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Species-Specific Effects of Herbivory on the Oviposition Behavior of the Moth *Manduca sexta*

Carolina E. Reisenman · Jeffrey A. Riffell · Kristin Duffy · Adrien Pesque · David Mikles · Brenna Goodwin

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Abstract In Southwestern USA, the jimsonweed *Datura wrightii* and the nocturnal sphinx moth *Manduca sexta* form a pollinator-plant and herbivore-plant association. While certain plant volatile organic compounds (VOCs) attract moths for oviposition, it is likely that other host-derived olfactory cues, such as herbivore-induced VOCs, repel moths for oviposition. Here, we studied the oviposition preference of female *M. sexta* towards intact and damaged host plants of three species: *D. wrightii*, *D. discolor* (a less preferred feeding resource but also used by females for oviposition), and *Solanum lycopersicum*–tomato–(used by moths as an oviposition resource only). Damage was inflicted to the plants either by larval feeding or artificial damage. Mated females were exposed to an intact plant and a damaged plant and allowed to lay eggs for 10 min. Oviposition preferences of females were highly heterogeneous in all cases, but a larger proportion of moths laid significantly fewer eggs on feeding-damaged and artificially damaged plants of *S. lycopersicum*. Many females also avoided feeding-damaged *D. discolor* and *D. wrightii* plants induced by treatment with methyl jasmonate. Chemical analyses showed a significant increase in the total amount of VOCs released by vegetative tissues of feeding-damaged plants, as well as species-specific increases in emission of certain VOCs. In particular, feeding-damaged *S. lycopersicum* plants emitted (-)-linalool, an odorant that repels moths for oviposition. Finally, the emission of *D. wrightii* floral VOCs, which are important in mediating feeding by adult moths (and hence pollination), did not change in plants damaged by larval feeding. We propose that the observed differential effects of herbivory on oviposition choice are due to different characteristics (i.e., mutually beneficial or parasitic) of the insect–plant interaction.

Keywords Herbivory · *Manduca sexta* · Oviposition · Solanaceae · GC-MS · Plant volatiles

Carolina E. Reisenman and Jeffrey A. Riffell equal contribution

C. E. Reisenman (✉) · K. Duffy · A. Pesque · D. Mikles · B. Goodwin
Department of Neuroscience, University of Arizona, 1040 E. Fourth St., Gould-Simpson 611, Tucson, AZ 85721-0077, USA
e-mail: carolina@neurobio.arizona.edu

J. A. Riffell
Department of Biology, University of Washington, Seattle, USA

Present Address:
K. Duffy
Center for Cognitive Neuroscience, Duke University, Durham, USA

Present Address:
A. Pesque
School of Veterinary Medicine, University of California, Davis, USA

Present Address:
D. Mikles
Leonard Miller School of Medicine, University of Miami, Miami, USA

Present Address:
B. Goodwin
University of Colorado School of Dental Medicine, Aurora, USA

Introduction

The most common types of insect–plant interactions include cases in which insects are pollinators, thus providing a benefit to the plant, and cases in which insects are folivores, therefore imposing a cost to the plant. Sometimes these two types of interactions occur within a single insect species. For instance, in Southwestern USA, the jimsonweed *Datura wrightii* (Solanaceae) and the nocturnal sphinx moth *Manduca sexta* (Sphingidae) form a pollinator–plant and herbivore–plant association (Adler and Bronstein, 2004). Flowers of *D. wrightii*, which bloom over a single night, are pollinated by adult moths (Alarcon et al., 2008; Riffell et al., 2008), and plants serve as

food resources for the larvae (Mechaber and Hildebrand, 2000). Although *D. wrightii* plants are highly self-compatible (Raguso et al., 2003; Bronstein et al., 2009), fruit set is increased in the presence of pollinators (Bronstein et al., 2009), and plants can tolerate high levels of defoliation and quickly re-grow after herbivory (personal observations).

Female *M. sexta* oviposit, and larvae feed almost exclusively, on plants of the family Solanaceae such as native and cultivated species of tobacco (*Nicotiana sp.*), tomato, native jimsonweeds (*Datura sp.*), and other solanaceous plants (e.g., eggplant, pepper, and potato) (Madden and Chamberlin, 1945; Yamamoto and Fraenkel, 1960; Tichenor and Seigler, 1980; del Campo et al., 2001). Female moths rely almost exclusively on olfactory cues to locate and identify host plants for oviposition (Sparks, 1969, 1973; Ramaswamy, 1988b). In many plants, however, the profile of volatile organic compounds (VOCs) is affected by physiological and environmental factors (Loughrin et al., 1994; Holopainen and Gershenzon, 2010). For instance, plants respond to herbivory with changes in chemistry and physiology that make them more resistant to further damage, such as induction of toxic metabolites that can poison attacking herbivores or slow their growth (Karban and Baldwin, 1997; Baldwin and Preston, 1999; Kessler and Halitschke, 2009). Plants also use indirect defenses, i.e., synthesize and release complex blends of VOCs that attract natural enemies of the herbivores (De Moraes et al., 1998; Turlings et al., 1998a; Baldwin and Preston, 1999; Paré and Tumlinson, 1999; Dicke and van Loon, 2000; Halitschke et al., 2000; Schnee et al., 2006). These VOCs, which include monoterpenes, sesquiterpenes, and aromatic compounds (Paré and Tumlinson, 1999), are produced *de novo* (Paré and Tumlinson, 1997), systemically (De Moraes et al., 1998), and in part are released just after the onset of herbivory (>24-hr post-attack; Kessler and Baldwin, 2001). Some quickly produced herbivore-induced VOCs also are released after artificial damage of plants, e.g., the so-called "green-leaf" VOCs that are biosynthesized *via* the lipoxygenase/lyase pathway (Paré and Tumlinson, 1999; Halitschke et al., 2000; De Moraes et al., 2001; Kessler and Baldwin, 2001). Feeding by larvae induces specific patterns of hormonal signaling in the plant, accumulation of secondary metabolites (which provides direct and indirect defenses), and gene transcription (Halitschke et al., 2000; De Moraes et al., 2001; Adler et al., 2006; McCall and Karban, 2006). In principle, avoiding such "induced" plants should be advantageous for a gravid ovipositing female, because these plants are likely to host both older larvae that could compete with her offspring and natural enemies attracted by the induced VOCs (De Moraes et al., 1998). For instance, *M. quinquemaculata* moths avoid ovipositing on feeding-damaged tobacco plants (Kessler and Baldwin, 2001). Whether females avoid ovipositing on feeding-damaged plants also depend on factors such as the plant species and the amount of damage (Landolt, 1993; Anderson and Alborn, 1999; Rojas, 1999; Dicke and van Loon, 2000), and in certain insect species oviposition is deterred by the VOCs emitted by larval frass (Anderson et al., 1992; Xu et al., 2006).

We have shown previously that the *D. wrightii* flower odor attracts females of *M. sexta* for oviposition (Reisenman et al., 2010). Having thus identified an important component that mediates attraction in this insect–plant interaction, here we investigated whether female moths avoid ovipositing on plants damaged by larval feeding. Although females should avoid ovipositing on such plants, we hypothesized that the effect of herbivory on the female's oviposition behavior depends on the characteristics of the insect–plant interaction. In order to test this prediction, we used three different solanaceous host plant species having different relationships with *M. sexta*: (1) the perennial *D. wrightii*, which has a reciprocally beneficial association with *M. sexta* (Bronstein et al., 2009); (2) the annual *Solanum lycopersicum* (tomato), which has a negative association with *M. sexta*, as females oviposit on these plants but do not pollinate their nectar-less flowers; and (3) the annual *D. discolor*, which is both an oviposition and a nectar resource for females, although their flowers are not as attractive as those of *D. wrightii* (unpublished observations).

Induction of plant VOCs upon herbivory has been shown in *D. wrightii* and in several species of tomato (Kant et al., 2004; Hare, 2007; Kessler and Halitschke, 2009). We, therefore, investigated whether herbivory changes the vegetative VOC profile of *D. wrightii*, *D. discolor,* and *S. lycopersicum*. In particular, (±)-linalool is one of the most common herbivore-induced vegetative VOCs in many plant species (Turlings and Tumlinson, 1992; Geervliet et al., 1997; Paré and Tumlinson, 1997; Kessler and Baldwin, 2001), including *D. wrightii* (Hare, 2007) and several species of tomato (e.g., Vercammen et al., 2001; Kant et al., 2004; Kessler and Halitschke, 2009). Moreover, since we recently found that (+)-linalool and (-)-linalool have opposite effects (attraction vs. repellence, respectively) on the oviposition behavior of *M. sexta* (Reisenman et al., 2010), we analyzed the enantiomeric composition of linalool produced by herbivore-induced plants. Finally, we analyzed whether the *D. wrightii* floral VOCs that are crucial in mediating feeding by adult moths (and hence pollinator attraction) are altered in plants damaged by larval feeding.

Methods and Materials

Animals Manduca sexta were reared in the laboratory on artificial diet (Bell and Joachim, 1976) supplemented with cholesterol, sugar, wheat germ, and linseed oil, under a long day regime (17:7 L: D) at 25–26 °C and at least 25 % relative humidity. The use of laboratory-reared animals is justified by previous investigations showing that the foraging behavior of laboratory-reared and wild-caught animals was comparable (Ramaswamy, 1988a; Riffell et al., 2008). Moths for

experiments were selected as pupal stage 16–17 (stages according to Sanes and Hildebrand, 1976) and maintained in a high humidity environment until eclosion to the adult stage and until experiments were conducted. Adults were not fed. Feeding in the adult stage has been reported to increase oviposition rates (Sasaki and Riddiford, 1984; Adler and Bronstein, 2004).

Behavioral Experiments Dual-choice experiments were conducted in screened flight cages (2×2×2 m). In each experiment, a control (undamaged) and a test (damaged) plant were positioned 1.5 m apart. Female moths (1.5 d-old) were mated and tested individually during the first 2–2.5 hr of the scotophase the following night. Each moth was allowed to lay eggs during 10 min after taking flight. Thereafter, the eggs that were oviposited on any part of each plant were carefully removed and counted. Eggs laid by each moth were kept separately for 10 d in order to check whether they were fertile. Results from tests in which a moth laid less than 15 eggs, or cases in which more than 50 % of the eggs did not hatch after 10 d, were discarded. The positions of the damaged and undamaged plants were alternated between tests. In most cases, each plant pair was tested with one moth only. In a few cases, if a moth laid a small number of eggs, then that same plant pair was tested with another moth during the same night after carefully removing the eggs oviposited by the first moth. Thus, in all figures and throughout the text, the number of moths tested was roughly similar to the number of plant pairs tested. Damaged plants were discarded; undamaged plants were re-used only after at least 10 d.

Plants Three species were used: *S. lycopersicum* (tomato, variety: money maker), *D. wrightii,* and *D. discolor*. All plants were grown in a greenhouse from seeds in 2 gallon pots (in the case of *S. lycopersicum* and *D. wrightii*) or 1 gallon pots (in the case of *D. discolor*) containing soil (Sunshine mix #3), vermiculite (Therm-O-Rock), and 20-grit sand commercial grade in a 3:2:1 ratio. Plants were 2–4 mo old at the time of the experiments, and had no flowers. Plants from the same cohort were paired to be as similar as possible in terms of height, foliage, and leaf size. The plant within a pair that was subjected to feeding or mechanical damage was sufficiently bigger than the plant that was left intact, so both control and damaged plant had the same overall size and appearance during tests. Because in most cases each plant pair was tested with a single moth, the number of plant pairs prepared and tested was roughly similar to the number of moths tested. Plants were subjected to the following treatments (see summary Table 1):

(1) Feeding damage: Five to seven 3rd–4th instars were allowed to feed on a restricted portion of each plant (this was achieved by enclosing the branch with a mesh cage) for 4 d. Cages and larval frass, but not larvae, were removed before tests. By weighing a subset of representative plants of average size, we calculated that 11.1±1.2 % of leaf tissue (mean ± SE, N=11) was eaten. This value is similar to that reported to be effective to elicit herbivore-induced responses (e.g., Hare, 2007; Kessler and Halitschke, 2009). Plants of all 3 species were subjected to this treatment.

(2) Mechanical damage: Pieces of leaf material from a restricted portion of plants [of size similar to that used in (1)] were cut with scissors at fixed time intervals (3–5 times per day) during 4 d. Only *D. wrightii* and *S. lycopersicum* were subjected to this treatment. In addition, a fabric pattern wheel was rolled over leaves in a restricted portion of *S. lycopersicum* plants (2 times per day during 4 d) to mimic the wound produced by caterpillar feeding (Halitschke et al., 2000; Schittko et al., 2000); this treatment did not remove leaf tissue.

(3) Application of methyl jasmonate: This was applied to *D. wrightii* plants to activate the jasmonic acid signaling pathway, which in many systems, including *D. wrightii*, induces plant defense responses (Hare and Walling, 2006). Three microliters of methyl jasmonate (Sigma-Aldrich, catalog no. 392707) were mixed with 20 mg of lanolin paste and applied to the apical side of a young leaf as previously described (Halitschke et al., 2000; Kessler and Baldwin, 2001; Hare and Walling, 2006; McCall and Karban, 2006). Plants were treated with methyl jasmonate on d 1, 3, and 5, and were tested in the evening of the 6th day. Each control plant received an application of 20 mg of lanolin paste with 3 μl of water in the same time intervals as plants treated with methyl jasmonate. In order to test whether the smell of methyl jasmonate itself influences moth oviposition behavior, we conducted an additional test. For this, a test glass slide with three applications of 3 μl methyl jasmonate in 20 mg of lanolin paste was placed directly on the soil of an intact plant during the tests; for control, a slide with three applications of 3 μl of water in 20 mg of lanolin paste was placed on the soil of another intact plant also during tests. The slides were treated on d 1, 3, and 5, and the moths' response to these plants with these slides was tested on the 6th day. Prior to the tests, the glass slides were kept under the same conditions (i.e., in the same greenhouse) as non-damaged plants.

Not all of the above treatments were conducted with all the 3 plant species, as new assays were designed based on experimental outcomes. Table 1 summarizes the experimental series (plant treatments) conducted with the different species. In all cases, damaged and undamaged plants were kept in different greenhouses in order to avoid possible plant–plant communication and VOC induction in undamaged plants (Arimura et al., 2000; Baldwin et al., 2002).

Collection and Analysis of Plant Volatiles We collected and analyzed VOCs released by intact and feeding-damaged

Table 1 Oviposition assays (plant treatments) conducted with *Manduca sexta* and different plant species (indicated with an "X")

Treatment	Plant species		
	Datura wrightii	*Datura discolor*	*Solanum lycopersicum*
No damage	X		
Feeding-damage	X	X	X
Feeding-damage (larvae removed before tests)			X
No damage, larvae present during tests			X
Mechanical damage (leaf removal)	X		X
Mechanical damage (leaf punctures)			X
Induction by methyl jasmonate	X		
Methyl jasmonate odor during tests	X		

plants of the 3 species. For this, plants were damaged as described; all larvae were removed from feeding-damaged plants before VOC collection. To study whether floral VOCs change in feeding-damaged plants, *D. wrightii* plants with flower buds that would open in about 4 d were subjected to herbivory as described above. Because larvae had access to vegetative tissues only, the flowers themselves were not damaged by herbivores. Floral VOCs were collected in the night when a flower opened (each *D. wrightii* flower opens for one night only); this occurred after 4 d of larval feeding.

To collect VOCs, single branches from each plant were enclosed in transparent vinyl oven bags (Reynolds) and cinched at 500-ml (Hare, 2007; Riffell et al., 2008). An automated collection system (AVCS; Analytical Research Systems, Gainesville, FL, USA) was used to pull overnight (ca. 12 hr, except in the case of some experiments with *S. lycopersicum*, see below) headspace air through sorbent-cartridge traps at a flow rate of 1.9 L/min. Filtered air (1.9 L/min) was injected into the bags. Traps were constructed by packing 100 mg of Super Q adsorbent (Altech, mesh size 80–100) into borosilicate glass tubes (7 mm outer diam.) plugged with silanized glass wool. Trapped volatiles were eluted from cartridges using 400 μl of *n*-hexane and analyzed using gas chromatography with mass spectrometric detection (GC-MS). Eluted volatiles were stored at −80 °C until used. For analysis, 1 μl of sample was subjected to GC-MS using a system comprised of an HP 7890A GC and a 5975C Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA). Two GC columns (J&W Scientific, Folsom, CA, USA) were used: DB1 (30 m, 0.25 mm, 0.25 μm) and Chiral SilB (30 m, 0.25 mm, 0.25 μm), and helium was used as carrier gas at a constant flow of 1 cc/min. For the DB1 column, the initial oven temperature was 50 °C for 4 min, followed by a heating gradient of 10 °C/min to 220 °C, which was held isothermally for 6 min. For the Chiral SilB column (used to identify enantiomers of linalool), the initial oven temperature was 50 °C for 4 min, followed by a heating gradient of 2 °C/min to 120 °C, followed by 10 °C/min to 220 °C, and then held isothermally for 6 min. Chromatogram peaks were identified tentatively through use of the NIST mass spectral library (ca. 120,000 spectra) and verified by chromatography with authentic standards (when available) and essential oils (see Tables 2, 3, 4 and 5). Standards were obtained from Sigma-Aldrich (>98 % purity except for *(E)*-ß-ocimene, which was 85 % pure). The peak area for each compound of interest was quantified using either an internal standard (*n*-nonyl acetate) or through a five-point standard (0.1 ng to 1 mg) of the synthetic odorants and expressed in units of ng/h and as percentage of total emissions. Peak areas for each compound were integrated using ChemStation software (Agilent Technologies, Palo Alto, CA, USA) and are presented in terms of ng/h. Since linalool could be detected only in trace amounts in *S. lycopersicum* plants, we conducted additional collections from whole plants (2 intact and 2 damaged) during 48 hr under a 12:12 L:D cycle; traps were eluted in this case with 600 μl of hexane. VOCs were analyzed according to non-metric Multidimensional Scaling (NMDS) based on the presence and absence of volatiles in the headspace samples. Analyses were performed using Matlab (v7.12; Mathworks Inc., Natick, MA USA).

Statistical Analyses Sign tests were conducted to test whether females laid more eggs on one of the two plants of a pair (Zar, 1999). *Chi-square* heterogeneity tests were conducted to evaluate whether the behavior of moths within an experimental group was homogeneous (Sokal and Rohlf, 1995). In order to illustrate variability in the oviposition preference of individual females, we calculated an oviposition index for each female as: (number of eggs oviposited in the intact plant–number of eggs oviposited in the damaged plant)/(number of eggs oviposited in the intact plant + number of eggs oviposited in the damaged plant). Oviposition indexes >0 and <0, respectively, indicate preference for the intact or the damaged plant, and oviposition indexes ≈ 0 indicate no preference for either plant.

Because *chi-square* heterogeneity tests were significant in all cases, we conducted *chi-square* goodness of fit tests to

Table 2 GC-MS analysis of the *Datura wrightii* volatiles from feeding-damaged and undamaged plants (N=7 plants per treatment)

	Treatment	
	Undamaged	Damaged
Emission rate (ng/h)*	162.2 (53.30)	424.4 (150.97)
Compound (ng/h)		
3-Hexanone	6.1 (1.8)	4.1 (1.0)
2-Hexanone	9.6 (3.1)	16.8 (4.1)
3-Hexanol	6.0 (1.7)	6.5 (2.0)
2-Hexanol	3.5 (0.8)	4.7 (1.6)
(*Z*)-2-Methyl-4-Hexen-3-ol	0.8 (0.3)	1.8 (1.0)
5-Methyl-1-heptanol	2.8 (1.2)	1.7 (0.1)
Nonanal	6.0 (1.4)	3.2 (1.2)
Decanal	1.4 (0.1)	1.3 (0.2)
Tetradecane	1.9 (0.1)	2.2 (0.2)
Hexadecane	3.4 (0.8)	2.5 (0.2)
Octadecane*	0.8 (0.1)	2.1 (0.4)
Eicosane*	3.5 (0.2)	2.6 (0.1)
Styrene	2.0 (0.8)	1.44 (0.1)
Benzaldehyde*	tr.	4.7 (1.3)
Acetophenone*	0.1 (0.1)	4.1 (3.7)
Methyl salicylate*	4.4 (0.1)	7.3 (1.7)
Benzothiazole*	tr.	1.44 (0.12)
p-Cresol*	0.6 (0.4)	3.5 (0.5)
3-Carene	0.4 (0.2)	1.0 (0.1)
α-Pinene*	tr.	1.1 (0.1)
***(D)*-Limonene**	4.3 (0.5)	3.1 (0.8)
(*E*)-β-Ocimene*	1.3 (0.5)	11.9 (0.4)

Shown are the mean values and SE (in parentheses) in ng/h

*t-tests: $P<0.05$ in all cases

tr: denotes trace levels of the volatile

VOCs in bold are those identified by authentic standards and/or essential oils

Table 3 GC-MS analysis of the *Datura discolor* volatiles from feeding-damaged (N=6) and undamaged plants (N=5)

	Treatment	
	Undamaged	Damaged
Emission rate (ng/h)*	111.7 (45.5)	438.7 (201.9)
Compound (ng/h)		
3-Hexanone	0.2 (0.2)	0.9 (0.2)
3-Hexanol*	0.3 (0.2)	1.7 (0.6)
2-Hexanone	2.6 (0.4)	2.0 (0.6)
2-Methyl-2-hexene	0.7 (0.5)	1.0 (0.2)
(Z)-3-Hexen-1-ol	1.2 (0.3)	1.42 (0.4)
Nonanal*	1.1 (0.4)	7.3 (3.9)
Decanal	0.3 (0.1)	0.2 (0.1)
2-Tetradecene*	0.4 (0.2)	3.4 (1.1)
Tetradecane	0.2 (0.1)	0.2 (0.1)
Pentadecane	2.7 (1.1)	2.6 (0.3)
Hexadecane	2.9 (0.9)	1.3 (0.3)
Octadecane*	tr.	0.6 (0.2)
Styrene*	0.3 (0.1)	1.2 (0.4)
Benzaldehyde*	tr.	2.3 (0.9)
Acetophenone*	0.6 (0.4)	2.4 (1.2)
Methyl salicylate*	tr.	1.3 (1.0)
(D)-Limonene	1.9 (1.0)	4.4 (1.2)
(E)*-ß-Caryophyllene	2.9 (1.1)	8.0 (1.5)

Shown are the mean values and SE (in parentheses) in ng/h

*t-tests: $P<0.05$ in all cases

tr: denotes trace levels of the volatile

VOCs in bold are those identified by authentic standards and/or essential oils

evaluate whether the distribution of eggs by each moth was different from the expected 50 % random distribution (Sokal and Rohlf, 1995). Thus, the oviposition preference of each female could be statistically characterized as random (no preference for either plant), preference for the intact plant, or preference for the damaged plant (right panels in Figs. 1, 2, 3 and 4). Because many *chi-square* tests were conducted, we used the false discovery rate method to control the proportion of false positives (Benjamini and Hochberg, 1995). Individual P-values were compared with a $(i/m).Q$ threshold, where i is the i^{th} observed P-value (ordered from smallest to largest), m is the total number of tests in each experimental series, and Q is the assigned false discovery rate (0.05). *Kruskal-Wallis* ANOVA or *Mann–Whitney U* tests were used to compare the total number of eggs laid by females across plant species and/or treatments (Zar, 1999). In all cases differences were considered significant if $P<0.05$.

Results

Oviposition Behavior of M. sexta on Feeding-Damaged Plants When *M. sexta* females were presented with two intact plants, eggs were distributed randomly between them (*sign test,* Z=0.61, N=25 moths, $P>0.5$; Fig. 1a). However, the oviposition preference of females changed, depending on the plant species, when they were presented with an intact and a feeding-damaged plant (Fig. 1). As a group, moths avoided ovipositing on feeding-damaged tomato plants (*sign test,* Z=3.1, N=38 moths, $P<0.005$, Fig. 1d), but not on feeding-damaged *D. wrightii* plants (*sign test,* Z=1.22, N=43 moths, $P>0.1$; Fig. 1b). Although as a group females seemed to show a relatively strong preference for ovipositing on intact over feeding-damaged *D. discolor* plants (61±5 % of the eggs were

Table 4 GC-MS analysis of the *Solanum lycopersicum* volatiles released from feeding-damaged and undamaged plants (N=8 per treatment)

	Treatment	
	Undamaged	Damaged
Emission rate (ng/h)*	427.6 (148.9)	963.3 (245.4)
Compound (ng/h)		
Ethyl butyrate*	1.9 (1.5)	20.9 (2.0)
(Z)-3-Hexenal	4.2 (1.9)	35.3 (26.2)
1,3,5-cycloheptatriene	17.7 (9.8)	261.5 (150.8)
Nonanal	16.2 (3.2)	21.5 (4.2)
Decanal	9.0 (6.4)	26.0 (14.3)
Pentadecane	2.8 (1.9)	10.9 (5.3)
Hexadecane	6.9 (3.2)	7.3 (1.2)
Heptadecane	15.0 (3.9)	14.3 (6.3)
Octadecane	5.9 (2.3)	15.6 (7.0)
Acetophenone*	0.6 (0.3)	358.9 (344.1)
Methyl salicylate	3.6 (1.6)	10.2 (4.8)
Benzothiazole*	2.5 (1.6)	36.9 (21.3)
α-Pinene	21.8 (10.5)	51.3 (15.0)
2-Carene	141.8 (64.7)	113.5 (39.3)
α-Thujene	156.9 (138.0)	40.5 (25.5)
α-Terpinene	6.6 (4.6)	47.1 (42.4)
***(D)*-Limonene**	70.2 (33.7)	344.8 (237.5)
β-Phellandrene	363.5 (177.1)	460.6 (102.9)
***(E)*-ß-Caryophyllene**	17.2 (12.8)	65.4 (24.7)
Humulene*	2.5 (2.5)	13.6 (4.0)
Enantiomeric composition (ng/h)–48 hr collection[b]		
(-)-Linalool	0.8 (0.5)	14.1 (3.2)
(+)-Linalool	0.7 (0.4)	1.2 (0.8)
Enantiomeric composition (ng/h)–12 hr collection[b]		
(-)-Linalool	nd	tr. (<0.1)
(+)-Linalool	nd	nd

Shown are the mean values and SE (in parentheses) in ng/h

*t-tests: $P<0.05$ in all cases

tr. denotes trace levels of the volatile

nd denotes not detected

[a] Using a DB5MS column

[b] Using a Cyclosil-B column

VOCs in bold are those identified by authentic standards and/or essential oils

Table 5 GC-MS analysis of the *D. wrightii* floral volatiles from feeding-damaged and undamaged plants (N=8 flowers per treatment)

	Treatment	
	Undamaged	Damaged
Emission rate (ng/h)	233.5 (39.2)	238.5 (92.9)
Compound (%)[a]		
β-Myrcene	0.4 (0.1)	0.5 (0.1)
Benzaldehyde	0.6 (0.3)	0.12 (0.0)
Benzyl alcohol	22.4 (7.6)	12.6 (3.6)
***E*-β-Ocimene**	37.7 (6.2)	34.8 (4.3)
Methyl benzoate*	0.4 (0.2)	1.2 (0.2)
(±)-Linalool	0.6 (0.2)	0.7 (0.1)
Methyl salicylate	1.3 (0.6)	2.1 (0.5)
Nerol	2.5 (1.2)	3.4 (0.6)
Geraniol*	31.5 (3.5)	41.3 (2.4)
***(E)*-β-Caryophyllene**	0.2 (0.1)	0.1 (0.0)
α-Farnesene	2.4 (1.4)	3.2 (1.1)
Enantiomeric composition (%)[b]		
(-)-Linalool	56.7 (1.1)	57.9 (1.8)
(+)-Linalool	43.3 (1.1)	42.1 (1.8)

Shown are the mean values and SE (in parentheses) in ng/h

*t-tests: $P<0.05$ in all cases

[a] Using a DB5MS column

[b] Using a Cyclosil-B column

VOCs in bold are those identified by authentic standards and/or essential oils

oviposited on intact plants), the differences were not statistically significant (*sign test,* Z=1.1, N=20 moths, $P>0.1$; Fig. 1c). In all cases, however, the behavior of females was highly heterogeneous (*chi-square heterogeneity tests*; $P>0.05$ in all cases). While many females had oviposition indexes close to zero when they were offered two intact plants (Fig. 1a, middle panel), the distribution of oviposition indexes changed when moths were tested with intact vs. feeding-damaged plants (Fig. 1b–d, middle panels). When presented with two intact plants, 84 % of females distributed eggs randomly (*chi-square tests*, $P>0.05$). In experiments where an intact vs. a feeding-damaged plant was offered, 56–70 % of the moths, depending on the plant species, distributed eggs randomly (*chi-square tests*, $P>0.05$; Fig. 1b–d, right panels). In the case of experiments with tomato and *D. discolor*, 24 % and 30 % of females, respectively, laid significantly more eggs in the intact plant, while only 8 % and 0 % of females laid more eggs in the damaged plants (*chi-square tests*, in all cases $P<0.05$). In contrast, in the case of experiments with *D. wrightii*, similar percentages of moths laid more eggs on either the intact or the feeding-damaged plant (26 % and 19 %, respectively; $P<0.05$ in both cases). Regardless of the plant species, we found that females laid on average the same total number of eggs (46.8± 1.9, mean ± SE, N=126 moths; *Kruskal-Wallis ANOVA,* H= 2.82, df=3, $P>0.1$). This demonstrates that females actively preferred (or not) one of the two plants rather than ovipositing fewer eggs overall.

Because females avoided ovipositing on tomato plants, both at the population and individual scale, we conducted additional tests in which larva were removed from feeding-damaged plants just before the tests. We found that as a group

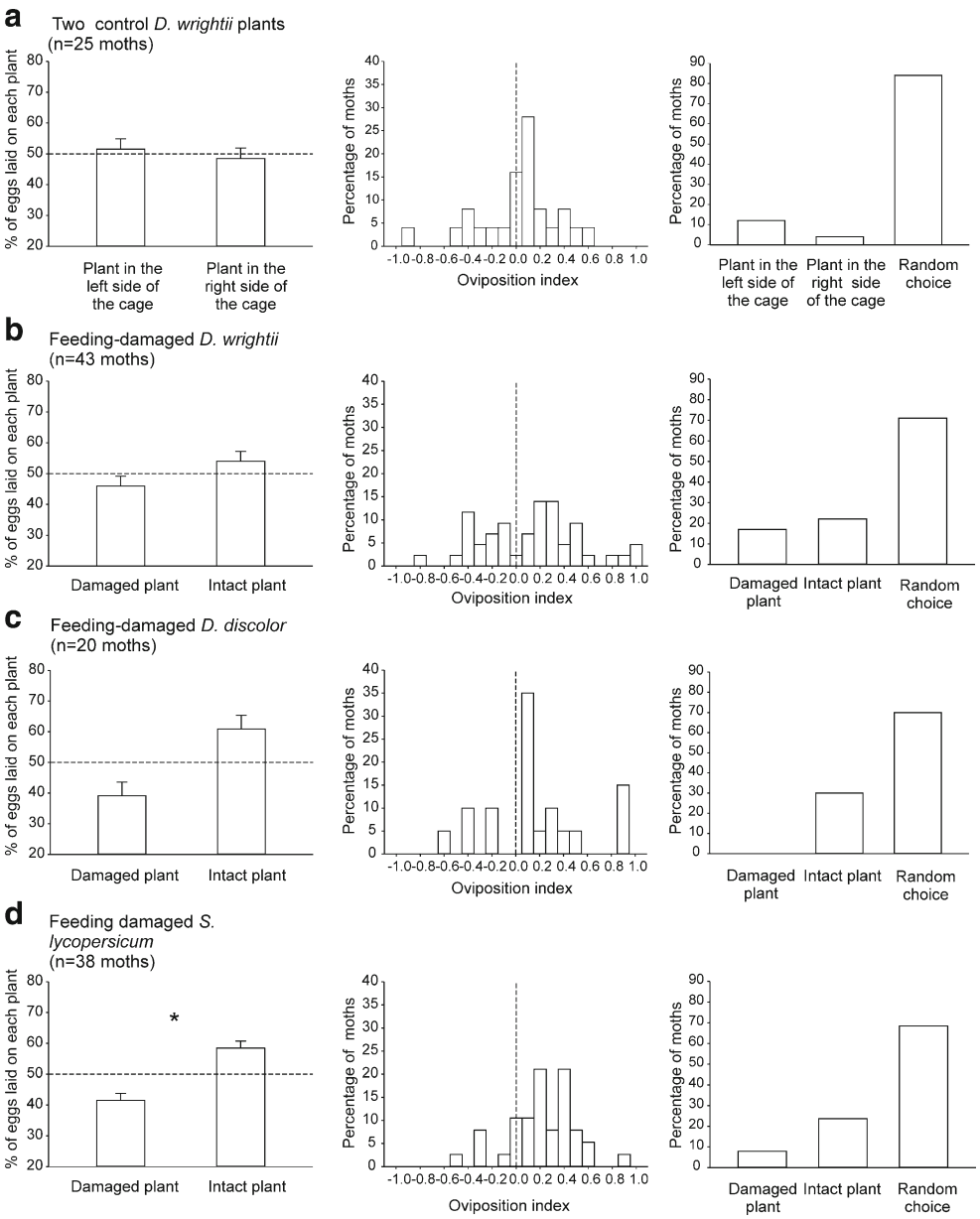

Fig. 1 The oviposition behavior of *Manduca sexta* towards feeding-damaged plants depends on the plant species. (**a**) Each female was allowed to oviposit on 2 intact (undamaged) plants of *Datura wrightii*, (**b**) an intact and a feeding-damaged plant of *D. wrightii*, (**c**) an intact and a feeding-damaged plant of *D. discolor*, or (**d**) an intact and a feeding-damaged plant of *Solanum lycopersicum*. Each plant pair was used only once. The graphs on the left show the mean percentage ± SE of eggs laid on each plant. The line at the 50 % level indicates no preference for either plant. The *asterisk* indicate statistically significant differences (*sign test*, $P<0.005$). Middle panels: distribution (percentage of females) of individual preference indexes in 0.1 bins. Oviposition indexes >0 and <0 indicate preference for the intact or the damaged plant, respectively; the *vertical line* at 0 indicates no preference for either plant. Right panels: percentage of moths that laid significantly more eggs on the intact plant, on the damaged plant, or that distributed eggs randomly

females distributed eggs randomly between the two plants (*sign test,* $Z=0.97$, $N=17$ moths, $P>0.1$, Fig. 2a). Although a relatively larger proportion of moths showed slightly positive oviposition indexes (Fig. 2a, middle panel), 61 % of the moths distributed eggs randomly (*chi-square tests*, $P>0.05$), and similar number of moths laid more eggs in either the intact or the damaged plant (22 % and 17 %, respectively; $P<0.05$ in all cases, Fig. 2a, right panel). In light of these results, we tested whether the sole presence of larvae on intact plants affected the oviposition behavior of females. In experiments in which an intact vs. an intact plant with larvae added were offered, we found that females distributed eggs randomly between plants (*sign test,* $Z=0.68$, $N=15$ moths, $P>0.1$, Fig. 2b), with most moths showing oviposition indexes close to zero (Fig. 2b, middle panel). Eighty three-percent of females distributed eggs randomly between the two plants (*chi-square tests*, $P>0.05$), and 17 % of moths laid more eggs in the plant without larvae ($P<0.05$). As before, females in these two experimental groups (Fig. 2) laid on average the same number of eggs (63.5±5, mean ± SE, $N=32$ moths; *Mann–Whitney U test,* $U=103.5$, $N=15$, 17 moths, $P>0.1$).

Oviposition Behavior of M. sexta on Mechanically Damaged Plants When females were exposed to an intact and a mechanically damaged plant, their oviposition preferences again depended on the species. Females distributed eggs randomly between intact and mechanically damaged *D. wrightii* plants (*sign test,* $Z=0.35$, $N=20$ moths, $P>0.5$; Fig. 3a), but they strongly avoided ovipositing on mechanically damaged tomato plants (*sign test,* $Z=2.83$, $N=19$ moths,

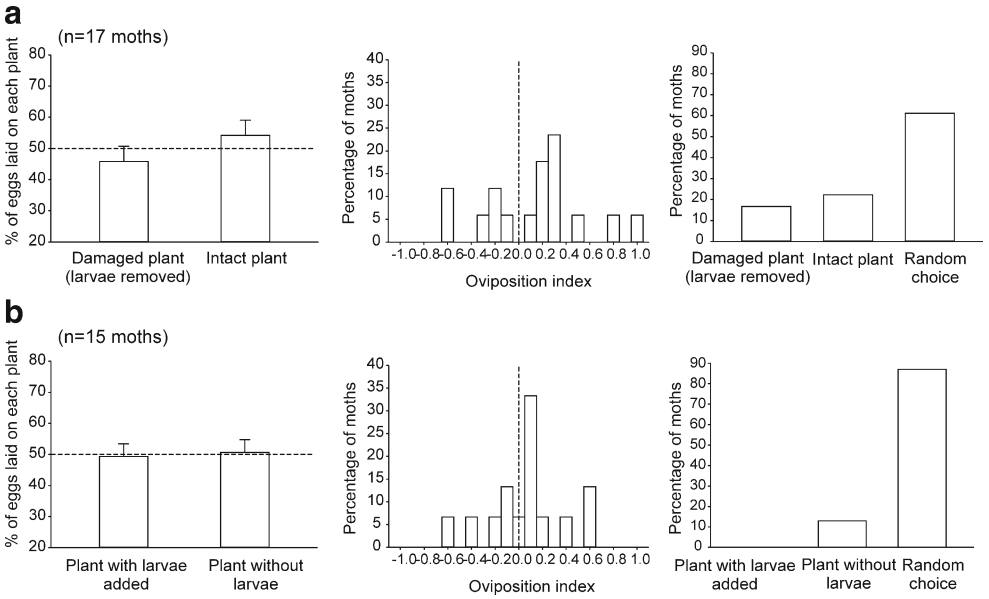

Fig. 2 Effects of the presence of conspecific larvae on plants in the oviposition behavior of *Manduca sexta*. (**a**) Each female was allowed to oviposit on an intact and a feeding-damaged tomato plant, but larvae were removed from the damaged plants just before tests. (**b**) Each female was allowed to oviposit on 2 intact tomato plants, but larvae were added to 1 of the plants just before tests. Each plant pair was used only once. The graphs on the left show the mean percentage ± SE of eggs laid on each plant. The line at 50 % indicates no preference for either plant. Middle panels: distribution (percentage of females) of individual preference indexes in 0.1 bins; the *vertical line* at 0 indicates no preference for either plant. Right panels: percentage of moths that laid significantly more eggs (**a**) on the intact plant, on the feeding-damaged plant, or (**b**) on the plant with added larvae, or that distributed eggs randomly

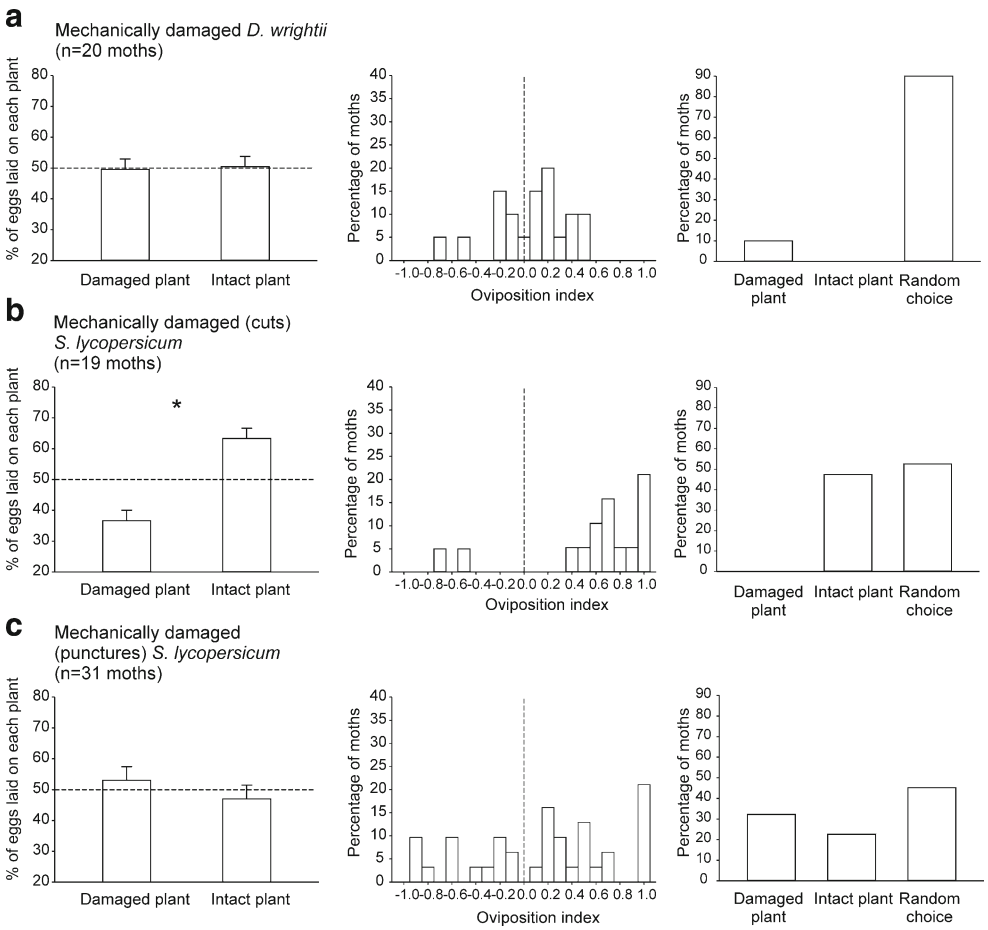

Fig. 3 The oviposition behavior of *Manduca sexta* towards mechanically damaged plants depends on the plant species. Each female was allowed to oviposit on an intact and a mechanically damaged plant of (**a**) *Datura wrightii* or (**b**–**c**) *Solanum lycopersicum*. (**b**) *S. lycopersicum* plants were damaged by cutting small pieces of leafs with scissors or (**c**) by inflicting small punctures with a fabric pattern at fixed time intervals. Each plant pair was used only once. The graphs on left show the mean percentage ± SE of eggs laid on each plant. The line at the 50 % indicates no preference for either plant. The *asterisks* indicate statistically significant differences (*Sign test*, $P<0.005$). Middle panels: distribution (percentage of females) of individual preference indexes in 0.1 bins; the *vertical line* at 0 indicates no preference for either plant. Right panels: percentage of moths that laid significantly more eggs on the intact plant, on the mechanically damaged plant, or that distributed eggs randomly

$P<0.05$; Fig. 3b). Females did not avoid ovipositing on tomato plants that were mechanically damaged by producing small punctures with a fabric pattern (*sign test,* $Z=0.36$, $N=31$ moths, $P>0.5$, Fig. 3c). Again, in all cases, the behavior of females was highly heterogeneous (*chi-square heterogeneity tests*; $P>0.05$ in all cases; Fig. 3, middle panels). Ninety-percent of females distributed eggs randomly in experiments with *D. wrightii* plants (*chi-square tests*, $P>0.05$, Fig. 3a, right panel). In contrast, in experiments with tomato, 47 % of the moths laid more eggs on intact plants *(*$P<0.05$), and 53 % distributed eggs randomly *(*$P>0.05$) (Fig. 3b, right panel). These differences in the proportion of females choosing intact vs. damaged tomato plants were not observed when plants were damaged with the fabric pattern (Fig. 3c, right panel). Again, we found that females laid on average the same total number of eggs regardless of the species or treatment (49.9±2.6, mean ± SE, $N=70$ moths; *Kruskal-Wallis ANOVA,* $H=1.92$, $df=2$, $P>0.1$).

Oviposition Behavior of M. sexta on Methyl Jasmonate Induced D. wrightii Plants Given that females did not avoid ovipositing on either feeding- or mechanically-damaged *D. wrightii* plants, we investigated whether application of methyl jasmonate, an elicitor of defense responses in many systems including *D. wrightii* (Hare and Walling, 2006), was able to induce the release of oviposition deterrent VOCs. Although 58.1±7.8 % (mean ± SE) of the eggs were oviposited on intact plants, differences were not statistically significant (*sign test,* $Z=1.48$, $N=29$ moths, $P>0.1$; Fig. 4a). However, most moths (41 %) laid more eggs on the intact plant (*chi-square tests*, $P<0.05$; Fig. 4a, right panel). The rest of the moths either distributed eggs randomly (38 %) or laid more eggs on treated plants (21 %). In tests in which just the odor of methyl jasmonate was offered in combination with an intact plant (but the plant itself was not treated), females distributed eggs randomly (*sign test,* $Z=0.22$, $N=20$ moths, $P>0.5$; Fig. 4b). Furthermore, oviposition indexes were distributed along the spectrum of positive and negative values (Fig. 4b, middle panel). Most moths (55 %) distributed eggs randomly (*chi-square tests*, $P>0.05$), and similar proportions of females preferred the plant with the odor or the odorless plant (25 and 20 %, respectively; $P<0.05$; Fig. 4b, right panel). As before, females laid on average the same total number of eggs regardless of the treatment (45.3±2.7, mean ± SE, $N=49$; *Mann–Whitney U test,* $U=222.5$, $N=20$, 27 moths; $P>0.1$).

Analysis of the VOCs Released by Vegetative Tissues of Damaged Plants It has been shown that herbivory induces the release of VOCs in several systems and that herbivory-induced plants receive fewer eggs from *M. quinquemaculata* moths (Kessler and Baldwin, 2001). We thus analyzed the VOCs released by vegetative tissues of intact and larva-damaged plants of *D. wrightii*, *D. discolor,* and *S. lycopersicum*. Overall, 18–22 different compounds were detected in the headspace of each plant species *(*$N=22$ in *D. wrightii*, $N=18$ in *D. discolor*, and $N=22$ in *S. lycopersicum*). Six compounds (methyl salicylate, nonanal, decanal, octadecane, acetophenone, and *(D)*-limonene) were released by intact plants of all three species (Tables 2, 3 and 4). Six other compounds (3-hexanone, 3-hexanol, tetradecane, hexadecane, styrene, and benzaldehyde) were released by intact plants of both *D. wrightii* and *D. discolor* (Tables 2 and 3). In intact plants, emission rates were the highest in *S. lycopersicum* plants (ca. 423 ng/hr, Table 4), followed by *D. wrightii* (ca. 162 ng/h, Table 2) and *D. discolor* (ca. 112 ng/h, Table 3). Damage by *M. sexta* larvae significantly increased the total emission of VOCs 2.2–3.9 times, depending on the plant species (Tables 2, 3 and 4, Fig. 5). Some compounds, which were undetectable or detected at trace levels in undamaged plants, were produced by vegetative tissues of larva-damaged plants of *D. wrightii* and *D. discolor*: benzaldehyde in both plant species (Tables 2 and 3); benzothiazole and α-pinene in *D. wrightii* (Table 2), and octadecane and methyl salicylate in *D. discolor* (Table 3). In addition, damage by larvae led to a significant increase of the emission of acetophenone in all species (Tables 2, 3 and 4); of *p*-cresol, α-pinene, and *(E)*-ß-ocimene in *D. wrightii* (Table 2); of nonanal, 2-tetradecene, octadecane, and *(E)*-ß-caryophyllene in *D. discolor* (Table 3); and of ethyl butyrate, 1,3,5-cycloheptatriene, benzothiazole, *(E)*-ß-caryophyllene, and (-)-linalool in *S. lycopersicum* (Table 4). The components that increased their emission rates the most in feeding-damaged plants were *(E)*-ß-ocimene in *D. wrightii* (9.1 times, Table 2); and acetophenone (628 times) and (-)-linalool (17.6 times) in *S. lycopersicum* (in this species the emission of three other components increased >10 times in larva-damaged plants; Table 4). Finally, we found that feeding damage significantly increased the emission of (-)-linalool (but not of (+)-linalool) in *S. lycopersicum* (Table 4).

Analysis of VOCs Released by Floral Tissues of Herbivore-Induced D. wrightii Plants Because *D. wrightii* floral odorants attract adult *M. sexta* for feeding (Riffell et al., 2008), pollinator attraction could be compromised if floral VOCs are altered in feeding-damaged plants. In order to evaluate this possibility, we analyzed if herbivory changed floral VOCs, focusing on the floral VOCs that are important to mediate feeding by adult *M. sexta* (Riffell et al., 2008; Reisenman et al., 2010). Damage by *M. sexta* larvae did not significantly increase the total emission of floral VOCs (Table 5). However, out of 11 compounds analyzed, feeding damage significantly increased the emission rate of two components, methyl benzoate (~3 times) and geraniol (1.3 times, Table 5). Although geraniol participates in mediating feeding by adult moths, none of the three floral VOCs that

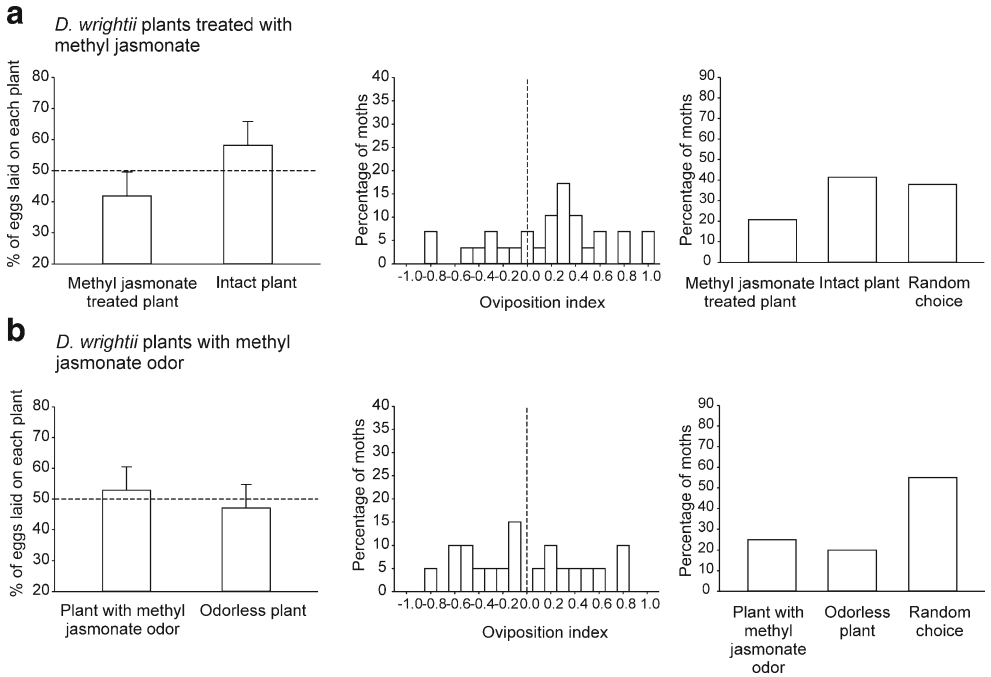

Fig. 4 *Manduca sexta* females do not avoid ovipositing on methyl jasmonate treated *D. wrightii* plants. (**a**) Each female was offered an intact plant and a plant that was treated with methyl-jasmonate or (**b**) 2 intact plants, one of which was combined with just the odor of methyl jasmonate during tests. Each plant pair was used only once. Shown are the mean percentage ± SE of eggs laid on each plant. The line at the 50 % level indicates no preference for either plant. Middle panels: distribution (percentage of females) of individual preference indexes in 0.1 bins; the *vertical line* at 0 indicates no preference for either plant. Right panels: percentage of moths that laid significantly more eggs (**a**) on the intact plant, on the chemically-induced plant or (**b**) on the plant with methyl jasmonate odor, or that distributed eggs randomly

are necessary and sufficient to mediate feeding behavior *by M. sexta* males (benzaldehyde, benzyl alcohol, and (±)-linalool; Riffell et al., 2009) increased their emission rates in feeding-damaged plants.

Discussion

We found that female *M. sexta* avoid ovipositing on tomato plants damaged by con-specific herbivores and to a certain

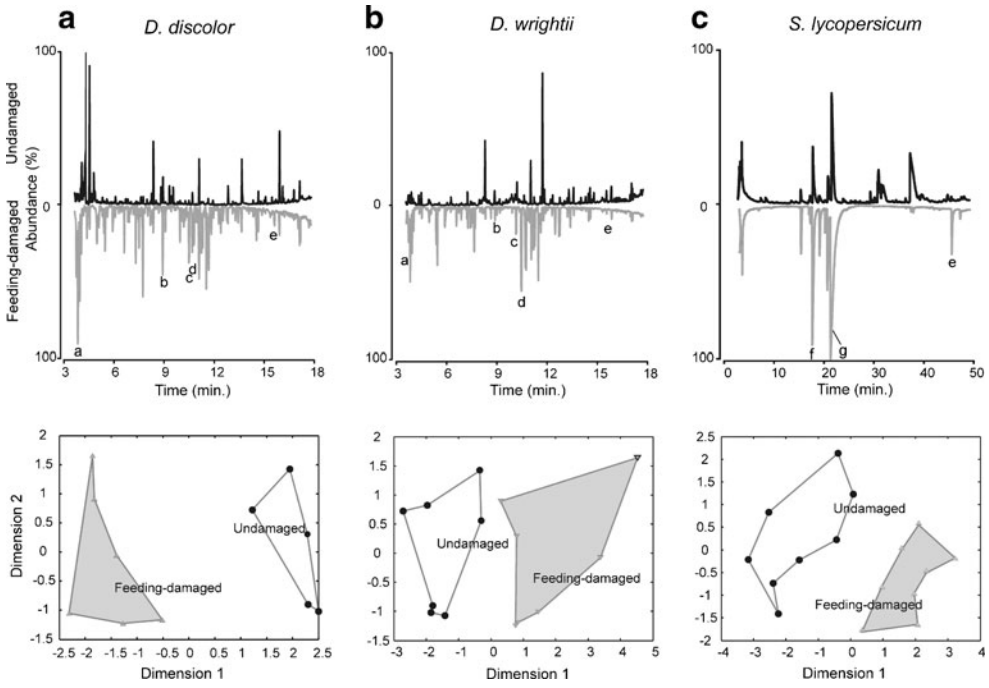

Fig. 5 VOC profiles of feeding-damaged and undamaged plants. Ion chromatograms (top) and NMDS plots (bottom) of the (**a**) *Datura discolor,* (**b**) *D. wrightii*, and (**c**) *Solanum lycopersicum* vegetation headspace of feeding-damaged (*gray, triangles*), and undamaged (*white, circles*) plants. Constituents of the vegetative headspace include 2-hexanone (**a**), *(D)*-limonene (**b**), nonanal (**c**), (*E*)-β-ocimene (**d**), *(E)*-ß-caryophyllene (**e**), carene (**f**), and β-phellandrene (**g**). The chromatogram for *S. lycopersicum* was from a GC-MS run on a chiral column. NMDS plots (bottom) are based on a presence/absence tentatively identified volatiles from the vegetative headspace in the different treatments (feeding-damaged, undamaged)

extent on feeding-damaged *D. discolor* plants, but did not avoid ovipositing on *D. wrightii* plants damaged by larval feeding (Fig. 1). Females also avoided ovipositing on mechanically damaged tomato plants (Fig. 3b), and to a certain degree, on *D. wrightii* plants treated with methyl jasmonate (Fig. 4), an elicitor of plant defenses. In all cases, the behavior of females within a group was highly heterogeneous (Figs. 1, 2, 3 and 4, middle and left panels). It is likely that these distinct behavioral preferences towards different hosts are caused by between-plant species differences in the production and emission of herbivore-induced VOCs.

Larval feeding damage of plants significantly changed the composition and quantity of VOCs released by vegetative tissues (Tables 2, 3 and 4, Fig. 5). Feeding-damaged plants produced VOCs that have been shown to be signal molecules in other plant systems. For instance, methyl salicylate and benzothiazole also have been shown to be emitted by damaged lima beans (Bruin et al., 1992; Mithöfer et al., 2005) and ash leaf maple plants (Ping et al., 2001a, b), respectively. In particular, methyl salicylate is a common VOC emitted by damaged plants that participates in interplant airborne signaling. Moreover, we found that levels of acetophenone were increased in the headspace of all three plant species (Tables 2, 3 and 4), which may indicate an increase in secondary defensive compounds, since phenols are a common defense in vegetation and floral tissues (Delvas et al., 2011). Similarly, the increases in the emission of certain terpenoids (e.g., α-pinene, *(E)*-ß-ocimene, and *(E)*-ß-caryophyllene) in the headspace of feeding-damaged plants (Tables 2, 3 and 4) are common in many plant species, and have been shown to be attractive to parasitic wasps (Turlings et al., 1995).

Interestingly, we found that the emission of (-)-linalool (but not (+)-linalool) was increased in feeding-damaged tomato plants (Table 4). Because this odorant repels *M. sexta* for oviposition (Reisenman et al., 2010), these results suggest that (-)-linalool, alone or together with other induced VOCs (Table 4), might mediate the observed oviposition avoidance of feeding-damaged tomato plants (Fig. 1d). Avoidance of plants releasing increased rates of (-)-linalool would be advantageous for gravid females, as it has been shown that (±)-linalool is involved in plant defense and can attract generalist predators that feeds upon *M. sexta* eggs (Kessler and Baldwin, 2001). It would be interesting to study whether this effect (attraction of egg predators) is enantiospecific.

It is possible that other induced VOCs, in addition to (-)-linalool, contribute to mediate avoidance of feeding-damaged plants. In *D. wrightii*, feeding by *Lema daturaphila* (the main herbivore of this plant in southern California) or application of methyl jasmonate increased the production of several VOCs, including *(E)*-ß-caryophyllene, *(Z)*-3-hexenyl acetate, (±)-linalool, and ß-selinene (Hare, 2007). We did not find any of these components in *D. wrightii* plants damaged by *M. sexta* larvae (Table 2). The differences in VOC emissions from *D. wrightii* plants damaged by *L. daturaphila* and *M. sexta* may be due to herbivore-specific induction of VOCs. Another parameter that might explain these results is that VOC emission follows a daily rhythm (e.g., Loughrin et al., 1994; De Moraes et al., 2001). While Hare (2007) collected VOCs over a 24-hr-period, we collected VOCs during the scotophase only, that is when ovipositing female *M. sexta* are active (for the chiral analysis we conducted two additional confirmatory experiments in which induced VOCs were collected during 48 hr because (-)-linalool was detected at trace levels in 2 out of 7 nighttime collections). This is important, as it has been shown that night-exclusive herbivore-induced tobacco VOCs are repellent to ovipositing moths (De Moraes et al., 2001). Furthermore, application of methyl jasmonate in *Nicotiana attenuata* (tobacco) or insect damage in cotton increases (±)-linalool emission during the day only (Paré and Tumlinson, 1997; Halitschke et al., 2000), which coincides with the period during which the natural enemies of the herbivores are active. Previous studies have shown that (±)-linalool emission is increased in herbivore-induced tomato plants (Kant et al., 2004; Kessler and Halitschke, 2009), and that a monoterpene synthase that produces (-)-linalool is induced by insect damage (van Schie et al., 2007). However, it is not clear whether linalool emission changes according to the light–dark cycle in tomato plants. Thus, the possibility that (-)-linalool also is released during the day by tomato plants remains to be investigated. If that were the case, it could be proposed that (-)-linalool has two distinct functions, one as a plant defense, attracting the natural enemies of the herbivores (Dicke et al., 1990), and the other as an oviposition repellent (Reisenman et al., 2010), helping moths to distinguish between intact and infested plants.

Interestingly, we found that the presence of larvae on tomato was necessary but not sufficient to repel moths from oviposition. Neither feeding-damaged plants from which larvae were removed, nor undamaged plants with larvae added, were strongly avoided by ovipositing females (Fig. 2). In many cases, females avoid ovipositing on host-plants already occupied (Rojas, 1999; see Almohamad et al., 2010), which has the obvious advantage of increasing the survival of their offspring. Most commonly, chemical cues derived from eggs, larvae, or larval tracks have been implicated in this behavior (reviewed by Almohamad et al., 2010). Importantly, in our experiments, we excluded the possibility that larval frass caused oviposition avoidance (Anderson et al., 1992; Xu et al., 2006). Also, females did not avoid ovipositing on herbivore-induced *D. wrightii* plants (which had larvae present during tests, Fig. 1b), which supports the finding that neither larvae "*per se*" nor any remaining larval frass may mediate oviposition

avoidance. Further experiments are necessary to unravel which type/s of larva-derived cues is/are involved in mediating oviposition avoidance.

We found that females strongly avoided ovipositing on wounded tomato plants (damaged by cutting pieces of leafs, but not by producing punctures, Fig. 3b–c), but not on mechanically damaged *D. wrightii* plants (Fig. 3a). As with feeding-damaged plants, differences across species have been reported previously. For instance, the moth *Mamestra brassicae* also oviposits less on mechanically damaged tomato plants (Rojas, 1999), but *Spodoptera littoralis* moths do not avoid mechanically damaged cotton plants (Anderson and Alborn, 1999). Typically (but not always, see Baldwin et al., 2002), mechanical damage induces an immediate release of VOCs that are chemically different from those induced by herbivory, and they include six-carbon alcohols, aldehydes, and acetates (Turlings et al., 1998b).

Because females did not avoid ovipositing on herbivore-induced *D. wrightii* plants, we treated plants with methyl jasmonate. This hormone has been shown to induce direct responses in *D. wrightii* (Hare and Walling, 2006) and the release of VOCs similar to those produced by feeding by the herbivore *L. daturaphila* (Hare, 2007). Although as a group females did not avoid ovipositing on plants treated with methyl jasmonate, 41 % of females laid significantly more eggs on intact plants (Fig. 4a), while only 26 % of females laid more on the intact plant in the case of experiments with feeding-damaged plants (Fig. 1b). These results suggest that damage by *M. sexta* larvae and methyl jasmonate application may induce the production of different VOCs (Baldwin et al., 2002). In particular, it has been shown that application of methyl jasmonate in *D. wrightii* induces the production of racemically uncharacterized linalool (Hare, 2007).

In *D. wrightii*, pollination by adult *M. sexta* benefits plants as it increases fruit set (Bronstein et al., 2009). Thus, it is reasonable to ask whether pollinator attraction in this system is affected by herbivory (Karban, 2011). A plausible hypothesis predicts that in order to optimize fitness, plants under herbivory attack should limit changes in floral scent and still attract pollinators (Kessler and Halitschke, 2009). In agreement with this, we found that the *D. wrightii* floral VOCs that are crucial to mediate feeding by adult *M. sexta* (Riffell et al., 2008) remained unchanged in herbivore-induced plants (Table 5; it is possible, however, that in our experiments herbivory did not occur at a critical time necessary to affect floral development and hence floral VOCs). This result suggests that odor-mediated attraction of moths for nectar-feeding in this system would not be affected by larval herbivory. It is possible also that other floral VOCs (not investigated here) change in feeding-damaged plants, having deterrent oviposition effects. In *N. suaveolens*, foliar herbivory by *M. sexta* larvae did not alter emission rates of floral VOCs either (Effmert et al., 2008), but in *Solanum peruvianum* (a species of wild tomato), herbivory affects floral chemistry, having negative effects on pollinator attraction (Kessler and Halitschke, 2009).

We hypothesize that the different oviposition preferences of *M. sexta* females towards herbivore-induced plants of different species might be due to the different relationships between *M. sexta* and these host-plants, i.e., larval feeding damage of *S. lycopersicum*, but mutually beneficial relationship in the case of *D. wrightii* (serving as food plant for larvae and as a nectar source for adults). These differential plant–insect relationships likely are mediated by differences in the VOCs released by these different species. The mutually beneficial association between *D. wrightii* and *M. sexta* is emphasized further by the finding that floral VOCs that are important to mediate attraction of adults–and hence pollination–remained unchanged in herbivore-induced plants.

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