

# Identification of Key Plant-Associated Volatiles Emitted by *Heliothis virescens* Larvae that Attract the Parasitoid, *Microplitis croceipes*: Implications for Parasitoid Perception of Odor Blends

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**Abstract** Herbivores emit plant-associated volatile organic compounds (VOCs) after feeding on plants. These plant-associated VOCs can be used by parasitoids to locate their hosts. It is hypothesized that certain compounds play key roles in the attractiveness of host-associated odor blends. The larval parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae) and its herbivore host, *Heliothis virescens* (Lepidoptera: Noctuidae), a major pest of cotton plant were used as model species to identify key compounds mediating attraction of parasitoids to hosts. Comparative GC/MS analyses of cotton-fed vs. artificial diet-fed hosts indicated that 12 of 17 compounds in the headspace of *H. virescens* larvae were exclusive to plant-fed hosts, and thus considered to be plant-associated. In order to identify key attractive compounds, a full blend of 15 commercially available synthetic compounds was modified by removing each of the 10 plant-associated compounds emitted by host larvae. In Y-tube olfactometer bioassays testing parasitoid responses to modified blends, 1-octen-3-ol, decanal, (*E*)- $\beta$ -caryophyllene,  $\alpha$ -humulene,  $\alpha$ -farnesene, and  $\beta$ -pinene were identified as key compounds contributing to attractiveness of the natural blend of VOCs emitted by cotton-fed hosts. The results showed that while various host-associated compounds act in concert to serve as useful host location cues, only a fraction of the natural blend mediates attraction in parasitoids. Furthermore, the role of a compound is better assessed in the context of other compounds, and odor blends are better perceived as a whole rather than as individual components.

**Keywords** Olfactometer · Gas chromatography-mass spectrometry · Contextual perception · Background odors

## Introduction

Foraging natural enemies rely on a plethora of semiochemicals from a plant-host complex for location, recognition, and acceptance of hosts. However, only a fraction of host-associated odor blends are attractive or ecologically relevant to natural enemies, thus, the need to identify key compounds that mediate behavioral responses in these species (Clavijo McCormick et al. 2014; D'Alessandro and Turlings 2005; D'Alessandro et al. 2009; van Dam et al. 2010). In the plant-host complex, informative volatile cues for parasitoid host location can be emitted by plants when infested by herbivores (De Moraes et al. 1998; Fürstenberg-Hägg et al. 2013; Pare and Tumlinson 1999; Peñaflor et al. 2011; Turlings and Wäckers 2004; Uefune et al. 2012) or be emitted by herbivores and their frass after feeding on plants (Alborn et al. 1995; Chucho et al. 2006; de Rijk et al. 2016; Elzen et al. 1984). Arguably, plants have larger biomass and produce more abundant volatiles than herbivorous insects (Turlings et al. 1995). However, once parasitoids find a host plant patch and make an appropriate landing on a plant, herbivore host-specific odors become critical short-range cues in the later phase of the host location process (Afsheen et al. 2008; Colazza et al. 2014; De Rijk et al. 2013). The choice between plant volatiles vs. host-specific odors presents a 'detectability' vs. 'reliability' dilemma to foraging natural enemies (Vet and Dicke 1992). Host-specific cues are more important to relatively specialized parasitoids whose hosts are generalist herbivores. This is due to the high number of chemical profile patterns that such parasitoids would have to detect and recognize if solely depending on host plant cues (Wajnberg et al.

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2008). According to optimal foraging theory, parasitoids are expected to select hosts that show the greatest fitness attribute so as to maximize their chances of progeny survival (Chesnais et al. 2015; Goubault et al. 2003; Steiner et al. 2007). In the field, herbivore hosts that recently fed on plants may be momentarily deemed more suitable by parasitoids than those that did not recently feed on plants.

Herbivores can acquire plant chemicals in their diet for further physiological or metabolic processes, or sequester toxic ones for defensive purposes (Despres et al. 2007). However, certain plant-associated compounds emitted by herbivores can serve as kairomones when used by parasitoids as odor cues to locate their hosts. In fact, it has been demonstrated that herbivore diet affects olfactory responses of parasitoids to host-specific odors (Ed Sauls et al. 1979; Elzen et al. 1984; Hofstetter and Raffa 1997; Nettles 1980; Reis et al. 2003; Sullivan et al. 2000). For instance, the parasitoid, *Microplitis croceipes* showed greater attraction to cowpea-fed *Helicoverpa zea* larvae compared with artificial diet-fed hosts (Ed Sauls et al. 1979). In a similar study, *Campoletis sonorensis* showed greater attraction to cotton-fed *Heliothis virescens* larvae compared with artificial diet-fed hosts (Elzen et al. 1984). In both studies, the increased attraction of parasitoids was attributed to plant-associated odors emitted by their hosts. Similarly, Nettles (1980) reported that *H. virescens* larvae that were otherwise not attractive to the parasitoid, *Eucelatoria* sp. (Tachinidae) became attractive after feeding on okra leaves. However, not all the components of such odor blends mediate attraction in parasitoids; and demonstrating the role of each compound in odor mixtures can be tasking (Schnee et al. 2006). The important question of which particular volatile compounds play key roles in the attraction of parasitoids to plant-fed hosts remains to be answered in several plant-herbivore-parasitoid systems that have been studied. Chemical ecologists are interested in differentiating useful cues from ‘background noise’, with regards to the insects’ use of host-associated odor blends. Previous studies have used a combination of chemical ecology techniques (Beyaert et al. 2010; Cha et al. 2012; Isberg et al. 2016; Logan et al. 2008; Molnár et al. 2015; Nojima et al. 2003), molecular techniques (Kappers et al. 2005; Schnee et al. 2006), and a bioinformatics approach (van Dam et al. 2010) to identify key compounds or blend fractions mediating attraction of foraging insects to host odors. Isolation and functional identification of specific compounds that mediate attraction of parasitoids to host-associated odors will increase our knowledge of odor perception and discrimination in parasitoids. In addition, this serves as a critical step in the quest for improved monitoring strategies and effectiveness of parasitoids as biocontrol agents.

In the present study, we hypothesized that certain plant-associated compounds play key roles in the attraction of parasitoids to VOC blends emitted by herbivores that recently fed

on a plant. *Microplitis croceipes*, a relatively specialized larval endoparasitoid of *Heliothis virescens*, which is an important pest of cotton and other economic crops in the US, was used as model species to identify key compounds that mediate its attraction to plant-fed hosts. Using a combination of head-space volatile collection coupled with a four-choice olfactometer bioassay, gas chromatography coupled with mass spectrometry (GC/MS), and Y-tube olfactometer bioassays, we established that only a fraction of the VOC blend emitted from the body and frass of cotton-fed *H. virescens* larvae mediates attraction of *M. croceipes*. To the best of our knowledge, this is the first study to identify compounds that mediate attraction of *M. croceipes* to plant-fed *H. virescens*, by using a combination of chemical ecology techniques. Here, we present a reductionist approach whereby a full blend is reduced by a single component in order to determine the relevance of the missing compound. We propose that the role of a compound is better assessed in the context of other compounds in an odor blend. The implication of the current results to perception and use of host-associated odors in parasitoids is discussed.

## Methods and Materials

**Insects** Cocoons of *M. croceipes* were provided by the USDA-ARS Insect Biology and Population Management Research Laboratory (Tifton, GA, USA). Rearing procedures were similar to those described by Lewis and Burton (1970) with modifications described by Morawo and Fadamiro (2014a). Briefly, *M. croceipes* were reared on 2nd-3rd instars of *Heliothis virescens*, and adult wasps were supplied with 10 % sugar water upon emergence. The two host groups, cotton-fed and artificial diet-fed *H. virescens* larvae, were reared initially on pinto bean artificial diet (Shorey and Hale 1965). The cotton-fed group was removed from diet, cleaned, and placed on a cotton plant to feed for 24 h. Larvae were cleaned initially by a soft camel hair brush and then allowed to walk over a moistened paper towel to remove diet crumbs and frass. The artificial diet-fed group was allowed to feed continuously on artificial diet. Before use in bioassays, host larvae from both groups were cleaned of frass, leaf, or diet crumbs as described above. All parasitoids used for behavioral bioassays were 2–5-d-old females that were presumed mated but inexperienced with oviposition. The general rearing conditions for all insects were  $25 \pm 1$  °C,  $75 \pm 5$  % RH and 14:10 h (L:D) photoperiod.

**Plants** Cotton (*Gossypium hirsutum*, var. max 9) plants were grown according to Morawo and Fadamiro (2014a) in growth chambers (Entomology & Plant Pathology, Auburn University) at 26.6 °C day, 25.6 °C night, and 60 % relative humidity. Care was taken to avoid fungal infection or insect infestation prior to use of plants in experiments. 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 deliberately infested with *H. virescens* were 4–5-wk-old.

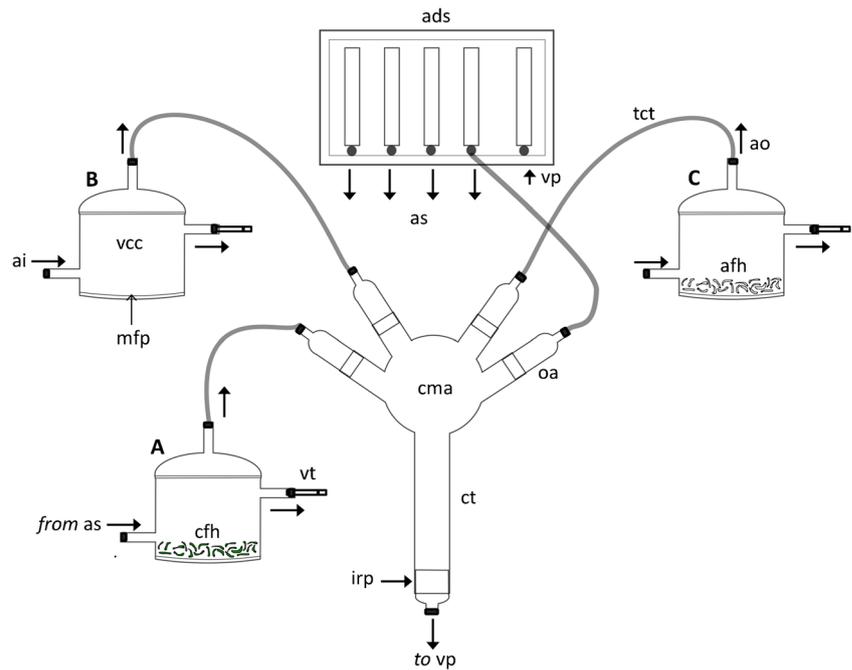
**Oviposition Choice Tests** Oviposition choice tests were conducted in two separate experiments (1 and 2) using a 9 cm petri dish arena. In experiment 1, each female parasitoid was presented with 5 cotton-fed and 5 artificial diet-fed hosts. *Heliothis virescens* larvae were positioned randomly before introducing a single parasitoid into the center of the arena, and a new petri dish was used for each replicate. Once introduced into the petri dish, a female parasitoid was allowed 15 min to oviposit (parasitize) 10 times, and the frequency of successful oviposition made in either host group was recorded. There was no need to physically mark the larvae to differentiate plant-fed hosts from artificial diet-fed hosts because *H. virescens* larvae that recently fed on foliage took up the green color. This visual method also was used to ensure that each *H. virescens* larvae considered plant-fed actually consumed plant tissues. In order to confirm that female *M. croceipes* were not showing affinity for already parasitized hosts, a second experiment, similar to the one described by Elzen et al. (1984) was conducted. In experiment 2, one cotton-fed and one artificial diet-fed host larva was presented to each parasitoid in a petri dish. After one type of host was stung, it was replaced with another one of its kind until the wasp had oviposited 10 times. The results of the two oviposition choice experiments were compared. Experiments were arranged in a randomized complete block design. Each test was replicated 10 times (1 wasp per replicate) and repeated at least 2 times.

**Headspace Volatile Collection Coupled with Four-Choice Olfactometer Bioassay** In order to identify plant-associated volatiles emitted by *H. virescens* larvae, headspace VOCs were collected from cotton-fed and artificial diet-fed larvae for profile comparison. Headspace volatile collection was coupled with four-choice olfactometer tests so as to match behavioral responses of parasitoids to the chemistry of headspace volatiles. The set-up was similar to that described by Morawo and Fadamiro (2014a) with slight modifications (Fig. 1). A group of cotton-fed hosts was cleaned and placed inside a volatile collection chamber (400 ml). Similarly, a group of artificial diet-fed hosts also was cleaned and placed inside a second chamber. The set-up included a third (control) chamber containing only moistened filter paper. The fourth arm of the olfactometer was connected directly to the air delivery system (air control). Purified (charcoal-filtered) and humidified air was let into each chamber at 300 ml/min. Each chamber had two outlets: one was connected to an olfactometer arm while the other was connected to a trap containing 50 mg of Super-Q (Alltech Associates, Deerfield, IL, USA).

To avoid mix-up of volatiles in the olfactometer, air was drawn by a vacuum pump from the bottom of the olfactometer at 1300 ml/min. The set-up was arranged such that odors from both host groups were passed through opposite arms, separated by each of the two control arms. Parasitoids were released individually through the bottom of the olfactometer and given up to 10 min to make a choice. For instance, after a female *M. croceipes* made a choice, the insect was removed, and 1 min elapsed before replacing with another one. A parasitoid was recorded to have made a choice for the odor offered through an arm when it moved into an extension of the arm and remained there for at least 15 s. The olfactometer was rotated 90° after testing four insects. The apparatus was cleaned with acetone after testing 12 insects. Parasitoids were used once, and non-responders (< 6 %) were excluded from data analyses. In a separate set-up, volatiles were trapped from 250 mg of frass produced by cotton-fed larvae so as to differentiate compounds exclusive to host body from those present in frass. In all experiments, headspace volatiles were collected for 6 h after it was determined in preliminary studies that this duration was optimal for trapping VOCs from host larvae and frass without subjecting them to desiccation. Experiments were completely randomized and repeated at least four times on different days between 0900 and 1700 h.

**GC/MS Analyses** Headspace volatiles trapped from host larvae complex (larvae and frass) as well as from frass alone were analyzed by GC/MS as described by Morawo and Fadamiro (2014a). Super-Q traps were eluted with 200 µl of methylene chloride, and the resulting extracts were stored in a freezer (at –20 °C) until use. Identification and quantification of headspace volatiles was done using an Agilent 7890 A GC coupled to a 5975C Mass Selective Detector, with an HP-5 ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). For each headspace volatile extract, 1 µl was injected into the GC/MS in splitless mode. The GC was programmed as follows: inject at 40 °C, hold at 40 °C for 2 min, and then increased by 5 °C/min to 200 °C for a total of 40 min. The temperature of both injector and detector was set at 200 °C. Mass spectra were obtained using electron impact (EI, 70 eV). Compounds were identified according to their retention times and mass spectra, in comparison with a NIST 98 library (National Institute of Standards and Technology, Gaithersburg, MD, USA) and commercially available synthetic standards (purity 95–99 %) obtained from Sigma-Aldrich® Chemical Co. (St. Louis, MO, USA) and Alfa Aesar® (Ward Hill, MA, USA) (Table 1). As a synthetic standard of α-bergamotene was not commercially available, its identity was confirmed by comparing with published GC profiles of cotton headspace volatiles (Loughrin et al. 1994; McCall et al. 1994; Ngumbi et al. 2009). For quantification, external calibration curves were made with standard solutions of representative compounds (α-pinene, 1-octen-3-ol, and (E)-β-caryophyllene) (Zebelo et al. 2014).

**Fig. 1** A simplified overview of headspace volatile collection coupled with four-choice olfactometer set-up. Each of three chambers A, B, C have one air inlet and two outlets. One outlet is connected to an arm of the olfactometer as an odor source, while the other arm is connected to a volatile trap. Purified air is directly passed into the fourth arm of the olfactometer. Description of parts: *ads*, air delivery system; *tct*, Teflon connecting tube; *ao*, air outlet; *vp*, vacuum pump; *as*, air source; *vcc*, volatile collection chamber; *ai*, air inlet; *afh*, artificial diet-fed hosts; *mfp*, moistened filter paper; *oa*, olfactometer arm; *cma*, choice making area; *vt*, volatile trap; *ct*, central tube; *cfh*, cotton-fed hosts; *irp*, insect release point



**Y-Tube Olfactometer Bioassays** Attraction of parasitoids to synthetic blends and single VOCs was tested in a Y-tube olfactometer (Analytical Research Systems Inc., Gainesville, FL, USA) set-up similar to that previously described by Ngumbi and Fadamiro (2012). A full synthetic blend of 15 compounds identified in the

headspace of cotton-fed *H. virescens* was formulated in hexane at 0.01, 0.05, and 0.1  $\mu\text{g}/\mu\text{l}$  concentration, according to component proportions in the natural blend. Odor blends were delivered on Whatman No. 1 filter paper strips (25  $\times$  7 mm) at 0.1, 0.5, and 1  $\mu\text{g}$  doses. The doses were selected as ecologically relevant based on results of GC/MS analyses of total amounts of volatiles emitted by cotton-fed *H. virescens* larvae in 6 h, and in consideration of the maximum time (10 min) given each parasitoid to make a choice. The full blend (at the most attractive dose of 1  $\mu\text{g}$ ) then was modified by removing each of the 10 compounds exclusive to cotton-fed hosts to make separate blends. In addition, attraction of parasitoids to select single compounds (1-octen-3-ol and decanal) was tested at 0.01 and 0.1  $\mu\text{g}$  doses. Single compounds were tested at relatively lower doses compared to the full blend to simulate ecologically relevant concentrations. The experimental conditions and procedure for testing individual wasps were similar to those earlier described for four-choice olfactometer bioassays. After synthetic compounds were applied to filter paper strips, 10 s elapsed to allow solvent evaporation and avoid initial steepness in the release rate of odor stimuli. For Y-tube olfactometer bioassays, the apparatus was rotated 180°, and filter paper strips were changed after testing 4 insects. Parasitoids were used once, and non-responders (< 7 % in all experiments) were excluded from data analyses. Insects chose between one arm carrying odor from a synthetic blend or single compound, and the other arm carrying hexane (control).

**Table 1** Information on purity and supplier of the synthetic compounds used in this study

Compound	Purity (%)	Supplier
$\alpha$ -Pinene	98	Sigma-Aldrich <sup>a</sup>
$\beta$ -Pinene	99	Sigma-Aldrich <sup>a</sup>
1-Octen-3-ol	98	Alfa Aesar <sup>b</sup>
3-Octanone	98	Alfa Aesar <sup>b</sup>
Myrcene	>95	Sigma-Aldrich <sup>a</sup>
Limonene	97	Sigma-Aldrich <sup>a</sup>
2-Ethylhexanol	>99	Sigma-Aldrich <sup>a</sup>
Decanal	96	Alfa Aesar <sup>b</sup>
Tridecane	>99	Sigma-Aldrich <sup>a</sup>
Tetradecane	>99	Alfa Aesar <sup>b</sup>
( <i>E</i> )- $\beta$ -Caryophyllene	>98.5	Sigma-Aldrich <sup>a</sup>
$\alpha$ -Humulene	>96	Sigma-Aldrich <sup>a</sup>
$\alpha$ -Farnesene <sup>c</sup>	-	Sigma-Aldrich <sup>a</sup>
Bisabolene <sup>c</sup>	-	Alfa Aesar <sup>b</sup>
$\alpha$ -Bisabolol	>95 %	Sigma-Aldrich <sup>a</sup>

<sup>a</sup> Sigma-Aldrich® Chemical Co. St. Louis, MO, USA

<sup>b</sup> Alfa Aesar®, Ward Hill, MA, USA

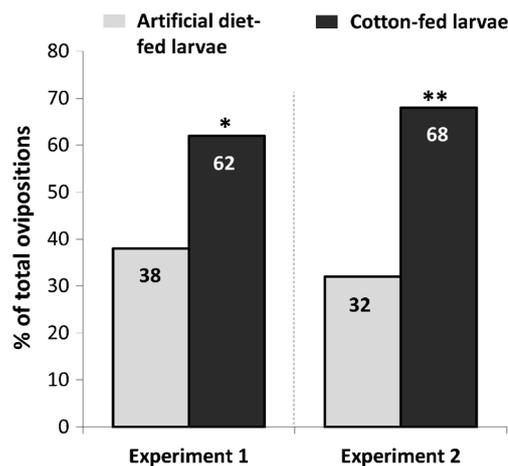
<sup>c</sup> Compounds are mixture of isomers

**Data Analyses** The deviation of oviposition choice of parasitoids from a 50:50 % distribution between two different groups of hosts was analyzed using a *Chi-square* goodness of fit test. Four-choice olfactometer data were modeled as a response count with *Logistic Regression* and slopes separated by Proc Logistic Contrast in SAS. The model adequacy was confirmed with a *Likelihood Ratio* test (Wajnberg and Haccou 2008). For visual comparison, both oviposition and olfactometer data were presented as percentages on charts. Amount of volatile compounds trapped from groups of 50 host larvae was expressed in ng, while amount of volatiles trapped from frass produced by cotton-fed hosts was expressed in ng/50 mg of frass. Significant differences in the amounts of each compound emitted by cotton-fed and artificial diet-fed larvae were established using *Wilcoxon-Mann-Whitney* test. A two-sided *binomial* test was used to compare parasitoid responses to stimulus vs. control in Y-tube olfactometer. *Chi-square* goodness of fit test was used to analyze the deviation of responses to modified blends from the 79:21 % (stimulus: control) recorded for full blend. The test was conducted in order to determine if the removal of a compound significantly affects attraction of parasitoids to the full blend. All analyses were performed using SAS 9.2 with 0.05 level of significance.

## Results

**Oviposition Choice of *M. croceipes*** In oviposition choice tests, female *M. croceipes* parasitized more cotton-fed larvae than artificial diet-fed larvae. In experiment 1, each female parasitoid was presented with 5 cotton-fed and 5 artificial diet-fed hosts. In experiment 2, one cotton-fed and one artificial diet-fed host larva was presented to each parasitoid. In total, 62 % ( $\chi^2 = 5.76$ ;  $P = 0.016$ ) of total oviposition was made into cotton-fed larvae when parasitoids were simultaneously presented with 5 *H. virescens* larvae from each of two host groups (Experiment 1) (Fig. 2). Similarly, 68 % ( $\chi^2 = 12.96$ ;  $P < 0.001$ ) of total oviposition was made into cotton-fed larvae when parasitoids were simultaneously presented with one *H. virescens* larva from each of two host groups (Experiment 2) (Fig. 2). A comparison of results obtained from both experiments indicates that incidence of superparasitism in experiment 1 did not significantly influence oviposition choice of parasitoids.

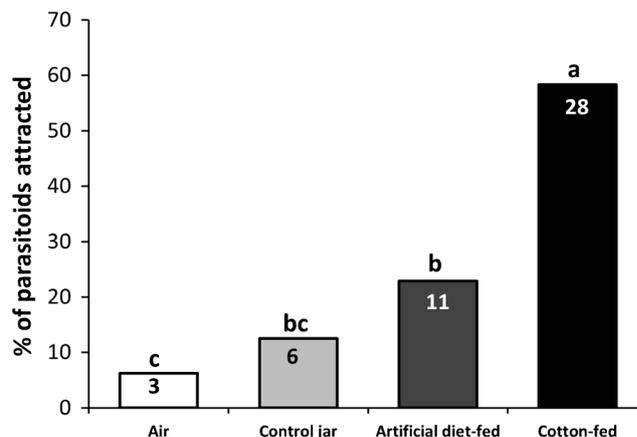
**Parasitoid Preference between Cotton-Fed and Artificial Diet-Fed Hosts in a Four-Choice Olfactometer** In four-choice olfactometer bioassays, female *M. croceipes* showed a clear preference for cotton-fed hosts over artificial diet-fed hosts ( $\chi^2 = 11.79$ ,  $df = 1$ ,  $P < 0.001$ ) (Fig. 3). In total, 58 % of parasitoids chose the arm offering odors from cotton-fed hosts, 23 % of parasitoids chose artificial-diet fed hosts, and 19 % chose the control arms (combined). This result suggests



**Fig. 2** Oviposition choice of female *Microplitis croceipes* between cotton-fed and artificial diet-fed *Heliothis virescens* larvae in a petri dish arena. Experiment 1: Each parasitoid was presented with 5 cotton-fed and 5 artificial diet-fed hosts and allowed to parasitize 10 times. Experiment 2: Each parasitoid was presented with 1 cotton-fed and 1 artificial diet-fed host and allowed to parasitize once, after which the parasitized host was replaced with another one of its type until the parasitoid had parasitized 10 times.  $N = 10$  wasps per method. Numbers inside bars indicate actual number of parasitized hosts. Asterisks (\*) indicate significant deviation from a 50:50 % distribution for total number of ovipositions made into the two types of host ( $P < 0.05$ ; *Chi-square* goodness of fit test)

that *M. croceipes* can distinguish between host larvae fed different diets, and are more attracted to *H. virescens* larvae that recently fed on cotton.

**GC/MS Analyses of Host Headspace VOCs** A comparison of headspace volatile profiles of cotton-fed larvae complex (larvae and frass), artificial diet-fed larvae complex, and frass of cotton-fed larvae showed qualitative and quantitative



**Fig. 3** Preference of female *Microplitis croceipes* between cotton-fed and artificial diet-fed *Heliothis virescens* larvae in four-choice olfactometer. Attraction to treatments was modeled as binary response counts and represented on the chart as percentage of total responding wasps. Numbers inside bars indicate actual number of wasps attracted to each arm of olfactometer.  $N = 48$  wasps. Values (%) having no letter in common are significantly different ( $P < 0.05$ ; Proc Logistic Regression Contrast)

differences in the composition. In total, 17 compounds (1 unknown) were consistently detected in the headspace of *H. virescens* larvae but not found in control (moistened filter paper). These included the monoterpenes,  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, and limonene; the alcohols, 1-octene-3-ol and 2-ethylhexanol; the carbonyl compounds, 3-octanone and decanal; the alkanes, tridecane and tetradecane; the sesquiterpenes, (*E*)- $\beta$ -caryophyllene,  $\alpha$ -bergamotene,  $\alpha$ -humulene,  $\alpha$ -farnesene, bisabolene, and  $\alpha$ -bisabolol (Table 2). More compounds (all 17) were detected in the headspace of cotton-fed larvae complex (larvae + frass), and generally in greater amounts compared with artificial diet-fed larvae. Only five compounds, 3-octanone, limonene, 2-

ethylhexanol, tridecane, and tetradecane, were consistently detected in the headspace of artificial diet-fed larvae complex. Thus, the remaining 12 compounds were exclusive to cotton-fed larvae. Comparing VOC profiles of cotton-fed larvae complex to that of their frass alone, it can be inferred that 1-octene-3-ol, myrcene, and decanal were exclusively released from the body of cotton-fed *H. virescens* larvae.

**Attraction of Parasitoids to Synthetic VOC Blends and Single Compounds in a Y-Tube Olfactometer** Attraction of parasitoids to formulated odor blends was dose-dependent. Compared to control (hexane), parasitoids showed the greatest attraction to the full (15-component) blend at the highest dose

**Table 2** Composition of headspace volatile organic compounds (VOCs) emitted by artificial diet-fed *Heliothis virescens* larvae, cotton-fed larvae and frass of cotton-fed larvae

ID <sup>a</sup>	Compound	Artificial Diet-fed larvae complex <sup>b</sup>		Cotton-fed larvae complex <sup>b</sup>		Frass (Cotton-fed larvae) only <sup>c</sup>	
		Amount <sup>d</sup> (ng)	Rel. %	Amount <sup>d</sup> (ng)	Rel. %	Amount <sup>e</sup> (ng)	Rel. %
1	$\alpha$ -Pinene <sup>f</sup>	0	0	863.5 $\pm$ 68.6 *	15.1	33.3 $\pm$ 15.1	12.1
2	$\beta$ -Pinene <sup>f</sup>	0	0	91.3 $\pm$ 10.5 *	1.6	2.8 $\pm$ 1.7	1.1
3	1-Octen-3-ol <sup>f, g</sup>	0	0	79.5 $\pm$ 29.2	1.4	0	0
4	3-Octanone	43.4 $\pm$ 15.8	7.6	42.8 $\pm$ 14.9	0.8	0	0
5	Myrcene <sup>f, g</sup>	0	0	153.0 $\pm$ 34.0 *	2.7	0	0
6	Unknown <sup>f</sup>	0	0	72.4 $\pm$ 13.5 *	1.2	10.5 $\pm$ 5.6	3.8
7	Limonene	246.6 $\pm$ 24.0	41.5	519.4 $\pm$ 147.2 *	9.1	0	0
8	2-Ethylhexanol <sup>h</sup>	24.4 $\pm$ 9.4	4.2	126.5 $\pm$ 23.1 *	2.2	3.3 $\pm$ 1.1	1.3
9	Decanal <sup>f, g</sup>	0	0	54.2 $\pm$ 19.1 *	1.0	0	0
10	Tridecane	169.2 $\pm$ 60.1	28.8	355.0 $\pm$ 106.5	6.2	0	0
11	Tetradecane	106.8 $\pm$ 40.2	17.8	140.5 $\pm$ 39.2	2.4	0	0
12	( <i>E</i> )- $\beta$ -Caryophyllene <sup>f</sup>	0	0	1673.5 $\pm$ 221.9 *	29.2	124.5 $\pm$ 13.6	45.0
13	$\alpha$ -Bergamotene <sup>f</sup>	0	0	39.6 $\pm$ 9.0 *	0.7	2.9 $\pm$ 0.5	1.1
14	$\alpha$ -Humulene <sup>f</sup>	0	0	367.6 $\pm$ 51.6 *	6.5	28.8 $\pm$ 4.1	10.5
15	$\alpha$ -Farnesene <sup>f</sup>	0	0	44.0 $\pm$ 7.7 *	0.8	4.0 $\pm$ 0.8	1.4
16	Bisabolene <sup>f</sup>	0	0	496.6 $\pm$ 55.2 *	8.6	41.2 $\pm$ 7.6	14.8
17	$\alpha$ -Bisabolol <sup>f</sup>	0	0	450.9 $\pm$ 71.6 *	7.9	24.4 $\pm$ 7.0	8.9

The identity of all compounds was confirmed by NIST library and comparison with synthetic standards (purity 95–99 %), with one exception. Identity of compound 13 was confirmed by NIST library and comparison with published GC profiles of cotton headspace volatiles

<sup>a</sup> In order of elution during gas chromatography

<sup>b</sup> Volatiles were collected for 6 h from 50 third instar *Heliothis virescens* that were continuously fed artificial diet (artificial diet-fed) or fed cotton foliage 24 h prior to VOC collection (cotton-fed). It is assumed that trapped volatiles were released from host body and frass produced during the 6 h of volatile collection

<sup>c</sup> Volatiles were collected for 6 h from 250 mg of frass excreted by cotton-fed larvae that had been separated from larvae, exuviae or leaf crumbs

<sup>d</sup> Amounts (ng) are mean  $\pm$  SEM of four replicates

<sup>e</sup> Amounts (ng/50 mg of frass) are mean  $\pm$  SEM of four replicates

<sup>f</sup> Compounds detected only in cotton-fed larvae and or their frass

<sup>g</sup> Compounds detected in the complex of cotton-fed larvae (larvae + frass) but not in their frass

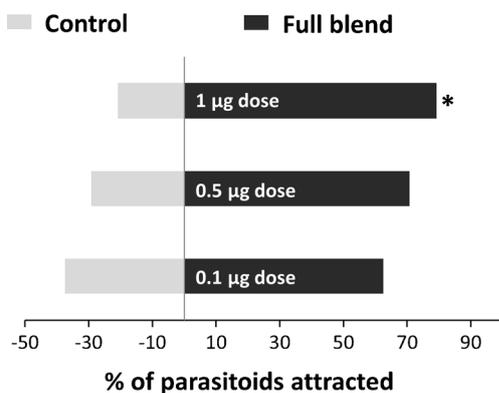
<sup>h</sup> Compound was detected in all three treatments

\* Asterisks indicate significant difference between mean amount of each compound emitted by artificial diet-fed and cotton-fed host complexes ( $P < 0.05$ ; Wilcoxon-Mann-Whitney test)

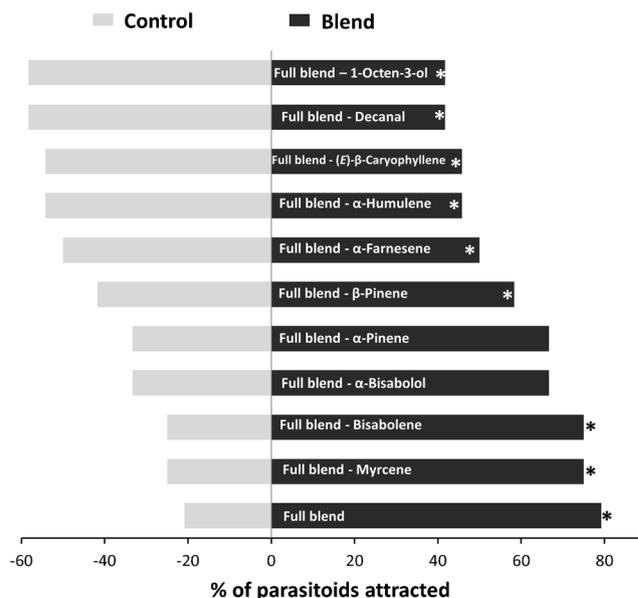
of 1  $\mu\text{g}$  (79 %,  $P = 0.007$ , *Binomial* test). Parasitoids showed reduced attraction to the full blend at the lower doses of 0.5  $\mu\text{g}$  (71 %,  $P = 0.06$ , *Binomial* test) and 0.1  $\mu\text{g}$  (63 %,  $P = 0.31$ , *Binomial* test) (Fig. 4). Thus, the 1  $\mu\text{g}$  dose was considered optimal, and was used in subsequent bioassays with volatile blends. Parasitoids could not significantly discriminate the odor blend from control when 1-octen-3-ol (42 % of parasitoids chose blend), decanal (42 %), (*E*)- $\beta$ -caryophyllene (46 %),  $\alpha$ -humulene (46 %),  $\alpha$ -farnesene (50 %),  $\beta$ -pinene (58 %),  $\alpha$ -pinene (67 %), or  $\alpha$ -bisabolol (67 %) were removed (Fig. 5). Furthermore, a goodness of fit test indicated that attraction of parasitoids to blends from which 1-octen-3-ol ( $\chi^2 = 20.16$ ,  $df = 1$ ,  $P < 0.001$ ), decanal ( $\chi^2 = 20.16$ ,  $df = 1$ ,  $P < 0.001$ ), (*E*)- $\beta$ -caryophyllene ( $\chi^2 = 15.91$ ,  $df = 1$ ,  $P < 0.001$ ),  $\alpha$ -humulene ( $\chi^2 = 15.91$ ,  $df = 1$ ,  $P < 0.001$ ),  $\alpha$ -farnesene ( $\chi^2 = 12.17$ ,  $df = 1$ ,  $P < 0.001$ ), or  $\beta$ -pinene ( $\chi^2 = 6.18$ ,  $df = 1$ ,  $P < 0.01$ ) had been removed was significantly less than the level of attraction elicited by the full blend (79:21 %; blend: control %). In contrast, removal of myrcene and bisabolene had no significant effect on the attraction of parasitoids. When presented as a single compound, decanal did not elicit significant attraction in parasitoids at 0.01 and 0.1  $\mu\text{g}$  doses. However, parasitoids showed significantly (75 %,  $P = 0.02$ , *Binomial* test) greater attraction to 0.01  $\mu\text{g}$  dose of 1-octen-3-ol, compared to the control (Fig. 6).

## Discussion

Based on the results from a combination of techniques used, it was evident that only a fraction of the VOCs emitted by cotton-fed *H. virescens* larvae mediates attraction in *M. croceipes*. In agreement with a previous study (Ed Sauls et al. 1979), *M. croceipes* preferred cotton-fed *H. virescens* larvae to artificial diet-fed larvae in oviposition choice tests.

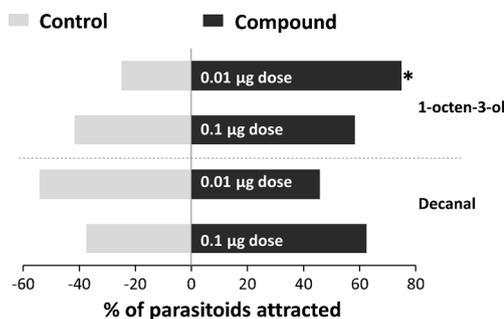


**Fig. 4** Attraction of female *Microplitis croceipes* in Y-tube olfactometer when given a choice between hexane (control) and full (15-component) synthetic blend of volatiles emitted by cotton-fed *Heliothis virescens* larvae. Volatile blend was tested at three doses (0.1, 0.5, and 1  $\mu\text{g}$ ).  $N = 24$  wasps per test. Asterisks (\*) indicate significant difference between stimulus and control ( $P < 0.05$ ; *Binomial* test)



**Fig. 5** Attraction of female *Microplitis croceipes* in Y-tube olfactometer when given a choice between hexane (control) and the full (15-component) or modified (14-component) synthetic blend of volatiles emitted by cotton-fed *Heliothis virescens* larvae. A blend was modified by removing one of ten plant-associated compounds from the full blend. Blends were tested at optimum dose of 1  $\mu\text{g}$ .  $N = 24$  wasps per test. Asterisks (\*) outside bars indicate significant difference between stimulus and control ( $P < 0.05$ ; *Binomial* test). Asterisks (\*) inside bars indicate significant deviation from the 79:21 % (stimulus: control) response recorded for full blend ( $P < 0.05$ ; *Chi-square* goodness of fit test)

Similarly, an effect of herbivore plant diet on olfactory responses of various parasitoids to their hosts has been previously reported (Elzen et al. 1984; Hofstetter and Raffa 1997; Reis et al. 2003; Sullivan et al. 2000). In the present study, parasitoids showed the greatest attraction to plant-fed hosts in four-choice olfactometer bioassays, indicating that odor played a role in their oviposition preference. Furthermore, comparative GC/MS analyses showed that certain VOCs were exclusively emitted by plant-fed larvae and their frass, thus highlighting the role of plant-associated compounds in the attraction of parasitoids to their herbivore hosts.



**Fig. 6** Attraction of female *Microplitis croceipes* in Y-tube olfactometer when given a choice between hexane (control) and 1-octen-3-ol or decanal. Single compounds were tested at two doses (0.01 and 0.1  $\mu\text{g}$ ).  $N = 24$  wasps per test. Asterisks (\*) indicate significant difference between stimulus and control ( $P < 0.05$ ; *Binomial* test)

As expected, the full 15-component blend of volatiles emitted by *H. virescens* elicited the greatest attraction in the Y-tube olfactometer, supporting the claim that odor perception in insects is optimized with blends (Bruce and Pickett 2011; Takemoto and Takabayashi 2015; van Wijk et al. 2011). The contribution of a compound to the attractiveness of an odor blend can be more accurately determined in the context of other blend components. Thus, a reductionist approach was used to determine the contribution of each compound in the present study. In general, attraction of parasitoids to the full blend was reduced when a component was removed. Removal of 1-octen-3-ol, decanal, (*E*)- $\beta$ -caryophyllene,  $\alpha$ -humulene,  $\alpha$ -farnesene, or  $\beta$ -pinene significantly reduced attraction of parasitoids to the full blend. These results highlight the important roles played by these compounds in mediating attraction of *M. croceipes* to *H. virescens* larvae. In contrast, the removal of myrcene, bisabolene,  $\alpha$ -bisabolol, and  $\alpha$ -pinene had no significant effect on attractiveness of full blend, suggesting that they may not be critical components of the attractive blend, or that they only play minor roles.

(*E*)- $\beta$ -Caryophyllene,  $\alpha$ -humulene,  $\alpha$ -farnesene,  $\beta$ -pinene, and decanal have been detected in the headspace of uninfested and/or herbivore-infested cotton in previous studies (De Moraes et al. 1998; Loughrin et al. 1994; Magalhães et al. 2012; Morawo and Fadamiro 2014a; Ngumbi et al. 2009; Röse and Tumlinson 2004; Röse et al. 1998; Sobhy et al. 2015; Turlings et al. 1995). However, these compounds are ubiquitous and not restricted to cotton plant. In fact, the roles of these compounds have been investigated in other plant-herbivore-parasitoid tritrophic interactions. For instance,  $\beta$ -pinene has been identified as chemical cues from larval frass of Cerambycids used for host location by the parasitoid *Dastarcus helophoroides* (Wei et al. 2013). Similarly, (*E*)- $\beta$ -caryophyllene was identified as the key volatile emitted by stink bug-infested bean (Colazza et al. 2004) and aphid-infested tomato (Sasso et al. 2007) that mediates attraction of the parasitoids, *Trissolcus basalis* and *Aphidius ervi*, respectively. In another study, where transgenic *Arabidopsis* was used to express production of maize sesquiterpenes, farnesene was identified as one of two compounds mediating attraction of the parasitoid, *Cotesia marginiventris* (Schnee et al. 2006). We are not aware of any previous study in which  $\alpha$ -humulene was identified as a key volatile mediating attraction of parasitoids. However,  $\alpha$ -humulene has been previously reported as a component of cotton-fed *H. virescens* odor that elicits attraction in *Camponotus sonorensis* (Elzen et al. 1984).

Removal of decanal or 1-octen-3-ol diminished the attractiveness of the full blend to the greatest extent, suggesting that the two compounds play a major role in attraction of *M. croceipes* to the host-emitted odor blend. Comparative GC/MS analyses showed that the two compounds were exclusively emitted from the body of cotton-fed host larvae, suggesting their use as reliable cues for host discrimination in

parasitoids. Previous studies have reported decanal as a component in the blend of VOCs released from the greater wax moth (Dweck et al. 2010) and scales of codling moth (Delury et al. 1999) that attracts the parasitoids, *Bracon hebetor* and *Ascogaster quadridentata*, respectively. In addition, it has been reported that decanal mediates attraction (Isberg et al. 2016) or host discrimination (Logan et al. 2008) in certain hematophagous insects, further suggesting its ubiquitous nature. 1-Octen-3-ol is another ubiquitous compound commonly associated with fungal odors (Steiner et al. 2007). In the present study, care was taken to avoid fungal infection on plants, and no visible moldiness was observed. Regardless, comparative GC/MS analyses suggest that 1-octen-3-ol emitted by cotton-fed host larvae was plant-associated. The present results indicate that 1-octen-3-ol is a key component of the attractive kairomone used by *M. croceipes* to locate cotton-fed *H. virescens*. In general, more parasitoids chose 1-octen-3-ol or decanal over the control when tested as single compounds but parasitoids only showed significant attraction to 0.01  $\mu$ g dose of 1-octen-3-ol. Conversely, a 0.1  $\mu$ g dose of 1-octen-3-ol, as well as 0.01 and 0.1  $\mu$ g doses of decanal did not elicit significant attraction in parasitoids. Previous studies also have reported instances in which a compound was not attractive to an insect when tested alone, but elicited attraction when presented in the context of a blend (Beyaert et al. 2010; Morawo and Fadamiro 2014b; Webster et al. 2010).

Furthermore, the present results suggest the relevance of background odors and importance of contextual perception of key compounds in attractive odor blends. Three mechanisms of odor recognition have been proposed for foraging insects: (i) species-specific odor recognition in which a few compounds associated with a particular species or group of closely related species are used to establish presence of host (Visser 1986), (ii) ratio-specific odor recognition in which insects detect certain ubiquitous volatiles and use their relative ratios to discriminate hosts (Visser 1986), and (iii) whole blend odor recognition in which all of the blend components are perceived as a whole in what seems to be an all-or-none situation (Clavijo McCormick et al. 2012). In the present study, behavioral responses of *M. croceipes* to full and modified blends in Y-tube olfactometer bioassays suggest a ratio-specific odor recognition pattern. Odor discrimination in *M. croceipes* was affected by the presence/absence of six ubiquitous compounds which were formulated in ratios that mimic the natural blend. Two of the six compounds, 1-octen-3-ol and decanal affected attractiveness of the full blend to the greatest extent. However, the relevance of key compounds was most apparent in the context of other blend components. Other plant-associated compounds not considered key to parasitoid attraction may serve as background odors that enhance olfactory contrast of key volatiles. Similarly, five other compounds, 3-octanone, limonene, 2-ethylhexanol (a widespread contaminant), tridecane, and tetradecane, whose relevance

was not directly investigated in the present study, may play a role in providing context to the blend as a whole. Such roles may include masking the effect of another repellent component or enhancing the contrast of an attractive component. The importance of background odors to odor perception and recognition in several insect species, including parasitoids (Beyaert et al. 2010; Clavijo McCormick et al. 2012, 2014; Mumm and Hilker 2005; Schröder and Hilker 2008; Wajnberg et al. 2008), house fly (Kelling et al. 2002) and *Drosophila* (Faucher et al. 2006) have been discussed.

In summary, natural enemies of herbivores often face the dilemma of choosing between the use of plant odors (more detectable) and host-specific odors (more reliable) (Vet and Dicke 1992). It is possible that this dilemma is optimally resolved by using long range cues from plant volatiles to find a host patch, and short-range cues from plant-associated volatiles emitted by hosts to locate and recognize suitable hosts (Colazza et al. 2014). Certain compounds may contribute in a major way to the attractiveness of host-associated odor blends. However, a complete blend, including less attractive components serving as background or masking odors, often is required for optimum perception and recognition of odors in parasitoids. The more an attractive component contrasts with the background, the greater the effectiveness of the cue (Wajnberg et al. 2008). In the present study, cotton plant, *H. virescens* and *M. croceipes* were used as model species to identify and characterize the role of key compounds emitted by plant-fed hosts in mediating attraction of parasitoids. Future studies should investigate interspecific differences in emission of plant-associated VOCs by herbivores, and the possible effect on host preference in natural enemies.

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## References

- Afshen S, Xia W, Ran L, Chuan-Shu Z, Yong-Gen L (2008) Differential attraction of parasitoids in relation to specificity of kairomones from herbivores and their by-products. *Insect Sci* 15:381–397
- Alborn HT, Lewis WJ, Tumlinson JH (1995) Host-specific recognition kairomone for the parasitoid *Microplitis croceipes* (Cresson). *J Chem Ecol* 21:1697–1708
- Beyaert I, Wäschke N, Scholz A, Varama M, Reinecke A, Hilker M (2010) Relevance of resource-indicating key volatiles and habitat odour for insect orientation. *Anim Behav* 79:1077–1086
- Bruce TJA, Pickett JA (2011) Perception of plant volatile blends by herbivorous insects - finding the right mix. *Phytochemistry* 72:1605–1611
- Cha DH, Adams T, Rogg H, Landolt PJ (2012) Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing *Drosophila*, *Drosophila suzukii*. *J Chem Ecol* 38:1419–1431
- Chesnais Q, Ameline A, Doury G, Le Roux V, Couty A (2015) Aphid parasitoid mothers don't always know best through the whole host selection process. *PLoS One* 10:1–16
- Chuche J, Xuéreb A, Thiéry D (2006) Attraction of *Dibrachys cavus* (Hymenoptera: Pteromalidae) to its host frass volatiles. *J Chem Ecol* 32:2721–2731
- Clavijo McCormick A, Unsicker SB, Gershenzon J (2012) The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends Plant Sci* 17:303–310
- Clavijo McCormick A, Gershenzon J, Unsicker SB (2014) Little peaks with big effects: establishing the role of minor plant volatiles in plant-insect interactions. *Plant Cell Environ* 37:1836–1844
- Colazza S, McElfresh JS, Millar JG (2004) Identification of volatile synomones, induced by *Nezara viridula* feeding and oviposition on bean spp., that attract the egg parasitoid: *Trissolcus basalidis*. *J Chem Ecol* 30:945–964
- Colazza S, Cusumano A, Lo Giudice D, Peri E (2014) Chemo-orientation responses in hymenopteran parasitoids induced by substrate-borne semiochemicals. *BioControl* 59:1–17
- D'Alessandro M, Turlings TCJ (2005) *In situ* modification of herbivore-induced plant odors: a novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. *Chem Senses* 30:739–753
- D'Alessandro M, Brunner V, von Mérey G, Turlings TCJ (2009) Strong attraction of the parasitoid *Cotesia marginiventris* towards minor volatile compounds of maize. *J Chem Ecol* 35:999–1008
- De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. *Nature* 393:570–573
- de Rijk M, Dicke M, Poelman EH (2013) Foraging behaviour by parasitoids in multiherbivore communities. *Anim Behav* 85:1517–1528
- de Rijk M, Krijn M, Jenniskens W, Engel B, Dicke M, Poelman EH (2016) Flexible parasitoid behaviour overcomes constraint resulting from position of host and nonhost herbivores. *Anim Behav* 113:125–135
- Delury NC, Gries R, Gries G, Judd GJR, Khaskin G (1999) Moth scale-derived kairomones used by egg-larval parasitoid *Ascogaster quadridentata* to locate eggs of its host, *Cydia pomonella*. *J Chem Ecol* 25:2419–2431
- Despres L, David J, Gallet C (2007) The evolutionary ecology of insect resistance to plant chemicals. *Trends Ecol Evol* 22:298–307
- Dweck HKM, Svensson GP, Gündüz EA, Anderbrant O (2010) Kairomonal response of the parasitoid, *Bracon hebetor* Say, to the male-produced sex pheromone of its host, the greater waxmoth, *Galleria mellonella* (L.). *J Chem Ecol* 36:171–178
- Ed Sauls C, Nordlund DA, Lewis WJ (1979) Kairomones and their use for management of entomophagous insects VIII. Effect of diet on the kairomonal activity of frass from *Heliothis zea* (Boddie) larvae for *Microplitis croceipes* (Cresson). *J Chem Ecol* 5:363–369
- Elzen GW, Williams HJ, Vinson SB (1984) Role of diet in host selection of *Heliothis virescens* by parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *J Chem Ecol* 10:1535–1541
- Faucher C, Forstreuter M, Hilker M, de Bruyne M (2006) Behavioral responses of *Drosophila* to biogenic levels of carbon dioxide depend on life-stage, sex and olfactory context. *J Exp Biol* 209:2739–2748
- Fürstenberg-Hägg J, Zagrobelny M, Bak S (2013) Plant defense against insect herbivores. *Int J Mol Sci* 14:10242–10297
- Goubault M, Plantegenest M, Poinot D, Cortesero AM (2003) Effect of expected offspring survival probability on host selection in a solitary parasitoid. *Entomol Exp Appl* 109:123–131
- Hofstetter RW, Raffa KF (1997) Effects of host diet on the orientation, development, and subsequent generations of the gypsy moth (Lepidoptera: Lymantriidae) egg parasitoid *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae). *Environ Entomol* 26:1276–1282

- Isberg E, Bray DP, Birgersson G, Hillbur Y, Ignell R (2016) Identification of cattle-derived volatiles that modulate the behavioral response of the biting midge *Culicoides nubeculosus*. *J Chem Ecol* 42:24–32
- Kappers IF, Aharoni A, van Herpen TWJM, Luckerhoff LLP, Dicke M, Bouwmeester HJ (2005) Genetic engineering of terpenoid metabolism attracts bodyguards to *Arabidopsis*. *Science* 309:2070–2072
- Kelling FJ, Ialenti F, Den Otter CJ (2002) Background odour induces adaptation and sensitization of olfactory receptors in the antennae of houseflies. *Med Vet Entomol* 16:161–169
- Lewis WJ, Burton RL (1970) Rearing *Microplitis croceipes* in the laboratory with *Heliothis zea* as host. *J Econ Entomol* 63:656–658
- Logan JG, Birkett MA, Clark SJ et al (2008) Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. *J Chem Ecol* 34:308–322
- Loughrin JH, Manukian A, Heath RR, Turlings TCJ, Tumlinson JH (1994) Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. *Proc Natl Acad Sci U S A* 91:11836–11840
- Magalhães DM, Borges M, Laumann RA, Sujii ER, Mayon P, Caulfield JC, Midega CAO, Khan ZR, Pickett JA, Birkett MA, Blassioli-Moraes MC (2012) Semiochemicals from herbivory induced cotton plants enhance the foraging behavior of the cotton boll weevil, *Anthonomus grandis*. *J Chem Ecol* 38:1528–1538
- McCall PJ, Turlings CJ, Loughrin J, Provioux AT, Tumlinson JH (1994) Herbivore-induced volatiles from cotton (*Gossypium hirsutum* L.) seedlings. *J Chem Ecol* 20:3039–3050
- Molnár BP, Tóth Z, Fejes-Tóth A, Dekker T, Kárpáti Z (2015) Electrophysiologically-active maize volatiles attract gravid female european corn borer. *Ostrinia nubilalis* *J Chem Ecol*:997–1005
- Morawo T, Fadamiro H (2014a) Duration of plant damage by host larvae affects attraction of two parasitoid species (*Microplitis croceipes* and *Cotesia marginiventris*) to cotton: implications for interspecific competition. *J Chem Ecol* 40:1176–1185
- Morawo T, Fadamiro H (2014b) Attraction of two larval parasitoids with varying degree of host specificity to single components and a binary mixture of host-related plant volatiles. *Chemoecology* 24:127–135
- Mumm R, Hilker M (2005) The significance of background odour for an egg parasitoid to detect plants with host eggs. *Chem Senses* 30:337–343
- Nettles WC (1980) Adult *Eucelatoria* sp.: response to volatiles from cotton and okra plants and from larvae of *Heliothis virescens*, *Spodoptera eridania*, and *Estigmene acrea*. *Environ Entomol* 9:759–763
- Ngumbi E, Fadamiro H (2012) Species and sexual differences in behavioural responses of a specialist and generalist parasitoid species to host-related volatiles. *Bull Entomol Res* 102:710–718
- Ngumbi E, Chen L, Fadamiro HY (2009) Comparative GC-EAD responses of a specialist (*Microplitis croceipes*) and a generalist (*Cotesia marginiventris*) parasitoid to cotton volatiles induced by two caterpillar species. *J Chem Ecol* 35:1009–1020
- Nojima S, Jr CL, Morris B, Zhang A, Roelofs W (2003) Identification of host fruit volatiles from hawthorn (*Crataegus* spp.) attractive to hawthorn-origin *Rhagoletis pomonella* flies. *J Chem Ecol* 29:321–336
- Pare PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. *Plant Physiol* 121:325–332
- Peñaflor MFGV, Erb M, Miranda LA, Werneburg AG, Bento JMS (2011) Herbivore-induced plant volatiles can serve as host location cues for a generalist and a specialist egg parasitoid. *J Chem Ecol* 37:1304–1313
- Reis J, Oliveira L, Garcia P (2003) Effects of the larval diet of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) on the performance of the parasitoid *Glyptapanteles militaris* (Hymenoptera: Braconidae). *Environ Entomol* 32:180–186
- Röse USR, Tumlinson JH (2004) Volatiles released from cotton plants in response to *Helicoverpa zea* feeding damage on cotton flower buds. *Planta* 218:824–832
- Röse USR, Lewis WJ, Tumlinson JH (1998) Specificity of systemically released cotton volatiles as attractants for specialist and generalist parasitic wasps. *J Chem Ecol* 24:303–319
- Sasso R, Iodice L, Cristina Digilio M, Carretta A, Ariati L, Guerrieri E (2007) Host-locating response by the aphid parasitoid *Aphidius ervi* to tomato plant volatiles. *J Plant Interact* 2:175–183
- Schnee C, Köllner TG, Held M, Turlings TCJ, Gershenzon DJ (2006) The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *Proc Natl Acad Sci U S A* 103:1129–1134
- Schröder R, Hilker M (2008) The relevance of background odor in resource location by insects: a behavioral approach. *Bioscience* 58:308
- Shorey HH, Hale RL (1965) Mass-rearing of the larvae of nine Noctuid species on a simple artificial medium. *J Econ Entomol* 58:522–524
- Sobhy IS, Erb M, Turlings TCJ (2015) Plant strengtheners enhance parasitoid attraction to herbivore-damaged cotton via qualitative and quantitative changes in induced volatiles. *Pest Manag Sci* 71:686–693
- Steiner S, Erdmann D, Steidle JLM, Ruther J (2007) Host habitat assessment by a parasitoid using fungal volatiles. *Front Zool* 4:3
- Sullivan BT, Pettersson EM, Seltnmann KC, Berisford CW (2000) Attraction of the bark beetle parasitoid *Roptrocercus xylophagorum* (Hymenoptera: Pteromalidae) to host-associated olfactory cues. *Environ Entomol* 29:1138–1151
- Takemoto H, Takabayashi J (2015) Parasitic wasps *Aphidius ervi* are more attracted to a blend of host-induced plant volatiles than to the independent compounds. *J Chem Ecol* 41:801–807
- Turlings TCJ, Wäckers F (2004) Recruitment of predators and parasitoids by herbivore-injured plants. *Adv Insect Chem Ecol* 2:21–75
- Turlings TC, Loughrin JH, McCall PJ, Rose USR, Lewis WJ, Tumlinson JH (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proc Natl Acad Sci U S A* 92:4169–4174
- Uefune M, Kugimiya S, Sano K, Takabayashi J (2012) Herbivore-induced plant volatiles enhance the ability of parasitic wasps to find hosts on a plant. *J Appl Entomol* 136:133–138
- van Dam NM, Qiu B, Hordijk CA, Vet LEM, Jansen JJ (2010) Identification of biologically relevant compounds in aboveground and belowground induced volatile blends. *J Chem Ecol* 36:1006–1016
- van Wijk M, de Bruijn PJA, Sabelis MW (2011) Complex odor from plants under attack: Herbivore's enemies react to the whole, not its parts. *PLoS One* 6:e21742
- Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annu Rev Entomol* 37:141–172
- Visser J (1986) Host odor perception in phytophagous insects. *Annu Rev Entomol* 31:121–144
- Wajnberg É, Haccou P (2008) Statistical tools for analyzing data on behavioral ecology of insect parasitoids. In: Wajnberg É, Bernstein C, van Alphen J (eds) Behavioral ecology of insect parasitoids: from theoretical approaches to field. Blackwell Publishing Ltd, Oxford, UK, pp. 402–429
- Wajnberg É, Bernstein C, van Alphen J (2008) Behavioral ecology of insect parasitoids: from theoretical approaches to field applications. Wiley-Blackwell, Malden, MA
- Webster B, Bruce T, Pickett J, Hardie J (2010) Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. *Anim Behav* 79:451–457
- Wei J-R, Lu X, Jiang L (2013) Monoterpenes from larval frass of two Cerambycids as chemical cues for a parasitoid, *Dastarcus helophoroides*. *J Insect Sci* 13:59
- Zebelo S, Piorowski J, Disi J, Fadamiro H (2014) Secretions from the ventral eversible gland of *Spodoptera exigua* caterpillars activate defense-related genes and induce emission of volatile organic compounds in tomato, *Solanum lycopersicum*. *BMC Plant Biol* 14:140