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Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize

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Abstract Plant volatiles function as important signals for herbivores, parasitoids, predators, and neighboring plants. Herbivore attack can dramatically increase plant volatile emissions in many species. However, plants do not only react to herbivore-inflicted damage, but also already start adjusting their metabolism upon egg deposition by insects. Several studies have found evidence that egg deposition itself can induce the release of volatiles, but little is known about the effects of oviposition on the volatiles released in response to subsequent herbivory. To study this we measured the effect of oviposition by *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) moths on constitutive and herbivore-induced volatiles in maize (*Zea mays* L.). Results demonstrate that egg deposition reduces the constitutive emission of volatiles and suppresses the typical burst of inducible volatiles following mechanical damage and application of caterpillar regurgitant, a treatment that mimics herbivory. We discuss the possible mechanisms responsible for reducing the plant's signaling capacity triggered by *S. frugiperda* oviposition and how suppression of volatile organic compounds can influence the interaction between the plant, the herbivore, and other organisms in its environment. Future studies should consider oviposition as a potential modulator of plant responses to insect herbivores.

Keywords Fall armyworm · Herbivory · Manipulation strategy · Plant defenses

Abbreviations

VOCs	Volatile organic compounds
HIPVs	Herbivore-induced plant volatiles
GLVs	Green leaf volatiles
DMNT	(3*E*)-4,8-Dimethyl-1,3,7-nonatriene
SA	Salicylic acid
JA	Jasmonic acid
SEM	Scanning electron microscopy

Introduction

Many plants release volatile organic compounds (VOCs), which are exploited as signals for host selection by herbivores (Bernays and Chapman 1994). In response to herbivore attack, plants produce a quantitatively and qualitatively different VOC blend. These herbivore-induced plant volatiles (HIPVs) can play an important role as foraging cues of parasitoids and predators (Dicke et al. 1990; Turlings et al. 1990). They also mediate interactions with other herbivores (De Moraes et al. 2001; Kessler and Baldwin 2001) and plants (Engelberth et al. 2004).

In many cases, feeding is not the first contact between plants and herbivores. Most Lepidoptera deposit their eggs

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directly on the host plant, making oviposition the primary encounter. From the plants' perspective, mobilizing defenses upon egg deposition may represent an effective strategy to reduce herbivory, as the resistance mechanisms can be activated before the onset of feeding (Hilker et al. 2002; Hilker and Meiners 2006). Indeed, it has been demonstrated that plants after contacting eggs can grow neoplasms, initiate tissue necrosis (hypersensitive response), or produce ovicidal substances in order to kill eggs or isolate hatching larvae from plant tissue (Blackmeer et al. 1994; Seino et al. 1996; Balbyshev and Lorenzen 1997; Doss et al. 2000; Hilker and Meiners 2002). After oviposition, plants can also release volatiles that are attractive to egg parasitoids (Hilker and Meiners 2002) or change chemicals on the leaf surface that arrest egg parasitoids (Fatouros et al. 2005, 2007). Since this discovery (Meiners and Hilker 1997), such indirect defenses elicited by oviposition have been reported for some plant-insect systems (Meiners and Hilker 2000; Wegener et al. 2001; Hilker et al. 2002; Mumm et al. 2003; Fatouros et al. 2008). In other systems, it is shown that the combination of insect oviposition and feeding is necessary to trigger the emission of attractive volatiles to egg parasitoids (Colazza et al. 2004; Conti et al. 2010).

On the other hand, herbivores have developed intricate strategies to suppress plant defensive responses (Musser et al. 2002), and it has been proposed that they may do so already during oviposition in order to give to offspring an optimal start upon emergence (Hilker and Meiners 2010). Following this idea, it has recently been demonstrated that the application of crushed *Pieris brassicae* L. (Lepidoptera: Pieridae) eggs to *Arabidopsis thaliana* (L.) activates SA-dependent defenses, which function against pathogens, but enhances larval growth of *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) (Bruessow et al. 2010). Yet, little is known about the possible suppression of plant defenses by insect eggs. Especially the possibility that herbivores may suppress HIPVs at oviposition has not yet been considered in detail. The only exception is the recent important observation by Bruce et al. (2010) that stemborer oviposition reduces the emission of (*Z*)-3-hexenyl acetate in an African grass.

Therefore, the current study aimed at assessing if oviposition by the noctuid moth *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) alters the constitutive and herbivore-induced volatile release of maize.

Materials and methods

Insects and plants

Larvae of *S. frugiperda* were collected from maize fields in Brazil and reared on artificial diet (Greene et al. 1976) until pupation. Adults were then transferred to cylindrical rearing cages (10 cm diameter and 21 cm height) for mating and oviposition. They were fed on water solution of 10% honey (v/v). Cages were covered by paper in which moths laid egg masses. Papers containing eggs were collected and replaced daily. Rearing was maintained under controlled conditions (25 ± 3°C, 70 ± 10% RH, 14:10 h L/D). Maize plants (*Zea mays* L., var. Delprim, Delley Semences et Plantes SA, Delley, Switzerland) were sown in plastic pots (10 cm high, 4 cm diameter) filled with commercial potting soil (Ricoter Aussaaterde, Aarberg, Switzerland) and placed in a climate chamber (23°C, 60% RH, 16:8 h L/D, 50,000 lm/m^2). Plants used for the experiments were 10–12 days old and had between two and three fully developed leaves.

Oviposition treatment

To obtain oviposition-treated plants, maize plants were put in nylon cages together with three 3- to 4-day-old *S. frugiperda* females for one night. The next day, plants containing egg masses (2–4 egg masses on each plant) were selected for experiments.

Scanning electron microscopy (SEM)

Two days after oviposition, eggs were removed from oviposition-treated plant and leaf areas were excised. The material was transferred to a 1% solution of OsO_4 and incubated for 12 h in an airtight container at room temperature. After that, samples were dried using Silica gel (SiO_2) for 48 h before being mounted on stubs and gold-sputtered (SDC-050 Sputter Coater, BAL-TEC). The samples were analyzed on a Zeiss LEO 435 VP scanning electron microscope.

Volatile sampling and identification

To assess if oviposition influences VOC release, we measured volatile emission released by: (1) plants with (oviposition-treated) and without eggs (control) for two consecutive days and (2) plants with and without eggs that were induced by scratching and application of *S. frugiperda* regurgitant (regurgitant-treated). To measure changes in constitutive volatiles, five oviposition-treated and control plants were sampled for 4 h on the first day and second day after oviposition (15:00–19:00), and on the night from first to second day (19:00–9:00). Eggs were left on the plant during the experiment.

To measure the effect of oviposition on HIPVs, eggs were removed from plants 48 h after oviposition to avoid damage by emerging larvae. Subsequently, five oviposition-treated and control plants were simultaneously induced by scratching approximately 1 cm^2 of leaf tissue on each side of the middle lamella of each true leaf with a

razor blade. A total of 10 μL of *S. frugiperda* regurgitant per plant was then applied to the wounds to mimic a caterpillar attack. This treatment has previously been shown to elicit a similar response as herbivore attack (Turlings et al. 1993a) and ensured that HIPV induction was not confounded by differences in larval feeding patterns. Directly after induction, the plants were transferred to the volatile sampling system described in detail by Turlings et al. (1998). Volatiles were trapped using Super-Q® filters and extracted at 1.5, 3, 4.5, 6, 7.5, 9 and 12 h after induction. Volatile identification and quantification was carried out as previously described (D'Alessandro and Turlings 2005). Induced volatiles were grouped based on their biochemical origin: (1) Green leaf volatiles (GLVs): (Z)-3-hexanal, (*E*)-3-hexen-1-ol, (*E*)-2-hexanal and (*Z*)-3-hexen-1-ol acetate; (2) aromatic compounds: benzyl acetate, phenethyl acetate and indole; (3) mono- and homoterpenes: *β*-myrcene, linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and geranyl acetate; (iv) Sesquiterpenes: (*E*)-*β*-caryophyllene, (*E*)-α-bergamotene and (*E*)-*β*-farnesene.

Statistical procedures

The data of quantities of emitted volatiles was analyzed using a repeated measures analysis of variance (ANOVA) with treatment as the main factor and period (1.5, 3, 4.5, 6, 7.5, 9 and 12 h) as the repeated factor. For the GLVs that were emitted only at one time interval (1.5 h), one-way ANOVA was used for analysis. Levene's and a Kolmorogov–Smirnov test were carried out to determine heteroscedasticity of error variance and normality of the data. All analyses were performed using the software SYSTAT 13 for Windows.

Results

S. frugiperda eggs are firmly "glued" to the plant

SEM analysis showed that *S. frugiperda* egg masses (Fig. 1a) are firmly attached to the plant surface (Fig. 1b–f). Removal of egg masses from the leaf surface revealed

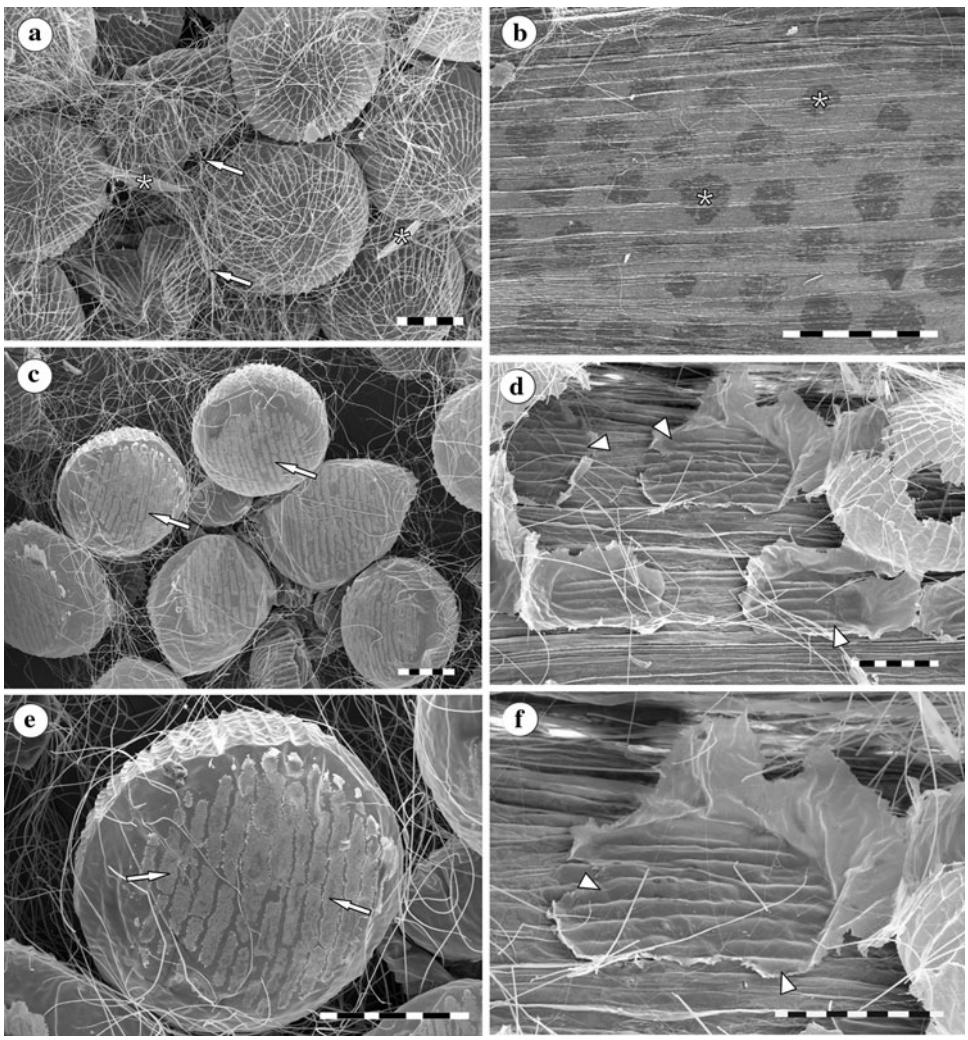

Fig. 1 SEM images showing a *Spodoptera frugiperda* double layer egg mass **a** covered by abdominal setae (*arrow*) and wing scales (*asterisk*). **b** Dark round marks (*asterisk*) on the leaf surface after the removal of egg masses. **c**, **e** Basal part of eggs with wax (*arrow*) from the leaf surface after removal of eggs. **d**, **f** Attachment of chorion of egg basal portion to the rough leaf surface (*arrowhead* oviposition site). *Scale bars* 200 μm (**a**, **c**, **d–f**), 1,000 μm (**b**)

round darkened areas where leaf wax had been removed due to the accessory gland excretions of *S. frugiperda*, which functions as glue (Fig. 1b). Accessory gland material and leaf surface wax were found on the basal part of the eggs (Fig. 1c, e), confirming the close contact between egg masses and plant cuticle.

Oviposition suppresses constitutive VOCs

Maize plants (*Zea mays* L., var. Delprim) constitutively release only β-myrcene and linalool in detectable amounts. Data of volatile emissions met the assumptions of normality and homogeneity of variances and thus could be analyzed using parametric test. Oviposition-treated and control maize plants emitted β-myrcene in equal amounts (Fig. 2a; Tables 1, 2; ANOVA RM between-subject: $F_{1,6} = 0.18$, $P = 0.681$, within-subject: $F_{2,12} = 0.13$, $P = 0.878$). However, oviposition-treated plants emitted linalool in lower amounts in comparison with control plants over time (Fig. 2b; Tables 1, 2; ANOVA RM between-subject: $F_{1,6} = 33.95$, $P = 0.001$, within-subject: $F_{2,12} = 7.00$, $P = 0.035$). On the night between first and

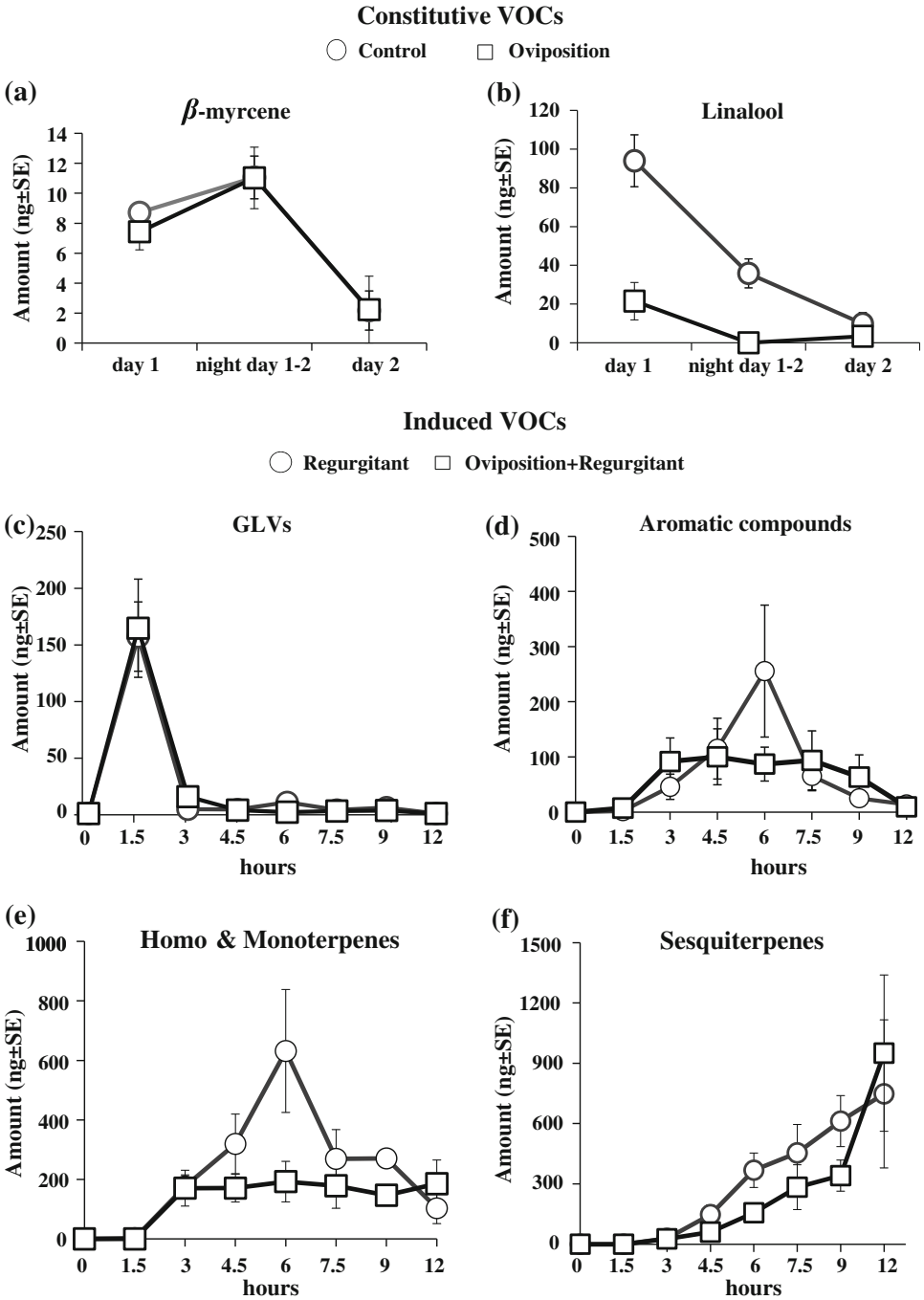

Fig. 2 Constitutive and herbivore-induced volatiles release by maize induced by *Spodoptera frugiperda* oviposition in time course. **a**, **b** Release pattern of constitutive volatiles from non-induced maize plants (control) and oviposition-treated plants (oviposition) along 2 days; β-myrcene (**a**), linalool (**b**). **c**–**f** Release pattern of herbivore-induced volatiles from regurgitant-treated plants (regurgitant) and plants on which the moths had oviposited and were treated with regurgitant (oviposition + regurgitant) at time points up to 12 h after treatment; green leaf volatiles (GLVs; **c**); aromatic compounds (**d**); homo-and monoterpenes (**e**); sesquiterpenes (**f**)

second day, no linalool was detected in the headspace of oviposition-treated plants, while this compound was detected in the headspace of control plants. Measurements were interrupted at emergence of *S. frugiperda* larvae beginning around 48 h after oviposition.

Oviposition suppresses HIPVs

After leaves were scratched and treated with caterpillar regurgitant, the plants emitted green leaf volatiles (GLVs) in equal amounts independent of whether they had carried eggs or not (Fig. 2c; Tables 1, 2; ANOVA RM between-subject: $F_{1,6} = 0.72$, $P = 0.429$, within-subject: $F_{5,30} = 1.61$, $P = 0.426$). Likewise, both treatments emitted in equal amounts the individual GLVs (*E*)-2-hexanal, (*E*)-3-hexen-1-ol, 2-hexen-1-ol, which were detected only at the first time point (1.5 h), and the (*Z*)-3-hexen-1-ol acetate (Table 2). The overall emission of aromatic compounds was also similar for oviposition + regurgitant-treated and regurgitant-treated plant (Fig. 2d; Tables 1, 2; ANOVA RM between-subject: $F_{1,6} = 0.67$, $P = 0.809$, within-subject: $F_{6,24} = 1.05$, $P = 0.417$) The individual aromatic compounds indole, benzyl acetate and phenethyl acetate were emitted in equal amounts by treatments (Table 2). In regard to monoterpenes and homoterpenes, oviposition + regurgitant-treated plants emitted similar amounts comparing to regurgitant-treated plants (Fig. 2e; Tables 1, 2; ANOVA RM between-subject: $F_{1,4} = 10.73$, $P = 0.047$, within-subject: $F_{6,24} = 2.44$, $P = 0.066$). However, within this group of volatiles, linalool, DMNT and geranyl acetate were the compounds that were released in significantly lower amounts by oviposition + regurgitant-treated in comparison to regurgitant-treated plants (Tables 1, 2; ANOVA RM within-subject: linalool $F_{5,20} = 2.63$, $P = 0.049$, DMNT $F_{6,24} = 2.56$, $P = 0.048$, geranyl acetate $F_{5,20} = 2.70$, $P = 0.046$). Oviposition + regurgitant-treated and regurgitant-treated plants released similar amounts of sesquiterpenes over time (Fig. 2f; Tables 1, 2; ANOVA RM between-subject: $F_{1,4} = 7.94$, $P = 0.048$, within-subject: $F_{5,20} = 1.47$, $P = 0.242$).

Discussion

This study reveals that oviposition by *S. frugiperda* suppresses the emission of several constitutive and herbivore-induced volatiles in maize. The SEM images reveal that *S. frugiperda* eggs are in close contact with the plant cuticle and are accompanied by accessory glandular secretion, which glues the egg chorion to the leaf (Fig. 1) (Nordlund et al. 1987). The underlying mechanisms of the observed volatile suppression are still under investigation, but recent findings may indicate how insect oviposition can change plant defenses. Bruessow et al. (2010) suggested that egg-derived elicitors could readily diffuse into the leaves, which may lead to suppression of insect resistance mechanism. Alternatively, fall armyworm egg masses are generally dense and cover part of photosynthetic tissue what can alter the plant physiology and thus affect plant volatile emission. One central question that remains unresolved however is *why* herbivores might suppress plant VOCs. Here, four hypotheses are proposed that may explain the phenomenon in an evolutionary context.

The reduction in HIPVs reflects a general suppression of plant defenses

Plants have evolved complex defense mechanisms to resist herbivores, which, in response, developed behavioral and/or physiological adaptations (Després et al. 2007). The suppression of maize volatiles invoked by oviposition may therefore be indicative of a manipulative strategy by *S. frugiperda* to reduce the plants general defensive capacity. One possibility proposed by Bruessow et al. (2010) is that oviposition induces salicylic acid (SA)-dependent defenses, which may lead to a suppression of oxylipin-mediated responses to herbivory controlled by the jasmonic acid (JA) pathway. There is ample evidence that jasmonic acid is involved in the induction of maize volatile emissions (Ozawa et al. 2000; Schmelz et al. 2003). Thus, it could be expected that suppression of the jasmonic acid pathway negatively affects volatile release and could also interfere in direct defenses.

In a separate experiment it was also assessed the effect of oviposition on maize direct defenses against *S. frugiperda* larvae (Figure s1). In contrast to what has been reported for the generalist *S. littoralis* (Erb et al. 2009), it was found that development of *S. frugiperda* larvae is not affected by maize direct defenses. Analogous to the unaffected performance of *P. brassicae* feeding on *Arabidopsis*, the more specialized and well-adapted *S. frugiperda* larvae are likely to be tolerant to maize defenses, making suppressive effects of oviposition hard to detect. Therefore, as induced direct defenses seem to be of little importance for *S. frugiperda*, it is unlikely that their suppression conveys a fitness benefit to the herbivore. However, as described below, the general suppression, including volatile signals, may have advantages for the herbivore by altering the plant's interaction with the environment.

The suppression of HIPVs reduces the attraction of predators and/or parasitoids

Spodoptera frugiperda oviposition suppressed emission of herbivore-induced monoterpenes and homoterpenes (Table 1; Fig. 2e). Given that terpenes are major constituents of herbivore-induced volatile blends, it is likely that they serve as host-location cues by parasitoids and/or

Table 1 Emission (ng ± SE) of volatiles released by regurgitant-treated plants (regurgitant) and oviposition + regurgitant-treated plants (oviposition + regurgitant) at 1.5, 3, 4.5, 6, 7.5, 9 and 12 h after induction using *Spodoptera frugiperda* regurgitant

Volatiles detected	Regurgitant							Oviposition + regurgitant						
	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h	12 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h	12 h
(*E*)-2-hexanal	9.97 ± 2.67	–	–	–	–	–	–	17.16 ± 4.94	–	–	–	–	–	–
(*E*)-3-hexen-1-ol	79.62 ± 15.55	–	–	–	–	–	–	78.06 ± 21.37	–	–	–	–	–	–
2-Hexen-1-ol	3.97 ± 1.10	–	–	–	–	–	–	6.76 ± 2.76	–	–	–	–	–	–
(*Z*)-3-hexen-1-ol acetate	64.07 ± 14.85	3.75 ± 1.14	3.64 ± 1.94	9.93 ± 5.77	3.07 ± 1.92	5.19 ± 3.11	–	63.13 ± 18.2	14.98 ± 6.57	2.95 ± 1.91	1.14 ± 0.28	2.16 ± 0.45	2.63 ± 1.34	–
Total GLVs	157.63 ± 30.91	3.75 ± 1.14	3.64 ± 1.94	9.93 ± 5.77	3.07 ± 1.92	5.19 ± 3.11	0	165.11 ± 43.76	14.98 ± 6.57	2.95 ± 1.91	1.14 ± 0.28	2.16 ± 0.45	2.63 ± 1.34	0
β-myrcene	–	3.54 ± 0.93	3.99 ± 1.65	7.18 ± 3.68	2.59 ± 1.20	1.23 ± 0.38	–	–	3.45 ± 1.89	2.71 ± 1.24	1.97 ± 0.95	0.63 ± 0.13	0.36 ± 0.15	–
Linalool	–	30.98 ± 6.99	66.47 ± 20.57	162.65 ± 50.05	70.97 ± 28.94	85.84 ± 17.00	50.70 ± 30.17	–	27.62 ± 8.34	33.07 ± 5.65	46.88 ± 17.36	51.95 ± 24.03	39.47 ± 9.38	40.13 ± 15.78
(3*E*)-4,8-dimethyl-1,3,7-nonatriene	2.17 ± 0.79	138.16 ± 33.27	237.41 ± 77.09	433.93 ± 144.58	180.91 ± 64.22	168.59 ± 19.38	95.61 ± 47.81	–	137.01 ± 50.57	130.81 ± 41.49	138.35 ± 51.23	118.30 ± 50.65	102.67 ± 22.90	136.01 ± 54.66
Geranyl acetate	–	3.41 ± 1.61	11.33 ± 3.92	27.88 ± 10.10	14.29 ± 6.45	14.56 ± 2.69	7.27 ± 4.43	–	3.95 ± 2.07	3.94 ± 1.45	6.32 ± 2.00	7.83 ± 3.49	3.94 ± 1.55	9.20 ± 9.2
Total mono- and homoterpenes	2.17 ± 0.79	176.09 ± 39.09	319.21 ± 100.24	631.65 ± 206.82	268.77 ± 98.40	270.23 ± 29.90	153.60 ± 79.14	0	174.40 ± 62.30	170.54 ± 46.72	192.49 ± 68.25	178.72 ± 75.70	146.46 ± 31.43	185.35 ± 79.64
Indole	1.95 ± 1.53	41.11 ± 21.13	103.03 ± 51.48	230.56 ± 117.08	55.53 ± 25.40	13.44 ± 6.34	–	1.03 ± 0.70	82.87 ± 38.97	90.61 ± 50.64	76.13 ± 31.51	86.98 ± 54.69	55.89 ± 41.69	–
Benzyl acetate	–	0.99 ± 0.52	0.99 ± 0.77	0.79 ± 0.39	–	–	–	–	2.37 ± 1.13	4.29 ± 1.67	–	–	–	5.52 ± 5.52
Phenethyl acetate	–	2.91 ± 0.90	8.13 ± 3.33	19.93 ± 10.68	9.11 ± 4.55	11.65 ± 2.36	13.90 ± 7.37	–	3.26 ± 1.41	3.95 ± 2.46	10.87 ± 5.31	6.79 ± 4.23	7.61 ± 4.23	4.00 ± 4.00
Total aromatic compounds	1.95 ± 1.53	45.92 ± 22.89	115.03 ± 55.06	255.59 ± 119.56	65.46 ± 26.71	25.10 ± 7.95	13.90 ± 7.37	1.03 ± 0.70	87.77 ± 40.66	100.23 ± 50.69	87.00 ± 30.72	93.77 ± 53.53	63.51 ± 40.28	9.52 ± 4.94
(*E*)-β-caryophyllene	–	1.54 ± 0.63	5.94 ± 2.93	11.51 ± 3.71	14.21 ± 2.30	24.21 ± 9.71	40.22 ± 22.37	–	4.06 ± 2.51	4.44 ± 1.37	6.93 ± 2.04	10.49 ± 3.86	15.00 ± 3.39	23.80 ± 12.16
(*E*)-α-bergamotene	–	9.77 ± 2.54	44.78 ± 13.96	113.44 ± 25.95	137.00 ± 43.51	182.93 ± 34.65	205.55 ± 95.48	–	8.51 ± 3.38	19.68 ± 5.81	48.24 ± 13.53	83.60 ± 32.33	105.19 ± 24.25	272.47 ± 106.78
(*E*)-β-farnesene	–	17.39 ± 5.71	93.90 ± 30.91	242.05 ± 56.35	293.56 ± 93.45	402.05 ± 83.71	478.99 ± 238.91	–	14.01 ± 7.16	35.07 ± 13.07	99.62 ± 28.00	182.01 ± 4.29	217.29 ± 50.10	629.71 ± 274.26
Total sesquiterpenes	0	28.70 ± 8.78	144.63 ± 47.28	367.00 ± 79.19	444.77 ± 138.53	609.21 ± 123.51	724.77 ± 356.07	0	26.58 ± 12.77	59.21 ± 19.90	154.79 ± 42.55	276.11 ± 107.41	316.56 ± 65.36	925.99 ± 392.47

Table 2 Results of repeated measures ANOVA on effects of treatment (regurgitant-treated and oviposition + regurgitant-treated plants), time and time × treatment on maize volatile emission

Plant volatile	Treatment			Time			Time × treatment		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Constitutive compounds									
β-Myrcene	1,6	0.18	0.681	2,12	28.12	0.002	2,12	0.13	0.878
Linalool	1,6	33.95	0.001	2,12	10.04	0.018	2,12	7.00	0.035
Herbivore-induced compounds									
(*E*)-2-Hexanal[a]	1,14	1.85	0.196						
(*E*)-3-Hexen-1-ol[a]	1,14	0.01	0.953						
2-Hexen-1-ol[a]	1,14	1.05	0.323						
(*Z*)-3-Hexen-1-ol acetate	1,6	0.80	0.405	5,30	4.58	0.189	5,30	2.92	0.274
Total GLVs	1,6	0.72	0.429	5,30	3.99	0.212	5,30	1.61	0.426
β-Myrcene	1,6	4.4	0.081	4,24	1.89	0.145	4,24	0.75	0.565
Linalool	1,4	8.22	0.046	5,20	2.99	0.036	5,20	2.63	0.049
(3*E*)-4.8-Dimethyl-1.3.7-nonatriene	1,4	5.98	0.050	6,24	5.49	0.001	6,24	2.56	0.048
Geranyl acetate	1,4	6.88	0.059	5,20	3.68	0.016	5,20	2.70	0.046
Total mono- and homoterpenes	1,4	10.73	0.047	6,24	3.07	0.03	6,24	2.44	0.066
Indole	1,6	0.18	0.688	5,30	2.89	0.03	5,30	1.72	0.161
Benzyl acetate	1,6	1.30	0.297	2,12	0.38	0.692	2,12	0.36	0.706
Phenethyl acetate	1,4	3.34	0.141	5,20	1.31	0.298	5,20	2.42	0.072
Total aromatic compounds	1,6	0.67	0.809	6,24	3.66	0.01	6,24	1.05	0.417
(*E*)-*β*-Caryophyllene	1,4	1.13	0.347	5,20	2.47	0.068	5,20	0.27	0.923
(*E*)-*α*-Bergamotene	1,4	1.19	0.336	5,20	5.05	0.004	5,20	0.74	0.603
(*E*)-*β*-Farnesene	1,4	0.94	0.387	5,20	4.65	0.006	5,20	0.63	0.678
Total sesquiterpenes	1,4	7.94	0.048	5,20	5.05	0.004	5,20	1.47	0.242

[a] Values of *df*, *F* and *P* are referent to one-way ANOVA

predators (Schnee et al. 2006). Their suppression may therefore be a strategy to reduce the risk of attracting natural enemies of the herbivores. Small differences in HIPV blend composition can be crucial for natural enemy recognition of host attack (De Moraes et al. 1998), and given the importance of blends rather than single compounds in host-finding by parasitoids (D'Alessandro and Turlings 2006), suppressing HIPV emission seems likely to benefit the herbivore. Considering that the suppression is partial, it is likely that natural enemies can still detect the HIPV signals. In fact, contrary to our proposed advantage to suppress volatile emissions at oviposition, Bruce et al. (2010) propose that the change they observe in volatile ratios due to suppression of a green leaf volatile in African grass after stemborer oviposition may provide an important cue for larval parasitoids. This would mean that volatile suppression might be informative to natural enemies and therefore not a useful strategy of herbivores to escape from parasitism. Indeed, parasitic wasps are highly adaptable and during the co-evolutionary process may have adapted to an avoidance strategy by their host and even use it to their advantage. This adaptability is also evident from their ability to learn by association, which provides tremendous flexibility in their responses (Turlings et al. 1993b; Vet et al. 1995), allowing them to adapt their responses to short-term changes in volatile composition.

The suppression of VOCs reduces intraspecific competition

Spodoptera frugiperda female moths are responsive to linalool (Malo et al. 2004), which is one of the main constitutive compounds released by undamaged maize (D'Alessandro et al. 2006). Moreover, linalool is a key compound that attracts sixth-instar *S. frugiperda* larvae (Carroll et al. 2006). Suppression of the emission of linalool was observed at day 1, and during the following night. As *S. frugiperda* moths are night-active, nocturnal suppression of linalool in particular may camouflage plants that carry eggs and may reduce oviposition by conspecific. In addition, the attraction of late-instar larvae may be reduced, which would decrease the risk of cannibalism for the younger larvae feeding on maize. Interestingly, Bruce et al. (2010) have recently reported that stemborer oviposition suppresses the constitutive release of (*Z*)-3-hexenyl acetate from African grass. These changes are likely to

disrupt host selection by stemborer female moths. Thus, the hypothesis that oviposition-mediated manipulation of host-plant odor may reduce intraspecific competition deserves further attention.

The suppression of VOCs is a non-adaptive, physiological consequence of the reduction in photosynthetic activity

Oviposition is known to reduce plant photosynthesis, partially by covering a part of the leaf surface, but also via a yet unknown mechanism (Schröder et al. 2005; Velikova et al. 2010). As the production of many HIPVs, particularly terpenoids, depends on plant photosynthesis (Paré and Tumlinson 1997), it is possible that the observed phenomenon is not adaptive per se, but simply the result of a physiological constraint, as has been observed for other aspects of plant-insect interactions (Erb et al. 2010). The fact that linalool was also suppressed during the night, however, suggests that the suppression also occurs independently of the plant's photosynthetic activity. Future research should aim at combining VOC and photosynthesis measurements to clearly disentangle the two effects.

Final considerations

Many studies have addressed volatile release of plants after herbivore attack. Yet, in a natural situation, oviposition usually precedes feeding. This study has shown that herbivore oviposition not only suppresses constitutive volatiles, as found by Bruce et al. (2010), but also herbivore-induced volatiles. This indicates that egg deposition may influence a plant's responses to herbivory and affect the interactions with associated organisms. We suggest that egg deposition should be included in future studies on herbivore-induced plant volatiles. Failing to do so may result in biased and potentially erroneous interpretation of results.

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