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Volatiles of *Solena amplexicaulis* (Lam.) Gandhi Leaves Influence Attraction of Two Generalist Insect Herbivores

Nupur Sarkar[1] · **Amarnath Karmakar**[1] · **Anandamay Barik**[1]

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Abstract *Epilachna vigintioctopunctata* Fabr. (Coleoptera: Coccinellidae) and *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) are important pests of *Solena amplexicaulis* (Lam.) Gandhi (Cucurbitaceae), commonly known as creeping cucumber. The profiles of volatile organic compounds from undamaged plants, plants after 48 hr continuous feeding of adult females of either *E. vigintioctopunctata* or *A. foveicollis*, by adults of both species, and after mechanical damaging were identified and quantified by GC-MS and GC-FID analyses. Thirty two compounds were detected in volatiles of all treatments. In all plants, methyl jasmonate was the major compound. In Y-shaped glass tube olfactometer bioassays under laboratory conditions, both insect species showed a significant preference for complete volatile blends from insect damaged plants, compared to those of undamaged plants. Neither *E. vigintioctopunctata* nor *A. foveicollis* showed any preference for volatiles released by heterospecifically damaged plants *vs.* conspecifically damaged plants or plants attacked by both species. *Epilachna vigintioctopunctata* and *A. foveicollis* showed attraction to three different synthetic compounds, linalool oxide, nonanal, and *E*-2-nonenal in proportions present in volatiles of insect damaged plants. Both species were attracted by a synthetic blend of 1.64 μg linalool oxide + 3.86 μg nonanal + 2.23 μg *E*-2-nonenal, dissolved in 20 μl methylene chloride. This combination might be used as trapping tools in pest management strategies.

Keywords *Solena amplexicaulis* · Volatiles · Coleoptera · Coccinellidae · *Epilachna vigintioctopunctata* · Chrysomelidae · *Aulacophora foveicollis* · olfactometer bioassay

Introduction

Epilachna vigintioctopunctata Fabr. (Coleoptera: Coccinellidae) is a polyphagous insect that feeds on different plant families such as Solanaceae (potato, tomato, brinjal), Cucurbitaceae (melon, cucumber, gourds, pumpkin) and Fabaceae (soya, haricot beans); while *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) consumes leaves of squash, pumpkin, cucumber, bottle gourd, luffa, spine gourd, and water melons (Cucurbitaceae) (Choudhuri et al. 1983; Khan et al. 2011; Mukherjee et al. 2015a; Rahaman and Prodhan 2007; Singh and Gill 1979). Both *E. vigintioctopunctata* and *A. foveicollis* also feed on *Solena amplexicaulis* (Lam.) Gandhi (syn: *Melothria heterophylla*) (Cucurbitaceae) in India, Bangladesh, and Vietnam (Choudhuri et al. 1983; Khan et al. 2011; Rahaman and Prodhan 2007; Singh and Gill 1979). *Solena amplexicaulis* is cultivated for production of fruits, and young leaves also are consumed as vegetables in developing countries (Nagarani et al. 2014). Different studies have shown that the whole plant has antimicrobial, anti-inflammatory, and hepatoprotective properties, and is a potential source of natural antioxidants (Karthika and Paulsamy 2014; Karthika et al. 2012; Nantachit and Tuchinda 2009; Venkateshwarlu et al. 2011). The larvae of *E. vigintioctopunctata* pass through four instars (12–16 d) on leaves to complete larval development. After

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✉ Anandamay Barik
anandamaybarik@yahoo.co.in

[1] Ecology Research Laboratory, Department of Zoology, The University of Burdwan, Burdwan 713 104, West Bengal, India

pupation (4–7 d), adults feed on leaves for 4–5 wk; whereas larvae of *A. foveicollis* feed on roots of the plant through four instars (17–21 d), and after pupation (12–13 d) in soil, adults feed on leaves for 8–9 wk. Damage on leaves by both species causes drying of branches and shoots of the plant, but typically does not kill the entire plant. Currently, chemical measures (pyrethroids, organophosphates) are applied to control both species (Sinha and Chakrabarti 1983). To reduce the yield losses, caused by adults and environmental risks associated with insecticide application, it is necessary to develop ecofriendly strategies, which can be included in integrated pest management programs (IPM). Hence, identification of volatiles from *S. amplexicaulis* plants causing attraction of both insect species may be helpful in developing IPM strategies such as baited traps.

Insect herbivores detect volatile organic compounds (VOCs) *via* olfactory sensilla present on the antenna to locate their host plants (Bruce et al. 2005; De Moraes et al. 1998; Piesik et al. 2011; Tasin et al. 2005; Visser 1986). Some of the VOCs in the blends are ubiquitous in different plants, but the specific qualitative and quantitative compositions of VOCs differ among plants, by which an insect can discriminate between host plants and non-host plants (Bruce and Pickett 2011; Bruce et al. 2005; Schoonhoven et al. 2005; Tasin et al. 2005; Wang et al. 2014; Wenda-Piesik et al. 2010). Production of volatiles by a host plant may differ both qualitatively and quantitatively due to feeding damage caused by different insect herbivores, which might induce different plant responses (Dicke and Hilker 2003; Gouinguené et al. 2003; Röse and Tumlinson 2005; Turlings et al. 1998; Wei et al. 2006). In the field, *E. vigintioctopunctata* and *A. foveicollis* may attack *S. amplexicaulis* leaves individually or simultaneously. Hence, it is interesting to observe the nature of volatile blends released by *S. amplexicaulis* plants attacked by either *E. vigintioctopunctata* or *A. foveicollis* concurrently. *Aulacophora foveicollis* females display short-range attraction to substances of low volatility, such as long-chain alkanes and fatty acids to leaves and flowers of *Momordica cochinchinensis* Spreng (Mukherjee and Barik 2014; Mukherjee et al. 2013, 2015b) and *S. amplexicaulis* (Karmakar and Barik 2016; Karmakar et al. 2016), however, low boiling volatiles from *M. cochinchinensis* leaves causing attraction of *A. foveicollis* also have been identified (Mukherjee et al. 2015a).

In the present study, volatiles from undamaged (UD) and mechanically damaged (MD) *S. amplexicaulis* plants, and plants after continuous feeding by either *A. foveicollis* or *E. vigintioctopunctata* and adult females of both species were collected, identified, and quantified by gas chromatography coupled with mass spectrometry (GC-MS) and by gas chromatography with flame ionization detection (GC-FID). Behavioral responses of *E. vigintioctopunctata* and *A. foveicollis* to volatile blends from UD and insect-damaged (ID) *S. amplexicaulis* plants were examined using a Y-shaped glass tube olfactometer bioassay. As insects frequently employ 3–10 compounds as chemical cues to locate their host plants (Bruce and Pickett 2011; Bruce et al. 2005), we studied the role of individual synthetic volatiles, followed by a combination of synthetic compounds to which insects had been previously attracted, in comparison to insect damaged *S. amplexicaulis* plants as an olfactory cue to *E. vigintioctopunctata* and *A. foveicollis*.

Methods and Materials

Plant Material Seeds of *S. amplexicaulis* were germinated on moist filter papers. Each seed with cotyledon was planted in a pot containing ca. 1500 cm^3 of sterilized soil [organic matter 5.3 ± 0.2 % (± Standard Error), pH 7.7, collected from the Crop Research Farm, University of Burdwan (23°16′ N, 87°54′ E), West Bengal, India] and held under natural conditions (photoperiod 13 L: 11 D at 30–35 ° C) for 2 mo (June–July, 2014). A whole plant with the pot was covered with a clear plastic dome [120 cm (height) × 80 cm (diam)] to prevent insect attacks and unintentional infection. Plants were provided with water every other day. Five to six-wk-old plants (about 100–120 cm height) were used for volatile collections.

Volatile Collection Plants were placed into environmental chambers (27 ± 1 °C, 65 ± 5 % RH, and 13 L: 11D) for collection of volatiles from undamaged (UD), mechanically damaged (MD) [10 leaves of a plant were wounded twice with hole punch (each hole 0.5 cm diam), and volatiles were collected right after wounding], and insect damaged (ID) plants. There were 3 damage treatments caused by insect feeding: (a) *E. vigintioctopunctata* females, (b) *A. foveicollis* females, and (c) females of both species feeding simultaneously. For individual feeding damage treatments, 10 adult females of either *E. vigintioctopunctata* or *A. foveicollis* were allowed to feed separately on *S. amplexicaulis* plants containing 10 leaves, and volatiles were collected 48 h after the insects had started to feed; whereas 5 adult females of *E. vigintioctopunctata* and 5 of *A. foveicollis* were allowed to feed continuously on an intact *S. amplexicaulis* plant containing 10 leaves, and volatiles were collected 48 h after both species had started to feed. To encourage immediate feeding after being placed to the plants, insects were provisioned with water and starved for 12 h. Plants with 10 undamaged leaves or plants after insect feeding were placed individually in 4 L closed glass domes with Teflon bases leaving only a small opening for the stem of the plant. Cotton balls were loosely plugged around the stem of the plant to prevent any abrasion by the Teflon bases. Volatiles from all treatments ($N = 5$ replicates for each treatment) were collected over 10 h during the light phase of a photoperiod between 8.00 and 18.00. Charcoal-filtered air

was pushed (6 L min^{-1}) into the top of the closed chamber and pulled (1 L min^{-1}) through 4 collector traps (150 mm long × 5 mm o.d.), each containing 100 mg of HayeSep Q (80–100 mesh, Sigma Aldrich, Germany) as an adsorbent, inserted around the base of the closed glass chamber.

Volatiles were eluted from the adsorbent by extraction with 800 μl methylene chloride and concentrated to 200 μl under a gentle stream of nitrogen. One hundred μl of each extract were used for olfactometer bioassays, and the remaining 100 μl were used for chemical analyses. For olfactometer bioassays, 20 μl of an aliquot (equivalent to the amount of volatiles released by a plant in ca. 1 h) were applied to Whatman No. 41 filter paper (1 cm^2). For quantification through GC, nonyl acetate was added as an internal standard (IS), at 1 μg μl^{-1}. All solvents used were purchased from Sigma Aldrich.

Analysis of Volatiles Volatile samples from each treatment were analyzed by using an Agilent 6890 GC coupled to a 5973 Mass Selective Detector equipped with a SE-30 capillary column (Agilent; Palo Alto, CA, USA; length: 30 m × 0.32 mm × 0.25-μm film thickness). Helium was the carrier gas. One μl sample was injected with a split ratio of 1:5. The oven temperature program was initially 50 °C held for 3 min, then raised at 3.75 °C/min to 240 °C, and finally held for 5 min. The MS parameters were 250 °C at the interface, the ionization energy was 70 eV, scan speed approximately 1 s. The identity of the volatile compounds was confirmed by comparison of their mass spectra with those in a data base (NIST, 2008) and by authentic standards.

For quantification of compounds, 5 volatile samples from each treatment were analyzed with a Techcomp GC (Em Macau, Rua De Pequim, Nos. 202A-246, Centro Financeiro F7, Hong Kong) model 7900 coupled to a flame ionization detector with a SE-30 column (Agilent; Palo Alto, CA, USA; length: 30 m × 0.32 mm × 0.25-μm film thickness) (same temperature conditions as for GC-MS analysis). The carrier gas was nitrogen with a flow rate of 17 ml/min. The injector port temperature was 280 °C. One μl sample was injected with a split ratio of 1:5. Components were characterized and quantified against the retention times of authentic standards, which were purchased from Sigma Aldrich.

Chemicals HayeSep Q (80–100 mesh), 2-hexanol (99 %), 1-hexanol (≥99.5 %), *E*-2-hexen-1-ol (96 %), 2-heptanone (99 %), α-pinene (≥99 %), benzaldehyde (≥99 %), 1-heptanol (≥99.5 %), sabinene (75 %), 1-octen-3-ol (≥98 %), 3-octanone (≥98 %), 3-octanol (99 %), 2-octanol (99 %), benzyl alcohol (99.8 %), ocimene (*cis*/*trans*-mixture) (≥90 %), linalool oxide (furanoid structure) (≥97 %), 1-octanol (≥99 %), nonanal (97 %), limonene oxide (97 %), *Z*-3-nonen-1-ol (95 %), *E*-2-nonenal (97 %), 1-nonanol (≥98 %), decanal (≥98 %), nerol (≥97 %), geraniol (98 %), 1-undecanol (99 %), 1-dodecanol (≥98 %), α-farnesene (mixture of isomers), 1-tridecanol (97 %), methyl jasmonate (≥95 %), 1-pentadecanol (99 %), phytol (≥97 %), and 1-octadecanol (99 %) were purchased from Sigma Aldrich, Germany.

Insects *Epilachna vigintioctopunctata* and *Aulacophora foveicollis* were collected by a light trap from brinjal (*Solanum melongena* L.) and bottle gourd [*Lagenaria siceraria* (Molina) Standl.] plants, respectively, grown in the Crop Research Farm of this University. *Epilachna vigintioctopunctata* and *A. foveicollis* were maintained in the leaves from which they were collected, and were kept in separate glass jars (1 L) covered with fine-mesh nylon nets at 27 ± 1 °C, 65 ± 10 % RH and 12:12, L:D photoperiod in a Biological Oxygen Demand incubator (ADS instruments and Tech.; Calcutta, India). A moist piece of cotton was placed around the cut ends of leaves followed by wrapping with aluminum foil to prevent water loss or withering. Fresh leaves were given daily by replacing the previous one.

Olfactometer Bioassays Females of *E. vigintioctopunctata* and *A. foveicollis* were used in bioassays, as females employ olfactory cues both for feeding and oviposition. Insects (2–3-wk-old) were provisioned with water and starved for 12 h prior to use in olfactometer bioassays. The behavioral responses of females to volatiles were investigated in a Y-shaped glass tube olfactometer (15 cm stem and arms long, 0.6 cm radius, 45° Y angle) (Mukherjee et al. 2015a). The stem of the olfactometer was connected to a small glass vial (starting grid, 1 cm radius × 3 cm long) containing small holes (for passing air through the olfactometer) in which test insects were released. Each arm of the olfactometer was connected to a glass-made micro kit adapter fitted into a glass vial (1 cm radius × 3 cm long). One glass vial contained a piece (1 cm^2) of Whatman No. 41 filter paper moistened with 20 μl of volatiles, while the other glass vial contained a filter paper of the same size moistened with the same amount of the control solvent (methylene chloride). Charcoal-filtered air was pushed into the system at 300 ml min^{-1}. All connections between different parts of the set-up consisted of silicon tubing.

The effectiveness of volatiles as attractants was evaluated in the laboratory at 27 ± 1 °C, 70 ± 3 % RH, and a light intensity of 150 lux. For each experiment, 20 μl of a volatile sample and the control solvent were applied to separate filter paper pieces, allowed to evaporate, and introduced into the glass vials before the first insect was released into the olfactometer. One adult female was introduced into the starting grid, which was then attached to the stem of the olfactometer and exposed to a test sample consisting of 20 μl of one of the various volatiles (natural samples, individual synthetic compounds or synthetic blends) on a filter paper in one glass vial, and 20 μl of the control solvent (methylene chloride) on a filter paper in another. The choice behavior of each female in

response to volatiles or individual synthetic compounds or synthetic blends was observed for 2 min. In preliminary assays, neither insect species was attracted to methylene chloride. The olfactory responses of the insects were recorded as one of the three categories choosing between methylene chloride or the treatment volatiles or 'non-responding' (individuals remained in the common arm of the Y-tube by the end of the observation period) (Magalhães et al. 2012; Mukherjee et al. 2015a; Sarkar et al. 2015). After testing 5 insects, the olfactometer set-up was cleaned with hexanes followed by acetone and dried in an oven at 60 ± 5 °C before further use. The position of the two arms was systematically changed in order to avoid positional bias. Each experiment with one volatile sample was conducted until a total of 90 naïve females had responded.

Bioassay 1 Behavioral responses of *E. vigintioctopunctata* were tested to volatiles obtained from UD or conspecifically (*E. vigintioctopunctata*) damaged or heterospecifically (*A. foveicollis*) damaged or damage caused by both species feeding simultaneously on *S. amplexicaulis* against the solvent control. Similarly, behavioral responses of *A. foveicollis* were tested to volatiles from UD or conspecifically (*A. foveicollis*) damaged or heterospecifically (*E. vigintioctopunctata*) damaged or damaged by both species feeding simultaneously on *S. amplexicaulis* against the control.

Bioassay 2 Behavioral responses of *E. vigintioctopunctata* were tested to volatiles obtained from conspecifically (*E. vigintioctopunctata*) damaged or heterospecifically (*A. foveicollis*) damaged, or damaged by both species feeding simultaneously on *S. amplexicaulis* plants against volatiles obtained from UD plants. Responses of *A. foveicollis* were tested to volatiles obtained from conspecifically (*A. foveicollis*) damaged or heterospecifically (*E. vigintioctopunctata*) damaged or damaged by both species feeding simultaneously on *S. amplexicaulis* against volatiles obtained from UD plants.

Bioassay 3 Behavioral responses of *E. vigintioctopunctata* were tested to volatiles in the following combinations: (i) conspecifically (*E. vigintioctopunctata*) damaged plants *vs.* heterospecifically (*A. foveicollis*) damaged plants, (ii) heterospecifically (*A. foveicollis*) damaged plants *vs.* damage caused by both species feeding simultaneously on *S. amplexicaulis*, and (iii) conspecifically (*E. vigintioctopunctata*) damaged plants *vs.* damage caused by both species feeding simultaneously on *S. amplexicaulis*.

Similarly, behavioral responses of female *A. foveicollis* were recorded to volatiles of: (i) conspecifically (*A. foveicollis*) damaged plants *vs.* heterospecifically (*E. vigintioctopunctata*) damaged plants, (ii) heterospecifically (*E. vigintioctopunctata*) damaged plants *vs.* damage caused by both species feeding simultaneously on *S. amplexicaulis*, and (iii) conspecifically (*A. foveicollis*) damaged plants *vs.* damage caused by both species feeding on *S. amplexicaulis*.

Bioassay 4 Pure synthetic compounds, equivalent to the amounts of individual VOCs detected among the volatiles of UD and ID plants (10 h volatile collection), were dissolved in 200 μl methylene chloride, and 20 μl of this solution (equivalent to the amounts released by a plant during ca. 1 h) were tested against the same amount of the solvent control to quantify responses of both insect species.

Seven synthetic compounds, (1-heptanol, 3-octanol, 2-octanol, linalool oxide, 1-octanol, nonanal, *Z*-3-nonen-1-ol, and *E*-2-nonenal) to which both insect species had shown behavioral responses were dissolved in 200 μl methylene chloride in naturally occurring proportions, and 20 μl of this solution (equivalent to the amounts released by each type of *S. amplexicaulis* during ca. 1 h) were tested for behavior-mediating capacity against *E. vigintioctopunctata* in comparison to the solvent control. The same set of experiments was carried out with *A. foveicollis*, however, Z-3-nonen-1-ol, to which only this species had shown positive response, was added to the synthetic blend. Furthermore, 20 μl of synthetic blends consisting of linalool oxide, nonanal, and *E*-2-nonenal (both insect species had shown clear attraction to these three compounds) were tested against 20 μl solvent control (Supplementary Table 1).

Bioassay 5 Again, 20 μl of volatiles, obtained when both species were feeding simultaneously on *S. amplexicaulis,* were tested against a synthetic compound or a synthetic blend, qualitatively and quantitatively equivalent to the amounts of volatiles released when both species were feeding simultaneously on *S. amplexicaulis* during ca. 1 hr.

Statistical Analyses Data on total amounts of VOCs and amounts of individual VOCs from UD, MD, and ID *S. amplexicaulis* plants were log (x + 1) transformed prior to performing statistical analyses. The log (x + 1) transformed data for the total amount of volatiles and amount of individual VOCs present in undamaged, mechanically damaged, and insect damaged *S. amplexicaulis* plants were subjected to Levene's test for homogeneity of variance with respect to treatments. Following this, one-way ANOVA was conducted to compare the treatment effects on total and individual VOCs. If an *F*-value of one-way ANOVA were found significant, data were subjected to a *post hoc* Tukey test using SPSS software (SPSS 16.0; SPSS Inc., Chicago, IL, USA). Data obtained on responses of insects to natural VOCs, individual synthetic compounds, and synthetic blends were analyzed based on the null hypothesis whether the response of beetles differed

significantly from 1:1, i.e., *chi-square* goodness of fit analysis (Malik and Barik 2015; Magalhães et al. 2012; Sarkar and Barik 2015). Insects that did not respond by selection of either arm of the olfactometer (non-responders) were excluded from the analyses.

Results

Thirty two compounds were detected in volatiles of undamaged (UD), mechanically damaged (MD), and insect damaged (ID) *S. amplexicaulis* plants (Table 1, Supplementary Fig 1). Levene's test (W) for homogeneity of variance indicated that total amounts of VOCs from UD, MD, and ID plants were homogenous conforming to application of ANOVA ($W = 1.024$; $df = 4, 20$; $P = 0.419$). Total volatile emissions were significantly higher in both species feeding on *S. amplexicaulis* plants followed by *E. vigintioctopunctata* damaged plants and MD plants, *A. foveicollis* damaged plants, and UD plants (Table 1). Levene's test for homogeneity of variance indicated that the data set for individual VOCs in UD, MD, and ID *S. amplexicaulis* plants were homogenous,

Table 1 Qualitative and quantitative composition (GC-FID Analysis) of volatile organic compounds (VOCs) emitted by *Solena amplexicaulis* plants (μg/10 hr) under various conditions: A = Undamaged plants, B = Mechanically damaged plants

Compounds	A	VOCs released by plants after 48 h of continuous feeding of			B	$F_{4, 20}$
		E. vigintioctopunctata	*A. foveicollis*	Both species		
2-Hexanol	9.51 ± 0.09^{a}	10.72 ± 0.08^{a}	22.57 ± 0.17^{b}	22.26 ± 0.08^{b}	14.54 ± 0.24^{c}	1567.68
1-Hexanol	16.99 ± 0.11^{a}	21.58 ± 0.15^{b}	30.12 ± 0.49^{c}	23.78 ± 0.12^{d}	37.62 ± 0.36^{e}	994.10
E-2-Hexen-1-ol	24.31 ± 0.22^{a}	24.75 ± 0.22^{a}	44.35 ± 0.53^{b}	43.13 ± 0.74^{b}	51.61 ± 0.76^{c}	765.68
2- Heptanone	32.60 ± 0.44^{a}	35.02 ± 0.69^{b}	47.43 ± 0.79^{c}	46.62 ± 0.68^{c}	99.90 ± 1.20^{d}	828.63
α-Pinene	13.63 ± 0.07^{a}	15.51 ± 0.17^{b}	21.79 ± 0.26^{c}	17.37 ± 0.09^{d}	41.51 ± 0.90^{e}	1238.13
Benzaldehyde	26.63 ± 0.35^{a}	30.08 ± 0.24^{b}	33.56 ± 0.29^{c}	30.87 ± 0.33^{b}	63.28 ± 0.72^{d}	1058.29
1-Heptanol	21.35 ± 0.16^{a}	21.72 ± 0.12^{a}	32.57 ± 0.41^{b}	23.10 ± 0.08^{c}	42.94 ± 0.83^{d}	722.31
Sabinene	25.75 ± 0.17^{a}	32.93 ± 0.37^{b}	36.16 ± 0.29^{c}	33.43 ± 0.47^{b}	54.54 ± 0.89^{d}	528.56
1-Octen-3-ol	13.49 ± 0.16^{a}	20.31 ± 0.11^{b}	24.18 ± 0.28^{c}	21.74 ± 0.08^{d}	34.20 ± 0.42^{e}	1174.85
3-Octanone	5.32 ± 0.08^{a}	5.57 ± 0.09^{a}	6.84 ± 0.06^{b}	5.67 ± 0.08^{a}	12.01 ± 0.17^{c}	570.96
3-Octanol	25.39 ± 0.37^{a}	26.52 ± 0.14^{b}	26.74 ± 0.27^{b}	26.62 ± 0.25^{b}	11.41 ± 0.21^{c}	908.18
2-Octanol	177.89 ± 1.41^{a}	191.17 ± 1.81^{b}	200.81 ± 1.56^{c}	189.90 ± 1.57^{b}	387.44 ± 2.37^{d}	1634.20
Benzyl alcohol	23.50 ± 0.54^{a}	25.09 ± 0.21^{b}	32.56 ± 0.39^{c}	25.72 ± 0.32^{b}	64.75 ± 0.80^{d}	876.16
Ocimene	11.74 ± 0.12^{a}	12.52 ± 0.08^{b}	20.48 ± 0.26^{c}	17.89 ± 0.17^{d}	37.01 ± 0.38^{e}	2084.61
Linalool oxide	6.48 ± 0.08^{a}	13.57 ± 0.09^{b}	17.26 ± 0.17^{c}	16.39 ± 0.12^{d}	17.39 ± 0.19^{c}	1769.95
1-Octanol	12.48 ± 0.08^{a}	12.54 ± 0.09^{a}	15.36 ± 0.20^{b}	14.06 ± 0.09^{c}	32.52 ± 0.22^{d}	2415.32
Nonanal	32.47 ± 0.46^{a}	37.79 ± 0.52^{b}	40.92 ± 0.62^{c}	38.55 ± 0.38^{b}	85.87 ± 0.43^{d}	1014.11
Limonene oxide	6.83 ± 0.09^{a}	7.71 ± 0.08^{b}	10.36 ± 0.14^{c}	8.57 ± 0.09^{d}	11.61 ± 0.12^{e}	322.82
Z-3-Nonen-1-ol	2.21 ± 0.08^{a}	3.01 ± 0.09^{b}	3.35 ± 0.06^{b}	3.14 ± 0.09^{b}	4.68 ± 0.09^{c}	96.44
E-2-Nonenal	12.63 ± 0.17^{a}	14.41 ± 0.08^{b}	22.73 ± 0.22^{c}	22.29 ± 0.24^{c}	41.17 ± 0.88^{d}	1206.33
1-Nonanol	9.92 ± 0.13^{a}	11.07 ± 0.05^{b}	16.21 ± 0.17^{c}	13.78 ± 0.09^{d}	11.34 ± 0.10^{b}	466.59
Decanal	5.66 ± 0.08^{a}	6.49 ± 0.07^{b}	10.55 ± 0.10^{c}	10.15 ± 0.07^{cd}	9.67 ± 0.11^{d}	643.10
Nerol	151.59 ± 1.11^{a}	160.24 ± 1.87^{b}	185.61 ± 1.84^{c}	181.28 ± 1.39^{c}	229.77 ± 1.81^{d}	315.82
Geraniol	42.33 ± 0.37^{a}	42.57 ± 0.34^{a}	57.46 ± 0.54^{b}	56.17 ± 0.92^{b}	54.78 ± 0.90^{b}	152.95
1-Undecanol	14.30 ± 0.11^{a}	20.69 ± 0.12^{b}	21.91 ± 0.20^{c}	20.76 ± 0.16^{b}	17.38 ± 0.12^{d}	546.22
1-Dodecanol	3.77 ± 0.04^{a}	5.83 ± 0.06^{b}	6.50 ± 0.06^{c}	6.14 ± 0.08^{bc}	4.11 ± 0.10^{d}	277.20
α-Farnesene	4.49 ± 0.06^{a}	5.53 ± 0.09^{b}	9.31 ± 0.07^{c}	8.44 ± 0.09^{d}	6.80 ± 0.09^{e}	542.66
1-Tridecanol	10.67 ± 0.13^{a}	12.90 ± 0.09^{b}	14.16 ± 0.11^{c}	13.17 ± 0.09^{b}	11.27 ± 0.20^{a}	107.38
Methyl jasmonate	431.25 ± 2.48^{a}	1105.95 ± 9.31^{b}	476.47 ± 2.00^{c}	1186.82 ± 7.63^{d}	435.05 ± 2.73^{a}	6613.62
1-Pentadecanol	6.27 ± 0.08^{a}	8.78 ± 0.09^{b}	11.04 ± 0.07^{c}	10.29 ± 0.08^{d}	24.37 ± 0.15^{e}	3071.46
Phytol	232.43 ± 2.18^{a}	238.70 ± 1.99^{a}	275.39 ± 1.86^{b}	262.50 ± 2.14^{c}	247.98 ± 2.46^{d}	64.97
1-Octadecanol	8.49 ± 0.08^{a}	8.80 ± 0.06^{a}	11.67 ± 0.14^{b}	10.16 ± 0.07^{c}	24.58 ± 0.11^{d}	2738.42
Total	1422.41 ± 5.98^{a}	2190.06 ± 12.24^{b}	1786.44 ± 5.16^{c}	2410.80 ± 9.98^{d}	2223.07 ± 7.59^{b}	2643.79

(Mean ± SE, $N = 5$). Different superscript letters in the same entry indicate significant differences ($P < 0.05$)

Table 2 Responses of females of *Epilachna vigintioctopunctata* to natural volatile organic compounds (VOCs) released by undamaged and insect damaged *Solena amplexicaulis* plants or to synthetic compounds and synthetic blends equivalent to those released by undamaged and insect damaged plants (T1) *vs.* the solvent control (T2: CH_2Cl_2)

Treatment (T1)	Insects responded		Non-	$\chi 2$	*P* values
	T1	T2	responders	(*df* = 1)	
Volatiles from *S. amplexicaulis* plants					
Undamaged	61	29	6	11.38	<0.001
Feeding by *E. vigintioctopunctata*	66	24	4	19.6	<0.001
Feeding by *Aulacophora foveicollis*	69	21	2	25.6	<0.001
Feeding by both species	68	22	3	23.51	<0.001
Synthetic VOCs (μg in 20 μl CH_2Cl_2) in similar amounts as released by undamaged *S. amplexicaulis* plants					
a. 1-Heptanol (2.14 μg)	50	40	6	1.11	0.292
b. 3-Octanol (2.54 μg)	42	48	7	*0.04*	*0.527*
c. 2-Octanol (17.79 μg)	47	43	6	0.18	0.637
d. Linalool oxide (0.65 μg)	57	33	4	6.4	0.011
e. 1-Octanol (1.25 μg)	49	41	5	0.71	0.399
f. Nonanal (3.25 μg)	57	33	4	6.4	0.011
g. *E*-2-Nonenal (1.26 μg)	54	36	4	3.6	0.058
a + b + c + d + e + f + g*	60	30	4	10	0.002
d + f *	59	31	3	8.71	0.003
Synthetic VOCs (μg in 20 μl CH_2Cl_2) in similar amounts as released by plants on which *E. vigintioctopunctata* is feeding					
a. 1-Heptanol (2.17 μg)	50	40	5	1.11	0.292
b. 3-Octanol (2.65 μg)	40	50	5	*1.11*	*0.292*
c. 2-Octanol (19.12 μg)	47	43	7	0.18	0.673
d. Linalool oxide (1.36 μg)	62	28	4	12.84	<0.001
e. 1-Octanol (1.25 μg)	49	41	6	0.71	0.399
f. Nonanal (3.78 μg)	55	35	5	4.44	0.035
g. *E*-2-Nonenal (1.44 μg)	55	35	4	4.44	0.035
a + b + c + d + e + f + g*	65	25	2	17.78	<0.001
d + f + g*	64	26	3	16.04	<0.001
Synthetic VOCs (μg in 20 μl CH_2Cl_2) in similar amounts as released by plants on which *A. foveicollis* is feeding					
a. 1-Heptanol (3.26 μg)	46	44	7	0.04	0.834
b. 3-Octanol (2.67 μg)	40	50	6	*1.11*	*0.292*
c. 2-Octanol (20.08 μg)	48	42	6	0.4	0.527
d. Linalool oxide (1.73 μg)	63	27	4	14.4	<0.001
e. 1-Octanol (1.54 μg)	50	40	5	1.11	0.292
f. Nonanal (4.09 μg)	55	35	4	4.44	0.035
g. *E*-2-Nonenal (2.27 μg)	56	34	4	5.38	0.020
a + b + c + d + e + f + g*	68	22	3	23.51	<0.001
d + f + g*	67	23	3	21.51	<0.001
Synthetic VOCs (μg in 20 μl CH_2Cl_2) in similar amounts as released by plants on which both species are feeding					
a. 1-Heptanol (2.31 μg)	50	40	5	1.11	0.292
b. 3-Octanol (2.66 μg)	40	50	6	*1.11*	*0.292*
c. 2-Octanol (18.99 μg)	47	43	6	0.18	0.673
d. Linalool Oxide (1.64 μg)	63	27	3	14.4	<0.001
e. 1-Octanol (1.41 μg)	50	40	4	1.11	0.292
f. Nonanal (3.86 μg)	55	35	5	4.44	0.035
g. *E*-2-Nonenal (2.23 μg)	56	34	5	5.38	0.020
a + b + c + d + e + f + g*	68	22	3	23.51	<0.001
d + f + g*	67	23	3	21.51	<0.001

Each test was carried out with at least 90 females

χ^2 and *P* values in italics are for control solvent loaded air flow

* Indicates compounds as mixture in the amounts given in the different entries

conforming to application of ANOVA (Supplementary Table 2). Methyl jasmonate (MeJa) was predominant among volatiles of UD, MD, and ID plants (Table 1). Phytol was the second most abundant component among volatiles of UD and ID plants; whereas 2-octanol was the second most abundant volatiles released by MD plants. *Z*-3-Nonen-1-ol was least abundant in volatiles of UD and ID plants, while 1-dodecanol was least abundant among volatiles of MD plants (Table 1). Among all compounds, geraniol did not differ significantly in amounts released by MD plants, *A. foveicollis* damaged plants, and when both species were feeding simultaneously on *S. amplexicaulis*; whereas 1-nonanol did not differ significantly in amounts between *E. vigintioctopunctata* damaged plants and MD plants. Methyl jasmonate was similarly abundant among volatiles of UD and MD plants, but its emission was higher in both species feeding simultaneously on plants followed by *E. vigintioctopunctata* damaged plants and *A. foveicollis*

Table 3 Responses of *Aulacophora foveicollis* to volatile organic compounds (VOCs) released by undamaged and insect damaged *Solena amplexicaulis* plants or by synthetic compounds and synthetic blends equivalent to those released by undamaged and insect damaged plants (T1) *vs.* the solvent control (T2: CH_2Cl_2. Each test was carried out with at least 90 females

Treatment (T1)	Insects responded		Non-	$\chi 2$	*P* values
	T1	T2	responders	(*df* = 1)	
Undamaged	63	27	4	14.4	<0.001
Feeding by *Epilachna vigintioctopunctata*	71	19	3	30.04	<0.001
Feeding by *A. foveicollis*	73	17	2	34.84	<0.001
Feeding by both insects	73	17	2	34.84	<0.001
Synthetic VOCs (μg in 20 μl CH_2Cl_2) in similar amounts as released by undamaged *S. amplexicaulis* plants					
a. 1-Heptanol (2.14 μg)	52	38	6	2.18	0.14
b. 3-Octanol (2.54 μg)	41	49	7	*0.71*	*0.399*
c. 2-Octanol (17.79 μg)	49	41	6	0.71	0.399
d. Linalool oxide (0.65 μg)	59	31	4	8.71	0.003
e. 1-Octanol (1.25 μg)	51	39	5	1.6	0.206
f. Nonanal (3.25 μg)	57	33	4	6.4	0.011
g. *Z*-3-Nonen-1-ol (0.22 μg)	46	44	6	0.04	0.834
h. *E*-2-Nonenal (1.26 μg)	55	35	5	4.44	0.035
a + b + c + d + e + f + g + h*	62	28	3	12.84	<0.001
d + f + h*	62	28	3	12.84	<0.001
Synthetic VOCs (μg in 20 μl CH_2Cl_2) in similar amounts as released by plants on which *E. vigintioctopunctata* is feeding					
a. 1-Heptanol (2.17 μg)	52	38	3	2.18	0.14
b. 3-Octanol (2.65 μg)	41	49	6	*0.71*	*0.399*
c. 2-Octanol (19.12 μg)	51	39	4	1.6	0.206
d. Linalool oxide (1.36 μg)	63	27	2	14.4	<0.001
e. 1-Octanol (1.25 μg)	51	39	5	1.6	0.206
f. Nonanal (3.78 μg)	57	33	4	6.4	0.011
g. *Z*-3-Nonen-1-ol (0.30 μg)	48	42	6	0.4	0.527
h. *E*-2-Nonenal (1.44 μg)	56	34	5	5.38	0.020
a + b + c + d + e + f + g + h*	68	22	2	23.51	<0.001
d + f + h*	66	24	3	19.6	<0.001
Synthetic VOCs (μg in 20 μl CH_2Cl_2) in similar amounts as released by plants on which *A. foveicollis* is feeding					
a. 1-Heptanol (3.26 μg)	48	42	7	0.4	0.527
b. 3-Octanol (2.67 μg)	41	49	6	*0.71*	*0.399*
c. 2-Octanol (20.08 μg)	52	38	5	2.18	0.14
d. Linalool oxide (1.73 μg)	66	24	3	19.6	<0.001
e. 1-Octanol (1.54 μg)	51	39	5	1.6	0.206
f. Nonanal (4.09 μg)	56	34	4	5.38	0.020
g. *Z*-3-Nonen-1-ol (0.34 μg)	48	42	6	0.4	0.527
h. *E*-2-Nonenal (2.27 μg)	58	32	5	7.51	0.006
a + b + c + d + e + f + g + h*	71	19	2	30.04	<0.001
d + f + h*	70	20	3	27.78	<0.001
Synthetic VOCs (μg in 20 μl CH_2Cl_2) in similar amounts as released by plants on which both species are feeding					
a. 1-Heptanol (2.31 μg)	52	38	5	2.18	0.14
b. 3-Octanol (2.66 μg)	41	49	6	*0.71*	*0.399*
c. 2-Octanol (18.99 μg)	51	39	4	1.6	0.206
d. Linalool oxide (1.64 μg)	66	24	3	19.6	<0.001
e. 1-Octanol (1.41 μg)	51	39	5	1.6	0.206
f. Nonanal (3.86 μg)	56	34	4	5.38	0.020
g. *Z*-3-Nonen-1-ol (0.31 μg)	48	42	7	0.4	0.527
h. *E*-2-Nonenal (2.23 μg)	58	32	5	7.51	0.006
a + b + c + d + e + f + g + h*	71	19	2	30.04	<0.001
d + f + h*	70	20	2	27.78	<0.001

χ^2 and *P* values in italics are for control solvent loaded air flow

* Indicates compounds as mixture in the amounts given in the different entries

damaged plants. The other identified VOCs displayed different patterns among volatiles of UD, MD, and ID plants (Table 1).

Bioassay 1 Both *E. vigintioctopunctata* and *A. foveicollis* were attracted by volatiles from UD or conspecifically damaged plants or heterospecifically damaged plants or on *S. amplexicaulis* when tested against the solvent control (Tables 2 and 3).

Bioassay 2 *Epilachna vigintioctopunctata* displayed attraction to volatiles from conspecifically (*E. vigintioctopunctata*) damaged plants or heterospecifically (*A. foveicollis*) damaged

plants or both species feeding on *S. amplexicaulis* plants (tested against volatiles from UD plants - Table 4).

Aulacophora foveicollis showed attraction to volatiles from conspecifically (*A. foveicollis*) damaged plants or heterospecifically (*E. vigintioctopunctata*) damaged plants or by VOCs released when both species were feeding simultaneously on *S. amplexicaulis* plants (tested against volatiles from UD plants—Table 5).

Bioassay 3 *Epilachna vigintioctopunctata* did not show a significant preference to VOCs released when both species were feeding simultaneously on *S. amplexicaulis* when tested against conspecifically (*E. vigintioctopunctata*) damaged plants, heterospecifically (*A. foveicollis*) damaged plants against VOCs released when both species were feeding simultaneously, and heterospecifically damaged plants against conspecifically damaged plants (Table 4).

Aulacophora foveicollis did not display a significant preference to volatiles released when both species were feeding simultaneously on *S. amplexicaulis* when tested against heterospecifically (*E. vigintioctopunctata*) damaged plants, conspecifically (*A. foveicollis*) damaged plants against VOCs released when both species were feeding simultaneously on *S. amplexicaulis*, and conspecifically damaged plants against heteropecifically damaged plants (Table 5).

Bioassay 4 *Epilachna vigintioctopunctata* showed behavioral responses to 7 individual synthetic volatiles, 1-heptanol, 3-octanol, 2-octanol, linalool oxide, 1-octanol, nonanal, and *E*-2-nonenal; whereas *A. foveicollis* displayed behavioral responses to these 7 compounds and in addition to *Z*-3-nonen-1-ol.

Epilachna vigintioctopunctata displayed attraction to linalool oxide, nonanal, and *E*-2-nonenal in similar amounts present in volatiles released by UD and ID plants during ca. 1 h against the solvent control (Table 2). The insect showed attraction to a synthetic blend of 7 compounds (1-heptanol, 3-octanol, 2-octanol, linalool oxide, 1-octanol, nonanal, and *E*-2-nonenal) or a synthetic blend of 3 compounds (linalool oxide, nonanal, and *E*-2-nonenal) in similar amounts and proportions present in volatiles of UD and ID plants against the solvent control (Table 2).

Aulacophora foveicollis displayed attraction to linalool oxide, nonanal, and *E*-2-nonenal in similar amounts present in volatiles of UD and ID plants during ca. 1 h against the solvent

Table 4 Responses of *Epilachna vigintioctopunctata* to natural volatile organic compounds (VOCs) released by undamaged *Solena amplexicaulis* plants *vs.* insect damaged plants, differently treated insect damaged plants tested against each other, volatiles released when both species feed on the plants *vs.* synthetic components or synthetic blends equivalent to volatiles released when both species feed on the plants

Comparison		Insects responded		Non-	$\chi 2$	*P* values
T1	T2	T1	T2	responders	(*df* = 1)	
Plants after continuous feeding by	Undamaged plants					
Epilachna vigintioctopunctata	Undamaged plants	55	35	6	4.44	0.035
Aulacophora foveicollis	Undamaged plants	57	33	4	6.4	0.011
Both species	Undamaged plants	57	33	4	6.4	0.011
A. foveicollis feeding on the plants	*E. vigintioctopunctata* feeding on the plants	52	38	6	2.18	0.14
A. foveicollis feeding on the plants	Both species feeding on the plants	48	42	5	0.4	0.527
Both species feeding on the plants	*E. vigintioctopunctata* feeding on the plants	52	38	5	2.18	0.14
Natural VOCs released, when both species are feeding on the plants	Synthetic VOCs and mixtures equivalent to volatiles released, when both species are feeding on the plants					
	a. 1-Heptanol (2.31 μg)	66	24	3	19.6	<0.001
	b. 3-Octanol (2.66 μg)	71	29	2	30.04	<0.001
	c. 2-Octanol (18.99 μg)	66	24	4	19.6	<0.001
	d. Linalool oxide (1.64 μg)	51	39	5	1.6	0.206
	e. 1-Octanol (1.41 μg)	65	25	3	17.78	<0.001
	f. Nonanal (3.86 μg)	56	34	4	5.38	0.020
	g. *E*-2-Nonenal (2.23 μg)	55	35	5	4.44	0.035
	a + b + c + d + e + f + g*	47	43	2	0.18	0.673
	d + f + g*	49	41	3	0.71	0.399

Each test was carried out with at least 90 female

* Indicates compounds as mixtures in the amounts given in the different entries

Table 5 Responses of *Aulacophora foveicollis* to natural volatile organic compounds (VOCs) released by undamaged *Solena amplexicaulis* plants *vs.* insect damaged plants, differently treated insect damaged plants tested against each other, volatiles released when both species feed on the plants *vs.* synthetic components or synthetic blends similar to volatiles released, when both species feed on the plants

Comparison		Insects responded		Non-	$\chi 2$	*P* values
T1	T2	T1	T2	responders	(*df* = 1)	
Plants after continuous feeding by	Undamaged plants					
Epilachna vigintioctopunctata	Undamaged plants	56	34	4	5.38	0.020
A. foveicollis	Undamaged plants	58	32	2	7.51	0.006
Both species	Undamaged plants	59	31	2	8.71	0.003
A. foveicollis feeding on the plants	*E. vigintioctopunctata* feeding on the plants	53	37	4	2.84	0.092
Both species feeding on the plants	*A. foveicollis* feeding on the plants	49	41	5	0.71	0.399
Both species feeding on the plants	*E. vigintioctopunctata* feeding on the plants	54	36	3	3.6	0.058
Natural VOCs released, when both species are feeding on the plants	Synthetic VOCs and mixtures equivalent to volatiles released, when both species are feeding on the plants					
	a. 1-Heptanol (2.31 μg)	68	22	3	23.51	<0.001
	b. 3-Octanol (2.66 μg)	75	15	2	40	<0.001
	c. 2-Octanol (18.99 μg)	70	20	2	27.78	<0.001
	d. Linalool oxide (1.64 μg)	55	35	4	4.44	0.035
	e. 1-Octanol (1.41 μg)	70	20	2	27.78	<0.001
	f. Nonanal (3.86 μg)	66	24	3	19.6	<0.001
	g. *Z*-3-Nonen-1-ol (0.31 μg)	72	18	2	32.4	<0.001
	h. *E*-2-Nonenal (2.23 μg)	62	28	4	12.84	<0.001
	a + b + c + d + e + f + g + h*	46	44	3	0.04	0.834
	d + f + h*	48	42	3	0.4	0.527

Each test was carried out with at least 90 females

* Indicates compounds as mixture in amounts given in the different entries

control (Table 3). The insect showed attraction to a synthetic blend of 8 compounds (1-heptanol, 3-octanol, 2-octanol, linalool oxide, 1-octanol, nonanal, *Z*-3-nonen-1-ol, and *E*-2-nonenal) or a synthetic blend of 3 compounds (linalool oxide, nonanal, and *E*-2-nonenal) in similar amounts and proportions present in volatiles of UD and ID plants against the solvent control (Table 3).

Bioassay 5 *Epilachna vigintioctopunctata* were attracted by VOCs released when both species were feeding simultaneously on *S. amplexicaulis* tested against 7 individual synthetic compounds (1-heptanol, 3-octanol, 2-octanol, linalool oxide 1-octanol, nonanal, and *E*-2-nonenal) in equivalent amounts as present among volatiles and released during ca. 1 h when both species were feeding simultaneously on plants (Table 4). Similarly, the insects did not show a preference when the volatiles released when both species feeding simultaneously were tested against a synthetic blend of the before mentioned 7 compounds or a synthetic blend of 3 compounds (linalool oxide, nonanal, and *E*-2-nonenal) (Table 4).

Aulacophora foveicollis were attracted by VOCs released when both species feeding simultaneously on *S. amplexicaulis* were tested against 8 individual synthetic compounds (1-heptanol, 3-octanol, 2-octanol, linalool oxide 1-octanol, nonanal, *Z*-3-nonen-1-ol, and *E*-2-nonenal) in equivalent amounts as present among volatiles and released during ca. 1 h when both species were feeding simultaneously on plants (Table 5). Similarly, the insects did not show a preference when the volatiles released when both species feeding simultaneously, were tested against a synthetic blend of the before mentioned 8 compounds or a synthetic blend of 3 compounds (linalool oxide, nonanal, and *E*-2-nonenal) (Table 5).

Discussion

Plants emit volatiles even when they are not attacked or mechanically damaged, and these volatiles act as chemical signals for neighboring plants, which influence growth pattern and biomass allocation (Kegge and Pierik 2010; Ninkovic 2003; Ninkovic et al. 2009; Piesik et al. 2010; Schoonhoven et al. 2005). Plants sense the environment by detecting the volatiles released from neighboring plants and thus adapt to the environment by appropriate morphological and

physiological responses (Kegge and Pierik 2010; Ninkovic et al. 2009). This study reveals that total amounts of volatile emissions were higher from ID and MD plants than from UD *S. amplexicaulis* plants. Quantitatively, amounts of two compounds (3-octanol and *Z*-3-nonen-1-ol) did not significantly differ in the VOCs between *A. foveicollis* and *E. vigintioctopunctata* damaged plants. Except MeJa, which was two times more abundant in volatiles released by *E. vigintioctopunctata* damaged plants as compared to *A. foveicollis* damaged plants, the other 29 identified VOCs were released in higher amounts by *A. foveicollis* damaged plants than by those damaged by *E. vigintioctopunctata*. This finding reveals that feeding on plants by two insect species separately may result in different emissions of VOCs. Methyl jasmonate from injured plants induces a defensive reaction through air in neighboring UD plants (Creelman and Mullet 1997; Farmer and Ryan 1990; Zhang et al. 2015), indicating that damaged *S. amplexicaulis* plants may induce a more intensive reaction in the defense of neighboring plants after attack by *E. vigintioctopunctata* than by *A. foveicollis*. The major VOCs emitted by fruits and vines of *M. charantia* L. (Cucurbitaceae) have been found to contain myrtenol, (*Z*)-3-hexenol, benzyl alcohol, 1-penten-3-ol, (*Z*)-2-pentenol, (*E*)-2-hexenal, and *cis*-sabinol, and the fly *Dacus cucurbitae* showed attraction to these volatiles (Binder et al. 1989). In another study, 1-tridecanol was detected in higher amounts followed by phytol in *M. charantia* leaf volatiles, and *E. dodecastigma* were attracted by individual geraniol, 1-tridecanol, and phytol (Sarkar et al. 2015). Twenty two compounds were identified in volatiles of *M. cochinchinensis* plants after 120 h of continuous feeding of *A. foveicollis*, and phytol was the major compound, followed by linalool and geranyl linalool in the VOCs of this plant (Mukherjee et al. 2015a). In the present study, 2-heptanone, sabinene, 2-octanol, ocimene, limonene oxide, *Z*-3-nonen-1-ol, *E*-2-nonenal, nerol, geraniol, 1-undecanol, 1-dodecanol, farnesene, 1-tridecanol, methyl jasmonate, 1-pentadecanol, and 1-octadecanol were detected among volatiles of UD and ID *S. amplexicaulis*, but these were not detected in volatiles of *M. cochinchinensis*. Hence, this study supports the hypothesis that variation in volatile compounds occurs between plant species (Schoonhoven et al. 2005).

Here, we also confirmed that herbivore feeding may result in an increase in total emissions of VOCs depending on the type of feeding damage (Magalhães et al. 2012; Paré and Tumlinson 1996; Piesik et al. 2013; Röse and Tumlinson 2004). Results of our olfactometer bioassays show that the two insect species used in this study discriminate between complete volatile blends released by UD *S. amplexicaulis* or after damage caused by conspecifics or by heterospecifics or by both species feeding simultaneously. Feeding of either *E. vigintioctopunctata* or *A. foveicollis* or of both species on *S. amplexicaulis* resulted in increased emissions of several compounds including benzyl alcohol that was also present in the complete volatile blend of UD plants. However, *E. vigintioctopunctata* and *A. foveicollis* did not show attraction to synthetic benzyl alcohol. This compound has been shown to act as part of the plant defense reaction, induced by herbivory and may cause attraction of natural enemies of these insect pests (De Moraes et al. 1998; Paré and Tumlinson 1999; Tabata et al. 2011). However, increased emissions of linalool oxide, nonanal, and *E*-2-nonenal from ID *S. amplexicaulis* plants might induce further attraction of both insect species to the ID plants as both species obviously use these three compounds for host location. Empirical evidence suggests that the affixed natural ratio between 3 and 10 compounds in plant-released volatile blends may determine the specificity of this chemical signal for insects (Bruce and Pickett 2011; Bruce et al. 2005; Tasin et al. 2006; Webster et al. 2010). Insect attraction may decline when the ratio of key compounds, typical for the volatiles of a host plant, are replaced by different ratios of the same compounds (Bruce and Pickett 2011; Bruce et al. 2005; De Moraes et al. 1998). Host plant discrimination by adult herbivorous insects depends on responses of olfactory receptor neurons to the ratio of compounds in the volatile blend (Riffell et al. 2009a; b). Visual cues from the host plant might also likely play a role in the attraction (Bahlai et al. 2008; Beyaert et al. 2010), but these cues were not considered in the present study.

Our results document that a synthetic blend of 1.64 μg linalool oxide, 3.86 μg nonanal, and 2.23 μg *E*-2-nonenal, dissolved in 20 μl methylene chloride, in similar amounts and proportions present among volatiles of plants after 48-h continuous feeding by both insect species, might facilitate the development of much needed eco-friendly trapping tools for pest management of *E. vigintioctopunctata* and *A. foveicollis*. Bioassays in a greenhouse to evaluate responses of *E. vigintioctopunctata* and *A. foveicollis* to blends of these three synthetic volatile compounds are necessary to corroborate their attractiveness shown in the present study.

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