

Duration of Plant Damage by Host Larvae Affects Attraction of Two Parasitoid Species (*Microplitis croceipes* and *Cotesia marginiventris*) to Cotton: Implications for Interspecific Competition

Tolulope Morawo · Henry Fadamiro

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Abstract Volatile organic compounds (VOCs) released by herbivore-damaged plants can guide parasitoids to their hosts. The quantity and quality of VOC blends emitted by plants may be affected by the duration of plant damage by herbivores, which could have potential ramifications on the recruitment of competing parasitoids. We used two parasitoid species, *Microplitis croceipes* and *Cotesia marginiventris* (Hymenoptera: Braconidae), to address the question of whether duration of plant damage affects parasitoid use of plant VOCs for host location. Both wasp species are larval endoparasitoids of *Heliothis virescens* (Lepidoptera: Noctuidae), an important pest of cotton. Attraction of the two parasitoid species to odors emitted by undamaged (UD), fresh (6 h infestation) damage (FD), and old (24 h infestation) damage (OD) cotton plants infested by *H. virescens* larvae was investigated using a headspace volatile collection system coupled with four-choice olfactometer bioassay. Both sexes of *M. croceipes* showed a preference for FD- and OD-plant odors over UD-plants. On the other hand, more *C. marginiventris* females were attracted to UD- and FD-plants than to OD-plants. GC/MS analyses showed qualitative and quantitative differences in the VOC profiles of UD, FD, and OD-plants, which may explain the observed preferences of the parasitoids. These results suggest a temporal partitioning in the recruitment of *M. croceipes* and *C. marginiventris* to *H. virescens*-damaged cotton, and may have potential implications for interspecific competition between the two parasitoid species.

Keywords Plant volatiles · Host location · Four-choice olfactometer · Gas chromatography–mass spectrometry · Interspecific competition

Introduction

Herbivore-damaged plants emit odors that often guide parasitoids to their hosts. Undamaged plants constitutively release small amounts of certain volatile organic compounds (VOCs), whose emissions often increase due to herbivore damage. Additional VOCs with new identities are released as infestation proceeds (Boland et al. 1998; Pare and Tumlinson 1997; Rose and Tumlinson 2004). The composition of VOC profiles depends on several factors including plant and pest species; pest density; type and duration of damage (Dicke et al. 2009). These qualitative and quantitative differences are believed to generate important host recognition codes for natural enemies (De Bruyne and Baker 2008; De Moraes et al. 1998; McCormick et al. 2012; Smith 1998). Of particular interest are the differences between the odors emitted by the same plant species at different stages of herbivore damage. Female parasitoids typically forage for food, mate and host while males are concerned with feeding and mating. Among other types of cues, an olfactory cue is probably the most useful signal for parasitoids in locating these resources. Plant odors play a unique role in the parasitoid-herbivore host interaction. Both plants and herbivores strongly influence the release of plant odors, but plants ultimately dictate the relevance of the signals transmitted (Turlings et al. 1995). At the third trophic level, parasitoids probably have evolved to use these plant-related signals for host location.

Several parasitoid species have overlapping host ranges (*i.e.*, parasitoids utilizing similar host species) and geographic

T. Morawo · H. Fadamiro (✉)
Department of Entomology & Plant Pathology, Auburn University,
Auburn, AL 36849, USA
e-mail: fadamhy@auburn.edu

T. Morawo
e-mail: tom0002@auburn.edu

distribution, thus necessitating the need for development of competitive strategies. Parasitoids with similar ecological and life strategies are more likely to engage in the strongest interspecific competition for limited host resources. For example, competition is more likely to occur in coexisting parasitoid species. Likewise, the competition between two larval parasitoid species that are able to utilize similar host instars is expected to be stronger than the competition between those that utilize markedly different host instars. Interspecific competitions can be extrinsic (between host-seeking adult female parasitoid species) or intrinsic (between developing immatures of parasitoids after multiparasitism) (Cusumano et al. 2012). The effectiveness of parasitoid host-searching strategy, developmental rates, host stage preference, and co-evolutionary ties with host species are crucial factors that determine interspecific competitive advantages. Among other factors, the order of competitive oviposition, largely dependent on the timing of parasitoid recruitment may determine the outcome of interspecific competitions (De Moraes et al. 1999; De Moraes and Mescher 2005; Harvey et al. 2013; Mohamad et al. 2011; Tillman and Powell 1992). Larval parasitoids may use various olfactory cues such as plant odors and herbivore host-specific odors (e.g., frass and exuviae) to locate their hosts. However, parasitoid recruitment is influenced largely by their relative attraction to various plant-related odors (Turlings et al. 1995).

Specifically, undamaged cotton is known to routinely release a few stored (constitutive) terpenes such as α -pinene, β -pinene, and myrcene. A few hours after herbivore infestation, the amount of constitutive terpenes released is increased. In addition, green leaf volatiles (GLVs) such as hexanal, (*Z*)-3-hexenal, and (*Z*)-3-hexenol also are released. The latter stage of herbivore damage (≥ 24 h) is characterized by the release of several acyclic terpenes such as (*E*)- β -ocimene, (*E*)- β -farnesene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, and 4,8,12-trimethyl-1,3,7,11-tridecatetraene. In addition, indole (aromatic compound), hexenyl acetates, isomeric hexenyl butyrates, and 2-methyl butyrates are also released (De Moraes et al. 1998; Loughrin et al. 1994; Mc Call et al. 1994; Magalhaes et al. 2012; Pare and Tumlinson 1999; Rose et al. 1996, 1998; Rose and Tumlinson 2004). Plant VOCs vary in chemical and physical properties. Compounds in the same chemical groups usually are synthesized *via* a similar pathway. Excessive VOC synthesis may have implications on plant fitness as energy cost is associated with driving chemical pathways (Gershenson 1994). Physical properties such as molecular mass and volatility determine how far a compound travels with time. This may have implications on parasitoid recruitment over long and short distances.

Herbivore feeding activities can inflict substantial amount of damage within a short time, leading to reduced plant growth/development and mortality (Coley 1987). To avoid excessive damage, plants often initiate or reinforce various

defense strategies early on herbivore attack. These defenses may be direct (physical and chemical deterrence) or indirect (recruitment of natural enemies). To gain a competitive advantage, some parasitoid species may have evolved to respond to odors released by plants early on in herbivore damage. Interspecific competition among natural enemies influences plant-herbivore interactions, population dynamics and the community structure (Harvey et al. 2013). Thus, the question of whether the duration of herbivore damage determines parasitoid recruitment to plants concerns the fitness of both plants and parasitoids. For example, De Moraes and Mescher (2005) reported that *C. marginiventris* dominated intrinsic competitions with *M. croceipes* when *C. marginiventris* oviposited first or simultaneously with *M. croceipes*. Thus, it can be hypothesized that females of *C. marginiventris* possess complementary behavior that helps them gain an early head start in such competitions.

In the present study, a four-choice olfactometer was coupled with a headspace volatile collection system such that the actual plant odors that elicited behavioral responses in parasitoids were collected in real time. Based on the results of previous studies on the outcomes of intrinsic (De Moraes et al. 1999; De Moraes and Mescher 2005) and extrinsic (De Moraes et al. 1999; De Moraes and Lewis 1999) interspecific competition among test parasitoids, and previous studies (Chen and Fadamiro 2007; Cortesero et al. 1997; Ngumbi et al. 2009, 2010, 2012) on responses of these parasitoids to host-related odors, it is hypothesized that *M. croceipes* will show relatively greater attraction to old damage (OD) plants (24 h infestation), while *C. marginiventris* will show relatively greater attraction to undamaged (UD) and fresh damage (FD) plants (6 h infestation). Finally, GC/MS analyses of headspace volatiles from UD-, FD-, and OD-plants were conducted to offer possible chemical explanation for the observed responses of the parasitoids.

Methods and Materials

Insects *M. croceipes* and *C. marginiventris* were reared in our laboratory (Auburn University AL, USA) on *Heliopsis virescens* larvae. Parent cultures of *M. croceipes* and *C. marginiventris* were provided by USDA-ARS Insect Biology and Population Management Research Laboratory (Tifton, Georgia, USA) and USDA-ARS Insect Behavior and Biocontrol Research Unit (Gainesville, Florida, USA), respectively. The rearing procedures were similar to those described by Lewis and Burton (1970). Upon emergence, adult wasps were transferred to aerated plastic cages ($\sim 30 \times 30 \times 30$ cm) and supplied with 10 % sugar water. For parasitization, female wasps (2–5 days-old) were supplied with 2nd–3rd instars of *H. virescens* in the ratio 1 female to 20 larvae. Mated, naïve (untrained) parasitoids (aged 2–5 days-old) were

used in the behavioral bioassays. Larvae of *H. virescens* were reared on pinto bean artificial diet (Shorey and Hale 1965). The general rearing conditions for all insects were 25 ± 1 °C, 75 ± 5 % RH and 14:10 h (L:D) photoperiod.

Plants Cotton (*Gossypium hirsutum*, var. max 9) plants were grown in growth chambers (Entomology & Plant Pathology, Auburn University) at 26.6 °C day, 25.6 °C night, and 60 % relative humidity. 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. Plants deliberately infested with *H. virescens* were 4–5-weeks-old.

Infestation For each trial, three treatment plants were tested: undamaged cotton plants (UD), fresh (6 h infestation) damaged cotton plants (FD), and old (24 h infestation) damage plants (OD). To induce VOC emissions from plants, 20 second instar larvae of *H. virescens* were allowed to feed on cotton plants for the previously stated time durations. Each plant, with the feeding larvae (as would be expected in nature) was placed in a 5 L volatile collection jar (Analytical Research Systems, Inc., Gainesville, FL, USA). To reduce contamination, pots with soil were wrapped with aluminum foil.

Coupled Headspace Volatile Collection-Olfactometer Bioassay Headspace VOCs from undamaged (UD) and host-damaged [FD (6 h infestation) and OD (24 h infestation)] cotton plants were collected according to the protocol used by Ngumbi et al. (2009), but with few modifications. The collection commenced after caterpillar infestation of FD- and OD-plants had continued for 6 and 24 h, respectively. Each of the three treatment plants was placed in separate jars. A fourth jar with no plant (control) was included in the set-up. The four-choice olfactometer used for bioassays was previously described by Morawo and Fadamiro (2014). Coupling of headspace volatile collection and olfactometer bioassay was according to Turlings et al. (2004) with slight modifications. Each jar had two air outlets: one outlet was connected to an olfactometer arm, and the other was connected to a trap containing 50 mg of Super-Q (Alltech Associates, Deerfield, IL, USA). A purified and humidified air stream of 400 ml/min was passed through all jars at room temperature for a 2 h collection period. Preliminary experiments and previous studies showed that 2 h was sufficient time to trap VOCs from cotton plants, especially since infestation had proceeded earlier for some hours. Air carrying plant odor (olfactory stimulus) from the jars was passed into each of the four arms of the olfactometer at 400 ml/min through Teflon® tubes, while the vacuum suck was set at 1600 ml/min to avoid a mix-up of odors.

Naive mated wasps of each sex of each parasitoid species (2–5-days-old) were released individually into the olfactometer from the bottom of the central tube. After testing four

insects, the olfactometer was rotated 90° to avoid any error from position effect, and the entire set-up was cleaned (with acetone) after testing 20 insects. For instance, after a female *M. croceipes* made a choice, the insect was removed and replaced with another female *M. croceipes* until four insects had been tested. Wasps were used only once and discarded. About 20–32 parasitoids were tested in separate bioassays for each replicate of plant treatments. Headspace volatile collection was replicated four times, while a total of 86–107 parasitoids were bioassayed for each species and sex. A wasp that did not make a choice after 15 min of exposure was recorded as ‘No choice’ and was not included in the data analyses (<10 % in all experiments). Most responding wasps made choices within a few minutes of exposure. A parasitoid was recorded to have made a choice for the odor offered through an arm when it got into the extension tube and remained there for at least 15 s. Bioassays of different species and sexes were carried out in a randomized complete block design on different days between 0900 and 1700 hr.

GC/MS Analyses The trapped headspace volatiles of cotton were eluted with 200 µl of methylene chloride and the resulting extracts were stored in a freezer (at –20 °C) until use. Identification and quantitation of headspace volatiles was done using an Agilent 7890A 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) according to the protocol used by Ngumbi et al. (2009). 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® Chemical Co. (St. Louis, MO, USA). Synthetic standards of (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene were obtained from Max Planck Institute for Chemical Ecology, Jena, Germany (Contact: Dr. Stefan Bartram). Identity of other compounds whose synthetic standards were unavailable was confirmed by comparing with published GC profiles of cotton headspace volatiles (Loughrin et al. 1994; Mc Call et al. 1994; Ngumbi et al. 2009).

Data Analyses Attraction of parasitoids to each VOC was modeled as a binary response count and treatments were compared using Logistic Regression Analysis. The model adequacy for each set of experiment was confirmed with a Likelihood Ratio test (Wajnberg and Haccou 2008). Slopes

were separated using Proc. Logistic Contrast in SAS. For data presentation, parasitoid attractions to VOCs were represented on charts as percentages of total wasps that responded due to varying sample sizes. Significant differences in the amounts of each volatile component emitted by treatment plants were established by using Kruskal-Wallis one-way analysis of variance, followed by Sidak’s multiple comparison test. The significance level was adjusted by the Sidak method to: $\alpha' = 0.0169 [\alpha' = 1 - (1 - \alpha)^{1/k}; \alpha' = 1 - (1 - 0.05)^{1/3} = 0.0169]$ (Rose and Tumlinson 2004). All analyses were performed using SAS 9.2 with 0.05 level of significance.

Results

Effect of Duration of Caterpillar Damage on Attraction of Parasitoids Female *M. croceipes* were more attracted ($\chi^2 = 13.71; P < 0.001; N = 85$) to odors from fresh damage (FD) and old damage (OD) plants than to odors from undamaged (UD) plants or the control (Fig. 1). Numerically, more females chose FD-plants than OD-plants. Males were more attracted ($\chi^2 = 22.77; P < 0.001; N = 106$) to herbivore-damaged and undamaged plants than to the control, but did not discriminate among plant treatments (Fig. 2). For *C. marginiventris*, females showed greater attraction to UD- and FD-plants than to the control, ($\chi^2 = 8.71; P < 0.03; N = 95$) (Fig. 1). Males showed a trend similar to conspecific females (Fig. 2).

Species Differences in Response Comparing species, female *M. croceipes* were more attracted ($\chi^2 = 4.18; P < 0.041; N = 167$) than female *C. marginiventris* to OD-plant odors (Fig. 1). In contrast, female *C. marginiventris* showed greater attraction ($\chi^2 = 3.88; P < 0.048; N = 167$) to UD-plant odors, compared to

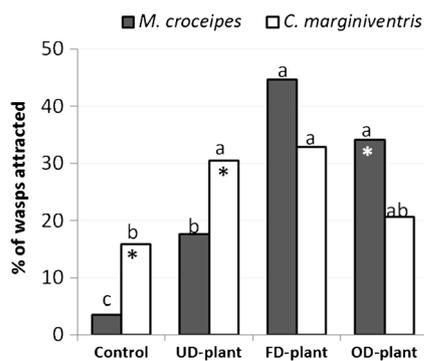


Fig. 1 Species differences in the attraction of females of *Microplitis croceipes* and *Cotesia marginiventris* to odors released by undamaged (UD-plants) cotton plants vs. plants damaged by *Heliothis virescens* caterpillars for 6 h (FD-plants) or 24 h (OD-plants). Values (%) having no letter in common are significantly different ($P < 0.05$; Proc. Logistic Regression). Attraction to VOCs was modeled as binary response counts and represented on the chart as percentage of total responding wasps. Asterisks (*) indicate significant differences between species for each odor source. ($P < 0.05$; Proc. Logistic Regression Contrast)

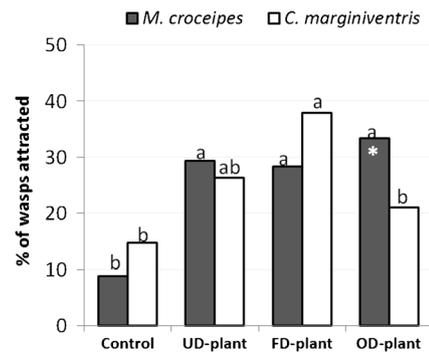


Fig. 2 Species differences in the attraction of males of *Microplitis croceipes* and *Cotesia marginiventris* to odors released by undamaged (UD-plants) cotton plants versus plants damaged by *Heliothis virescens* caterpillars for 6 h (FD-plants) or 24 h (OD-plants). Values (%) having no letter in common are significantly different ($P < 0.05$; Proc. Logistic Regression). Attraction to VOCs was modeled as binary response counts and represented on the chart as percentage of total responding wasps. Asterisks (*) indicate significant differences between species for each odor source. ($P < 0.05$; Proc. Logistic Regression Contrast)

female *M. croceipes* (Fig. 1). Similarly, male *M. croceipes* were more attracted ($\chi^2 = 4.16; P < 0.041; N = 201$) than male *C. marginiventris* to odors from OD-plants (Fig. 2). Females of both species were highly attracted to FD-plant odors.

GC/MS Analyses Analyses of headspace volatiles emitted from UD-, FD-, and OD-plants simultaneously trapped during olfactometer bioassays revealed qualitative and quantitative differences in composition. Generally, more compounds were detected, and at relatively greater amounts in the headspace of OD-plants than in FD-plants or UD-plants (Fig. 3). The headspace of UD-plants contained the least number of VOCs, usually at the lowest amounts. In total, twenty-four VOC components were identified in this study. These included several terpenes such as α -pinene, β -pinene, myrcene, (*E*)- β -caryophyllene, (*E*)- β -ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (*E,E*)- α -farnesene, α -humulene, β -elemene; GLVs [e.g., (*Z*)-3-hexenal, (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate]; and aromatic compounds such as indole (Table 1). Comparing the treatments, α -pinene and myrcene were prominent components detected in the headspace of UD-plants. GLVs were barely detectable in the headspace of UD-plants. The amount of α -pinene and myrcene emitted increased in herbivore-damaged (FD and OD) plants. In addition, (*Z*)-3-hexenal, (*Z*)-3-hexenol, β -pinene, limonene, (*E*)- β -caryophyllene, and α -humulene were detected in the headspace of FD-plants. Certain components including (*Z*)-3-hexenyl acetate, (*E*)- β -ocimene, DMNT, (*Z*)-3-hexenyl butyrate, (*Z*)-3-hexenyl-2-methyl butyrate, indole, (*E,E*)- α -farnesene, and β -elemene, were found only in significant amounts in the headspace of OD-plants (Table 1).

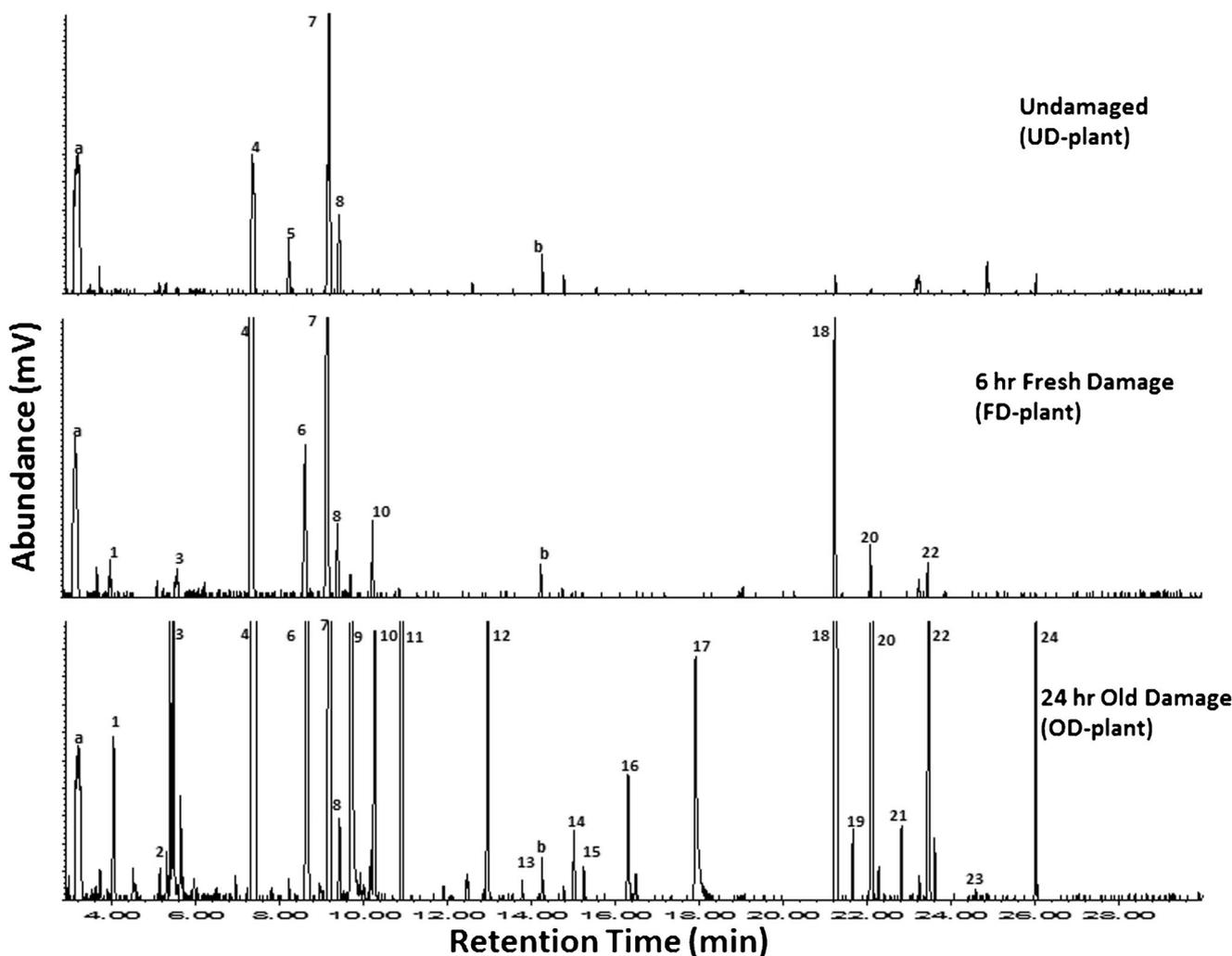


Fig. 3 Typical chromatograms of headspace volatiles released by undamaged, fresh damage (6 h infestation) and old damage (24 h infestation) cotton plants. 20 second instar larvae of *Heliothis virescens* were used for infestation. Volatiles were trapped for 2 h. Peak identities: 1, (*Z*)-3-hexenal; 2, (*E*)-2-hexenal; 3, (*Z*)-3-hexenol; 4, α -pinene; 5, Benzaldehyde; 6, β -pinene; 7, Myrcene; 8, α -phellandrene; 9, (*Z*)-3-hexenyl acetate; 10, Limonene; 11, (*E*)- β -ocimene; 12, (*E*)-4,8-dimethyl-1,3,7-

nonatriene (DMNT); 13, (*E*)-2-hexenyl butyrate; 14, (*Z*)-3-hexenyl butyrate; 15, (*Z*)-2-hexenyl butyrate; 16, (*Z*)-3-hexenyl-2-methyl butyrate; 17, Indole; 18, (*E*)- β -caryophyllene; 19, (*E,E*)- α -farnesene; 20, α -humulene; 21, β -elemene; 22, γ -bisabolene; 23, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); 24, β -bisabolol. Peaks labeled (a) and (b) are unidentified. Relative % composition is based on identified compounds

Discussion

Results of the present study show that inexperienced *M. croceipes* and *C. marginiventris* differ in their attraction to odors from cotton plants infested for different duration by *H. virescens*. According to Turlings et al. (2005), clarity, specificity, and timing are yardsticks for measuring the suitability of signals that would serve as effective host location cues for natural enemies. Plant odors can serve as effective host location cues for parasitic wasps, especially considering their role in both long and short-range attraction (De Moraes and Lewis 1999). In the present study, female *M. croceipes* were significantly more attracted to odors from fresh damage (FD) and old damage (OD) plants than to odors from undamaged (UD) plants, partly supporting our initial hypothesis.

Conspecific males did not significantly discriminate among odors from the treatment plants. Since the female sex parasitizes the host, it is expected that they possess a greater ability to discriminate between odors from undamaged and herbivore-damaged plants, compared to conspecific males. On the other hand, female *C. marginiventris* did not significantly discriminate among the various plant odors, suggesting their use of more general odor cues for host location. This result is consistent with the findings of Cortesero et al. (1997) in which *C. marginiventris* showed no preference between undamaged and *Helicoverpa zea*-damaged cotton squares. However, Hoballah and Turlings (2005) reported that inexperienced female *C. marginiventris* showed preference for fresh damage over old damage maize plants. Although more females of this species were attracted to FD-plants (33 % of total) than to OD-

Table 1 Composition of headspace volatile organic compounds (VOCs) emitted by undamaged cotton plants vs. fresh damage (6 h infestation) and old damage (24 h infestation) cotton plants infested by *Heliothis virescens* caterpillars

ID	Compound ¹	Undamaged ²		Fresh Damage ²		Old Damage ²	
		Amount ³ ng/g of fwt.	Rel. %	Amount ³ ng/g of fwt.	Rel. %	Amount ³ ng/g of fwt.	Rel. %
1	(Z)-3-hexenal ⁵	0 ^b	0	7.5±6.1 ^{ab}	0.7	29.2±6.4 ^a	0.3
2	(E)-2-hexenal ⁵	0 ^c	0	2.4±0.8 ^b	0.2	18.8±5.0 ^a	0.2
3	(Z)-3-hexenol ⁵	0 ^b	0	10.3±7.3 ^a	1.0	60.8±33.1 ^a	0.7
4	α-pinene ⁴	7.4±4.8 ^b	10.8	630.0±208.1 ^a	59.8	3264.3±923.2 ^a	36.4
5	Benzaldehyde ⁴	6.8±1.7 ^a	10.0	3.4±1.2 ^a	0.3	2.8±0.9 ^a	0.03
6	β-pinene ⁵	0 ^c	0	65.1±26.2 ^b	6.2	454.9±134.4 ^a	5.1
7	Myrcene ⁴	47.9±38.3 ^b	70.0	128.0±65.2 ^{ab}	12.2	714.7±258.8 ^a	8.0
8	α-phellandrene ⁴	3.6±2.1 ^a	5.3	3.5±2.1 ^a	0.3	4.3±2.5 ^a	0.1
9	(Z)-3-hexenyl acetate ⁵	0 ^b	0	6.7±6.7 ^b	0.6	1585.9±757.9 ^a	17.7
10	Limonene ⁵	0 ^b	0	52.2±19.8 ^a	5.0	206.2±62.8 ^a	2.3
11	(E)-β-ocimene ⁵	0 ^b	0	5.7±3.9 ^b	0.5	602.1±209.9 ^a	6.7
12	(E)-4,8-dimethyl-1,3,7-nonatriene ⁶	0 ^b	0	0 ^b	0	169.3±46.0 ^a	1.9
13	(E)-2-hexenyl butyrate ⁶	0 ^b	0	0 ^b	0	2.8±1.5 ^a	0.03
14	(Z)-3-hexenyl butyrate ⁶	0 ^b	0	0 ^b	0	70.0±55.7 ^a	0.8
15	(Z)-2-hexenyl butyrate ⁶	0 ^b	0	0 ^b	0	15.0±8.7 ^a	0.2
16	(Z)-3-hexenyl-2-methyl butyrate* ⁶	0 ^b	0	0 ^b	0	100.4±75.4 ^a	1.1
17	Indole ⁶	0 ^b	0	0 ^b	0	125.2±97.2 ^a	1.4
18	β-caryophyllene ⁵	0 ^c	0	103.5±55.1 ^b	9.8	1043.2±244.0 ^a	11.6
19	(E,E)-α-farnesene ⁶	0 ^b	0	0 ^b	0	7.6±3.3 ^a	0.1
20	α-humulene ⁵	0 ^c	0	18.3±10.8 ^b	1.7	270.7±59.1 ^a	3.0
21	β-elemene ⁵	0 ^b	0	0.3±0.3 ^b	0.03	15.1±6.0 ^a	0.2
22	γ-bisabolene* ⁵	0 ^c	0	10.0±8.0 ^b	1.0	195.4±61.6 ^a	2.2
23	(E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene ⁶	0 ^a	0	0 ^a	0	2.2±1.6 ^a	0.02
24	β-bisabolol ⁴	2.8±2.8 ^a	4.1	6.3±6.3 ^a	0.6	16.0±5.3 ^a	0.2

¹ In order of elution during gas chromatography

² Volatiles were collected for 2 h; *Heliothis virescens*-damaged plants were infested with 20 second instar larvae

³ Amounts (ng/g of plant fresh weight) are mean±SEM of four replicates

⁴ Compounds were detected in both undamaged and *Heliothis virescens*-damaged plants

⁵ Compounds were only detected in fresh and old damage plants

⁶ Compounds were only detected in old damage plants

Means across the same row followed by different letters are significantly different ($P < 0.05$; Kruskal-Wallis test followed by Sidak's multiple comparison test)

The identity of all compounds was confirmed by NIST library and comparison with synthetic standards (purity 95–99%), with two exceptions indicated by an *. Identities of compounds 16 and 22 were confirmed by NIST library and comparison with published GC profiles of cotton headspace volatiles

plants (21 % of total) in the present study, the difference was not statistically significant. Differences in materials (cotton vs. maize; *H. virescens* vs. *Spodoptera littoralis*) and methodology (plant induction via herbivory vs. use of regurgitant; relative durations of induction) may have contributed to slight discrepancy between results of the previous and present studies. We note that in both studies, female *C. marginiventris* showed poor innate attraction to old damage plants. On the other hand, both sexes of *M. croceipes* showed greater attraction to odors from OD-plants, compared to *C. marginiventris*.

Damage duration-dependent attraction of parasitoids to various plant odors has potential implications for parasitoid fitness and plant protection. At the third trophic level, parasitoids are subjected to interspecific competitions in overlapping niches. *Microplitis croceipes* and *C. marginiventris* share a similar life history, and are both larval endoparasitoids of *H. virescens*, a generalist herbivore on cotton, tobacco, flax, alfalfa, and many other field crops (Graham and Robertson 1970). Interspecific competition likely may occur between the two parasitoid species where host resources are limited. Compared to *M. croceipes*, female *C. marginiventris* showed

greater attraction to UD-plant odors. The amount of these VOCs increases significantly during the earliest stages of herbivore damage (De Moraes et al. 1998; Loughrin et al. 1994; Mc Call et al. 1994; Magalhaes et al. 2012; Pare and Tumlinson 1999; Rose et al. 1996, 1998; Rose and Tumlinson 2004). Therefore, *C. marginiventris* may randomly forage around intact plants, possibly making the first contact with caterpillar hosts in the earliest stages of plant infestation. Female *M. croceipes* may be recruited later if cues from FD- and OD-plants are mostly used.

In extrinsic competitions between female parasitoids, competitive head start due to earlier recruitment directly influences outcomes of intrinsic competitions between immature stages following multiparasitism (Harvey et al. 2013). Generally, it has been reported that egg number, developmental rate, oviposition order, and preferred host instar are important factors determining the outcome of intrinsic competitions (De Moraes et al. 1999; Harvey et al. 2013; Tillman and Powell 1992; van Strien-van Liempt 1983). Here, we further make the case that the timing of parasitoid recruitment, largely dependent on the relative attractiveness of plant odors, is a key factor that may determine the outcome of interspecific competitions. Females of both parasitoid species were similarly attracted to FD-plant odors, further indicating the possibility of a direct interference while searching for hosts (extrinsic interspecific competition) during the early stages (about 6 h) of herbivore damage. Such competitions may have implications on species population dynamics and coexistence of competing species. According to the competitive exclusion principle, two species occupying the same niche cannot coexist; a classic example being the interspecific displacement of less competitive species by more dominant ones when three *Opius* parasitoid species were introduced to control the oriental fruit fly in Hawaii (Clausen et al. 1965). Other hypotheses such as the dispersal-competition trade-off suggest that coexistence is possible among certain competing species through spatial partitioning of resources, especially when the inferior competitor disperses better than the superior one (Amarasekare 2000). Competing parasitoid species also may coexist through season-long temporal partitioning of resources (Mainali and Lim 2012). Here, we hypothesize that parasitoid coexistence may sometimes depend on short-term temporal partitioning of host resources.

When limited host resources are available to parasitoid species with similar life histories, differences in life strategy such as degree of host specialization also may influence extrinsic competitive advantages (De Moraes et al. 1999). From an evolutionary perspective, generalist parasitoids may have developed competitive abilities while broadening their host range over time (De Moraes and Mescher 2005). For instance, Iwao and Ohsaki (1996) reported that developing *Compsilura concinnata* (generalist) physically destroyed the larvae of *Epicampocera succincta* (specialist) during intrinsic competition.

Microplitis croceipes and *C. marginiventris* belong to the same family, and are both koinobiont solitary larval endoparasitoid of *H. virescens*. Relatively, *M. croceipes* can be considered a specialist parasitoid with a restricted range, utilizing *Heliothis/Helicoverpa* host species (Lewis and Snow 1971; Stadelbacher et al. 1984; Tillman 1996; Tillman and Laster 1995). On the other hand, *C. marginiventris* is considered a generalist with a broader host range, utilizing several species in the Lepidopteran family Noctuidae. De Moraes and Lewis (1999) reported that *Cardiochilis nigriceps*, a more specialized parasitoid on *H. virescens* was more efficient in locating individual hosts. However, *C. marginiventris*, a largely generalist parasitoid dominated intrinsic competitions with the more specialized *M. croceipes* (De Moraes and Mescher 2005), which dominated over the most specialized *C. nigriceps* (De Moraes et al. 1999). Differences in life history and strategy aside, relative size of females also may influence outcomes of extrinsic competitions as a result of direct interference (Mohamad et al. 2011). However, this has not been specifically reported in *M. croceipes* (relatively larger in size) and *C. marginiventris* competitive interactions.

In the present study, GC/MS analyses of headspace volatiles of UD-, FD-, and OD-plants were conducted to investigate the basis for the behavioral responses of parasitoids to the plant odors. UD- and FD-plants released certain terpenes and GLVs, which are relatively ubiquitous compounds released also by several plants including cotton and corn in response to damage by various herbivore species (Cortesero et al. 1997; D'Alessandro and Turlings 2005; Hoballah and Turlings 2005; Ngumbi et al. 2012). Due to the general nature of these odors, they may be useful for several host-seeking parasitoids, especially those with a relatively broad range of hosts. This is in agreement with the report of Cortesero et al. (1997), which suggested that the generalist parasitoid, *C. marginiventris* uses odor cues from recently damaged plants. Solitary parasitoid species such as *C. marginiventris* that dominate intrinsic interspecific competitions due to an early head start development in the host would also be expected to show complementary behavioral traits leading to early recruitment of females to host patches.

In contrast, odors from OD-plants are expected to convey more host specific signals. This is due to the specificity of the VOCs released by such old damage plants and possibly due to the accumulation of host-specific materials (e.g., frass and exuviae) over time (Cortesero et al. 1997; De Moraes and Mescher 2005; McCormick et al. 2012). In the present study, (*Z*)-3-hexenyl butyrate, (*Z*)-3-hexenyl-2-methyl butyrate, (*E*)- β -ocimene, (*E*)-4, 8-dimethyl-1, 3, 7-nonatriene (DMNT), (*E,E*)- α -farnesene, β -elemene, and indole were among the headspace components almost exclusive to OD-plants. Previous studies that used ^{13}C labeling and timed collection of headspace volatiles have shown that most of these compounds are synthesized *de novo* as a delayed

response to herbivore damage, offering a possible explanation for the relatively high attraction of specialist parasitoids to such VOCs (Boland et al. 1999; Dudareva et al. 2006; Loughrin et al. 1994; Magalhaes et al. 2012; Pare and Tumlinson 1997; Rose and Tumlinson 2004). In addition, the heavier and less volatile compounds released by OD-plants such as DMNT (homoterpene) do not travel far and likely mediate short-range attraction of parasitoids. On the other hand, most VOCs released by UD- and FD-plants such as α -pinene (monoterpene) and (*Z*)-3-hexenol (GLV) are lighter and more volatile, likely mediating long-range parasitoid attraction. This further suggests that *C. marginiventris* may be recruited earlier on by herbivore damage when using odor cues from UD- and FD-plants, while *M. croceipes* may be recruited later when using odor cues from HIPVs released by OD-plants. The role of HIPVs in parasitoid interspecific competitions also has been reported (see Poelman et al. 2013). In the study conducted by De Moraes and Mescher (2005), *M. croceipes* was inferior to *C. marginiventris* in intrinsic competitions when *C. marginiventris* oviposited first. Ideally, it would be expected that *M. croceipes* females compensate for this disadvantage by using constitutive and fresh-damage plant odors, thus achieving early recruitment. However, this may not always be the case in nature since the relatively specialized *M. croceipes* likely uses more host-specific odor cues (including HIPVs released by OD-plants later on herbivory) to locate a narrower range of hosts. This increases the chances of finding suitable hosts while using less energy, thus achieving an efficient means of host location. Such host foraging strategy, however, may come at the expense of losing out in intrinsic competitions with a species that benefits from an early head start. Similar trade-offs in host searching efficiency (De Moraes et al. 1999), adult size (Harvey et al. 2013) and avoidance of hyperparasitism (Poelman et al. 2013) in parasitoid interspecific competitions have been reported.

Although it has been argued that the entire blend, not individual components of plant volatiles generates better host location cues (van Wijk et al. 2011), we previously reported the attraction of naïve female *M. croceipes* to HIPVs including (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenyl butyrate, and the attraction of *C. marginiventris* to constitutive VOCs such as α -pinene and GLVs such as (*Z*)-3-hexenol (Morawo and Fadamiro 2014; Ngumbi et al. 2012; Ngumbi and Fadamiro 2012). These HIPVs were among other compounds found in the headspace of OD-plants in the present study. The synthesis of certain terpenes such as (*E*)- β -ocimene, DMNT, (*E*, *E*)- α -farnesene and β -elemene is mediated by the terpenoid pathway and requires extensive chemical reduction reactions (Gershenzon 1994). Thus, a substantial amount of energy is expended by plants to drive their production. This may explain why many plants release several compounds in this group as a delayed response, but not early on herbivore attack.

For plants infested by several species of herbivores, the release of generalized and metabolically less costly VOCs that attract multiple species of parasitoids early on in herbivore damage would be ideal from a plant fitness perspective.

Parasitoid experiences can affect their behavioral responses. Although the effect of experience was not the focus of this study, associative learning has been reported to play a role in the response of parasitoids to host-related odors (Dukas and Duan 2000; Lewis and Takasu 1990; Ngumbi et al. 2012). For instance, Ngumbi et al. (2012) reported that female *M. croceipes* and *C. marginiventris* that were trained to associate sugar water with test odors (single VOCs) showed increased attraction to some of the VOCs, with *C. marginiventris* benefitting more from the experience. Dukas and Duan (2000) also reported that under a favorable ecological system, associative learning had positive fitness effects on host location in *Biosteres arisanus*, a parasitoid of Mediterranean and oriental fruit flies. Consequently, parasitoids may enhance or modify their innate ability to cue in on different host-related odors through associative learning in the field, thus, providing them with competitive advantages. The effect of learning on the temporal partitioning of host resources as related to interspecific competition merits further study.

In summary, results of the present study showed both differences and similarities in the attraction of *M. croceipes* and *C. marginiventris* to odors from undamaged cotton and plants damaged for different durations by *H. virescens*. The results suggest temporal partitioning in the recruitment of parasitoids to herbivore-damaged plants. The implications of odor preferences on the timing of recruitment and possible interspecific competition between the parasitoid models used should be considered in integrated pest management strategies that seek to optimize the use of various parasitoids as biocontrol agents.

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