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Wound-induced green leaf volatiles cause the release of acetylated derivatives and a terpenoid in maize

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Abstract

Green leaf volatiles (GLVs), generally occurring C_6 alcohols, aldehydes and acetates from plants, play an important role in plant–plant communication. These compounds induce intact plants to produce jasmonic acid, and induce defense-related gene expression and the release of volatile compounds. Here, we address wound-induced GLVs cause the release of acetylated derivatives and a terpenoid, (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT) in intact maize, which may be a type of plant–plant interaction mediated by airborne GLVs. Upon exposure of intact maize seedlings to wound-induced GLVs, (*Z*)-3-hexenyl acetate was consistently the most abundant compound released. Exogenous application of individual alcohols and aldehydes mostly resulted in the release of corresponding acetate esters. C_6-alcohols with a double bond between the second and third, or the third and fourth carbon atoms, C_5- or C_6-aldehydes, and (*Z*)-3-hexenyl acetate triggered the release of DMNT. When (*Z*)-3-hexenyl acetate and hexyl acetate were used to treat maize seedlings, they were recovered from the plants. These data demonstrated that: (1) apart from direct adsorption and re-release of acetate esters, absorption and conversion of exogenous alcohols and aldehydes into acetate esters occurred, and (2) DMNT was induced by a range of aldehydes and unsaturated alcohols.

Keywords: *Zea mays*; Gramineae; Green leaf volatiles; Acetylated derivatives; Terpenoid

1. Introduction

The transfer of chemical information between organisms has been well established for a wide range of organisms (Stock et al., 1999; Tegoni et al., 2004; Waldman and Bishop, 2004; Estabrook and Yoder, 1998). Plants are in constant communication with a multitude of diverse organisms. For many plant species it has been demonstrated that they provide chemical information to carnivorous arthropods (see reviews by Vet and Dicke, 1992; Dicke and Van Loon, 2000). However, the idea of transfer of chemical information from damaged to undamaged plants has been received with skepticism for a long time. In a recent review, Dicke and Bruin (2001) conclude that there is good evidence that undamaged plants can adaptively respond to the chemical information emitted by damaged neighbors, and evidence is mounting that the transfer of chemical information from damaged plants to undamaged plants is possible (Dicke et al., 1990; Farmer and Ryan, 1990; Bruin et al., 1992; Shulaev et al., 1997; Agrawal, 2000; Arimura et al., 2000a,b; Dolch and Tscharntke, 2000; Karban et al., 2000; Dicke et al., 2003).

Both intra- and inter-specific signaling, as well as aerial and underground information transfer have been demonstrated (Bruin et al., 1992; Bruin et al., 1995; Karban et al., 2000; Karban, 2001; Chamberlain et al., 2001; Dicke and Dijkman, 2001; Bruin and Sabelis, 2001; Karban et al., 2003; Engelberth et al., 2004; Choh et al., 2004). The

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studies on plant-to-plant communication through chemicals are mostly concentrated on interactions between conspecifics (as reviewed by Dicke and Bruin, 2001). However, in ecological reality, diverse plant species usually co-occur in a patch. Also the agricultural practice of intercropping implies co-occurrence of different crop plant species. Therefore, interspecific chemical communication between plants is relevant for both natural and agricultural ecological systems.

Methyl jasmonate (MeJA) (**1**), methyl salicylate (MeSA) (**2**) and ethylene (**3**) have been proposed as potential signals for plant–plant communication (Farmer and Ryan, 1990; Bruin et al., 1995; O'Donnell et al., 1996; Shulaev et al., 1997; Dolch and Tscharntke, 2000; Preston et al., 2001; Tscharntke et al., 2001; Arimura et al., 2001). Recently, evidence has accumulated that green leaf volatiles (GLVs) are also important signal molecules in plant–plant interactions. A demonstration of the elaborate and fine-tuned responses of plants to GLVs comes from the assessment of gene expression in laboratory studies. Aerial treatment of *Arabidopsis* seedlings with 10 μM of (*E*)-2-hexenal (**4**) induced several genes known to be involved in the plant's defense response (Bate and Rothstein, 1998). Exposing detached lima bean leaves to the GLVs, (*Z*)-3-hexen-1-ol (**5**), (*E*)-2-hexenal (**4**), and (*Z*)-3-hexenyl acetate (**6**), resulted in the transcription of defense genes in the leaves (Arimura et al., 2001). In tomato, (*E*)-2-hexenal (**4**) triggered local and systemic volatile emissions (Farag and Paré, 2002), and in maize (*Z*)-3-hexenal (**7**), (*Z*)-3-hexen-1-ol (**5**), and (*Z*)-3-hexenyl acetate (**6**) induced the emission of sesquiterpenes (Engelberth et al., 2004). As GLVs are released from green plants in response to mechanical damage caused by herbivores, they are possibly involved in both intra- and inter-specific plant interactions.

In the current study, we address the release of volatile acetate esters from maize seedlings exposed to wound-induced plant volatiles which are predominantly composed of GLVs. We show that acetate esters are mainly produced through the conversion of the exogenous alcohols and aldehydes into the corresponding esters, and that direct adsorption and re-release of acetate esters is also involved. The induction of a terpenoid, (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT) (**8**) by aldehydes and alcohols was also reported.

2. Results and discussion

GLVs are released immediately from green plants after mechanical wounding and the onset of herbivory, and are considered typical wound signals (Hatanaka, 1993). Damaged maize leaves released large amounts of GLVs with (*Z*)-3-isomers as dominant components (Fig. 1A1). Similar results were revealed in damaged hot pepper and tobacco leaves (Fig. 1B1 and C1). In contrast, besides GLVs cotton leaves released a number of terpenoids after wounding (Fig. 1D1). These terpenoids are stored in glands located near the surface of cotton leaves and are released when the glands are ruptured (Elzen et al., 1985; Loughrin et al., 1994).

Four compounds, hexyl acetate (**9**), DMNT (**8**), (*Z*)-3-hexenyl acetate (**6**) and (*Z*)-3-hexen-1-ol (**5**), were emitted from maize seedlings exposed to wound-induced maize, hot pepper and tobacco volatiles (Fig. 1A2, B2 and C2). Maize seedlings exposed to wound-induced cotton volatiles emitted all the four compounds and another two GLVs, (*E*)-2-hexenyl acetate (**10**) and 1-hexanol (**11**), and some terpenoid compounds (Fig. 1D2). In all cases, (*Z*)-3-hexenyl acetate (**6**) was the major blend component emitted from maize seedlings exposed to wound-induced plant volatiles. This led to the question of the origin of the compound in intact maize seedlings. As suggested by Dicke et al. (1990) and Bruin et al. (1995), and found by Choh et al. (2004), two processes, active production and passive adsorption and re-release of volatiles, were both involved in the volatile emission from Lima bean leaves that have been exposed to volatiles from *Tetranychus urticae*-infested conspecific leaves. Therefore, three possible mechanisms may be involved in explaining the origin of (*Z*)-3-hexenyl acetate (**6**). (1) Exogenous plant volatiles induced the de novo synthesis and release of (*Z*)-3-hexenyl acetate (**6**) in intact maize seedlings; (2) exogenous (*Z*)-3-hexenyl acetate (**6**) was directly adsorbed onto maize seedlings and subsequently re-released; and (3) exogenous (*Z*)-3-hexenal (**7**) and (*Z*)-3-hexen-1-ol (**5**) were absorbed as precursors into maize seedlings and then converted into (*Z*)-3-hexenyl acetate (**6**).

To determine the possible involvement of the adsorption, conversion and re-release mechanism, plants were treated with individual pure compounds at a dosage of 50 μg for 1 h, and subsequently the headspace constituents of the plants were collected and analyzed. Control plants that were exposed to 5 μl of hexane released only very low amounts of volatiles (Fig. 2A). After treatment with C_6-alcohols for 1 h, maize seedlings released corresponding acetate esters, preserving the isomeric configuration, saturation, position of the double bond and position of hydroxyl group of the alcohols (Fig. 2B–J). Similarly, the corresponding acetate esters of C_5- and C_7-alcohols were recovered effectively after incubation of C_5- and C_7-alcohols with maize seedlings for 1 h (Fig. 2K and L). (*E*)-2-hexenyl acetate (**10**), hexyl acetate (**9**) and *n*-pentyl acetate (**12**) were recorded from volatiles of maize seedlings exposed to (*E*)-2-hexenal (**4**), hexanal (**13**), and *n*-pentanal (**14**), respectively (Fig. 2M–O), but no *n*-heptyl acetate (**15**) was detected from plants that were exposed to *n*-heptaldehyde (**16**) (Fig. 2P). When (*Z*)-3-hexenyl acetate (**6**) and hexyl acetate (**9**) were used to treat maize seedlings, they were recovered from the plants (Fig. 2Q and R). Therefore, our approach of treating maize seedlings with individual exogenous compounds clearly demonstrated that absorption and conversion of exogenous alcohols and aldehydes occurred. Direct adsorption and re-release might also have taken place.

From the average amounts of recovery acetate esters, a clear precursor preference among a range of alcohols and aldehydes was observed (Fig. 2). Primary alcohol was more efficiently converted to ester than secondary alcohols. Hexyl acetate (**9**) was emitted at levels one time higher than 2-hexyl acetate (**17**), and 9 times higher than 3-hexyl acetate (**18**) (Fig. 2B–D). The presence of a double bond tends to attenuate conversion efficiency of C_6-alcohols (Fig. 2E–J). In comparing isomers, (*Z*)-2-hexen-1-ol (**19**) was more efficiently turned over than (*E*)-2-hexen-1-ol (**20**) (*t*-test, $P = 0.018$; Fig. 2E and F), while the conversion efficiencies of (*Z*)-3-hexen-1-ol (**5**) and (*E*)-3-hexen-1-ol (**21**) showed no significant difference (*t*-test, $P = 0.283$; Fig. 2G and H). So, it is difficult to predict structural specificity on the basis of *cis* and *trans*-motifs. Compared with alcohols, the corresponding aldehydes had lower conversion rates. The compound (*E*)-2-hexenal (**4**) displayed a very low conversion rate to (*E*)-2-hexenyl acetate (**10**) (Fig. 2M), and *n*-heptaldehyde (**16**) was entirely unconvertible in maize plants (Fig. 2P). It seems that the volatility of (*E*)-2-hexenal (**4**) is not a major regulation for the low turnover rate of this compound in maize plants. When 100 nmol (*E*)-2-hexenal (**4**) was added to a 37×4 cm chamber, $\geqslant 95\%$ of which can be recovered within 2 min (Farag and Paré, 2002). Low

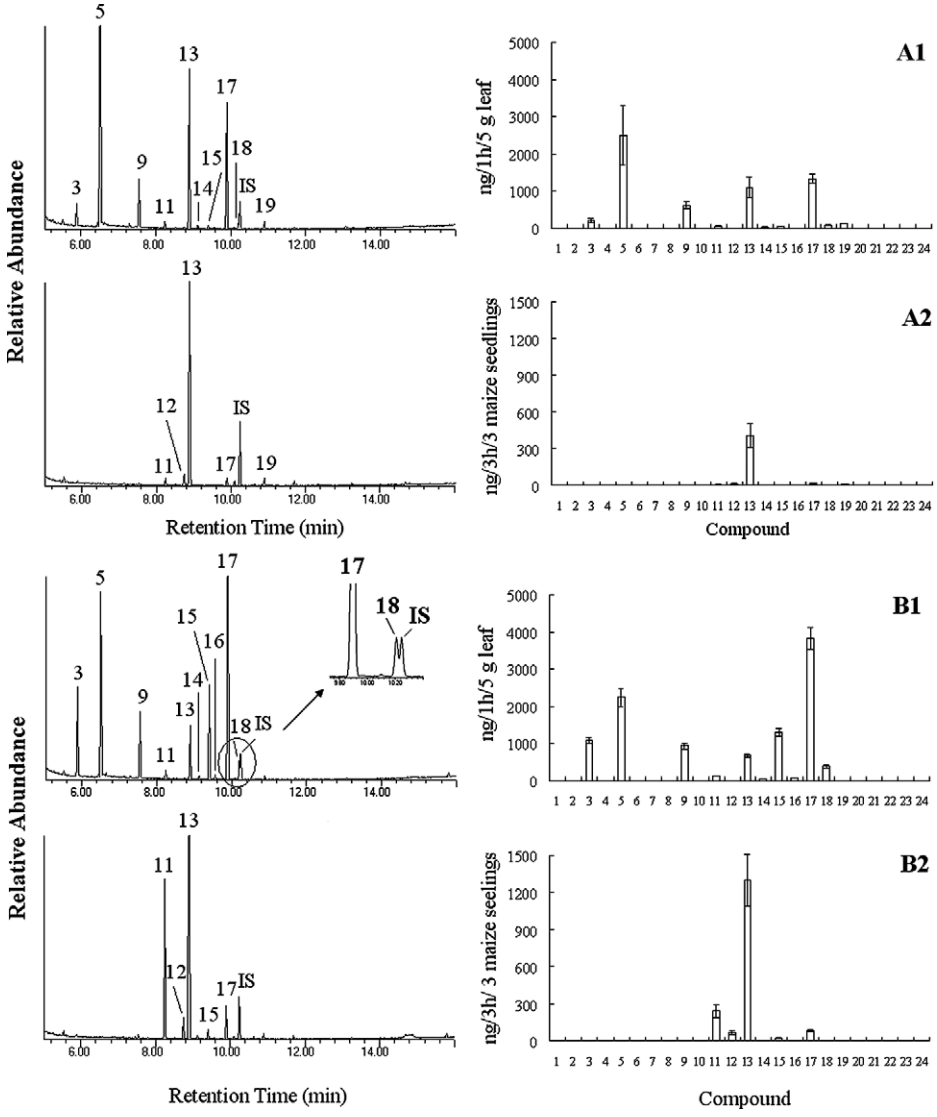

Fig. 1. Representative total ion current chromatograms of the headspace volatiles collected for 1 h from 5 g mechanically damaged maize leaf (A1), hot pepper leaf (B1), tobacco leaf (C1), and cotton leaf (D1); or collected for 3 h from three maize seedlings that were exposed to would-induced maize volatiles (A2), hot pepper volatiles (B2), tobacco volatiles (C2), and cotton volatiles (D2) each emitted from 5 g leaf. 1. α-Pinene (**31**); 2. Camphene (**32**); 3. Hexanal (**13**); 4. β-Pinene (**33**); 5. (*Z*)-3-hexenal (**7**); 6. β-Myrcene (**34**); 7. D-Limonene (**35**); 8. β-Phellandrene (**36**); 9. (*E*)-2-hexenal (**4**); 10. Ocimene (**37**); 11. Hexyl acetate (**9**); 12. (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT) (**8**); 13. (*Z*)-3-hexenyl acetate (**6**); 14. (*E*)-2-hexenyl acetate (**10**); 15. 1-Hexanol (**11**); 16. (*E*)-3-hexen-1-ol (**21**); 17. (*Z*)-3-hexen-1-ol (**5**); 18. (*E*)-2-hexen-1-ol (**20**); 19. 1,4-Dichlorobenzene (**38**); 20. Copaene (**39**); 21. α-Guaiene (**40**); 22. Caryophyllene (**41**); 23. α-Humulene (**42**); 24. α-Bulnesene (**43**). The internal standard tetradecane (**30**) is labeled with IS. The bar graph to the right of each chromatogram represents an average amount of each volatile from four replications (means ± SE). Notice the vertical axis scales are different.

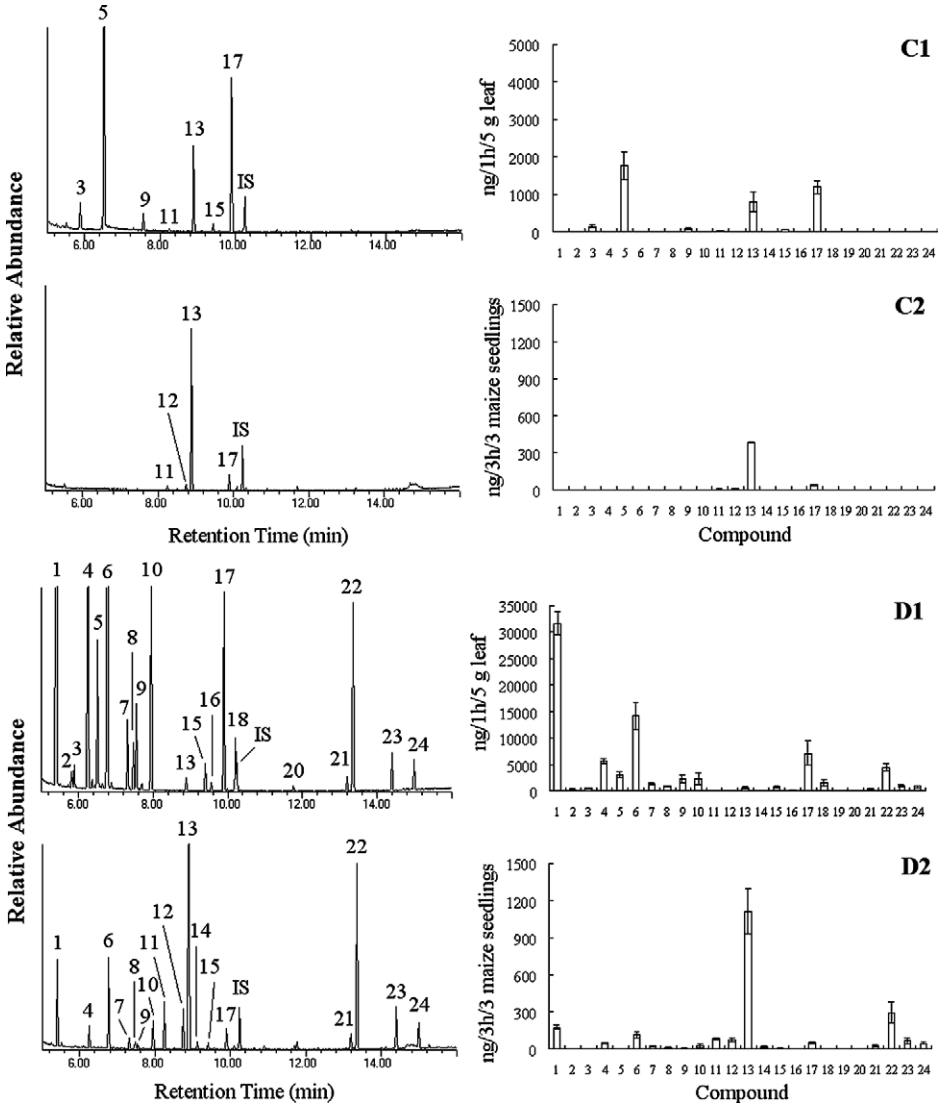

Fig. 1 (*continued*)

uptake rate, structural limitations of the related acetylating enzymes, or low release rate may explain the low recovery of (*E*)-2-hexenyl acetate (**10**).

Volatile esters are important components of the majority of fruits, such as ripe strawberry fruit (Zabetakis and Holden, 1997), and are part of floral scents (Dudareva et al., 1998). They are emitted from vegetative plant parts in response to stress or insect infestation (Mattiacci et al., 2001). The last step in the biosynthesis of esters is catalyzed by alcohol acyltransferases (AATs), which link alcohols to acyl moieties from acetyl-CoA. The specific detection of acetate esters from maize seedlings exposed to pure alcohols strongly suggests that there are alcohol acetyltransferases active in the vegetative parts of intact maize, which may be constitutively expressed or substrate-induced. As to the conversion of aldehydes into acetate esters, involvement of alcohol dehydrogenases, which catalyze the interconversion between aldehydes and alcohols is also implied. In many fruit species, the substrate specificity of the AATs toward tested substrates (both to alcohols and acyl-CoAs) appeared to be broad (Wyllie and Fellman, 2000; Shalit et al., 2001; Olias et al., 2002; Beekwilder et al., 2004). The AAT in maize also seems to have such broad substrate specificity.

As mentioned above, exposure of intact maize seedlings to blends of wound-induced plant volatile triggered the release of DMNT (**8**) (Fig. 1). Further experiments by using individual pure compounds to treat intact maize seedlings revealed that the emission of DMNT (**8**) in maize plants was also triggered by C_6-alcohols with a double bond between the second and third, or the third and fourth carbon atoms ((*Z*)-2-hexen-1-ol (**19**), (*E*)-2-hexen-1-ol (**20**), (*Z*)-3-hexen-1-ol (**5**) and (*E*)-3-hexen-1-ol (**21**)), aldehydes ((*E*)-2-hexenal (**4**), hexanal (**13**) and *n*-valeraldehyde (**22**)) and (*Z*)-3-hexenyl acetate (**6**) (Fig. 2). All saturated alcohols (1-hexanol (**11**), 2-hexanol (**23**), 3-hexanol (**24**), *n*-pentanol (**25**), *n*-heptanol (**26**)), unsaturated C_6-alcohols with a double bond between the fourth and fifth, or the fifth and sixth

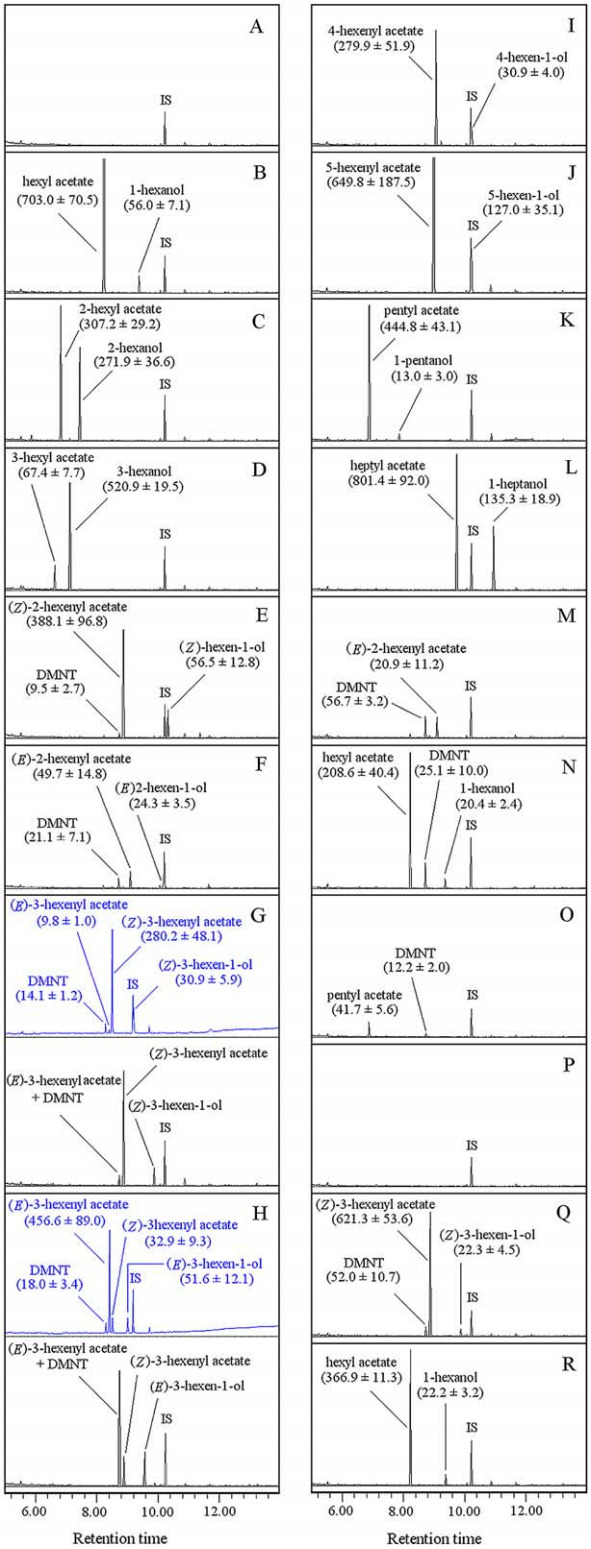

Fig. 2. Representative total ion current chromatograms and quantitative data of the headspace volatiles from maize seedlings treated with solvent (A), or pure compounds, 1-hexanol (**11**) (B), 2-hexanol (**23**) (C), 3-hexanol (**24**) (D), (*Z*)-2-hexen-1-ol (**19**) (E), (*E*)-2-hexen-1-ol (**20**) (F), (*Z*)-3-hexen-1-ol (**5**) (G), (*E*)-3-hexen-1-ol (**21**), (H), 4-hexen-1-ol (**27**) (I), 5-hexen-1-ol (**28**) (J), *n*-petanol (**25**) (K), *n*-heptanol (**26**) (L), (*E*)-2-hexenal (**4**) (M), hexanal (**13**) (N), *n*-valeraldehyde (**22**) (O), *n*-heptaldehyde (**16**) (P), (*Z*)-3-hexenyl acetate (**6**) (Q), hexyl acetate (**9**) (R). The internal standard tetradecane (**30**) is labeled with IS. (*E*)-3-hexenyl acetate (**44**) and DMNT (**8**) were not separated from each other on DB-WAX column (below chromatograms in G and H), but they can be separated on BP-20 column (Polyethylene Glycol 20000, 60 m × 0.25 mm ID, 0.25 μm film thickness, SGE Int. Pty. Ltd.) with GC-FID analysis under the same GC conditions as run on DB-WAX column (above chromatograms in G and H). Each collection was made for 3 h from three maize seedlings having been exposed for 1 h to individual compound at a dosage of 50 μg (in 5 μl of hexane) loaded on a filter paper. Data following each compound name represents the mean of four replications ± SE (ng/3 h/three maize seedlings).

carbon atoms (4-hexen-1-ol (**27**) and 5-hexen-1-ol (**28**)), heptaldehyde (**16**), and hexyl acetate (**9**) had no such an activity (Fig. 2). Farag et al. (2005) proposed that the presence of a double bond in C_6-alcohols is important in conferring the biological activity for triggering emission of plant volatile organic compounds. Our results further demonstrate that the position of the double bond in C_6-alcohols is another important factor for the induction of DMNT (**8**). As to aldehydes, those compounds with five and six-carbon chain length are good inducers for DMNT (**8**) elicitation, while extension of the chain length to seven carbon atoms resulted in an entire deactivation.

GLVs have been described as the inducer of defense-related processes in various plant species. However, the signaling mechanisms involved have not been clearly defined (Engelberth et al., 2004). Indeed, nothing is known about how plants perceive airborne GLVs. As pointed out by Bruin and Dicke (2001), how plants perceive chemical information from damaged neighbors deserves investigation. This perception may dependent on open stomata, or just a straight diffusion through the apolar waxy surface. At least, aqueous spray of (*Z*)-3-hexen-1-ol (**5**) was found to be effective in passing through the waxy coating of the maize leaf (Farag et al., 2005). Moreover, Farag and Paré (2002) found different C_6-compounds had different efficacies in activating volatile emission in tomato, but Engelberth et al. (2004) reported (*Z*)-3-hexenal (**7**), (*Z*)-3-hexen-1-ol (**5**) and (*Z*)-3-hexenyl acetate (**6**) had identical priming activity in maize. The current study revealed very significant differences of C_6-compounds in eliciting the emission of DMNT (**8**). The molecular basis of the structure–activity relationship of C_6-compounds in mediating interplant communication remains unclear. Nevertheless, the finding that exogenous GLVs induced jasmonic acid (**29**) in maize seedlings has shed a new light on a possible mechanism linking GLVs to defense responses in receiving plants (Engelberth et al., 2004).

Farag et al. (2005) recently reported that exogenous ^{13}C labeled (*Z*)-3-hexenol (**5**) could be converted to ^{13}C (*Z*)-3-hexenyl acetate (**6**) in undamaged maize plants. Our results further demonstrated the capability of intact maize seedlings to perceive and respond to a range of airborne GLVs by the conversion of alcohols and aldehydes into corresponding esters. This is a different type of response from the induction of terpenoid compounds (Farag and Paré, 2002; Engelberth et al., 2004). The ecological consequence of the specific release of acetate esters from volatile-exposed intact maize seedlings is an obvious area for subsequent research. Noticeably, (*Z*)-3-hexenyl acetate (**6**) was an effective attractant to a number of natural enemies of herbivorous insects, including the parasitoids *Microplitis croceips* (Whitman and Eller, 1992), *Aphidius ervi* (Du et al., 1998) and *Cotesia flavipes* (Ngi-Song et al., 2002), and the predators *Deraeocoris brevis*, *Orius tristicolor*, and *Stethorus punctum picipes* (James, 2003).

As in most studies of plant–plant communication, we exposed plants to a single dose of volatiles and enclosed them in a small volume of still air to increase the likelihood of detecting a response. Even though we chose 1 h to treat plants to reduce the possible effect of CO_2 depletion and to explore a quick response mechanism, the question remains how the results would translate to natural conditions. Nevertheless, the present results point to the possible involvement of wound-induced signals in mediating interplant communication through conversion in neighboring plants.

Since GLVs are commonly emitted by green tissues in plant in response to mechanical damage or herbivory, and undamaged plants can perceive and respond to these chemicals, there is no reason why plants would not exploit GLVs from heterospecific damaged plants. Obviously, GLV compounds play a key role in both intra- and interspecific plant-to-plant signaling. We suggest that active conversion and release of airborne GLVs by undamaged plants are of universal occurrence in plant–plant interactions, and agree with the notion that GLVs may act as fast-responding plant-to-plant airborne signals of mechanical damage (Arimura et al., 2001).

3. Concluding remarks

This study shows that wound-induced GLVs cause the release of acetylated derivatives and DMNT in intact maize seedlings. The specific release of acetate esters is involved in the adsorption and conversion of airborne alcohols and aldehydes, and direct adsorption and re-release of acetate esters might also have taken place. DMNT was induced by a range of aldehydes and unsaturated alcohols. Further experiments should be manipulated to exam the ecological consequence of the specific release of acetate esters and DMNT (**8**) from volatile-exposed intact maize seedlings.

4. Experimental

4.1. Plants and reagents

Maize (*Zea mays*) cultivar "Zhongdan-306", hot pepper (*Capsicum frutescens*) cultivar "78-9", tobacco (*Nicotiana tabacum*) cultivar "Putongyan" and cotton (*Gossypium hirsutum*) cultivar "Zhong-12" were grown in fields at the Institute of Zoology, the Chinese Academy of Sciences. Leaves obtained from about one month old maize and two months old hot pepper, tobacco and cotton, were used as volatile sources to treat maize seedlings. The maize, hot pepper, tobacco, and cotton cultivars were obtained, respectively, from Institute of Crop Breeding and Cultivation, Institute of Vegetables and Flowers, Institute of Crop Germplasm Resources, and Institute of Plant Protection, the Chinese Academy of Agricultural Sciences (CAAS). Maize seedlings were cultivated in 16 cm (diameter) × 15 cm (deep) flowerpots using fertilized soil obtained from Institute of Vegetables and Flowers, CAAS. The seedlings were kept outdoors for growth in natural condi-

tions with temperature 24–33 °C from June to August, 2004. Plant seedlings were watered every day. A net cage (3 m length, 3 m width, 2 m height) was used to prevent plants from infestation of naturally occurring herbivores. Two-week-old maize seedlings with 3–4 leaves were used for volatile exposure treatments.

Chemicals, 1-hexanol (**11**) (99%), (*E*)-2-hexen-1-ol (**20**) (95%), *n*-pentanol (**25**) (99%), *n*-heptanol (**26**) (99.5%), (*E*)-2-hexenal (**4**) (97%), hexanal (**13**) (98%), *n*-valeraldehyde (**22**) (98%), *n*-heptaldehyde (**16**) (95%), and hexyl acetate (**9**) (95%) were purchased from Fluka; 2-hexanol (**23**) (99%), 3-hexanol (**24**) (99%), 4-hexen-1-ol (**27**) (97%), and 5-hexen-1-ol (**8**) (99%) from Aldrich; and (*Z*)-2-hexen-1-ol (**19**) (95%), (*Z*)-3-hexen-1-ol (**5**) (98%), (*E*)-3-hexen-1-ol (**21**) (98%), and (*Z*)-3-hexenyl acetate (**6**) (97%) from Roth. Compounds were used individually to treat maize seedlings with a single dose of 50 μg in 5 μl hexane.

4.2. Volatiles from cut-leaf material of maize, hot pepper, tobacco and cotton

To determine the quantitative composition of volatile blends emitted by cut-leaf materials, 5 g of leaf material from maize, hot pepper, tobacco or cotton was cut into pieces of ca. 1 cm^2 and placed on a glass Petri dish (10 cm diameter), which was subsequently put into a jar for volatile collection.

4.3. Volatiles emitted from maize seedlings that were exposed to wound-induced plant volatiles or individual compounds

Volatiles emitted from cut-leaf materials or individual compounds were used to expose maize seedlings. For exposure to cut-leaf volatiles, 5 g of cut-leaf material cut as above from maize, hot pepper, tobacco or cotton was added to a 1 l cylinder with three intact maize seedlings, and the cylinder was immediately hereafter covered with aluminium foil. The cylinder was placed into an incubator at 25 ± 1 °C under a light intensity of 2000 lux. After 1 h, the maize seedlings were removed from the cylinder and directly transferred to a glass jar for volatile collection. For exposure to various individual compounds, the same procedure was used but instead of cut leaf material 50 μg (dissolved in hexane, 10 μg/μl) of each compound was pipetted onto a filter paper (2 × 2 cm) placed in the cylinder. As a control, only 5 μl of hexane was added to the filter paper.

4.4. Volatile collection and chemical analysis

Volatiles were collected in a glass jar (12 cm ID × 21 cm long) using a push-pull technique (compressed air and vacuum). Clean air was led through a water bubbler for humidification, a flowmeter for measuring and regulating the airflow, and a charcoal filter for purification. The moist and pure air then entered a jar at 300 ml/min from the lower part of the jar, passed over the plant materials, and then passed through an outlet in the top of the jar. The blend of volatiles was trapped in a glass tube (10 cm long, 6 mm diameter) that contained 25 mg of 80/100 mesh Super Q adsorbent (Altech Assoc., USA). The trap was connected through Teflon tube to the outlet of the jar at one end, and via another flow meter at the other end to a vacuum pump. During the collection, the jar containing the plant materials was kept on ice to keep the inner temperature 25 ± 2 °C, and two fluorescent lamps (each 40 W) were suspended over the jar to illuminate the plants, producing a light intensity of about 2000 lux. Two collection systems with the same treatments were used in parallel every time and the collections were run for 1 h for cut-leaf material and 3 h for maize seedlings exposed to cut-leaf material or individual compounds. Each treatment was repeated four times with fresh batches of plants.

After collection, the traps were rinsed with 150 μl redistilled hexane. As an internal standard tetradecane (**30**), 300 ng for the blend of cut-leaf volatiles and 100 ng for the blend of intact maize seedling volatiles were added. Identification and quantification of volatiles were carried out by coupled gas chromatography–mass spectrometry (GC–MS) on a Hewlett–Packard 6890 GC-5973 MSD. The GC was equipped with a DB-WAX column (Polyethylene Glycol 20000, 60 m × 0.25 mm ID; film thickness 0.15 μm). Helium was used as carrier gas with a constant flow of 26 cm/s. A 2 μl aliquot of cut-leaf volatile samples or 3 μl of intact maize seedling volatile samples was injected, and then split with a purge flow of 30 ml/min. The injector temperature was 250 °C and the GC–MS transfer line temperature was 280 °C, source 230 °C, quadrupole 150 °C, ionization potential 70 eV, and scan range 30–300 *m*/*z*. Following injection, the column temperature was increased from 55 to 200 °C at 8 °C/min, and held at 200 °C for 20 min. Compounds were identified by comparing mass spectra with those of authentic reference compounds and with NIST library spectra (Agilent Technologies, USA). Compounds were quantified by their total ion abundances relative to that of the internal standard.

4.5. Statistics

Analysis of variance and *t*-test ($P < 0.05$) were run using the SPSS 10.0 statistic software.

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