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Plant–plant interactions mediated by volatiles emitted from plants infested by spider mites

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Abstract

In an earlier study, we demonstrated plant–plant interactions mediated by volatiles released from lima bean leaves infested by spider mites (*Tetranychus urticae*) (Nature 406 (2000a) 512, Biochem. Biophys. Res. Commun. 277 (2000b) 305). In the present study, we further show that, under laboratory conditions, volatiles emitted from *T. urticae*-infested lima bean plants activate transcription of genes encoding pathogenesis-related proteins and phenylalanine ammonia-lyase in leaves of intact neighboring plants. This finding indicates that intact lima bean plants may be responsive to volatile signals. Further, as green leaf volatiles (GLVs) are released from green plants in response to mechanical damage caused by herbivores, we studied possible involvement of GLVs in plant–plant interaction. We found that (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate, induced the expression of defense genes in uninfested leaves. This finding suggests that GLVs may act as signal compounds in plant–plant interactions.

Keywords: Lima bean; Spider mites (*Tetranychus urticae*); Green leaf volatiles; Plant–plant interactions; Pathogenesis-related (PR) genes

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1. Introduction

Plants have developed a multitude of inducible defense mechanisms against aggressive biotic agents (Karban and Baldwin, 1997). Defensive action by plants, induced via specific signal transduction, may negatively affect a herbivore's physiology. Examples are the induction of protease inhibitors in potato and soybean plants (for review, see Koiwa et al., 1997) or of nicotine and alkaloids in tobacco plants in response to wounding or herbivory (Baldwin, 1988; Kahl et al., 2000). In addition to such direct induced defenses, plants may also defend themselves against herbivores indirectly by emitting specific blends of volatiles that attract carnivorous natural enemies of herbivores (Dicke et al., 1990b, 1999; Turlings et al., 1990; Takabayashi and Dicke, 1996; De Moraes et al., 1998). The tritrophic system consisting of lima bean plants (*Phaseolus lunatus*), spider mites (*Tetranychus urticae*) and predatory mites (*Phytoseiulus persimilis)* has been well studied by researchers seeking to determine whether it involves such indirect induction of defenses (Dicke et al., 1990b, 1999; Takabayashi and Dicke, 1996).

Plant volatiles induced by spider mites may affect not only predatory mites but also uninfested conspecific plants near infested plants. These neighboring plants have been observed to become more attractive to predatory mites (Dicke et al., 1990a; Bruin et al., 1992) and less susceptible to spider mites (Bruin et al., 1992; Arimura et al., 2000a). We previously reported observing the induction of expression of genes encoding the pathogenesis-related (PR) proteins: lipoxygenase (LOX, a key enzyme of the octadecanoid pathway; Bell et al., 1995), phenylalanine ammonia-lyase (PAL, an enzyme involved in the phenylpropanoid pathway), and farnesyl pyrophospate synthetase (FPS, an enzyme involved in the isoprene biosynthetic pathway) in uninfested lima bean leaves in the vicinity of leaves which had been infested with 100 *T. urticae* females for 1–3 days (Arimura et al., 2000a). The expression patterns of these genes that we observed were similar to those induced by exogenous jasmonic acid (JA), but expression was abolished by the LOX inhibitor salicylhydroxamic acid (SHAM) (Arimura et al., 2000a). These results suggest that the JA is one of the signals essential for expression of these genes in neighboring leaves. In the same study, three volatile terpenoids [β-ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and (*E*, *E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT)] elicited the expression of LOX, PAL and FPS genes. Some of the above mentioned volatiles were gradually emitted from lima bean leaves in response to *T. urticae* infestation (Arimura et al., 2000a). In addition, the amount of chlorophyll lost in leaves was less than that in leaves neighboring uninfested leaves. Interplant communication via airborne signals seems to have resulted in defensive responses to *T. urticae*. In our previous paper (Arimura et al., 2000a), we used detached leaves to study plant–plant interaction. However, intact plants and detached leaves may exhibit induced responses against herbivores in different ways (e.g., Dussourd and Denno, 1994). In this study, therefore, we investigated the interaction between intact *T. urticae*-infested and uninfested lima bean plants.

Green leaf volatiles (GLVs) are released from green plants in response to mechanical damage caused by herbivores. In previous reports, GLVs such as (*Z*)-3-

hexenol, (*E*)-2-hexenal, and (Z)-3-hexenyl acetate were recorded in the headspace of lima bean leaves infested by *T. urticae* (Dicke et al., 1990b, 1999, Arimura et al., 2000a). In our previous paper, we reported the possible involvement of *T. urticae*-induced volatile terpenoids in plant–plant interaction (Arimura et al., 2000a). In the present paper, we further study the possible involvement of GLVs in plant–plant interaction.

2. Materials and methods

2.1. Plants and mites

Lima bean plants (*P. lunatus* cv. Sieva) were grown in plastic pots (diameter = 12 cm, depth = 10 cm) in a climate-controlled greenhouse (25 ± 2°C, 50–70% R.H.). We used young potted plants with two primary leaves (2–3 week-old) for the experiments. The herbivorous mites (*T. urticae*) were obtained from a laboratory-maintained culture reared on kidney bean plants (*P. vulgaris* cv. Nagauzuramame) grown under the same conditions as described above for lima bean plants.

2.2. Plant–plant interaction mediated by herbivore-induced plant volatiles

We used potted lima bean plants for bioassays. We placed ca. 100 *T. urticae* females on each of the plant's primary leaves. In each experiment, two infested, potted plants (together in one plastic dish filled with water) were positioned on one side of a plastic airtight container (18 l) (Fig. 1). We then placed two uninfested potted lima bean plants on the other side of the container. The container was then sealed. To prevent invasion of the uninfested plants by *T. urticae*, we used wet cotton wool to partition the container into an infested-plant area and an uninfested-plant area. We used a cleaned container for each experimental replicate in order to exclude effects from the previous experiment or experimental replicate. Three different containers were used for this procedure after being washed with hot water and then with ethanol and dried at 65°C. The experimental setup was maintained at 25 ± 2°C, 50–70% R.H., and 16L-8D conditions (2,150 lx, fluorescent light) for up to three days. Prior to subsequent analyses, we checked the plants to ensure that the receiver leaves were never infested by *T. urticae*.

As the whole plants (or detached leaves; see Section 2.3) were confined in the sealed container for 1–3 days, the available CO_2 may have been depleted and thus several types of stress may have occurred. We checked for the effects of such stresses on the plants' photosynthetic activity by monitoring the photosynthesis yield (arbitrary unit) in attached and detached leaves, by using a photosynthesis yield analyzer (model: MINI-PAM, WALZ, Germany) ($n = 4$). The yields of an intact plant before and after the experiment (kept in the sealed container for three days) were 0.771 ± 0.005 and 0.752 ± 0.012, respectively ($P = 0.244$, Student's t-test). The yields of a detached leaf before and after the experiment (kept in the sealed container for one day) were 0.772 ± 0.004 and 0.774 ± 0.002, respectively ($P = 0.713$, Student's

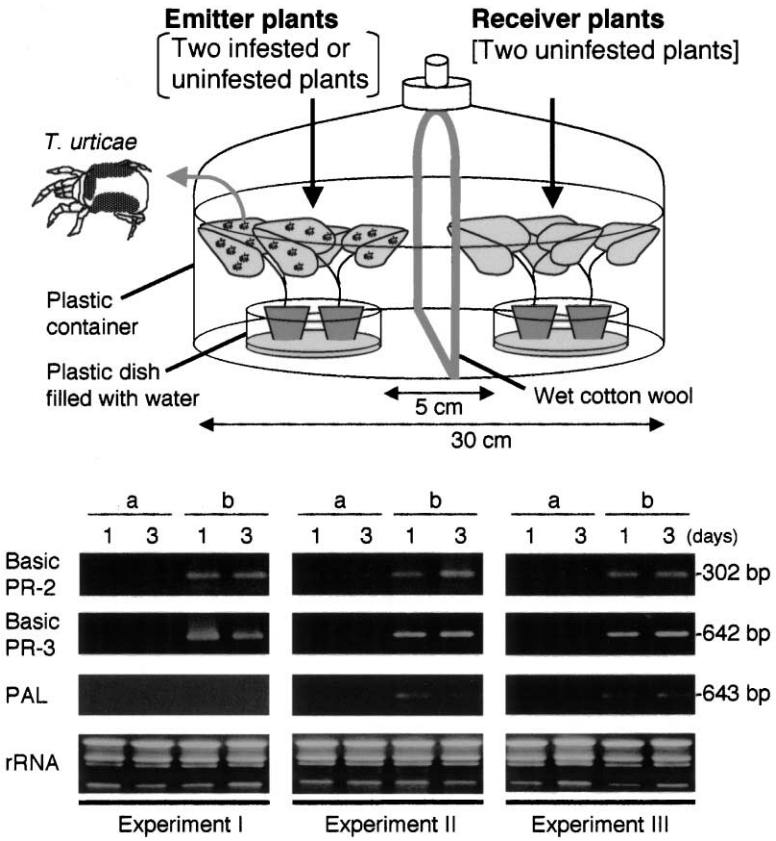

Fig. 1. Expression of defense genes in leaves of lima bean plants in response to herbivore-induced plant volatiles. Experimental setup used to analyze the effects of *T. urticae*-induced volatiles on gene expression in plant leaves. Two uninfested plants were exposed to volatiles released from uninfested (a) or *T. urticae*-infested (b) plants for one or three days, in a plastic container. Total RNA was isolated from the leaves of the plants, and the expression of basic PR-2, basic PR-3, and PAL genes was analyzed using RT-PCR.

t-test). Thus, no evidence was seen, which indicated that the photosynthetic activity of the plants was subject to stress.

2.3. *Effects of green leaf volatiles on uninfested lima bean leaves*

Three GLV compounds, (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate, were obtained from Tokyo Chemical Industry. Each detached leaf was kept in a glass vial (6 ml) filled with distilled water. Each GLV compound was dissolved in pure dichloromethane (1 μg per μl solution), and 10 μg of each was then impregnated into a piece of cotton wool. To compare the effect of GLVs with that of *T. urticae*-induced terpenoids on the gene expression of exposed leaves reported in Arimura

et al. (2000a), the amount of synthetic GLVs used for bioassay was the same as the amount of synthetic volatile terpenoids tested in Arimura et al. (2000a). After evaporation of the solvent, each piece of cotton wool was enclosed in a glass container (7 l) together with two detached leaves for 3 or 24 h. A piece of cotton wool containing only dichloromethane was used as the control. We used a cleaned container for each experimental replicate in order to exclude effects from the previous experiment or experimental replicate. Five different containers were used in this procedure after being washed as described in Section 2.2. The experimental setup was maintained at $25 \pm 2^{\circ}C$, 50–70% R.H., and 16L-8D conditions (2,150 lx, fluorescent light).

2.4. Chemical analysis

Potted lima bean plants with two primary leaves were infested with *T. urticae* (100 females per leaf) in plastic containers for one or three days. We transferred the infested plants to glass containers (2 l). Volatile compounds were drawn from the headspace of the container holding the infested plant into a glass tube packed with Tenax TA adsorbents (100 mg, mesh 20/35) for 1 h at a flow rate of 100 ml per min. The adsorbed compounds were eluted with 2 ml of diethyl ether, and *n*-eicosane (the internal standard) was then immediately added to the eluate. After the eluate was concentrated with a stream of gaseous N_2, it was injected into an injection port (250°C) of a GC-MS [GC: Hewlett Packard 6890 with an HP-5MS capillary column (i.d. = 0.25 mm, length = 30 m, film thickness = 0.25 μm); MS: Hewlett Packard 5973 mass selective detector, 70 eV]. The GC oven temperature was programmed to rise from 40°C (held for 5 min) to 280°C at 15°C/min. The compounds were identified by comparing their spectra with mass spectra in the database (Wiley), along with the data regarding herbivore-induced volatile compounds from lima bean plants.

2.5. RT–PCR analysis

Total RNA was extracted from two leaves by means of the acid guanidinium-phenol-chloroform method (Chomczynski and Sacchi, 1987). First-strand cDNA was synthesized from 1 μg of total RNA, using primers (5′-CTTGCACCATC-TATCTCTTC-3′, 5′-GATGCGGTCTTGAACCCTGC-3′, 5′-GGACTCTTTTGATT CTCATC-3′, 5′-TGACAATATCTCTATGACTG-3′, or 5′-TCCTCCAAATGCCT-CAAGTC-3′) which corresponded to the cDNA sequence of *P. vulgaris* acidic chitinase (nucleotides 336–355) (Margis-Pinheiro et al., 1991), basic chitinase (nucleotides 881–900) (Broglie et al., 1986), basic β-1,3-glucanase (nucleotides 864–883) (Edington et al., 1991), pLOX3 (nucleotides 1972–1991) (Meier et al., 1993), or PAL (nucleotides 937–956) (Edwards et al., 1985), respectively. The first-strand cDNA was amplified by adding Taq DNA polymerase (Takara, Japan) and primers (5′-AGCAACAACGTTAATGTTGC-3′, 5′-CTCAGCGCCCTCATATC-CAG-3′, 5′-TATGCTCTTTTCACTTCACC-3′, 5′-CTAGCAACAAACAGG-CAACT-3′, or 5′-AGGCTGCTGCCATTATGGAG-3′) which corresponded to the cDNA sequence of *P. vulgaris* acidic chitinase (nucleotides 105–124), basic

chitinase (nucleotides 259–278), basic β-1,3-glucanase (nucleotides 582–601), LOX3 (nucleotides 1260–1279), or PAL (nucleotides 314–333), respectively, as reported in the above-cited works. Amplification of cDNA of acidic chitinase, basic chitinase, basic β-1,3-glucanase, pLOX3 and PAL was, respectively, performed in 30, 25, 22, 23 and 25 cycles under optimum dynamic ranges before arriving at the plateau (the condition of each single cycle: 95°C for 30 s, 55°C for 60 s, and 72°C for 60 s). After the first-strand FPS cDNA was synthesized using a random nonamer primer, it was amplified with a pair of primers, 5′-CATGGATGACTCTCACACTC-3′ and 5′-AGTCATCCTGGACTTGAAAG-3′, which corresponded to nucleotides 405–424 and 801–820, respectively, in the sequence of maize FPS cDNA (Li and Larkins, 1996), in 35 cycles (the condition of each single cycle: 95°C for 30 s, 55°C for 60 s, and 72°C for 60 s). An equal amount of PCR products and 5 μg of total RNA were electrophoresed in agarose gels and detected by staining with ethidium bromide. No PCR products were detected when PCR was run without reverse transcription, and the sequence analysis confirmed that each cDNA product had been amplified from a transcription product of the lima bean gene. Each analysis was repeated three times.

3. Results and discussion

3.1. Plant–plant interaction mediated by herbivore-induced plant volatiles

In leaves of intact lima bean plants exposed to volatiles from *T. urticae*-infested plants for three days in the container (all three replicates), we detected transcripts of the genes for basic PR-2 (β-1,3-glucanase) and basic PR-3 (chitinase) (Fig. 1). We detected low levels of accumulation of PAL transcript in two of the three replicates. Transcripts of LOX, FPS, and acidic PR-4 genes were not detected (data not shown). In a control experiment in which uninfested lima bean plants were exposed to volatiles from other uninfested lima bean plants, we did not detect transcripts of any of the genes we assayed for in the receiver leaves. Thus, transcription of basic PR-2, basic PR-3, and PAL genes appears to have been specifically induced by volatiles from the infested plants. The data suggest that neighboring lima bean plants can respond to *T. urticae*-induced volatiles through activation of defense genes. However, the observed responses of the intact plants were not identical to those of detached leaves: In uninfested detached leaves exposed to volatiles from detached leaves infested by *T. urticae,* transcripts of PR-2, PR-3, PAL, LOX and FPS genes were all detected.

We found that potted lima bean plants emitted herbivore-induced volatiles when infested with *T. urticae* for 1–3 days (Fig. 2), and that these volatiles consisted mainly of terpenoids, DMNT and TMTT, which were also emitted by detached leaves infested with *T. urticae* (Fig. 2). (*E*)-β-Ocimene was detected in small amounts in the infested plants after one day, but was not detected after three days. The amounts of volatiles emitted from the infested potted plants were much smaller than those emitted from the infested detached leaves (Fig. 2). In addition, we did not detect release of GLVs from the infested plants, although previous studies have reported

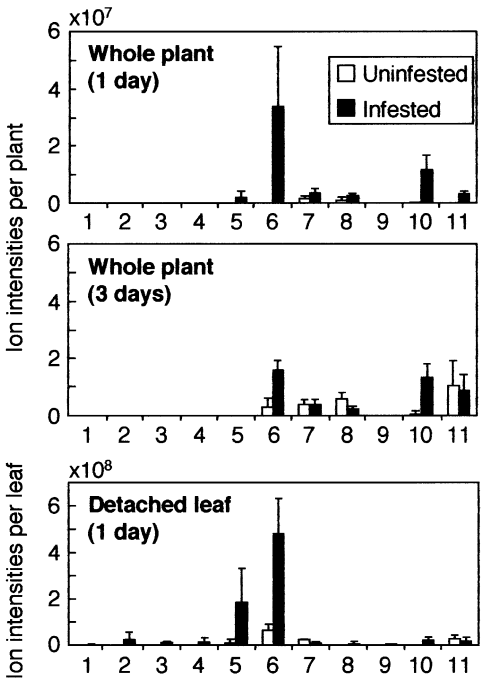

Fig. 2. Compounds identified in volatiles drawn from the headspace of containers holding whole plants or detached leaves. Whole lima bean plants and detached leaves which were infested with *T. urticae* or left uninfested were kept in a lidded plastic container for one or three days. Volatiles were collected in another container for 1 h, and then subjected to GC–MS analysis ($n = 4$). The data for volatiles from two detached leaves are taken from Arimura et al. (2000a). 1, (*Z*)-3-hexenol; 2, (*Z*)-3-hexenyl acetate; 3, limonene; 4, (*Z*)-β-ocimene; 5, (*E*)-β-ocimene; 6, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT); 7, α-copaene; 8, β-caryophyllene; 9, α-humulene; 10, (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); 11, methyl salicylate.

the detection of some GLVs (Dicke et al., 1990b, 1999; Arimura et al., 2000a). We previously observed the induction of transcription of defense genes in detached uninfested leaves by β-ocimene, DMNT and TMTT (Arimura et al., 2000a). Thus, our failure in the present study to detect expression of LOX and FPS genes in uninfested plants exposed to volatile emissions (from intact infested plants) containing β-ocimene, DMNT and TMTT may be due, in part, to their reduced amounts emitted.

3.2. *Effects of green leaf volatiles on uninfested lima bean leaves*

We exposed detached leaves to the vapors of synthesized preparations of the GLVs (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate, and assayed for transcripts of defense genes in the leaves (Fig. 3). GLVs are produced by green plants in response to mechanical damage (Dicke et al., 1990b; Matsui, 1998), exogenous JA

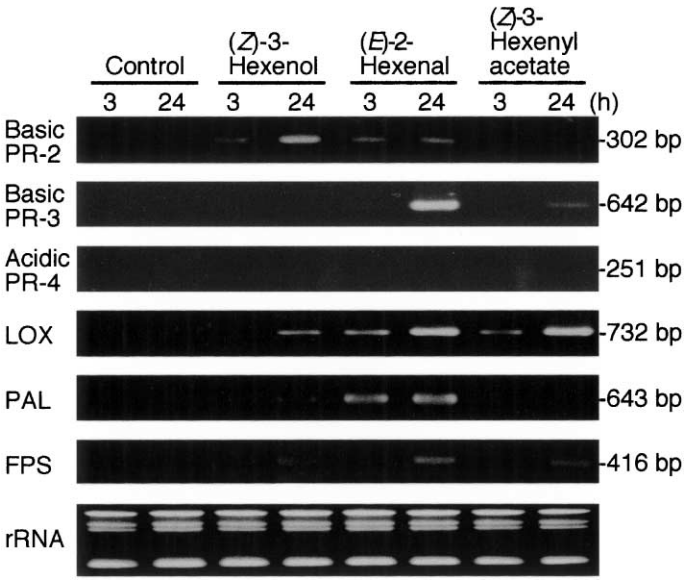

Fig. 3. Each of the three synthetic vapors shown here elicited the expression of defense genes. Two uninfested leaves were enclosed in a glass container together with a piece of cotton wool containing (*Z*)-3-hexenol, (*E*)-2-hexenal or (*Z*)-3-hexenyl acetate, dissolved in dichloromethane, for 3 or 24 h.

(Dicke et al., 1999), and damage resulting from herbivory (Dicke et al., 1990b, 1999; Turlings et al., 1990). Basic PR-2 and LOX genes were the only genes expressed to any great extent after exposure of lima bean leaves to (*Z*)-3-hexenol; very weak expression of PAL and FPS genes was induced. In contrast, (*E*)-2-hexenal induced the expression of five out of the six genes we assayed for. (*Z*)-3-Hexenyl acetate, which is one of the components of the volatiles released from detached leaves infested by *T. urticae* (Fig. 2), induced strong expression of the LOX gene and weak expression of the basic PR-3 and FPS genes, after a 24 h exposure period.

We previously reported that the GLVs emitted from detached lima bean leaves after infestation by 100 female *T. urticae* for 24 h were (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate (Arimura et al., 2000a, Fig. 2). In contrast, Dicke et al. (1990b, 1999) reported that heavily infested leaves from *T. urticae*-infested lima bean plants emitted large amounts of two GLVs: (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate. The discrepancy between these data and our current data may be due to differences in the conditions of the infested leaves; i.e., leaf age, number of spider mites per leaf, and duration of the infestation. Also, the environmental conditions of plants in this study and in the study by Dicke et al. (1990b) were different: we used a sealed container when preparing infested plants, whereas Dicke et al. (1990b, 1999) used a ventilated room.

GLVs are produced by the LOX pathway (Matsui, 1998). Since the GLV compounds are produced immediately after the leaf tissue is mechanically damaged (Hatanaka et al., 1987; Turlings et al., 1995; Matsui, 1998; Arimura et al., 2000a),

and because they are emitted from uninfested lima bean plants only in small amounts (Ozawa, R., unpublished data), they may act as fast-response plant-to-plant airborne signals of mechanical damage. Bate and Rothstein (1998) reported that aerial treatment of *Arabidopsis* seedlings with 10 μM concentrations of (*E*)-2-hexenal induced the transcription of several genes known to be involved in the plant's defense response, including phenylpropanoid-related genes and genes of the LOX pathway. However, transcription of genes encoding PR-1 or PR-2 was not induced. Hildebrand et al. (1993) found that (*E*)-2-hexenal and C_6 alcohols [(*E*)-2-hexenol and (*Z*)-3-hexenol] both reduce tobacco aphid fecundity on tobacco leaves. Interestingly, (*E*)-2-hexenal has both direct effects on aphid fecundity and indirect effects due to changes induced in the leaves upon which the aphids are feeding, while only indirect effects have been observed for (*E*)-2-hexenol. These data suggest that GLVs from wounded plants may play a role in both the direct and indirect defense of neighboring plants from herbivores.

3.3. Conclusion and future directions

Agrawal (2000) and Dicke and Bruin (2001) pointed out that the main weakness of our previous study (Arimura et al., 2000a) was that we used detached lima bean leaves for assays of plant–plant interactions. In this study we used potted lima bean plants, and detected responses similar to those of detached leaves. We found that intact plants also responded to the volatiles from *T. urticae*-infested conspecific plants. However, the expression patterns of defense genes were different from those observed in the detached leaves. Detached leaves may be more sensitive to *T. urticae*-infested leaf volatiles than intact plants.

Most previous studies of plant–plant interactions have been criticized (e.g., Fowler and Lawton, 1985) as suffering from problems of inadequate methodology. Recent advances in molecular biological technology have made it possible to reveal changes in gene expressions in response to volatiles (Shulaev et al., 1997; Bate and Rothstein, 1998; Arimura et al., 2000a, b). Experiments utilizing such technology have convincingly demonstrated that the existence of plant–plant interaction via airborne signals can occur in closed systems (Farmer and Ryan, 1990; Shulaev et al., 1997; Arimura et al., 2000a).

In a recent study using cDNA microarray technology, we observed changes in the transcription levels of many genes in response to herbivory and *T. urticae*-induced volatiles (Arimura et al., 2000b). These activated genes are involved in a broad range of functions, involving responses to pathogenesis and wounding, hormones, ethylene biosynthesis, flavonoid biosynthesis, (post-) transcriptional modifications, translation, chaperons, secondary signaling messengers, membrane transport, protein/peptide degradation, and photosynthesis. We therefore conclude that herbivore-induced volatiles elicit wide-ranging, marked changes in the metabolic processes of leaves.

Our findings raise several questions, the most important of which are as follows:

1. Are the plant–plant interactions detected in this study relevant to natural situations?

Our studies were carried out in a container in a laboratory, and this experimental condition could put stress on the test plants. However, as the photosynthetic yields of plants did not differ significantly before and after the experiment (see Section 2.2), the anticipated stress was not so strong as to affect the photosynthetic activity of the plants. A very important related question is whether the plant–plant interactions observed using our experimental system would also be observed under air-stream conditions, such as in a wind tunnel in the laboratory, or outdoors. Recently, sound experiments on plant–plant communication under outdoor conditions have been reported by Karban et al. (2000): Native tobacco plants downwind of wounded sagebrush plants are more resistant to herbivores than tobacco plants neighboring unwounded sagebrush. This effect was found to correlate with induced production of volatile methyl jasmonate in the sagebrush and increased production of polyphenol oxidase, a putative defense enzyme, in the tobacco.

2. How do plants perceive volatile signals?

In a previous study, we showed that three volatile terpenoids [β-ocimene, DMNT, and TMTT] elicited the expression of defense genes (Arimura et al., 2000a). In the present study, we showed that three GLVs [(*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate] also elicited the expression of defense genes (Fig. 3). However, the patterns of gene expression were different for each chemical. Furthermore, linalool did not induce gene expression (Arimura et al., 2000a). These data suggest the specificity in reception of these chemicals by plants. We have also reported that the expression of defense genes in receiver leaves is mediated by calcium influx into cells and by protein phosphorylation and dephosphorylation (Arimura et al., 2000a). However, details of these mechanisms remain elusive.

3. Herbivore specificity.

Tetranychus urticae-induced volatiles are specific and are different in chemical composition from wound-induced volatiles. In a previous study, we observed that responses in receiver leaves correlated with differences in composition (Arimura et al., 2000a). Ozawa et al. (2000) reported that lima bean leaves infested by caterpillars (*Spodoptera exigua*) emit blends of volatiles which are qualitatively and quantitatively different from those we have reported in studies of *T. urticae*-infested plants. It would be interesting to know whether these specific differences result in different responses in uninfested receiver leaves.

4. Functions of MeSA and ethylene.

Dicke et al. (1990b, 1999) and Ozawa et al. (2000) reported that lima bean plants infested by *T. urticae* emitted the volatile methyl salicylate (MeSA). MeSA is the most abundant compound from infested plants as seen in Dicke et al. (1999). In the present study, we also detected a small amount of MeSA among the volatiles emitted by the infested plants. In contrast, we did not detect significant amounts of this compound among the volatiles emitted from infested leaves in a previous study (Arimura et al., 2000a). This discrepancy needs to be investigated. We reported the induction of acidic PR-4 genes by gaseous MeSA (Ozawa et al., 2000). In the present experiment, we detected MeSA in the infested plants (Fig. 2), but we did not detect transcription of an acidic PR-4 gene in the exposed plant. Perhaps, MeSA only acts as an airborne signal mediating plant–plant interactions at higher concentrations.

High levels of MeSA emissions have been observed in tobacco plants infected by tobacco mosaic virus (TMV) (Shulaev et al., 1997). TMV resistance and the expression of a PR-1 gene are induced in intact tobacco plants by gaseous emissions of TMV-inoculated plants, but only by emissions containing MeSA. It has therefore been suggested that MeSA functions as an airborne signal that activates disease resistance and the expression of defense-related genes in neighboring plants (Shulaev et al., 1997). We recently observed ethylene emissions from lima bean leaves infested with *T. urticae*. (Arimura et al., unpublished data). As it has been reported that some defense genes are activated by ethylene (Xu et al., 1994; O'Donnell et al., 1996), ethylene is also thought to be one of the candidate airborne signals involved in plant–plant communication.

Plant–plant communication through the atmosphere is often referred to using the terms "talking plants" and "listening plants". Some scientists have suggested that such communication might be of great advantage to listening plants, in terms of defense against possible future damage from herbivores (Bruin Sabelis and Dicke, 1995; Karban and Baldwin, 1997; Agrawal, 2000). In a study using lima beans, both the talking plants and the listening uninfested plants were found to be attractive to the predatory mite *P. persimilis* (Dicke et al., 1990a). In addition, listening uninfested cotton and lima bean plants are characterized by reduced suitability as resources for spider mites (Bruin et al., 1992; Arimura et al., 2000a). Under such conditions, the predatory mites that are attracted to the uninfested plants (listening plants) can be expected to eventually move to the infested plants (talking plants). If this is the case, such plant–plant interactions may be beneficial to the talking plants as well as to the listening plants, if the presence of the listening plants enhances the total number of predators attracted (see also Bruin, Sabelis and Dicke, 1995). Cost-benefit analysis of plant–plant interactions is necessary in order to better understand the importance of these interactions in nature.

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