Emission of Herbivore-induced Volatiles in Absence of a Herbivore – Response of *Zea mays* to Green Leaf Volatiles and Terpenoids

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Green leaf volatiles (GLV), a series of saturated and monounsaturated six-carbon aldehydes, alcohols, and esters are emitted by plants upon mechanical damage. Evidence is increasing that intact plants respond to GLV by activating their own defense mechanisms, thus suggesting that they function in plant-plant communication. The present paper demonstrates that exposure of maize plants to naturally occurring GLV, including (*Z*)-3-, (*E*)-2- and saturated derivatives, induce the emission of volatile blends typically associated with herbivory. Position or configuration of a double bond, but not the functional group of the GLV influenced the strength of the emissions. (*Z*)-3-Configured compounds elicited stronger responses than (*E*)-2- and saturated derivatives. The response to (*Z*)-3-hexen-1-ol increased linearly with the dose between 200 and 1000 nmol per plant. Not only the naturally occurring (*E*)-2-hexenal, but also (*E*)-2-pentenal and (*E*)-2-heptenal induced maize plants, although to a lesser extend. Externally applied terpenoids [(3*E*)-4,8-dimethyl-1,3,7-nonatriene, β-caryophyllene, and (*E*)-β-farnesene] did not significantly increase the total amount of inducible volatiles in maize. Of three tested maize cultivars Delprim and Pactol responded much stronger than Attribut. Recovery experiments in the presence and absence of maize plants demonstrated that large proportions of externally applied GLV were assimilated by the plants, whereas (3*E*)-4,8-dimethyl-1,3,7-nonatriene was recovered in much higher amounts. The results furthermore suggested that plants converted a part of the assimilated leaf aldehydes and alcohols to the respective acetates. We propose that GLV not only can alert neighboring plants, but may facilitate intra-plant information transfer and can help mediate the systemic defense response in a plant.

Key words: Plant-plant Signaling, Green Leaf Volatiles, Herbivore-induced Volatile Organic Compounds

Introduction

It has been proposed that plants employ a strategy of indirect defence by attracting and arresting natural enemies of herbivores. This is achieved by offering food or housing and by releasing herbivore-induced volatile organic compounds (HI-VOC) that guide carnivores to their prey (Dicke and van Loon, 2000; Turlings and Wäckers, 2004). The composition of HI-VOC is highly variable among plant species (Takabayashi *et al.*, 1991), genotypes within a plant species (Gouinguené *et al.*, 2001), herbivore species (Turlings *et al.*, 1998), and developmental stage of the herbivore (Gouinguené *et al.*, 2003). They consist mainly of compounds originating from the enzymatic degradation of fatty acids (green leaf volatiles, GLV), the shikimic acid pathway, and the terpene pathways (mono-, sesqui-, and homoterpenes) (Paré and Tumlinson, 1999; Pichersky and Gershenzon, 2002). Production of HI-VOC is inducible, *i.e.*, plants increase volatile emission after herbivore attack (Paré and Tumlinson, 1999). Elicitors for this selective induction have been located in the regurgitant of the herbivores (Mattiacci *et al.*, 1995; Alborn *et al.*, 1997; Turlings *et al.*, 2000).

Direct and indirect defense responses in plants can be systemic: that is, also uninfested plant parts of infested plants activate their defense mechanisms (Turlings and Tumlinson, 1992). The mechanisms enabling this information transfer from damaged to undamaged parts within a plant are only poorly understood. In tomato (*Lycopersicon esculentum*), the polypeptide systemin along with hydraulic or electrical signals has been suggested to cause this effect (Schaller and Weiler, 2002). Since the transport speed of chemical signals within the vascular bundles is relatively low, volatile chemicals emitted from mechanically damaged parts of a plant might be more effective in mediat-

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ing systemic responses. In fact, evidence is increasing that exposure of intact plants to volatiles released from mechanically damaged plants or synthetic analogues induces defense responses and that this chemical information transfer is also possible between different plant individuals (plant-plant signaling) (Dicke *et al.*, 1990; Arimura *et al.*, 2000, 2001; Dolch and Tscharntke, 2000; Tscharntke *et al.*, 2001). The plants involved do not even have to belong to the same species (Karban *et al.*, 2000). This phenomenon has been referred to in the literature as examples of "talking plants" or "listening plants" and has been subject of a vivid discussion (Agrawal, 2000; Dicke and Bruin, 2001; Farmer, 2001; Baldwin *et al.*, 2002; Lerdau, 2002; Dicke *et al.*, 2003).

Volatile phytohormones like methyl jasmonate, methyl salicylate, and ethylene have been suggested to enable defense responses in receiving plants. Although methyl jasmonate is known to induce various defense responses (*e.g.*, Farmer and Ryan, 1990; Avdiushko *et al.*, 1995; Baldwin, 1998), the function of this compound as a general cue in plant-plant signaling is questionable because only few plants do emit this compound upon mechanical damage (Farmer, 2001). Methyl salicylate, which is released by tobacco after pathogen attack, induces defense responses in receiving intact tobacco plants (Shulaev *et al.*, 1997). Eposure of intact elder leaves to ethylene resulted in an induction of phenols and proteinase inhibitors (PI) (Tscharntke *et al.*, 2001). Herbivore-induced terpenoids are further candidates for signaling information about an impending herbivore attack to intact plants. Arimura *et al.* (2000, 2001) demonstrated that exposing intact lima bean (*Phaseolus lunatus*) leaves to volatiles from spider mite infested lima bean leaves as well as to β-ocimene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) or (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) resulted in the induction of defense-related genes. However, linalool did not induce any responses and nothing is known about the ability of inducible sesquiterpenes to induce in turn intact receiver plants.

Also GLV have been studied with respect to their ability to induce defense responses in intact plants. GLV, a series of saturated and monounsaturated six-carbon aldehydes, alcohols and esters of the latter, are emitted by green plants upon mechanical damage (Ruther, 2000). In the first step of GLV formation, lipoxygenase (LOX) peroxidizes linoleic or linolenic acid to the respecitve 13-hydroperoxy-derivatives, which are cleaved by a hydroperoxide lyase. The first detectable GLV are (*Z*)-3-hexenal (*Z*-3-al) (from linolenic acid) and hexanal (C_6-al) (from linoleic acid). Isomerization of *Z*-3-al leads to (*E*)-2-hexenal (*E*-2-al). Aldehydes may be reduced by alcohol dehydrogenase to the respective alcohols (C_6-ol, *Z*-3-ol, *E*-2-ol), which subsequently may be esterified (C_6-ac, *Z*-3-ac, *E*-2-ac) (Paré and Tumlinson, 1999). Alternatively, 13-hydroperoxylinolenic acid may be processed to the defense-related phytohormone jasmonic acid (JA) (Paré and Tumlinson, 1999).

Experiments with *Arabidopsis thaliana* (Bate and Rothstein, 1998), *P. lunatus* (Arimura *et al.*, 2001), and *Zea mays* (Farag *et al.*, 2005) showed that exposure to synthetic GLV induces several defense-related genes. Among the genes induced in all three studies were those involved in phenol biosynthesis (phenylalanine ammonia-lyase, PAL) and LOX. Furthermore, genes involved in ethylene biosynthesis are inducible by GLV in *P. lunatus* (Arimura, *et al.*, 2002) and those coding for PI were induced in maize (Farag *et al.*, 2005). Results from studies on genes involved in terpene biosynthesis were inconsistent. Hydroxymethylglutaryl-coenzyme A reductase was not inducible by *E*-2-al in *Arabidopsis* (Bate and Rothstein, 1998), whereas in lima bean farnesyl pyrophosphate-synthase (FPS) was (Arimura *et al.*, 2001). In contrast, FPS was not induced by *Z*-3-ol in maize (Farag *et al.*, 2005). Recent work on tomato revealed that exposure to GLV can affect emissions of inducible terpenoids (Farag and Paré, 2002). In maize, results are controversial: Engelberth *et al.* (2004) found an induction of sesquiterpenes and JA following exposure to gaseous (*Z*)-configured GLV and demonstrated also a priming effect, *i.e.*, the exposed plants emitted HI-VOC faster and in higher amounts when subsequently treated with caterpillar regurgitant. Ruther and Kleier (2005) demonstrated an induction of HI-VOC emission by *Z*-3-ol which was synergized by ethylene. In contrast, Farag *et al.* (2005), who sprayed watery *Z*-3-ol solutions directly on maize plants, did not detect sesquiterpene emissions.

In the present study, we investigated the influence of GLV structure (functional group, double bond, and chain length) and dose on the plant response and compared different maize cultivars with respect to their inducibility. The plant's responses to GLV were compared to responses to

three common inducible sesqui- and homoterpenes.

Results

Maize plants were exposed for 14 h to different chemicals in combined exposure/volatile collection chambers. Subsequently, headspace volatiles of treated plants and controls were collected for 8 h using a purge and adsorbent trap method. A total of 27 volatiles was identified in the headspace extracts of differently treated maize plants (Table I), among them GLV, saturated aldehydes with longer carbon chains (C_8–C_{10}), monoterpenoids, aromatic compounds, several sesquiterpenoids, and the two homoterpenes DMNT and TMTT. All of them had been described before as constituents of the HI-VOC bouquet of maize plants (Gouinguené *et al.*, 2001; Köllner *et al.*, 2004). The control plants emitted in general only low amounts of volatiles indicating that the experimental conditions did not stress the plants *per se.* Control plants of the cultivar Delprim, which was used for most of the experiments emitted significant amounts only of linalool, myrcene and the aldehydes octanal, nonanal, and decanal whereas all other compounds were absent or occurred only in traces. However, treatment of maize seedlings with synthetic plant volatiles resulted in many cases in a clear induction of volatile emission, which is described in more detail below.

Response to green leaf volatiles

All GLV applied at doses of 1 μmol for incubation of intact maize plants of the cultivar Delprim were able to induce the emission of HI-VOC when compared with the respective controls (Table I). Two-way-ANOVA revealed that the presence/configuration of a double bond had an impact on the total amount of inducible volatile emission (F = 7.2831; d.f. = 2, 36; P = 0.0022) but not the functional group of the applied GLV (F = 2.4162; d.f. = 2, 36; P = 0.1036). An interaction was not detectable (F = 0.2774; d.f. = 4, 36; P = 0.8906). *Post hoc* comparison by the LSD-test revealed that maize plants responded significantly stronger to (*Z*)-3-derivatives than to (*E*)-2-derivatives (P = 0.0214) and to saturated derivatives (P = 0.0006). The difference between (*E*)-2- and saturated derivatives was statistically not significant (P = 0.1811).

Volatile emission from Delprim seedlings correlated with an increasing dose of *Z*-3-ol (for total amounts Spearman's R^2 = 0.9100, P < 0.001) (Table II). Interestingly, aldehydes and alcohols applied to the headspace of maize seedlings at doses of up to 1 μmol were found only in relatively small amounts in the headspace extracts won by our purge and trap method (Table I). In many cases, the compounds were not detectable at all. When repeating the induction experiment applying *E*-2-al and *Z*-3-ol (100 μg) individually to collection chambers with and without maize plants, we found large amounts of the applied GLV in the control experiments without maize plants suggesting that the observed loss was caused by the presence of maize plants rather than by the breakthrough of the volatile compounds during headspace sampling. In contrast, the decrease of DMNT due to the presence of maize plants was not significant (Table III). The fact that maize plants actively or passively assimilate the GLV is further supported by the occurrence of higher amounts of possible conversion products of the respective applied GLV. For example, plants treated with *Z*-3-al showed a significant increase of *Z*-3-ol and *Z*-3-ac. Even more drastic was the effect when applying alcohols: a clear increase of the corresponding acetate in the volatile extracts was detectable (Tables I, II). This suggests that there is an active conversion in maize of externally applied gaseous GLV from aldehydes to alcohols and finally to acetates.

Response to (E)-2-aldehydes of differing chain lengths

Not only application of 1 μmol (*E*)-2-hexenal per plant but also equimolar amounts of (*E*)-2-pentenal and (*E*)-2-heptenal induced maize plants of the cultivar Delprim to release typical HI-VOC (Table IV). The volatile emission in response to the naturally occurring C_6-aldehyde was much stronger when compared to the C_5- and C_7-derivatives. However, only the difference to (*E*)-2-heptenal was statistically significant.

Response of different maize cultivars to Z-3-al

Treatment with 1 μmol *Z*-3-al induced all tested maize cultivars (Delprim, Pactol, and Attribut) to emit HI-VOC when compared with the respective controls (Table V). However, there were clear quantitative differences in the volatile patterns of the three cultivars. Delprim and Pactol emitted in general much higher amounts of inducible compounds than Attribut. Delprim tended to emit

Table I. Individual and total amounts of volatiles estimated in the headspace of maize plants treated for 14 h with 1 μmol per plant of different GLV. Values represent means ± SE of five replicates. Asterisks indicate significant differences between treatments within aldehydes, alcohols and acetates, respectively (Kruskall-Wallis-*H*-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Values with different lowercase letters are significantly different at $P < 0.05$ within each line for the different functional groups (Mann-Whitney-*U*-test).

	Aldehydes					Alcohols				
Compound	Control	(*Z*)-3	(*E*)-2	Saturated	*P*	Control	(*Z*)-3	(*E*)-2	Saturated	*P*
(*E*)-2-Hexenal	0a	0a	72 ± 13b[a]	0a	***	0	0	0	0	n.s.
(*Z*)-3-Hexen-1-ol	0a	184 ± 30b	0a	0a	***	0a	282 ± 13b[a]	0a	0a	***
(*E*)-2-Hexen-1-ol	0	0	0	0	n.s.[c]	0a	0a	134 ± 29b[a]	0a	***
1-Hexanol	0a	0a	34 ± 14ab	32 ± 6b	*	0a	0a	0a	232 ± 49b[a]	***
β-Myrcene	21 ± 1a	166 ± 23bc	195 ± 9b	82 ± 4c	**	12 ± 3a	278 ± 71b	77 ± 6bc	56 ± 6c	**
Octanal	19 ± 1a	31 ± 3ab	30 ± 3b	43 ± 2b	*	13 ± 2	19 ± 1	14 ± 1	25 ± 2	n.s.
(*Z*)-3-Hexenyl acetate	0a	622 ± 92b	94 ± 23bc	32 ± 4c	**	20 ± 7a	3367 ± 67b	27 ± 8a	12 ± 2a	**
Hexyl acetate	0a	10 ± 1b	54 ± 18b	217 ± 29c	***	0a	2 ± 1a	63 ± 17a	1255 ± 104b	**
(*E*)-2-Hexenyl acetate	0a	0a	95 ± 38b	0a	***	0a	2 ± 1a	312 ± 85b	0a	***
Linalool	359 ± 33a	1548 ± 162b	1408 ± 91b	944 ± 80b	**	222 ± 22a	1515 ± 149b	821 ± 85ab	847 ± 114ab	*
Nonanal	54 ± 4a	138 ± 16b	114 ± 10b	174 ± 12b	**	29 ± 2a	90 ± 10b	55 ± 4b	67 ± 4b	*
DMNT	9 ± 2a	542 ± 73b	443 ± 66b	84 ± 16c	***	2 ± 0a	614 ± 82b	208 ± 22c	44 ± 6d	***
Decanal	72 ± 9a	152 ± 8a	116 ± 13a	166 ± 15a	*	43 ± 3a	129 ± 9b	91 ± 6b	125 ± 9b	**
2-Phenylethyl acetate	0a	26 ± 4b	16 ± 4bc	4 ± 2c	**	0a	44 ± 8b	8 ± 2a	3 ± 1a	**
Indole	0a	22 ± 3b	0a	0a	***	0a	10 ± 5ab	0,4 ± 1a	3 ± 0b	*
Geranyl acetate	2 ± 0a	40 ± 6b	23 ± 6b	15 ± 4ab	**	0a	64 ± 10b	16 ± 2c	8 ± 1c	**
7-Epi-sesquithujene	0a	23 ± 6b	16 ± 2b	17 ± 3b	*	0a	8 ± 1b	3 ± 0c	1 ± 0ac	**
Sesquithujene	11 ± 3a	194 ± 34b	173 ± 30b	93 ± 16b	*	0a	58 ± 11b	28 ± 5b	26 ± 3b	**
β-Caryophyllene	4 ± 1a	200 ± 43bc	117 ± 16b	32 ± 3c	**	4 ± 1a	121 ± 16b	62 ± 3b	17 ± 2c	***
(*E*)-α-Bergamotene	12 ± 4a	745 ± 232b	299 ± 61b	69 ± 17c	**	4 ± 1a	336 ± 71b	100 ± 9b	14 ± 1c	***
Sesquisabinene	0a	52 ± 18b	13 ± 3b	12 ± 3b	**	0a	11 ± 3bc	4 ± 0c	0,4 ± 0,1ab	**
α-Humulene	0a	12 ± 2b	6 ± 1b	0a	***	0a	6 ± 1bc	3 ± 0c	1 ± 0ab	**
(*E*)-β-Farnesene	29 ± 9a	1038 ± 289c	485 ± 106bc	120 ± 33ab	**	9 ± 2a	489 ± 94b	142 ± 14b	21 ± 1a	**
β-Bisabolene	2 ± 1a	56 ± 13b	38 ± 8b	19 ± 4b	**	2 ± 1a	19 ± 4b	5 ± 1a	5 ± 1a	*
β-Sesquiphellandrene	2 ± 1a	87 ± 30b	25 ± 6b	6 ± 1a	**	1 ± 0a	28 ± 6b	7 ± 1b	1 ± 0a	**
(*E*)-Nerolidol	3 ± 0a	24 ± 5b	13 ± 1b	8 ± 1b	**	1 ± 0a	9 ± 1b	6 ± 0b	2 ± 0b	**
TMTT	7 ± 2a	191 ± 32b	120 ± 22b	47 ± 12c	**	2 ± 1a	88 ± 13b	35 ± 3c	12 ± 0d	***
Total[b]	459 ± 33a	4981 ± 910b	3403 ± 316b	1569 ± 173c	**	264 ± 24a	3716 ± 443b	1537 ± 115c	1077 ± 125c	**

[a] Compound added for induction.
[b] Without added compound.
[c] n.s., not significant.

higher amounts of monoterpenoids (β-myrcene, linalool, and geranyl acetate) than Pactol whereas the latter emitted higher amounts of some sesquiterpenoids [sesquithujene, β-bisabolene, and (*E*)-β-farnesene] even if the difference was not significant for the last compound. In accordance with Gouinguené *et al.* (2001), β-caryophyllene was not detectable in headspace extracts from Pactol.

Response to terpenoids

In contrast to the GLV, there was no clear evidence that the homoterpene DMNT or the sesquiterpenes β-caryophyllene and (*E*)-β-farnesene are able at doses of 1 μmol to induce a significant increase of the total amounts of HI-VOC (Table VI). Plants treated with DMNT released twice as much of total inducible compounds (applied compounds not included) than control plants. These differences, however, were not statistically significant. When looking at individual compounds, there was a slight inductive effect of DMNT regarding β-myrcene, geranyl acetate, and some of the sesquiterpenoids.

In contrast to the GLV experiments, applied compounds were recovered in very high amounts in the respective headspace extracts. α-Humulene and β-bisabolene occurring in mentionable amounts in the headspace of some sesquiterpene-treated plants turned out to be minor components of the applied reference chemicals rather than emitted by the plants.

Table I (cont.)

Acetates				
Control	(*Z*)-3	(*E*)-2	Saturated	*P*
0	0	0	0	n.s.
0a	345 ± 30b	0a	0a	***
0	0	0	0	n.s.
0a	0a	0a	45 ± 10a	*
26 ± 7	94 ± 8	84 ± 10	59 ± 3	n.s.
20 ± 5	18 ± 1	20 ± 2	37 ± 3	n.s.
2 ± 1a	2937 ± 128b[a]	11 ± 3a	5 ± 1a	**
1 ± 0a	9 ± 2a	5 ± 1a	801 ± 105b[a]	**
3 ± 1a	0a	124 ± 33b[a]	0a	**
365 ± 73a	1284 ± 93b	717 ± 69ab	773 ± 79ab	**
74 ± 20	93 ± 3	66 ± 5	110 ± 10	n.s.
11 ± 2a	282 ± 50b	176 ± 34bc	212 ± 2ac	**
97 ± 25	126 ± 4	99 ± 12	203 ± 17	n.s.
0a	15 ± 1b	4 ± 1a	1 ± 1a	**
0	14 ± 5	6 ± 1	3 ± 1	n.s.
3 ± 1a	34 ± 4b	5 ± 1a	6 ± 0a	**
3 ± 1	13 ± 2	6 ± 1	4 ± 1	n.s.
7 ± 1a	87 ± 32b	49 ± 10b	15 ± 2ab	*
12 ± 3a	297 ± 49b	102 ± 22bc	14 ± 2ac	**
7 ± 1a	247 ± 52b	134 ± 31ab	16 ± 2b	*
0	0	0	0	n.s.
0a	17 ± 3b	6 ± 1ab	1 ± 0a	**
29 ± 9	432 ± 101	238 ± 55	31 ± 4	n.s.
20 ± 3	32 ± 5	17 ± 2	18 ± 3	n.s.
3 ± 0	24 ± 5	12 ± 3	2 ± 0	n.s.
1 ± 0	10 ± 2	6 ± 1	1 ± 0	n.s.
5 ± 1a	76 ± 20b	51 ± 14ab	9 ± 1ab	*
401 ± 52a	2972 ± 346b	1606 ± 229bc	994 ± 84c	**

Discussion

The present study presents clear evidence that naturally occurring GLV irrespective of occurrence or configuration of a double bond are able to induce the emission of typical HI-VOC in maize (Turlings *et al.*, 1990; Gouinguené *et al.*, 2001; Köllner *et al.*, 2004). However, the strength of the inductive capability of GLV is clearly influenced by the double bond of the applied compounds. Farmer (2001) hypothesized that α,β-unsaturated carbonyls like *E*-2-al may play a particular role as Michael acceptors in the induction of defense responses in plants. However, this was not supported by our data since the (*Z*)-3-derivatives elicited in general comparable or even stronger volatile emissions. Also saturated GLV irrespective of the functional group were able to induce maize. Farag and Paré (2002) found that terpene induction by GLV treatment in tomato was triggered by both functional group and double bond. Induction of volatile monoterpenes was triggered three times higher by the conjugated *E*-2-al than by *Z*-3-al. The authors found also a dose-dependent response of tomato plants to *E*-2-al between 1 and 1000 nmol maximizing at a dose of 100 nmol. In our study using a similar experimental procedure, volatile emission in maize plants increased linearly with *Z*-3-ol dose between 200 and 1000 nmol per plant.

Engelberth *et al.* (2004) demonstrated the induction of sesquiterpenes in maize plants after exposure to gaseous *Z*-3-al, *Z*-3-ol, and *Z*-3-ac at doses of 20 μg (representing 204, 200, and 140 nmol, respectively). In contrast, Farag *et al.* (2005) were not able to detect the induction of sesquiterpene emission in maize plants sprayed with a watery solution of *Z*-3-ol (50 nmol) possibly due to the application technique, the relatively low dose or a short sampling time during volatile collection.

Our data show a clear influence of the maize cultivar on the response to GLV: Delprim and Pactol emitted much higher volatile amounts than Attribut. This is in accordance with studies addressing the impact of cultivars on the response of maize to actual caterpillar feeding where Delprim and Pactol were found to emit the highest amounts of HI-VOC (Gouinguené *et al.*, 2001). Attribut which was studied here for the first time responded much weaker to *Z*-3-al suggesting a strong variability within maize cultivars.

The mechanisms leading to the emission of GLV from green plants via the octadecanoid pathway are well investigated (Paré and Tumlinson, 1999). In contrast, little is known about the imission of GLV and other putative mediators of auto-signaling and plant-plant signaling (Baldwin *et al.*, 2002). Engelberth *et al.* (2004) demonstrated in their study the involvement of JA in plant responses to GLV: Maize plants responded to GLV incubation with an increase of JA. The role of JA in HI-VOC emission in maize is well established (Schmelz *et al.*, 2003a, b; Ozawa *et al.*, 2004). Thus, the octadecanoid pathway is probably the link between herbivore induction and indirect defense responses mediated by GLV. This is supported by Farag *et al.* (2005) showing that exposure of maize plants to *Z*-3-ol induced the transcription of the

Table II. Individual and total amounts of volatiles estimated in the headspace of maize plants treated for 14 h with different doses (0.2–1.0 μmol per plant) of *Z*-3-ol. Values represent means ± SE of five replicates. Asterisks indicate significant differences between treatments (Kruskall-Wallis-*H*-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Values with different lowercase letters are significantly different at $P < 0.05$ within each line (Mann-Whitney-*U*-test).

Compound	Control	0.2 μmol	0.5 μmol	1.0 μmol	*P*
(*E*)-2-Hexenal	0	0	0	0	n.s.[c]
(*Z*)-3-Hexen-1-ol[a]	34 ± 15a	163 ± 24a	167 ± 3a	282 ± 16b	**
(*E*)-2-Hexen-1-ol	0	0	0	0	n.s.
1-Hexanol	0	0	0	0	n.s.
β-Myrcene	4 ± 1a	59 ± 4b	78 ± 2bc	278 ± 71c	**
Octanal	15 ± 1a	9 ± 2ab	2 ± 1b	19 ± 1a	**
(*Z*)-3-Hexenyl acetate	9 ± 2a	760 ± 166b	2905 ± 259c	3367 ± 67c	***
Hexyl acetate	0	0	0	2 ± 1	n.s.
(*E*)-2-Hexenyl acetate	0	0	0	0	n.s.
Linalool	165 ± 19a	346 ± 40a	683 ± 18b	1515 ± 149b	**
Nonanal	33 ± 2a	29 ± 2a	34 ± 3a	90 ± 10b	*
DMNT	4 ± 2a	107 ± 7b	256 ± 15c	614 ± 82c	***
Decanal	42 ± 2a	28 ± 2a	37 ± 3a	129 ± 9b	**
2-Phenylethyl acetate	0a	10 ± 2b	27 ± 4b	44 ± 8b	**
Indole	0	0	0	10 ± 5	n.s.
Geranyl acetate	0a	7 ± 1b	36 ± 3c	64 ± 10c	***
7-Epi-sesquithujene	0a	0a	0a	8 ± 1b	***
Sesquithujene	1 ± 0a	34 ± 9b	53 ± 17b	58 ± 11b	**
β-Caryophyllene	3 ± 1a	97 ± 25b	64 ± 3b	121 ± 16b	**
(*E*)-*α*-Bergamotene	2 ± 1a	52 ± 10b	122 ± 8bc	336 ± 71c	**
Sesquisabinene	0a	0a	0a	11 ± 3b	**
α-Humulene	1 ± 0	5 ± 0	7 ± 0	6 ± 1	n.s.
(*E*)-*β*-Farnesene	4 ± 2a	84 ± 16b	200 ± 17bc	489 ± 94c	**
β-Bisabolene	0a	9 ± 1b	21 ± 3b	19 ± 4b	**
β-Sesquiphellandrene	0a	5 ± 1b	10 ± 1b	28 ± 6b	**
(*E*)-Nerolidol	0a	3 ± 1b	8 ± 2b	9 ± 1b	**
TMTT	1 ± 0a	23 ± 5b	42 ± 10b	88 ± 13b	**
Total[b]	188 ± 25a	849 ± 92b	1622 ± 48c	3716 ± 443d	***

[a] Compound added for induction.
[b] Without added compound.
[c] n.s., not significant.

Table III. Recovery rates (ng per sampling) of *E*-2-al, *Z*-3-ol, and DMNT in a purge and adsorbant trap arrangement. A dose of 100 μg of each compound was applied in the presence and in the absence of a maize plant to the volatile collection chamber. 14 h after application the headspace was sampled for 8 h. Values represent means ± SE. Asterisks indicate significant differences between treatments for each compound (Mann-Whitney-*U*-test, *** $P < 0.001$)

Compound	With plant	Without plant	*P*	*n*
(*E*)-2-Hexenal	40 ± 30	5200 ± 900	***	6
(*Z*)-3-Hexen-1-ol	70 ± 20	7600 ± 1600	***	6
DMNT	5100 ± 1200	8300 ± 1100	n.s.[a]	5

[a] n.s., not significant.

LOX gene, a key enzyme of the octadecanoid pathway. Another player is probably ethylene which is not only known to synergize typical HIVOC emission in maize after treatment with JA and volicitin (Schmelz *et al.*, 2003b) but also after exposure to *Z*-3-ol: Sesquiterpene emissions in intact maize plants exposed to *Z*-3-ol increased by 6-fold when additionally ethylene was present (Ruther and Kleier, 2005).

Nothing is known about how GLV enter the plant and how they act. Our data suggest that maize plants have a high affinity to GLV and may possess an adapted assimilation mechanism for these compounds because GLV were downright devoured by the plants. The drastic decrease of GLV after incubation with maize seedlings is probably not due to simple adsorption of the applied

Table IV. Individual and total amounts of volatiles estimated in the headspace of maize seedlings treated for 14 h with 1 μmol of (*E*)-2-configured aldehydes with different chain lengths. Values represent means ± SE of five replicates. Asterisks indicate significant differences between treatments for each compound (Kruskall-Wallis-*H*-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Values with different lowercase letters are significantly different at $P < 0.05$ within each line (Mann-Whitney-*U*-test).

	(*E*)-2-Aldehydes				
Compound	Control	C_5	C_6	C_7	*P*
(*E*)-2-Hexenal	0a	0a	73 ± 13b[a]	0a	***
(*Z*)-3-Hexen-1-ol	0	0	0	0	n.s.[c]
(*E*)-2-Hexen-1-ol	0	0	0	0	n.s.
1-Hexanol	0a	0a	34 ± 14b	0a	***
β-Myrcene	9 ± 1a	76 ± 7b	196 ± 9c	58 ± 7b	***
Octanal	14 ± 1a	20 ± 1ab	30 ± 3bc	53 ± 1c	**
(*Z*)-3-Hexenyl acetate	2 ± 1a	13 ± 2b	97 ± 23c	7 ± 1ab	**
Hexyl acetate	1 ± 0a	2 ± 1a	54 ± 18b	17 ± 2b	**
(*E*)-2-Hexenyl acetate	0a	0a	95 ± 38b	0a	***
Linalool	238 ± 38a	810 ± 108b	1408 ± 91b	878 ± 161b	*
Nonanal	34 ± 2a	62 ± 4b	114 ± 10bc	128 ± 6c	**
DMNT	1 ± 0a	174 ± 28bc	443 ± 66c	46 ± 6b	**
Decanal	53 ± 3a	94 ± 7b	116 ± 13bc	206 ± 10c	**
2-Phenylethyl acetate	0a	0a	16 ± 4b	0a	***
Indole	1 ± 0a	7 ± 1b	0a	5 ± 1b	**
Geranyl acetate	1 ± 0a	10 ± 2b	23 ± b	8 ± 1b	**
7-Epi-sesquithujene	1a	4 ± 1b	16 ± 2c	2 ± 0ab	*
Sesquithujene	3 ± 1a	34 ± 10b	173 ± 30c	29 ± 5b	**
β-Caryophyllene	2 ± 0a	34 ± 5b	117 ± 16c	9 ± 1b	**
(*E*)-α-Bergamotene	2 ± 1a	159 ± 29bc	299 ± 61c	28 ± 7b	**
Sesquisabinene	0a	7 ± 1bc	13 ± 3c	0a	**
α-Humulene	0a	2 ± 0b	6 ± 1c	9 ± 4bc	**
(*E*)-β-Farnesene	6 ± 1a	273 ± 53bc	485 ± 106c	67 ± 18b	**
β-Bisabolene	0a	11 ± 2b	38 ± 8b	8 ± 2b	**
β-Sesquiphellandrene	0a	13 ± 3bc	25 ± 6c	3 ± 1ab	**
(*E*)-Nerolidol	0a	5 ± 1b	13 ± 1c	2 ± 1ab	**
TMTT	2 ± 0a	40 ± 8bc	120 ± 22c	14 ± 3b	**
Total[b]	227 ± 43a	1674 ± 227bc	3403 ± 316c	1329 ± 217b	**

[a] Compound added for induction.
[b] Without added compound.
[c] n.s., not significant.

chemicals on the waxy plant surface because nonpolar compounds like DMNT and sesquiterpenes, which should adsorb even better on the waxy leaf surface, were recovered in much higher amounts from the headspace of exposed maize plants.

The response of maize plants is not restricted to naturally occurring compounds, but also compounds with shorter or longer chains elicited volatile emission even to a lower extend.

According to Baldwin *et al.* (2002), two modes of action are thinkable for putative signaling molecules after having entered the plant: (i) They may be perceived by receptors and subsequently trigger a signal cascade or (ii) their mode of action may depend on diffusion and accumulation in the plant tissue, which would probably require very high release rates in the emitter. Volatile receptors have hitherto only been described for ethylene (Baldwin *et al.*, 2002). Thus, future work has to focus on the fate of the GLV after having entered the plant. Experiments using radiolabeled GLV might be a promising approach to localize possible sites of action within the plant. Furthermore, the fact that GLV and the defense related phytohormone JA are derived from the same precursor during the octadecanoid pathway, *i.e.*, 13-hydroperoxylinolenic acid, deserves particular attention in future studies. Possibly the increase of one end product (GLV) due to external application favors the formation of the alternative end product (JA),

Table V. Individual and total amounts of volatiles estimated in the headspace of maize seedlings from different cultivars treated for 14 h with 1 mmol of *Z*-3-al. Values represent means ± SE of five replicates. Asterisks indicate significant differences between treated maize cultivars and the respective controls (Mann-Whitney-*U*-test, * $P < 0.05$, ** $P < 0.01$). Different lowercase letters indicate significant differences between treated cultivars at $P < 0.05$ (Mann-Whitney-*U*-test). *P*-levels of preceding Kruskall-Wallis-*H*-test are given in the last column.

	Delprim			Pactol			Attribut			Kruskall-Wallis
Compound	Control	Treatment		Control	Treatment		Control	Treatment		*P*
(*E*)-2-Hexenal	0	0		0	0		0	0		n.s.[b]
(*Z*)-3-Hexen-1-ol	0	184 ± 30**		0	116 ± 30**		0	94 ± 18**		n.s.
(*E*)-2-Hexen-1-ol	0	0		0	0		0	0		n.s.
1-Hexanol	0	0		0	0		0	0		n.s.
β-Myrcene	21 ± 1	166 ± 23**	a	7 ± 1	39 ± 5**	b	16 ± 2	19 ± 2	b	0.009
Octanal	19 ± 1	31 ± 3	a	8 ± 1	10 ± 2	b	8 ± 1	9 ± 1	b	0.025
(*Z*)-3-Hexenyl acetate	0	622 ± 92**		10 ± 3	1057 ± 295**		2 ± 1	1925 ± 314**		n.s.
Hexyl acetate	0	10 ± 1**		0	5 ± 2		0	2 ± 1		n.s.
(*E*)-2-Hexenyl acetate	0	0		0	0		0	0		n.s.
Linalool	359 ± 33	1548 ± 162**	a	17 ± 1	527 ± 106**	ab	25 ± 4	90 ± 18	a	0.013
Nonanal	54 ± 4	138 ± 16*	a	33 ± 5	40 ± 7	b	21 ± 1	34 ± 2*	b	0.018
DMNT	9 ± 2	542 ± 73**		14 ± 2	452 ± 56**		2 ± 1	223 ± 30**		n.s.
Decanal	72 ± 9	152 ± 8*	a	55 ± 7	61 ± 11	b	37 ± 2	68 ± 3*	b	0.02
2-Phenylethyl acetate	0	26 ± 4**		2 ± 1	6 ± 1		0	4 ± 1**		n.s.
Indole	0	22 ± 3**	a	1 ± 0	17 ± 3**	a	0	0	b	0.007
Geranyl acetate	2 ± 0	40 ± 6**	a	0	1 ± 0*	b	6 ± 2	8 ± 2	b	0.007
7-Epi-sesquithujene	0	23 ± 6*	a	5 ± 1	38 ± 3**	a	0	0	b	0.009
Sesquithujene	11 ± 3	194 ± 34**	a	90 ± 14	576 ± 42**	b	0	0	c	0.002
β-Caryophyllene	4 ± 1	200 ± 43**	a	0	0	b	3 ± 1	35 ± 3**	a	0.005
(*E*)-*α*-Bergamotene	12 ± 4	745 ± 232**	a	5 ± 1	844 ± 89**	a	0	8 ± 2**	b	0.006
Sesquisabinene	0	52 ± 18**	a	1	45 ± 5**	a	0	0	b	0,005
α-Humulene	0	12 ± 2**		1 ± 0	3 ± 0**		2 ± 1	3 ± 0		n.s.
(*E*)-*β*-Farnesene	29 ± 9	1038 ± 289**	a	16 ± 1	1789 ± 174**	a	2 ± 1	24 ± 8*	b	0.006
β-Bisabolene	2 ± 1	56 ± 13**	a	206 ± 36	964 ± 64**	b	0	6 ± 1**	c	0.004
β-Sesquiphellandrene	2 ± 1	87 ± 30*	ab	0	103 ± 10**	b	0	7 ± 1**	a	0.02
(*E*)-Nerolidol	3 ± 0	24 ± 5**	ab	0	25 ± 2**	b	1 ± 0	5 ± 1	a	0.04
TMTT	7 ± 2	191 ± 32**	a	17 ± 2	240 ± 18**	a	2 ± 0	40 ± 5	b	0.019
Total [a]	459 ± 33	4981 ± 910**	a	402 ± 50	5692 ± 499**	a	60 ± 8	477 ± 51**	b	0.008

[a] Without added compound.
[b] n.s., not significant.

Table VI. Individual and total amounts of volatiles estimated in the headspace of maize seedlings treated for 14 h with 1 μmol of different terpenes. Values represent means ± SE of five replicates. Asterisks indicate significant differences between treatments for each compound (Kruskall-Wallis-*H*-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Values with different lowercase letters are significantly different at $P < 0.05$ within each line (Mann-Whitney-*U*-test).

Compound	Control	DMNT	β-Caryophyllene	(*E*)-β-Farnesene	*P*
(*E*)-2-Hexenal	0	0	0	0	n.s.[d]
(*Z*)-3-Hexen-1-ol	0	0	0	0	n.s.
(*E*)-2-Hexen-1-ol	0	0	0	0	n.s.
1-Hexanol	0	0	0	0	n.s.
β-Myrcene	13 ± 2a	47 ± 3b	57 ± 7b	31 ± 3ab	*
Octanal	7 ± 1	14 ± 2	6 ± 1	6 ± 1	n.s.
(*Z*)-3-Hexenyl acetate	29 ± 12ab	36 ± 12b	5 ± 2ab	0a	*
Hexyl acetate	0	0	0	0	n.s.
(*E*)-2-Hexenyl acetate	0	0	0	0	n.s.
Linalool	347 ± 48	432 ± 48	340 ± 51	320 ± 87	n.s.
Nonanal	38 ± 3	49 ± 6	27 ± 3	31 ± 5	n.s.
DMNT	9 ± 3ac	11640 ± 1075b[a]	32 ± 8c	1 ± 1a	**
Decanal	31 ± 2	48 ± 4	20 ± 3	27 ± 3	n.s.
2-Phenylethyl acetate	0	0	0	0	n.s.
Indole	0	0	0	0	n.s.
Geranyl acetate	0a	11 ± 2b	2 ± 1ab	0a	**
7-Epi-sesquithujene	11 ± 3a	79 ± 29a	27 ± 6ab	0b	*
Sesquithujene	0	0	0	0	n.s.
β-Caryophyllene	13 ± 1a	35 ± 4b	27640 ± 2178c[a]	54 ± 15ab	**
(*E*)-α-Bergamotene	11 ± 3ab	131 ± 34b	33 ± 10b	0a	**
Sesquisabinene	0	0	0	0	n.s.
α-Humulene	0a	0a	1361 ± 69b[c]	0a	***
(*E*)-β-Farnesene	62 ± 12a	299 ± 74a	60 ± 17a	34138 ± 3385b[a]	**
β-Bisabolene	0a	0a	7 ± 1b	137 ± 17b[c]	**
β-Sesquiphellandrene	1 ± 0a	13 ± 3b	0a	0a	**
(*E*)-Nerolidol	0a	2 ± 1a	0a	0a	*
TMTT	15 ± 4	56 ± 13	21 ± 6	11 ± 1	n.s.
Total[b]	588 ± 60	1260 ± 184	638 ± 95	486 ± 93	n.s.

[a] Compound added for induction.
[b] Without added compound.
[c] Minor component of the added chemical.
[d] n.s., not significant.

which subsequently triggers gene expression for volatile emission as known from insect feeding.

Our results suggest that a proportion of the assimilated GLV is converted by the plant confirming recent results by Farag *et al.* (2005). Aldehydes appear to be reduced to the corresponding alcohols, a reaction which is known to be catalyzed by rather non-specific alcohol dehydrogenases in plants (Dudareva *et al.*, 2004). The alcohols in turn are subsequently acetylated by acetyl-CoA-dependent acyltransferases (D'Auria *et al.*, 2002). However, *Z*-3-ac has also been shown to be inducible in maize by treatment with caterpillar regurgitant (Turlings *et al.*, 2000) and thus might be additionally synthesized *de novo* by the plant.

Our data did not provide any clear evidence that also terpenoids are able to induce indirect defense responses in the receiver plants. Even if applied at a relatively high dose (1 μmol), neither the homoterpene DMNT nor the sesquiterpenes β-caryophyllene and (*E*)-β-farnesene elicited a significant increase of total amounts of inducible volatiles. There was a slight effect of DMNT when looking at individual components. However, this effect was far below the one observed for equimolar amounts of GLV. Arimura *et al.* (2000) found an inductive effect of DMNT in lima bean leaves on the transcriptional level. Five defense-related genes were induced after an incubation time of 3 h among them LOX, PAL, and FPS. However, after 24 h only one gene not related to indirect defense mechanisms (basic pathogenesis related protein 2) was induced. Gene expression experiments may reveal whether DMNT is able to induce maize

plants at the transcriptional level without becoming evident in the phenotype.

A maize plant responding to GLV by emitting HI-VOC may benefit in various ways from this reaction irrespective of whether these GLV are emitted from infested parts of the same plant (auto-signaling) or from neighboring plants (plant-plant signaling). HI-VOC are not only attractive for carnivores preying upon herbivores (Dicke and van Loon, 2000; Turlings and Wäckers, 2004) but have also been shown to directly defend plants by deterring insect females from oviposition (Kessler and Baldwin, 2001; de Moraes *et al.*, 2001). It has been demonstrated that these defense mechanisms may indeed increase plant fitness (Fritzsche-Hoballah and Turlings, 2001; Kessler and Baldwin, 2001). Furthermore, it has been demonstrated that maize plants pre-treated with GLV are able to respond faster and stronger to subsequent damage and regurgitant treatment which has been interpreted as a priming of the maize plants against pending herbivory (Engelberth *et al.*, 2004). From an evolutionary perspective the question arises why maize responds to GLV but not to terpenoids. GLV are unspecific cues being emitted by any green plant tissue upon any kind of mechanical damage whereas terpenoids are closer associated with actual insect feeding and thus, should indicate an impending herbivore attack more reliably. A possible explanation may be based on the kinetics of volatile emission: GLV are emitted by the octadecanoid pathway within seconds after mechanical damage (Pichersky and Gershenzon, 2002) whereas the induction of terpenoids occurs within hours (Turlings *et al.*, 1998). Thus, it is probably speed rather than reliability that has been selected during the evolution of plant-plant signaling.

The present study and earlier work have demonstrated that auto-signaling and plant-plant signaling can in principle be mediated through the vapour phase by GLV. However, behavioral studies have to be performed showing that plants exposed to GLV actually become attractive for parasitoids and predators or repel herbivores. Field studies are indispensible confirming the observed phenomenon under natural conditions using natural GLV sources, before it can be claimed that the information transfer between infested and unifested maize plants has an ecological relevance in nature.

Experimental

Plant material

Maize plants of the three cultivars Delprim, Pactol (provided by Ted Turlings) and Attribut (Saaten-Union, Hannover, Germany) were used for the experiments. The seeds were pre-welled in tap water for 3 d and subsequently planted individually in plastic pots (650 ml, 9 cm × 9 cm × 8 cm) filled with potting soil (Einheitserde Typ T, Werkverband e. V., Sinntal-Jossa, Germany). The seedlings were grown between October 2003 and February 2005 in a green house (60 m^2) at 20–25 °C and 60% relative humidity. Supplemental artificial lighting was provided between 6 and 22 h by 27 SON-T Agro 400 high-pressure sodium vapor lamps (Royal-Phillips Electronics, Eindhoven, The Netherlands). The plants were watered daily. For the experiments 3- to 4-weeks-old maize seedlings were used. At this time the plants were approximately 40 cm high and had 5–6 fully developed leaves.

Incubation/volatile collection chamber

Plant treatment and volatile collection were performed in a combined incubation/volatile collection chamber that was similar to that described by Turlings *et al.* (1998). It consisted of a glass cylinder (volume 8.8 l, 15-cm diameter, 50 cm high) that was closed at the top and the bottom by two teflon plates (20-cm diameter, 10 mm thick). Furrows (15-cm diameter, 2 mm deep) were millcut into both plates to prevent shift of the glass cylinder. The lower teflon plate had a hole in the centre (8-mm diameter) and was split into two parts. By closing the two parts loosly around the stem of the maize plant, the intact aboveground part of the plant was placed into the glass cylinder whereas the pot was kept outside. The upper teflon plate had two holes (5-mm diameter). For volatile collection (see below), charcoal-purified air was pumped at a flow rate of 1.2 l h^{-1} into the container via a teflon tube (5-mm outer diameter) that led through one of the holes. The volatile-laden headspace was sucked at a flow rate of 1 l h^{-1} through an adsorption tube (Charcoal Filter 5 mg, Gränicher and Quartero, Daumazan, France) that was connected to a mini vacuum pump (Neolab, Heidelberg, Germany) by another teflon tube leading through the second hole. Four identical incubation/volatile collection chambers were used simultaneously.

Plant treatments and volatile collection

General procedure

Unless otherwise stated, all experiments were done with maize plants of the cultivar Delprim. Plants were introduced into incubation/volatile collection chambers as described above. Incubation with the synthetic plant volatiles was started between 2 and 3 p.m. For this purpose, aliqouts of standard solutions of each compound dissolved in dichloromethane (10 μg μl^{-1}) were applied directly into the incubation chamber using a microsyringe. The solutions were applied onto the inner surface of the glass cylinder and direct contact with the plants was avoided. Experimental series consisted of differently treated plants (see below) and one control plant treated with equal amounts of solvent. Volatile collection started after an incubation time of 14 h and lasted 8 h. The volatiles were eluted from the adsorption tube by rinsing the charcoal layer with 25 μl of dichloromethane containing 50 ng μl^{-1} methyl nonanoate (Sigma-Aldrich, Deisenhofen, Germany) as an internal standard.

Response to green leaf volatiles

The response of maize plants to equimolar (1 μmol) amounts of nine common GLV was tested in the experimental series one to three. The tested chemicals were the aldehydes (*Z*)-3-hexenal, (*E*)-2-hexenal (95%), hexanal (98%) (series one), the alcohols (*Z*)-3-hexen-1-ol (98%), (*E*)-2-hexen-1-ol (96%), 1-hexanol (98%) (series two), and the esters (*Z*)-3-hexenyl acetate (98%), (*E*)-2-hexenyl acetate (98%), hexyl acetate (98%) (series three). Each series consisted of three treatments and one solvent control and was replicated five times (n = 5 for each test chemical). All chemicals except *Z*-3-al were purchased from Sigma-Aldrich (Deisenhofen, Germany). Since *Z*-3-al may spontaneously rearrange to *E*-2-al, it was freshly synthesized and purified by adsorption chromatography right before the experiments as described elsewhere (Ruther, 2000).

Dose-dependent response to *Z*-3-ol

The response of maize plants to three different doses of *Z*-3-ol was tested in the experimental series four. The series consisted of three treatments (1.0, 0.5, and 0.2 μmol) and one solvent control and was replicated five times (n = 5 for each dose).

Recovery of *E*-2-al, *Z*-3-ol, and DMNT with and without plants

The impact of the presence of a maize plant on the decrease of *E*-2-al, *Z*-3-ol, and DMNT during incubation and volatile sampling was tested in the experimental series five. Following the general procedure described above, we applied 100 μg of each compound to the different incubation/volatile collection chambers both in the presence and absence of a maize plant (n = 6 for *E*-2-al/*Z*-3-ol and n = 5 for DMNT). Quantification of the compounds was done as described below.

Response to (*E*)-2-aldehydes of differing chain lengths

The response of maize plants to equimolar amounts (1.0 μmol) of (*E*)-2-pentenal and (*E*)-2-heptenal was tested in the experimental series six. The series consisted of two treatments and one solvent control and was replicated five times (n = 5 for each compound). For comparison of the obtained data with the naturally occurring GLV *E*-2-al, data obtained in the experimental series one were used.

Response of different maize cultivars to *Z*-3-al

The response of maize plants of the cultivars Pactol and Attribut to *Z*-3-al was tested in the experimental series seven. This series consisted of two treatments (1.0 μmol *Z*-3-al with each cultivar) and two solvent controls (one for each cultivar) and was replicated five times (n = 5 for each cultivar). To allow for comparison with the cultivar Delprim, data obtained in the experimental series two were used.

Response to terpenoids

The response of maize plants to equimolar amounts (1.0 μmol) of the sesquiterpenes β-caryophyllene (87%, Sigma-Aldrich) and (*E*)-β-farnesene (99%) and the homoterpene (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (95%, provided by Stefan Schulz, Technical University of Braunschweig, Germany) was tested in the experimental series eight (n = 5 for each compound).

Chemical analysis

Volatile extracts were analysed by coupled gas chromatography-mass spectrometry (GC-MS). Analytical separations were performed on a Fisons

8060 GC, mass spectra were obtained on a Fisons MD800 quadrupole mass spectrometer. Analyses were carried out using a 30 m × 0.32 mm i.d. DB-5ms fused silica column, film thickness 0.25 μm (J&W Scientific, Folsom, CA) with helium as carrier gas (head pressure 10 kPa). The temperature program started at 40 °C, held for 4 min and ran with 3 °C min^{-1} to 280 °C. The column effluent was ionized by electron impact ionization (EI) at 70 eV. Injection volume was 1 μl (splitless). Linear retention indices were estimated by co-injection of a hydrocarbon mixture (C_7–C_{20}). The identification of compounds was based in most cases on comparison of mass spectra and linear retention indices with those of authentic reference compounds. The sesquiterpenoids (*E*)-α-bergamotene, β-sesquiphellandrene, sesquithujene, 7-epi-sesquithujene, and sesquisabinene were not available as reference chemicals. Their identification is based on comparison of both mass spectra and linear retention indices with those compiled in the essential oil library of Massfinder 3.12 scientific software (Detlef Hochmuth Consulting, Hamburg, Germany). Quantification of plant volatiles in the extracts was done by comparing the peak areas of individual compounds with the peak area resulting from the co-injection of the internal standard (50 ng μl^{-1}). Compounds used for induction were analyzed for minor contaminants to make sure that novel compounds found in the headspace were actually due the plant metabolism rather than impurities of the used chemicals.

Statistical analysis

The amounts of each compound estimated in the volatile extracts from the differently treated maize plants were analysed for each experimental series by a non-parametric Kruskall-Wallis-*H*-test followed by a Mann-Whitney-*U*-test for individual comparisons. For comparison of total amounts of plant volatiles emitted by differently treated maize plants, the amounts of mono-, sesqui-, and homoterpenoids, 2-phenylethyl acetate, and indole were summed up and compared by the same statistical procedure. GLV were not included in this comparison, although some of them are known to be inducible by insect feeding or regurgitant treatment, because these compounds were applied during treatment. Thus, it was not possible to distinguish between applied amounts of these compounds and those that were produced by the plant in response to the treatment. Total amounts of volatiles induced by different GLV in the experimental series one were additionally analysed by a two-way-ANOVA [factor 1: functional group, with classes aldehyde, alcohol, acetate; factor 2: double bond, with classes (*Z*)-3-, (*E*)-2-, saturated] to test whether there was a general impact of the functional group or a double bond on the plant response. In case the ANOVA indicated a significant effect, least significant difference (LSD) tests were used for *post hoc* comparison. Recovery rates of applied volatiles in the presence and absence of a maize plant were compared by a Mann-Whitney-*U*-test. All statistical analyses were done using Statistica 5.5 scientific software (StatSoft Inc., Tulsa, USA).

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