

CHAPTER FIVE

Variation in induced volatiles released from *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum* and *Vicia faba* in response to *Liriomyza* (Diptera: Agromyzidae) species damage

Abstract

Plants are known to emit a variety of volatile compounds due to wounding and herbivore damage. *Liriomyza* leafminers can be important pests of horticultural crops worldwide. This study identified volatile components emitted by whole plants of the following species: *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum* and *Vicia faba*. The treatments considered were healthy undamaged plants, mechanically damaged and leafminer damaged plants. Three *Liriomyza* species, viz. *L. huidobrensis*, *L. sativae* and *L. trifolii* were used to induce damage to these host plant species. *Liriomyza* damaged plants were used for volatile collection when the larvae had reached the third instar. Volatiles were analyzed using gas chromatography coupled with mass spectrometry (GC-MS). Among several constituents, (Z)-3-hexenyl acetate, (Z)-3-hexenol, and *n*-nonanal were emitted by *P. vulgaris*, *P. sativum* and *V. faba*. *Solanum lycopersicum* plant treatments resulted in the emission of mainly monoterpenes: 2- δ -carene, sabinene, α -pinene, β -phellandrene and the sesquiterpene (*E*)-caryophyllene. There was considerable qualitative variation within the same host plant species, with different damages mainly for *P. vulgaris*, *P. sativum* and *V. faba*. However, for all *S. lycopersicum* treatments, there were slight qualitative inter-treatment differences. Volatiles identified through reference to the MS library (Adams 2, chemcol and NIST 05a) need to be confirmed experimentally by comparing the spectra obtained with spectra of authentic internal standards and should be quantified. The volatile compounds identified here should also be tested for their behavioural activities in attracting natural enemies of *Liriomyza* spp.

Introduction

Plants have evolved a wide range of defensive (direct and indirect) tactics to protect them from attack by pathogens and herbivores (Mattiacci *et al.*, 2001; Hartmann, 2004; Kang *et al.*, 2009). Indirect defense may involve the recruitment and sustenance of natural enemies to a damaged plant via its induced volatiles (Wei & Kang 2006a, b; Kang *et al.*, 2009). A number of researchers have reported that volatile induction in plants can vary with herbivore species, instar and environmental conditions and have highlighted specificity in plant responses as an important signal to natural enemies for locating their hosts (Turlings *et al.*, 1990, 1995; De Moraes *et al.*, 1998; Kessler & Baldwin, 2001; Gouinguéné & Turlings, 2002; Gouinguéné *et al.*, 2003; Zhao & Kang 2002a, b, 2003; Rasmann *et al.*, 2005; Röse & Tumlinson, 2005).

The leafminers *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) are polyphagous and important pests of agricultural crops worldwide (Spencer, 1985; Murphy & LaSalle, 1999; EPPO, 2006; Burgio *et al.*, 2007). *Liriomyza huidobrensis* and *L. sativae* have been observed to differ in their feeding habits (Zhao & Kang, 2002a). The larvae of *L. huidobrensis* consume mesophyll in both palisade and spongy tissues (Parrella & Bethke, 1984; Wei *et al.*, 2000), whereas *L. sativae* larvae only feed on palisade tissue (Kang, 1996). Further, *L. huidobrensis* larvae frequently mine along the midribs of leaves, and late instars are usually found mining the lower surfaces of leaves or within petioles. This mining behaviour is distinctly different from the serpentine mines of *L. sativae* and *L. trifolii* on upper leaf surfaces (Parrella, 1987).

The difference in feeding habits of *Liriomyza* species may trigger the emission of different volatile blends. This has been observed in other herbivore species such as leaf-feeding caterpillars, spider mites, stem-boring caterpillars, and sucking insects (Turlings *et al.*, 1990, 1995; De Moraes *et al.*, 1998; Kessler & Baldwin, 2001). Studies by Dicke & Minkenbergh, (1991), Petitt *et al.* (1992), Finidori-Logli *et al.* (1996) and Zhao & Kang (2002a, b; 2003) have shown that volatiles emitted from plants with different types of damage can stimulate the behavioural or antennal responses of both leafminers and their parasitoids. Leafminer-induced volatile blends or individual compounds can thus be used to attract natural enemies of leafminers (Kang *et al.*, 2009).

Studies on herbivore-induced plant volatiles (HIPVs) have received increasing attention since the early 1990s. New developments in the interdisciplinary fields of biochemistry, physiology, and behavioural ecology have resulted in a growing knowledge of chemical ecology of plants, *Liriomyza* species, and their associated parasitoids (Kang *et al.*, 2009). The current study seeks to add knowledge to the already developing trends in this area of research. In this study, the differences that exist in volatile composition from host plant species attacked by three related leafminer species (*L. huidobrensis*, *L. sativae* and *L. trifolii*) with different feeding habits are determined.

Materials and Methods

Plants

The four plant species, *P. vulgaris* (variety Julia), *P. sativum* (variety Oregon Sugar Pod III), *S. lycopersicum* (variety Moneymaker) and *V. faba* (a local Kenyan open-pollinated variety), used in experiments were grown and maintained as described in Chapter 2. The average leaf areas of the plants before the experiments were: *P. vulgaris*, 111.68 cm², *P. sativum*, 76.65 cm², *S. lycopersicum* 98.43 cm² and *V. faba* 37.40 cm².

Leafminers

The three leafminer species, *L. huidobrensis*, *L. sativae* and *L. trifolii*, were obtained from the International Centre of Insect Physiology and Ecology (*icipe*) leafminer rearing facility. The colonies were maintained for 22-24 generations prior to experiments. *Liriomyza huidobrensis* was reared on *V. faba* while *L. sativae* and *L. trifolii* were reared on *P. vulgaris*.

Leafminer rearing for experimental purposes

In each experimental set, three potted plants of each of the plant species evaluated were infested with 50 four-day-old male and female leafminers (sex ratio 1:1), previously fed on a 10 % sucrose solution, in a cage for a period of 24 hours to allow for oviposition. The cage (50 cm × 50 cm × 45 cm) was fitted with a wire screen mesh on top for

ventilation. Infested potted plants were removed and transferred to another similar cage, free of leafminers, to monitor the development of larvae until the third-instar stage which was used in experiments. Any damage to the exposed plants was avoided as much as possible to prevent release volatiles not associated with the insect damage.

Experimental procedures: volatile collection and analysis

Plant treatments

The following plant treatments were selected for volatile collection and analysis: plants infested with third-instar larvae of *L. huidobrensis*: *P. vulgaris* (FrLH3), *P. sativum* (SNLH3), *S. lycopersicum* (TMLH3), *V. faba* (FBLH3); third-instar larvae of *L. sativae*: *P. vulgaris* (FrLS3), *S. lycopersicum* (TMLS3), *V. faba* (FBLS3); third-instar larvae of *L. trifolii*: *S. lycopersicum* (TMLT3) and *V. faba* (FBLT3). The following additional plant treatments were used for reference purposes: mechanically damaged plants: *P. vulgaris* (FrMC), *P. sativum* (SNMC), *S. lycopersicum* (TMMC), *V. faba* (FBMC); healthy undamaged plants of all plant species and a blank control (empty trapping bag).

All leafminer-infested plants were obtained as described above while the mechanical damage on the plants was induced by making four straight scratch lines using a sharp needle along the entire length of eight leaves per plant.

Plant volatile collections

A headspace sampling method using a mobile pump was used to collect volatiles from the different treatments. The volatile collection system consisted of an air suction pump (Air Cadet vacuum/pressure station, Cole Palmer Instrument Co., USA), a flow meter (Cole Palmer Instrument Co., USA), Reynolds[®] oven bag (turkey size 482 mm × 596 mm, Reynolds Kitchens, Richmond, VA) and Super Q adsorbent traps (Analytical Research Systems, Gainesville, FL).

For each volatile trapping process, bags were cleaned by baking them overnight in an oven at 120 °C before use. The oven-cleaned bags were then placed over plants and closed up around the stem of the branch with a strong PVC thread (Fig. 5.1). Airflow into the sampling bag was provided by two Teflon[®] tubes. One tube pushed air into the bag over the foliage while the other tube pulled air out of the bag through the Super Q adsorbent trap at the end and then through the flow meter at a rate of 265

ml/min. Volatiles were collected for 2 hours in the afternoon between 15:00 and 17:00. The adsorbent trap was removed, sealed with Teflon[®] tape and stored in a freezer at -20 °C until use. The experiment was replicated between 3 to 6 times per plant and *Liriomyza* species.



Fig. 5.1. Volatile collection system set-up placed on a bench in the greenhouse

Immediately after collection of volatiles, the number of mined leaves and leafminer larvae per plant were counted. The mean number of mined leaves and leafminer larvae per plant species were: FrLH3 (81 larvae/10 mined leaves), SNLH3 (36 larvae/17 mined leaves), TMLH3 (36 larvae/15 mined leaves), FBLH3 (92 larvae/17 mined leaves), FrLS3 (105 larvae/10 mined leaves), TMLS3 (31 larvae/15 mined leaves), FBLS3 (60 larvae/19 mined leaves), TMLT3 (30 larvae/16 mined leaves) and FBLT3 (61 larvae/14 mined leaves).

Plant volatile analysis

Super Q adsorbent traps were eluted with 200 μ l of GC/GC-MS-grade dichloromethane (Burdick & Jackson, Muskegon, Michigan, USA). Fifty microlitres of volatile samples were analyzed by gas chromatography-mass spectrometric methods. The analysis was carried out on an Agilent technology 7890A GC- with 5975C MSD. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV and emission current of 34.6 μ A. The temperature of the source was held at 230 °C (ion source), 150

°C (Quadrope) and multiplier voltage was 1106 V (35 to 280). The pressure of the ion source was held at 7×10^{-6} mBar. The spectrometer had a scan cycle of three scans per two seconds. The mass range was set at m/z 1-1050 and scan range was 38-550 m/z for the compounds. The instrument was calibrated using heptacosane (Perfluorotributylamine) [CF₃(CF₂)₃]₃N (Apollo scientific Ltd. UK). HP5 MS capillary column, 30 m × 0.25 mm (i.d) × 0.25 μm (film thickness) supplied by J & W Scientific was used. The GC-MS was linked to a computer with MS libraries (Adams 2, chemecol and NIST 05a). Identification of chemical components in the volatiles was based on the interpretation of the mass spectral and fragmentation patterns obtained within the data libraries (Adams 2, chemecol and NIST 05a) to obtain preliminary structural assignments.

Results

Across all host plant species treatments (undamaged plants, mechanically damaged plants and *Liriomyza* damaged plants), 52 different plant compounds were identified with the GC-MS analysis. The majority of compounds were produced by *L. trifolii* and *L. huidobrensis*- damaged *S. lycopersicum* and *L. huidobrensis*-damaged *V. faba* plants each with a total of 14, 13 and 13, respectively.

Undamaged *P. vulgaris* plants emitted four compounds (Table 1). Mechanically damaged plants only emitted two compounds, namely (Z)-3-hexenol and (Z)-3-hexenyl acetate. These two compounds were not, however, emitted by undamaged plants. *Phaseolus vulgaris* damaged by *L. huidobrensis* emitted three compounds, namely 2-hexanol, a green leaf volatile, 1,1-dimethyl-3-chloropropanol and 3-methoxy-1-butanol (Table 5.1). 2-hexanol was exclusively emitted in this treatment for this host plant species while the other two were also emitted by *P. vulgaris* damaged by *L. sativae*. *Phaseolus vulgaris* damaged by *L. sativae* emitted the highest number of compounds (11) (Table 5.1) compared to the same plant species damaged by either of *L. huidobrensis* and *L. trifolii*. With the exception of (Z)-3-hexenol, (Z)-3-hexenyl acetate, *n*-nonanal, 1,1-dimethyl-3-chloropropanol and 3-methyl-1-butanol, the compounds exclusively occurred in this treatment for this host plant species.

Undamaged *P. sativum* emitted seven compounds (three green leaf volatiles, two ketones and two aldehydes) (Table 5.2). Mechanically damaged snow pea plants emitted three green leaf volatiles and one ketone. The same green leaf volatile compounds were emitted by both the undamaged and the mechanically damaged *P. sativum* plants (Table 5.2). *Pisum sativum* plants damaged by *Liriomyza huidobrensis* emitted two aldehydes and two ketones. All the compounds except (*E*)-2-hexenal were also emitted by undamaged plants. Compounds emitted by mechanically damaged *P. sativum* and leafminer-damaged plants were exclusively different between the two plant treatments (Table 5.2).

Table 5.1. Composition of volatile blends emitted by *P. vulgaris* plants: healthy undamaged (UDFr), mechanically damaged (FrMC), plants with *L. huidobrensis* (FrLH3) and *L. sativae* (FrLS3) feeding damage at third instar; + indicates presence, - indicates absence.

Chemical compound	Plant treatment			
	UDFr	FrMC	FrLH3	FrLS3
Green leaf volatiles				
2-hexanol	-	-	+	-
(Z)-3-hexenol	-	+	-	+
(Z)-3-hexenyl acetate	-	+	-	+
3-penten-2-ol	+	-	-	-
Octan-3-ol	-	-	-	-
Aldehydes				
(E)-2-hexenal	-	-	-	+
(Z)-3-hexenal	-	-	-	+
<i>n</i> -nonanal	+	-	-	+
<i>n</i> -octanal	+	-	-	-
Ketones				
1-cyclopentyl-ethanone	-	-	+	-
3-methyl-2-butanone	-	-	-	+
Dodecane	-	-	-	+
6-methyl-5-hepten-2-one	-	-	-	+
3-hydroxy-3,5-dimethyl-2-hexanone	+	-	-	-
2, 6-dimethyl -4-hepten-3-one	-	-	+	-
3-methyl-6-methylene-octane	-	-	-	+
Pentadecane	-	-	-	+
2-pentanone	-	-	+	+
Other compounds				
1,1-dimethyl-3-chloro-propanol	-	-	+	+
3-methoxy-1-butanol	-	-	+	+
Total number of compounds	4	2	6	13

Table 5.2. Composition of volatile blends emitted by *P. sativum* plants: healthy undamaged (UDSN), mechanically damaged (SNMC) and plants with *L. huidobrensis* feeding damage at third instar (SNLH3) and blank control (BL); + indicates presence, - indicates absence.

Chemical compound	Plant treatment			
	UDSN	SNMC	SNLH3	BL
Green leaf volatiles				
2-hexanol	+	+	-	-
(Z)-3-hexenol	+	+	-	-
(Z)-3-hexenyl acetate	+	+	-	-
Aldehydes				
<i>n</i> -decanal	+	-	+	-
(<i>E</i>)-2-hexenal	+	-	+	-
Ketones				
1-cyclopentyl-ethanone	+	-	+	-
6-methyl-5-hepten-2-one	+	-	+	-
2-pentanone	-	+	-	-
Total number of compounds	7	4	4	NIL

Undamaged *S. lycopersicum* emitted 12 compounds, mainly monoterpenes and sesquiterpenes (Table 5.3). Mechanically damaged *S. lycopersicum* plants emitted six monoterpenes and two sesquiterpenes. The same chemical compounds were emitted in the two plant treatments except that *trans*-isolimonene, β -phellandrene, terpinolene and (*E*)-caryophyllene were not emitted by mechanically-damaged plants.

For *S. lycopersicum* plants, *L. trifolii*-damaged plants emitted the highest numbers of compounds, whereas the lowest was from those damaged by *L. sativae*. Undamaged and mechanically damaged *S. lycopersicum* plants also emitted mainly monoterpenes and sesquiterpenes. The compound β -phellandrene was only emitted by undamaged *S. lycopersicum* plants and *L. sativae*-damaged plants. There seemed to be an inverse relationship in emission patterns between the compounds β -phellandrene and sabinene, based on the observation that in treatments where sabinene was emitted (undamaged, mechanically damaged and *L. huidobrensis* and *L. trifolii*-damaged plants), β -phellandrene was not emitted at all (TMMCR, TMLH3 and TMLT3) (Table 5.3). Conversely, for TMLS3, where β -phellandrene was emitted, there were no concomitant emissions of sabinene.

The compounds myrcene, (*E*)- β -ocimene, α -phellandrene, α -terpinene, *ortho*-cymene and (*Z*)-3-hexenol or allo-ocemene were not emitted by either undamaged *S. lycopersicum* plants or mechanically damaged plants. These compounds were only emitted by leafminer- damaged plants (Table 5.3). There were slight qualitative variations in the compounds emitted by leafminer-damaged *S. lycopersicum* plants (Table 5.3).

Table 5.3. Composition of volatile blends emitted by *S. lycopersicum* plants: healthy undamaged (UDTM), mechanically damaged (TMMCR), plants with *L. huidobrensis* (TMLH3), *L. sativae* (TMLS3) and *L. trifolii* (TMLT3) feeding damage at third instar; + indicates presence, - indicates absence.

Chemical compound	Plant treatment				
	UDTM	TMMCR	TMLH3	TMLS3	TMLT3
Monoterpenes					
2- δ -carene	+	+	+	+	+
β -elemene	+	+	+	+	+
δ -elemene	+	+	+	+	+
<i>Trans</i> -isolimonene	+	-	+	+	+
Myrcene	-	-	+	+	+
(<i>E</i>)- β -ocimene	-	-	+	+	+
α -phellandrene	-	-	-	-	+
β -phellandrene	+	-	-	+	-
α -pinene	+	+	+	+	+
Sabinene	+	+	+	-	+
α -terpinene	-	-	+	-	-
γ -terpinene	+	+	-	-	+
Terpinolene	+	-	+	+	-
Sesquiterpenes					
Allo-aromadendrene	+	+		-	-
(<i>E</i>)-caryophyllene	+	-	+	+	+
α -humulene	+	+	+	+	+
Other Compounds					
<i>Ortho</i> -cymene	-	-	+	-	+
Allo-ocemene	-	-	-	-	+
Total number of compounds	12	8	13	11	14

For undamaged *V. faba* plants, only three compounds were emitted, all of which were green leaf volatiles (Table 5.4). Mechanically damaged plants emitted six volatile compounds (Table 5.4). Except for (*Z*)-3-hexenol which was produced in undamaged and mechanically- damaged plants, there were qualitative differences between undamaged plants and mechanically damaged plants.

There were qualitative variations in the volatile compounds emitted by the *V. faba* plants damaged by the three leafminer species. *Liriomyza huidobrensis* damaged *V. faba* plants emitted 13 compounds; *L. sativae*-damaged plants emitted five compounds while *L. trifolii* damaged plants emitted two compounds (Table 5.4).

The majority of compounds emitted by *L. huidobrensis* infested *V. faba* plants were green leaf volatiles and ketones (Table 5.4). Three monoterpenes and two other compounds were emitted by *V. faba* plants damaged by *L. sativae*. β - elemene was the only exclusive emitted compound for this plant treatment.

Overall, across all the different host plant and leafminer species, considerable qualitative variation in the volatiles emitted occurred between *S. lycopersicum* plant treatments and other host plant species treatments. Monoterpenes and sesquiterpenes were largely emitted in *S. lycopersicum* plant treatments as opposed to green leaf volatiles, aldehydes and ketones emitted in the other plant treatments. None of the compounds obtained by trapping from various plant treatments were obtained by trapping from the empty oven bags.

Table 5.4. Composition of volatile blends emitted by *V. faba* plants: healthy undamaged (UDFB), mechanically damaged (FBMC), plants with *L. huidobrensis* (FBLH3), *L. sativae* (FBLS3) and *L. trifolii* feeding damage at third instar (FBLT3). (+) indicates presence, (-) indicates absence.

Chemical compound	Plant treatment				
	UDFB	FBMC	FBLH3	FBLS3	FBLT3
Green leaf volatiles					
(Z)-3-hexenol	+	+	+	-	-
3-hexen-1-ol	+	-	-	-	-
(Z)-3-hexenyl acetate	-	+	-	-	-
(Z)-3-hexenyl hexanoate	+	-	-	-	-
1-nonen-3-ol	-	-	+	-	-
Octan-3-ol	-	-	+	-	-
Phenyl ethyl alcohol	-	-	+	-	-
Aldehydes					
<i>n</i> -nonanal	-	-	+	-	-
Octanal	-	-	+	-	-
Ketones					
1-cyclopentyl-ethanone	-	-	+	-	-
6-methyl-5- hepten-2-one	-	-	+	-	-
4-octanone	-	-	+	-	-
2-pentanone	-	+	-	-	-
Monoterpenes					
β -elemene	-	-	-	-	+
2- δ -carene	-	+	-	+	-
Limonene	-	-	-	+	-
(<i>E</i>)- β -ocimene	-	-	+	-	+
β -phellandrene	-	-	-	+	-
Sesquiterpenes					
(<i>E</i>)-caryophyllene	-	-	-	+	-
Other Compounds					
Decanoic acid	-	-	+	-	-
4-methyl-3-octene	-	-	+	+	-
3,6,6 trimethyl- cyclohex-2-enol	-	+	+	-	-
Total number of compounds	3	5	13	5	2

Discussion

The number of compounds emitted by *P. vulgaris* was low compared with previous studies involving bean plants. Finidori-Logli *et al.* (1996) identified 15 volatile components emitted by kidney bean (*Phaseolus vulgaris* L., Fabaceae) plants damaged by *L. trifolii*. Wei & Kang (2006a) identified 25 volatiles emitted by healthy, mechanically damaged and leafminer-damaged *P. vulgaris* plants. Although it was expected that different varieties of the same plant species would produce different plant volatile profiles, the current results show very low levels of volatile emission from *P. vulgaris* across all the host plant treatments.

In the current study, the volatile trapping time was shorter (2 hours) than the 10 hours as reported by Wei & Kang (2006a). The volatile trapping duration used in the current study was chosen to avoid trapping physiologically stress-induced compounds resulting from enclosing plants for prolonged periods during the volatile-trapping process.

Of the few volatiles compounds that were identified from mechanically damaged *P. vulgaris* plants in the current study, (Z)-3-hexenol and (Z)-3-hexenyl acetate were also identified by Wei & Kang (2006a) on bean plants. 2-hexanol was released exclusively by *P. vulgaris* infested with third-instar larvae of *L. huidobrensis*. This compound seems to be leafminer-induced, as it was not identified in either undamaged or mechanically damaged plants.

The green leaf volatiles (GLVs) always refer to six-carbon-chain-lengths alcohols, aldehydes and esters, while oximes are three to four carbon, nitrogen-containing compounds (Kang *et al.*, 2009). Oximes were not identified from any of the *P. vulgaris* plant treatments nor other plant treatments in the current study. Finidori-Logli *et al.* (1996) observed that the identification of oximes in trapped volatiles obtained from mined and artificially damaged *P. vulgaris* leaves was a rare phenomenon. However, Wei & Kang (2006a) and Wei *et al.* (2007) identified a sizeable number of oximes from leafminer-damaged bean plants. In the current study, oximes might have been emitted by the plants but probably in very small quantities that were beyond detection by the GC-MS analyses. In addition, the *P. vulgaris* variety 'Julia'

used in the current study may not have emitted oximes as opposed to varieties used in studies done elsewhere.

The compounds (Z)-3-hexenol and (Z)-3-hexenyl acetate were among the compounds emitted by *L. sativae* damaged *P. vulgaris* plants. Wei & Kang (2006b) observed that these two compounds elicited electroantennogram (EAG) responses by the generalist parasitoid *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) in an olfactometer bioassay. The presence of these compounds in *L. sativae*-damaged plants observed in the current study provides strong evidence of the emission of *Liriomyza* induced volatiles in bean plants.

Few studies have examined the volatile profiles of *P. sativum*, *S. lycopersicum* and *V. faba* in relation to *Liriomyza* damage. In the current study all *S. lycopersicum* plant treatments emitted mainly monoterpenes and sesquiterpenes. Wei *et al.* (2007) demonstrated that *S. lycopersicum* produced many monoterpenes and sesquiterpenes in relatively high concentrations compared to other host plant species. The differences in the volatile profiles of *S. lycopersicum* and other host plant treatments could be related to differences in the larval feeding habits of *Liriomyza* species. *L. huidobrensis* feeds in the palisade and spongy mesophyll (Parella & Bathke, 1984, Wei *et al.*, 2000; Wei & Kang 2006a), whereas larvae of *L. sativae* and *L. trifolii* only feed in palisade mesophyll (Kang, 1996; Salvo & Valladares, 2004).

There was a notable inverse relationship in the emission of the compounds sabinene and β -phellandrene mainly from *S. lycopersicum* treatments. β -phellandrene was only emitted by *L. sativae*-damaged plants and sabinene was absent. β -phellandrene and/or sabinene could have been a by-product of monoterpene synthases. For example, Shimada *et al.* (2004) reported that monoterpene synthase produces specific main products, e.g. β -phellandrene, whereas some synthases may or may not synthesize sabinene as a by-product. Shimada *et al.* (2004) further observed that monoterpene synthase may have unique mechanisms to control specific deprotonation or cyclization of carbocations to produce a wide diversity of monoterpenoids. The results of the current study require further investigation to determine the relationship between damage caused by *Liriomyza* larvae and β -phellandrene and sabinene.

In *S. lycopersicum* plant treatments, the compounds myrcene, (*E*)- β -ocimene, α -phellandrene, α -terpinene, *ortho*-cymene and (Z)-3-hexenol or allo-ocimene were likely

leafminer-induced as they were not emitted by undamaged and mechanically damaged plant treatments.

Overall, there were qualitative differences in the volatile compounds across all the plant treatments. Although Dicke (1999) observed that differences between volatile blends were greatest between plant species and smallest between individual plants of one species infested by different herbivores, in the current study this conclusion seems only to hold for *S. lycopersicum* plant treatments.

Most of the compounds identified here have been previously mentioned in the literature from undamaged plants. β -caryophyllene, α -humulene, allo-aromadendrene have been reported from undamaged *Dipterocarpus kerii* (Dipterocarpaceae) (Jantan, 1988). α -pinene, limonene, β -pinene, myrcene, β -phellandrene, γ -trepinene have also been identified from leaf essential oils of *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux and subsp. *macrocarpa* (Sm.) Ball (Adams, 1999). This suggests that the emission of these compounds by *S. lycopersicum* is not specifically due to wounding or *Liriomyza* feeding damage. However, Loughrin *et al.* (1994) and Röse *et al.* (1996) reported that low molecular weight monoterpenes, myrcene and β -ocimene are formed by *Arabidopsis thaliana* (L.) Heynh. and that these isoprenoids are produced by damaged plant leaves.

Kishimoto *et al.* (2005) studied the expression profiles of genes involved in defense responses upon exposing *A. thaliana* to volatiles. The compounds (*E*)-2-hexenal, (*Z*)-3-hexenol or allo-ocimene were shown to induce the genes expressing chalcone synthase (CHS), caffeic acid-O-methyltransferase (COMT), diacylglycerol kinase1 (DGK1), glutathione-S-transferase1 (GST1) and lipoxygenase2 (LOX2). These genes were also induced by mechanical wounding (Kishimoto *et al.*, 2005). GLVs, including (*E*)-2-hexenal, (*Z*)-3-hexenal and their corresponding alcohols, or esters, are produced from damaged plant tissues as products of fatty acid hydroperoxide lyase (HPL) from 13-hydroperoxides of linoleic or linolenic acid, as one of the branches of phytooxylipin pathway (Hatanaka, 1993). Arimura *et al.* (2001) also showed that (*E*)-2-hexenal, (*Z*)-3-hexenol or (*Z*)-3-hexenyl acetate could induce genes encoding basic pathogenesis-related proteins (PRs), LOX or phenylalanine ammonia lyase (PAL) in lima beans.

Although the above provides evidence for the role of plant volatile emissions in indirect plant responses to wounding and herbivore damage, the evolutionary significance of the production of such compounds in *Liriomyza*-susceptible host plant species is not clear. A susceptible host plant may suffer energy (resource) cost by emitting these compounds that may not be involved in triggering resistance expression genes. Therefore, the most likely reason for the emission of these plant compounds in susceptible host plant species is the attraction of natural enemies of *Liriomyza* species. This should be explored in further studies to test the volatile compounds identified in this study for their ability to attract major natural enemies of *Liriomyza*.

Discussion of the current findings is purely based on the identification of compounds by their MS spectra based on reference to published mass spectra data from (Adams 2, chemocol and NIST 05a) libraries. To confirm the identity of the compounds referred to in the current study, further studies are needed to compare the spectra of the compounds and retention times with those of synthetic standards. The quantitative variations of the compounds also should be taken into account and correlated to levels of leafminer damage.

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