001*	*0.002*	*0.677*	XXX	0.020	<0.001	<0.001	0.028	<0.001
α-Farnesene	*0.027*	*0.350*	*0.017*	*0.672*	*0.037*	*0.671*	*0.010*	*0.008*	XXX	0.120	0.014	0.776	<0.001
(+)-Limonene	*0.051*	*0.108*	*0.002*	*0.864*	*0.005*	*0.263*	*0.051*	*0.033*	*0.494*	XXX	0.396	0.054	0.021
(*E*)-β-Ocimene	*0.057*	*0.513*	*0.035*	*0.494*	*0.075*	*0.896*	*0.004*	*0.003*	*0.769*	*0.325*	XXX	0.004	0.097
α-Pinene	*<0.001*	*0.011*	*<0.001*	*0.305*	*0.001*	*0.036*	*0.306*	*0.186*	*0.097*	*0.321*	*0.050*	XXX	<0.001
Methyl salicylate	*0.495*	*0.775*	*0.376*	*0.173*	*0.571*	*0.457*	*0.002*	*0.001*	*0.275*	*0.097*	*0.395*	*0.014*	XXX

P values for males are in italics.

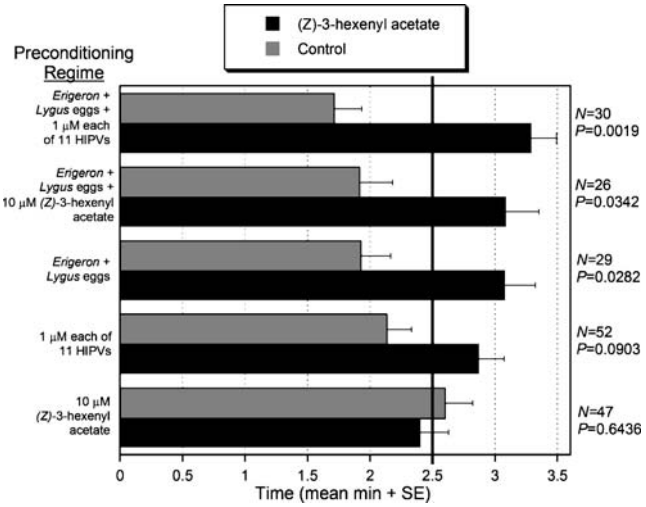

Fig. 2 Mean total time (+SE) that female *A. iole* wasps spent in treatment vs. control fields of four-arm olfactometer after exposure to different preconditioning regimes. Treatment odor was 100 μM (*Z*)-3-hexenyl acetate. *Solid vertical* line at 2.5 min indicates hypothetical value for equal response. *P* values correspond to paired *t* test

11 HIPVs, suggesting an additive or synergistic effect for the combination of *Erigeron* and host eggs alone or 1 μM of 11 HIPVs. In the simplest regime (10 μM (*Z*)-3-hexenyl acetate), the lack of behavioral response contrasted with EAG results that showed that *A. iole* females perceived (*Z*)-3-hexenyl acetate.

Long-Range Olfactory Response to HIPVs More naïve *A. iole* females were recovered from methyl salicylate (G=15.57; df=1; P<0.001) and α-farnesene (G=4.31; df=1; P<0.05) treatment compartments than controls; there was no difference in wasp numbers recovered from the (*Z*)-3-hexenyl acetate treatment and controls (G=3.61; df=1; P>0.05; Fig. 3). For this experiment, approximately 32% of the wasps were recaptured in the treatment and control compartments.

Field Parasitism Single-factor ANOVA revealed a significant HIPV treatment effect on parasitism (F=3.84; df=3, 36; P=0.018). Parasitism was greater in the α-farnesene (q=3.27; df=36; P=0.007) and (*Z*)-3-hexenyl acetate (q=2.42; df=36; P=0.053) treatments than in the untreated control. Mean parasitism ranged from 0.68% in the control to 2.57% in α-farnesene (Fig. 4). Parasitism was numerically but not significantly (P>0.05) greater for host eggs in the methyl salicylate treatment than the control (Fig. 4).

Discussion

An understanding of the chemical ecology of *A. iole* is essential before developing HIPV strategies for enhancing

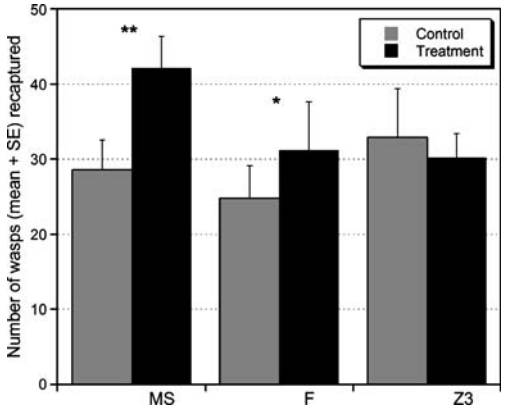

Fig. 3 Mean number (+SE) of female *A. iole* wasps recovered in treatment vs. control compartments of a horizontal wind tunnel. *Empty bars* indicate controls; *solid bars* indicate treatments [*MS* methyl salicylate, *F* α-farnesene, and *Z3* (*Z*)-3-hexenyl acetate]. Significant differences are noted by *asterisks* (*single asterisk* $P<0.05$, *double asterisk* $P<0.001$) using *G* test for goodness of fit. $N=6$ replicates per tested compound

biological control of *Lygus* species. This study used a variety of approaches to study the response of *A. iole* to HIPVs. Our EAG analyses on *A. iole* are the first that we are aware of on a mymarid, and we found that the strongest antennal responses were elicited by (*Z*)-3-hexenyl acetate and methyl salicylate. The greater female antennal responses may be adaptive given that these compounds are associated with the presence of host eggs. Male perception of HIPVs might reflect their use as cues for locating female conspecifics. Behavioral studies revealed that the response to (*Z*)-3-hexenyl acetate was dependent on wasp experience. In further behavioral studies, naïve *A. iole* females were responsive to methyl salicylate and α-farnesene but not to (*Z*)-3-hexenyl acetate. In a field

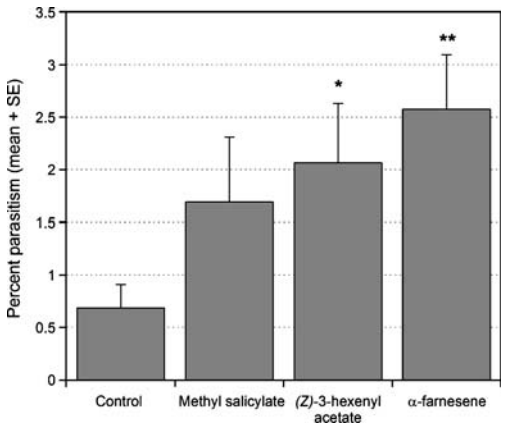

Fig. 4 Percent mean parasitism (+ SE) by *A. iole* of *L. lineolaris* eggs baited with single herbivore-induced plant volatiles in a cotton field. Significant differences for Dunnett's test of each treatment vs. the control are noted by *asterisks* [(*Z*)-3-hexenyl acetate, $P=0.053$; α-farnesene, $P=0.007$). $N=10$ replicates per tested compound and control

experiment, (*Z*)-3-hexenyl acetate and α-farnesene increased egg parasitism by *A. iole*.

Vet and Dicke (1992) proposed a conceptual framework for infochemical use in parasitoid–host and predator–prey interactions. An important issue in this framework is the reliability-detectability problem faced by foraging natural enemies, i.e., that cues from the host or prey are reliable but not easily detectable and, conversely, that signals from plants damaged by the host or prey are more easily detectable but not necessarily reliable. Detection of host-specific cues has important implications for the use of HIPVs in pest management, i.e., HIPVs must be attractive to natural enemies of the herbivore species that cause the injury. Recent studies recognize a growing list of parasitoids that can distinguish between host and non-host HIPVs (Du et al. 1996; Dicke 1999; Meiners et al. 2000; Moraes et al. 2005), including *A. iole*, which is attracted to cotton volatiles emitted after injury by its host, *L. hesperus*, but not by a non-host, *Spodoptera exigua* (Manrique et al. 2005). However, the present study also indicated that a single, relatively ubiquitous HIPV can be attractive to *A. iole* and can lead to increased parasitism. The green-leaf volatile, (*Z*)-3-hexenyl acetate; the sesquiterpene, α-farnesene; and the carboxylate ester, methyl salicylate, are all produced and released by plants in response to injury by *Lygus* species (Rodriguez-Saona et al. 2002; Blackmer et al. 2004; Williams et al. 2005) and by other herbivores (Turlings et al. 1991; Loughrin et al. 1995; Röse et al. 1996; Paré and Tumlinson 1998). Thus, it appears that *A. iole* can use either host-specific or general volatile cues in the process of host habitat location.

Under field conditions, the variation in production and emission of HIPVs by plants likely complicates the signal perceived and utilized by parasitoids for host location. However, associative learning, i.e., association of host-specific volatiles with the presence of hosts, is a possible solution to this problem (Vet and Groenewold 1990; Vet and Dicke 1992; Kaiser et al. 2003; Randlkofer et al. 2007; Schröder et al. 2008). Compared to larval parasitoids, little is known on the learning capabilities of host-plant-derived cues in egg parasitoids. Egg parasitoids are less expected to use plant-derived cues or to learn; instead, they are likely to use host-derived cues (i.e., host adult pheromones) that are highly predictable within and between generations during foraging (Vet et al. 1995). Recent studies have shown that egg parasitoids use not only short-distance (host-associated) cues but also may use long-distance volatiles, such as those associated with the host plant (Meiners and Hilker 1997; Hilker et al. 2002; Hilker and Meiners 2006). Because host-plant volatiles are highly detectable but less reliable than host-associated odors, learning might be critical for egg parasitoids that employ host-plant volatiles in host finding (e.g. Mumm et al. 2005). Schröder et al. (2008) hypothe-

sized a contextual learning sequence by a eulophid that is mediated by both a sugar food source and by the presence of host eggs of a suitable age. The present study and those by Conti et al. (1997) and Manrique et al. (2005) show that *A. iole*, a parasitoid that specializes in *Lygus* spp. eggs, uses host-plant volatiles and that both innate and learned responses are important in host finding. Naïve wasps responded to methyl salicylate and α-farnesene but needed to learn host-plant odors before responding to (*Z*)-3-hexenyl acetate. These three volatile compounds are common in many plant species, and one or more of them are induced in cotton, maize, and alfalfa following herbivory by *L. hesperus* (Rodriguez-Saona et al. 2002; Blackmer et al. 2004; Williams et al. 2005). It is, therefore, not surprising that *A. iole* uses more than one volatile cue to find hosts; still, a single compound was sufficient to attract wasps in both laboratory and field experiments. This was confirmed by the fact that several plant volatile compounds elicited an antennal response from *A. iole*, albeit at different amplitudes. Associative learning, however, was not a requisite for host-habitat location by *A. iole* given that naïve wasps responded to HIPVs under both laboratory and field conditions.

Field studies that address the applicability of using HIPVs for the protection of agricultural crops are limited but promising. Drukker et al. (1995) and Shimoda et al. (1997) reported higher densities of predators in the proximity of host-infested plants than near uninfested plants. Several investigators have applied signal molecules, such as jasmonic acid or methyl jasmonate, to plants and demonstrated increases in oxidative enzymes (Thaler 1999), proteinase inhibitors (Rodriguez-Saona et al. 2005), and HIPVs (Ockroy et al. 2001; Rodriguez-Saona et al. 2001). Thaler (1999) also reported increases in parasitism of *S. exigua* on induced plants versus controls. A series of field studies demonstrated that sachets of individual synthetic HIPVs could be used to attract and retain natural enemies in perennial crop systems (James 2003a,b; James and Price 2004; James and Grasswitz 2005). In particular, these studies showed that (*Z*)-3-hexenyl acetate, farnesene, and methyl salicylate could be used to manipulate behavior of beneficial insects under field conditions. These three compounds were attractive to numerous predators and parasitoids; methyl salicylate attracted the broadest diversity of insects and farnesene the least (James 2005; James and Grasswitz 2005). However, all compounds attracted the mymarid *Anagrus daanei* and other *Anagrus* species, although this effect was inconsistent. In our field study with the mymarid *A. iole*, we found that host eggs baited with (*Z*)-3-hexenyl acetate or α-farnesene suffered greater parasitism than untreated controls. The low overall rate of parasitism (approximately 2.6%) may be due to the unnaturally large number of host eggs present in a single patch and the propensity of *A. iole* to disperse its eggs among host patches. Wasps may also have been more strongly attracted to volatiles emitted by nearby cotton plants.

This study provides evidence that plant volatiles represent key semiochemical signals in the recognition of and attraction to host-associated patches by *A. iole*. Bloem and Yeargan (1982) reported that *Anaphes diana* (=*Patasson lameerei* Debauche) used its antennae to differentiate between volatiles from host-damaged vs. undamaged plants and from host vs. non-host frass. The authors proposed that volatiles from host-damaged plants and from host frass play important roles in the host-finding sequence of *A. diana*. It would be adaptive for host-specific natural enemies of *Lygus* to recognize and walk or fly to host-associated habitats. Prior work by Manrique et al. (2005) found that *A. iole* females can discriminate between plant odors emanating from *Lygus*-damaged vs. non-host damaged plants. Here, we identified several plant-produced compounds that, upon further development, might be useful tools to manipulate *A. iole* behavior to enhance *Lygus* suppression. Future recordings from antennal sensilla might help determine if the structurally similar compounds [(*E*)-3-hexen-1-ol vs. (*Z*)-3-hexen-1-ol] are detected by the same or different types of receptor cells and if specific receptor cells exist for certain compounds. Likewise, further lab and field studies of other plant- and host-associated compounds not included in this study, mixtures of compounds, and identification and behavioral testing of GC-EAG active compounds, would be worthwhile.

Based on our current understanding of *A. iole* host foraging, we propose the following host-finding sequence for this egg parasitoid. The proposed sequence does not exclude the possible roles of other sensory modalities (e.g., vision). This sequence is similar to that proposed for *A. diana* (Bloem and Yeargan 1982) and fits into the conceptual framework of Vet and Dicke (1992): (1) detection of the host habitat via host-specific HIPVs; (2) nonrandom and random searching for host eggs on the plant guided by physical and chemical (volatile and/or tactile) cues from host and plant; (3) recognition and acceptance of host eggs directed by physical and chemical (volatile and/or tactile) cues from host eggs and associated plant tissue.

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References

BACKUS, E. A., CLINE, A. R., ELLERSEICK, M. R., and SERRANO, M. S. 2007. *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: new methods and parameters for analysis of nonsequential electrical penetration graph data. *Ann. Entomol. Soc. Amer.* 100:296–310.

BIRKETT, A. M., CHAMBERLAIN, K., GUERRIERI, E., PICKETT, J. A., WADHAMS, L. J., and YASUDA, L. 2003. Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia formosa*. *J. Chem. Ecol.* 29:1589–1600.

BLACKMER, J. L., RODRIGUEZ-SAONA, C., BYERS, J. A., SHOPE, K. L., and SMITH, J. P. 2004. Behavioral response of *Lygus hesperus* to conspecifics and headspace volatiles of alfalfa in a Y-tube olfactometer. *J. Chem. Ecol.* 30:1547–1564.

BLOEM, K. A., and YEARGAN, K. V. 1982. Host-finding behavior of *Patasson lameerei*, (Hym., Chalcidoidea), a parasitoid of *Sitona* eggs (Col., Curculionidae). *Entomophaga* 1:93–97.

COBBEN, R. H. 1978. Evolutionary trends in Heteroptera. Part II. Mouthpart-structures and feeding strategies. *Meded. Landbouwhogesch. Wageningen.* 78–5:1–407.

COLAZZA, S., MCELFRESH, J. S., and MILLAR, J. G. 2004. Identification of volatile synomones, induced by *Nezara viridula* feeding and oviposition on bean spp., that attract the egg parasitoid *Trissolcus basalis*. *J. Chem. Ecol.* 30:945–964.

CONTI, E., JONES, W. A., BIN, F., and VINSON, S. B. 1996. Physical and chemical factors involved in host recognition behavior of *Anaphes iole* Girault, an egg parasitoid of *Lygus hesperus* Knight (Hymenoptera: Mymaridae; Heteroptera: Miridae). *Biol. Control* 7:10–16.

CONTI, E., JONES, W. A., BIN, F., and VINSON, S. B. 1997. Oviposition behavior of *Anaphes iole*, an egg parasitoid of *Lygus hesperus* (Heteroptera: Miridae; Hymenoptera: Mymaridae). *Ann. Entomol. Soc. Amer.* 90:91–101.

DICKE, M. 1999. Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomol. Exp. Appl.* 91:131–142.

DICKE, M., VAN BAARLEN, P., WESSELS, R., and DIJKMAN, H. 1993. Herbivory induces systemic production of plant volatiles that attract predators of the herbivore: extraction of endogenous elicitor. *J. Chem. Ecol.* 19:581–599.

DRUKKER, B., SCUTAREANU, P., and SABELIS, M. W. 1995. Do anthocorid predators respond to synomones from *Psylla*-invested pear trees under field conditions? *Entomol. Exp. Appl.* 77:193–203.

DRUKKER, B., BRUIN, J., and SABELIS, M. W. 2000. Anthocorid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. *Physiol. Entomol.* 25:260–265.

DU, Y.-J., POPPY, G. M., and POWELL, W. 1996. Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of *Aphidius ervii*. *J. Chem. Ecol.* 22:1591–1605.

HILKER, M., and MEINERS, T. 2006. Early herbivore alert: insect eggs induce plant defense. *J. Chem. Ecol.* 32:1379–1397.

HILKER, M., KOBS, C., VARAMA, M., and SCHRANK, K. 2002. Insect egg deposition induces *Pinus* to attract egg parasitoids. *J. Exp. Biol.* 205:455–461.

HUBER, J. T., and RAJAKULENDRAN, V. K. 1988. Redescription of and host-induced antennal variation in *Anaphes iole* Girault (Hymenoptera: Mymaridae), an egg parasite of Miridae (Hemiptera) in North America. *Can. Entomol.* 120:893–901.

HUNTER, M. D. 2002. A breath of fresh air: beyond laboratory studies of plant volatile-natural enemy interactions. *Agric. Forest Entomol.* 4:81–86.

JACKSON, C. G., and GRAHAM, H. M. 1983. Parasitism of four species of *Lygus* (Hemiptera: Miridae) by *Anaphes ovijentatus* (Hymenoptera: Mymaridae) and an evaluation of other possible hosts. *Ann. Entomol. Soc. Amer.* 76:772–775.

JAMES, D. G. 2003a. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *J. Chem. Ecol.* 29:1601–1609.

JAMES, D. G. 2003b. Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. *Environ. Entomol.* 32:977–982.

JAMES, D. G. 2005. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *J. Chem. Ecol.* 31:481–495.

JAMES, D. G., and PRICE, T. S. 2004. Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *J. Chem. Ecol.* 30:1613–1628.

JAMES, D. G., and GRASSWITZ, T. R. 2005. Synthetic herbivore-induced plant volatiles increase field captures of parasitic wasps. *BioControl* 50:871–880.

KAISER, L., PEREZ-MALUF, R., SANDOZ, J. C., and PHAM-DELEGUE, M.-H. 2003. Dynamics of odour learning in *Leptopilina boulardi,* a hymenopterous parasitoid. *Anim. Behav.* 66:1077–1084.

KARBAN, R., and BALDWIN, I. T. 1997. Induced Responses to Herbivory. Chicago University Press, Chicago.

KOVATS, E. 1958. Gas-chromatographische Charakterisierung organisher Verbindungen. *Helv. Chim. Acta* 41:1915–1932.

LEIGH, T. F., KERBY, T. A., and WYNHOLDS, T. F. 1988. Cotton square damage by the plant bug, *Lygus hesperus* (Hemiptera: Heteroptera: Miridae), and abscission rates. *J. Econ. Entomol.* 81:1328–1337.

LOUGHRIN, J. H., MANUKIAN, A., HEATH, R. R., and TUMLINSON, J. H. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.* 21:1217–1227.

MANRIQUE, V., JONES, W. A., WILLIAMS, L. III, and BERNAL, J. S. 2005. Olfactory responses of *Anaphes iole* (Hymenoptera: Mymaridae) to volatile signals derived from host habitats. *J. Ins. Behav.* 18:89–104.

MEINERS, T., and HILKER, M. 1997. Host location in *Oomyzus gallerucae* (Hymenoptera: Eulophidae), an egg parasitoid of the elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). *Oecologia* 112:87–93.

MEINERS, T., and HILKER, M. 2000. Induction of plant synomones by oviposition of a phytophagous insect. *J. Chem. Ecol.* 26:221–231.

MEINERS, T., WESTERHAUS, C., and HILKER, M. 2000. Specificity of chemical cues used by a specialist egg parasitoid during host location. *Entomol. Exp. Appl.* 95:151–159.

MORAES, M. C. B., LAUMANN, R., SUJII, C. P., and BORGES, M. 2005. Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical brown stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi*. *Entomol. Exp. Appl.* 115:227–237.

MUMM, R., TIEMANN, T., VARAMA, M., and HILKER, M. 2005. Choosy egg parasitoids: specificity of oviposition-induced pine volatiles exploited by an egg parasitoid of pine sawflies. *Entomol. Exp. Appl.* 115:217–225.

OCKROY, M. L. B., TURLINGS, T. C. J., EDWARDS, P. J., FRITZSCHE-HOBALLAH, M. E., AMBROSETTI, L., BASSETTI, P., and DORN, S. 2001. Response of natural populations of predators and parasitoids to artificially induced volatile emissions in maize plants (*Zea mays* L.). *Agric. Forest Entomol.* 3:201–209.

PARÉ, P. W., and TUMLINSON, J. H. 1998. Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochemistry* 47:521–526.

RANDLKOFER, B., OBERMAIER, E., M, and EINERS, T. 2007. Mother's choice of the oviposition site: balancing risk of egg parasitism and need of food supply for the progeny with an infochemical shelter? *Chemoecology* 17:177–186.

RODRIGUEZ-SAONA, C., CRAFTS-BRANDNER, S. J., PARÉ, P. W., and HENNEBERRY, T. J. 2001. Exogenous methyl jasmonate induces volatile emissions in cotton plants. *J. Chem. Ecol.* 27:679–695.

RODRIGUEZ-SAONA, C., CRAFTS-BRANDNER, S. J., WILLIAMS, L. III, and PARÉ, P. W. 2002. *Lygus hesperus* feeding and salivary gland extracts induce volatile emissions in plants. *J. Chem. Ecol.* 28:1733–1747.

RODRIGUEZ-SAONA, C., CHALMERS, J. A., RAJ, S., and THALER, J. S. 2005. Induced plant responses to multiple damagers: differential effects on an herbivore and its parasitoid. *Oecologia* 143:566–577.

RÖSE, U. S. R., MANUKIAN, A., HEATH, R. R., and TUMLINSON, J. H. 1996. Volatile semiochemicals released from undamaged cotton leaves. *Plant Physiol.* 111:487–495.

RUBERSON, J. R., and WILLIAMS, L. H. 2000. Biological control of *Lygus* spp.: a component of areawide management. *Southwest. Entomol. Suppl.* 23:96–110.

SANTOLAMAZZA-CARBONE, S., ILLAMOLA, A. R., and RIVERA, A. C. 2004. Host finding and host discrimination ability in *Anaphes nitens* Girault, an egg parasitoid of the *Eucalyptus* snout-beetle *Gonipterus scutellatus* Gyllenhal. *Biol. Control* 29:24–33.

SAS INSTITUTE, INC. 2003. *SAS/STAT* User's Guide, Release 9.1 edition. Cary, NC.

SCHRÖDER, R., WURM, L., VARAMA, M., MEINERS, T., and HILKER, M. 2008. Unusual mechanisms involved in learning of oviposition-induced host plant odours in an egg parasitoid? *Anim. Behav.* 75:1423–1430.

SHIMODA, T., TAKABAYASHI, J., ASHIRA, W., and TAKAFUJI, A. 1997. Response of predatory insect *Scolothrips takahashi* towards herbivore induced plant volatiles under laboratory and field conditions. *J. Chem. Ecol.* 23:2033–2048.

STEINBERG, S., DICKE, M., VET, L. E. M., and WANNINGEN, R. 1992. Response of the braconid parasitoid *Cotesia* (=*Apanteles*) *glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomol. Exp. Appl.* 63:163–175.

THALER, J. S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* 399:686–688.

TUMLINSON, J. H., LEWIS, W. J., and VET, L. E. M. 1993. How parasitic wasps find their hosts. *Sci. Am.* 268:100–106.

TURLINGS, T. C. J., TUMLINSON, J. H., HEATH, R. R., PROVEAUX, A. T., and DOOLITTLE, R. E. 1991. Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. *J. Chem. Ecol.* 17:2235–2251.

VET, L. E. M., and GROENEWOLD, A. W. 1990. Semiochemicals and learning in parasitoids. *J. Chem. Ecol.* 16:3119–3135.

VET, L. E. M., and DICKE, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37:141–172.

VET, L. E. M., VAN LENTEREN, J. C., HEYMANS, M., and MEELIS, E. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiol. Entomol.* 8:97–106.

VET, L. E. M., LEWIS, W. J., and CARDE, R. T. 1995. Parasitoid Foraging and Learning, pp. 65–101, in R. Cardé, and W. J. Bell (eds.). Chemical Ecology of Insects 2Chapman & Hall, New York.

WEI, J., WANG, L., ZHU, J., ZHANG, S., NANDI, O., and KANG, L. 2007. Plants attract parasitic wasps to defend themselves against insect pests by releasing hexenol. PLoS One 2(9):e852.

WHEELER, A. G. Jr. 2001. Biology of the Plant Bugs. Cornell University Press, Ithaca.

WILLIAMS, L. III, RODRIGUEZ-SAONA, C., PARÉ, P. W., and CRAFTS-BRANDNER, S. J. 2005. The piercing-sucking herbivores *Lygus hesperus* and *Nezara viridula* induce volatile emissions in plants. *Arch. Insect Biochem. Physiol.* 58:84–96.

ZAR, J. H. 1996. Biostatistical Analysis. 3rd edn.Prentice-Hall, Inc., Upper Saddle River, New Jersey.