ORIGINAL ARTICLE

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Volatiles released from bean plants in response to agromyzid flies

Abstract *Liriomyza sativae* Blanchard and *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) are two invasive flies in China that have caused economical damage on vegetables and ornamental plants. In this article, we report the profiles of emitted volatiles from healthy, mechanically damaged, and leafminer-damaged bean, *Phaseolus vulgaris* L., plants. Among 25 emitted volatiles identified, (*E*)-2-hexen-1-al, (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (*Z*)-3-hexenyl acetate, (*Z*)-3-hexen-1-ol, (*syn*)- and (*anti*)-2-methylpropanal oxime, (*syn*)-2-methylbutanal oxime, linalool, and (*E,E*)-α-farnesene were consistently released from damaged bean plants. Combined amounts of these nine compounds made up more than 70% of the total volatiles emitted from each treatment. No qualitative differences in volatile emission were found between bean plants damaged by the two fly species; however, amounts of several major compounds induced by *L. huidobrensis* damage were significantly higher than those from plants damaged by *L. sativae*. The mechanically damaged plants released a higher proportion of green leaf volatiles than plants in the other treatments, whereas leafminer-damaged plants produced more terpenoids and oximes. Furthermore, the volatile profiles emitted from plants, damaged by adult leafminers, by second instar larvae, and even the plants with empty mines left by leafminer larvae (the pupal stage) were significantly different. The identification of volatile oximes released from damaged plants was confirmed and is discussed in a behavioral and biological control context.

Keywords Invasive insect species · *Liriomyza* · Oximes · *Phaseolus* · Plant volatiles

Abbreviations CLP: Bean plants with cut leaves · CTRL: Clean bags · DMNT: (3*E*)-4,8-Dimethyl-1,3,7-nonatriene · GC–MS: Gas chromatography–mass spectrometry · Lh-EMP: Bean plants with empty mines left by *L. huidobrensis* larvae · Lh-FOP: Bean plants on which *Liriomyza huidobrensis* had fed and oviposited · Lh-LP: Bean plants infested with *L. huidobrensis* 2nd-instar larvae · Ls-EMP: Bean plants with empty mines left by *L. sativae* larvae · Ls-FOP: Bean plants on which *L. sativae* had fed and oviposited · Ls-LP: Bean plants infested with *L. sativae* 2nd-instar larvae · NPP: Bean plants with needle-punctured leaves · TMTT: (3*E*,7*E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene · UP: Undamaged bean plants

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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). Indirect defense may involve the recruitment and sustenance of natural enemies to a damaged plant via its induced volatiles. There have been many excellent examples reported by Vet and Dicke (1992) and Turlings and Wäckers (2004) (see references therein). Recently, Dicke and Hilker (2003) demonstrated that different

types of "attackers" can evoke different plant responses due to specific elicitors or due to wounding. Furthermore, induced plant volatiles can vary with plant species, plant genotype, and even with plant part (Turlings et al. 1998a; Fritzsche Hoballah et al. 2002; Gouinguené et al. 2003; Piechulla and Pott 2003; Röse and Tumlinson 2004). Volatile induction also can vary with herbivore species or instar, and potentially with environmental conditions (Gouinguené and Turlings 2002; Gouinguené et al. 2003). Together, this information suggests that specificity in plant response may act as an important signal to natural enemies for locating their hosts (Turlings et al. 1990, 1995; De Moraes et al. 1998; Kessler and Baldwin 2001; Zhao and Kang 2002a, b, 2003; Rasmann et al. 2005; Röse and Tumlinson 2005).

Agromyzid flies (Diptera: Agromyzidae) represent a very particular group of herbivorous insects. They are exclusively plant feeders and best known as leafminers in host plants within diverse plant communities (Spencer 1990). The majority of agromyzids (99.4%) show very narrow host plant selection (Spencer 1990). Among 300 agromyzid species in the genus *Liriomyza*, only five species are considered truly polyphagous (Spencer 1973; Parrella 1987). However, some of polyphagous *Liriomyza* species have a strong propensity for rapid host adaptation and exploitation. For example, the pea leafminer, *Liriomyza huidobrensis* (Blanchard); the vegetable leafminer, *Liriomyza sativae* Blanchard; and *Liriomyza trifolii* (Burgess) have been reported to invade almost all zoogeographical regions, causing serious damage on various crops and further extending their host range (Spencer 1973; Parrella 1987; Kang 1996; Wei et al. 2000).

It is thought that *L. sativae* and *L. huidobrensis* were introduced accidentally into China (Kang 1996). The vegetable leafminer mainly distributes in vegetable fields of southern China and in greenhouses in northern China, whereas the pea leafminer is widespread ranging from subtropical to temperate regions (Zhao and Kang 2000; Chen and Kang 2005). The pea leafminer has started to replace the vegetable leafminer as the predominant pest in all areas (Chen and Kang 2004, 2005). These insect pests can cause economic losses to host plants by both adult and larval infestations. During the adult stage, female flies use their ovipositor to penetrate the epidermis of host plant leaves and then lay eggs inside or feed on the wounded part of the plant, which can greatly reduce photosynthesis and eventually kill young plants (Wei et al. 2000). Although male flies are unable to puncture leaves, they occasionally feed in the wounds and oviposition punctures made available by females (Parrella and Bethke 1984). Mining larvae consume foliage while simultaneously dwelling inside leaves (Connor and Taverner 1997). In addition, two allied *Liriomyza* species have been observed to differ in feeding habits. The larvae of the pea leafminer consume mesophyll both in palisade and spongy tissues (Parrella and Bethke 1984; Wei et al. 2000), whereas vegetable leafminer larvae only feed on palisade tissue (Kang 1996).

The difference in feeding habit by the two *Liriomyza* species may trigger emission of different volatile blends, which has been found from herbivore species such as leaf-feeding caterpillars, spider mites, stem-boring caterpillars, and sucking insects (aphids) (see details in Turlings et al. 1998a).

Recent studies have shown that volatiles emitted from plants with different types of damage could stimulate the behavioral or antennal responses of both leafminers and their parasitoids (Dicke and Minkenberg 1991; Petitt et al. 1992; Finidori-Logli et al. 1996; Zhao and Kang 2002a, b, 2003). However, very little is known about whether differences exist in volatile composition from host plants attacked by allied leafminer species with different feeding habits. To determine how different treatments may change the volatile composition of bean, *Phaseolus vulgaris* L., plants, we conducted experiments to collect and analyze volatile compounds emitted from healthy, mechanically damaged, and leafminer-damaged bean plants. We have further compared the volatile blends emitted from plants damaged at different developmental stages of leafminers *L. sativae* and *L. huidobrensis*.

Materials and methods

Plants

Seeds of *P. vulgaris* (cv Naibai, Haizhong Vegetables Market, Beijing, China) were planted individually in 12-cm-diameter plastic pots with a mixture of peat and vermiculite (4:1). Plants were grown in environmental chambers (Conviron Co., Winnipeg, Manitoba, Canada) under $25 \pm 5°C$, 14-h:10-h light:dark (L:D) photoperiod and 60% RH. Approximately 2-week-old bean plants with two fully developed true leaves were used in all experiments.

Insects

Colonies of *L. huidobrensis* and *L. sativae* were reared on 2-week-old bean plants. Two cultures were maintained in separate environmental chambers (Donglian Co., Harbing, China) operating at $25 \pm 1°C$, a 14-h:10-h (L:D) photoperiod, and $70 \pm 10\%$ RH. Adult flies used in the experiments were 2–6 days old. The two cultures have been reared for 6 years.

Treatments

Ten treatments were conducted. (1) Clean bags (CTRL): the odor collected from a clean cooking bag (Reynolds cooking bags) was used as a control. (2) Healthy, undamaged bean plants (UP): plants were cultured and protected from any insect feeding and mechanical

damage in an environmental chamber. (3) Plants with cut leaves (CLP): 200 cuts (1 cm in length) were made on the upper surface of leaves of each bean plant with a razor blade to simulate the damage caused by second instar leafminers. (4) Plants with punctured leaves (NPP): 1,000 small punctures on the upper surface of the leaves of each plant were made with a needle (0.5 mm in diameter) to mimic damage caused by ovipositor of female flies. Immediately after artificial treatments, plants were placed inside an oven bag [see (1)] for volatile collection. (5) Plants on which *L. huidobrensis* females had fed and oviposited (Lh-FOP): three intact plants were enclosed in a cage (40×40×40 cm³) and exposed to 80–100 adult *L. huidobrensis* (sex ratio 1:1) in an environmental chamber for 4 h, usually between 6:00 and 10:00 a.m. (6) Plants on which *L. sativae* females had fed and oviposited (Ls-FOP, same methods as described in Lh-FOP). In treatments (5) and (6), no adult flies of either species were allowed on the plants during volatile collections. (7) Plants on which second instars of *L. huidobrensis* had fed (Lh-LP): three plants were exposed to 100–150 adult flies for 4 h so that they obtained a cohort of second instars after 4–5 days. (8) Plants on which second instars of *L. sativae* had fed (Ls-LP, same methods as described in Lh-LP). (9) Plants with empty leaf mines previously infested by *L. huidobrensis* larvae (Lh-EMP). (10) Plants with empty *L. sativae* larvae leaf mines (Ls-EMP). In treatments (9) and (10), three plants were exposed to 100–150 adult flies for 2 h so that a moderate number of empty mines per leaf were present after approximately 10 days when pupae on each plant were collected (approximately 150 pupae for the pea leafminer-infested leaves and 100 pupae for the vegetable leafminer-infested leaves). If the exposure time is longer than 2 h, a high density of larvae feeding on the leaves results in abscission of the leaves after 10 days.

Plant volatile collections

A headspace sampling method was used to collect volatiles from bean plants with different treatments described above. This method was similar to those described by Turlings et al. (1998a) and Andersson (2003), but with some modifications. Each potted plant was enclosed in a 40.6×44.4 cm² plastic oven bag (Reynolds, Richmond, Virginia, USA) sealed with self-sealing strips at the opening edge around each stem approximately 4–5 cm above soil level. The compressed air (Beijing Gas Main Plant, Beijing, China) was purified and humidified through three glass jars (500 ml) each filled with molecular sieve (0.5 nm, Beijing Chemical Company, Beijing, China), freshly activated charcoal (Beijing Chemical Company), and distilled water, respectively. The filtered and moisturized air was pushed into the bag at a rate of 500 ml/min, and then drawn from the bag via a collector (a glass tube with an internal diameter of 3 mm) containing 100 mg of Porapak Q (80–100 mesh, Supelco) on the bag at a rate of 400 ml/

min by a membrane pump (Beijing Institute of Labour Instruments, China) at the end of the system for 10 h (usually, from 10:00 a.m. until 8 p.m.). By using this positive/negative pressure system, we ensured that no ambient air was sucked into the bag. The collections were replicated 3–7 times. Volatile compounds were rinsed with 600 μl HPLC-grade dichloromethane (Tedia Company, Fairfield, Ohio, USA) added with a mixture of three internal standards (300 ng each of heptanoic acid, ethyl ester and dodecanoic acid, ethyl ester) for quantification. The aeration extracts were stored at −20°C until chemical analyses. After headspace sampling, the weight of the plant was measured immediately. Number of punctured holes, larvae, and mines were recorded by examining leaves under stereomicroscope or counted facing up to a light source.

Volatile analysis

Collected volatiles were analyzed and identified using a gas chromatography–mass spectrometry (GC–MS) system (Hewlett Packard 6890N GC model coupled with 5973 MSD), equipped with into a DB-WAX polydimethyl siloxane column (60 m×0.25 mm ID, 0.15-μm film thickness, Agilent Technologies, Palo Alto, CA, USA) and an DB-1 column (30 m×0.25 mm ID×0.25-μm film thickness, Agilent Technologies). For analyses using the DB-WAX column, the initial oven temperature was kept at 30°C for 2 min and then increased to 180°C at a rate of 5°C/min, followed with 10°C/min to 230°C. On DB-1 column, the GC oven temperature was kept at 30°C for 2 min and then increased to 200°C at a rate of 5°C/min, followed with a programmed rate at 20°C/min to 280°C. The inlet was operated under the splitless injection mode, and the injector temperature was maintained at 250°C with a constant flow rate at 1.0 ml/min of helium. The GC–MS electron impact source was operated in the scan mode with the MS source temperature at 230°C and the MS Quad at 150°C.

Volatile compounds were identified by comparing their retention times from analyses on two columns and spectra with synthetic standards (Table S1) and those in the NIST02 library (Scientific Instrument Services, Inc., Ringoes, NJ, USA). Confirmations of oximes were based on peak enhancement on coinjection on GC with authentic standards added in the headspace sample of Lh-LP (Fig. 1).

Statistical analysis

Data were analyzed with statistical program SPSS (version 11.0, SPSS Inc., Chicago, IL, USA). Student's *t*-test was used to compare relative emissions between NPP and CLP, or Lh-LP and Ls-LP, or Lh-EMP and Ls-EMP. This test was also used to compare absolute quantity of volatile emissions between Lh-EMP and Ls-EMP. Analysis of variance (ANOVA) followed by Tu-
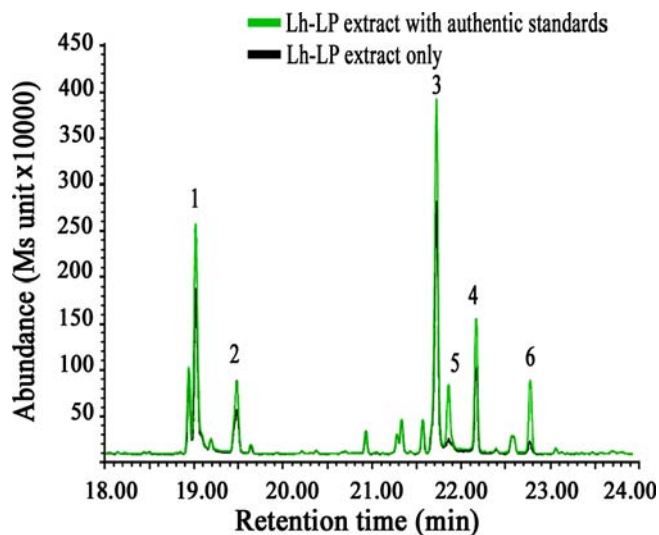
Fig. 1 Confirmations of oximes by coinjection of headspace sample collected from bean plants infested with second instars of *L. huidobrensis* (Lh-LP) with authentic standards on a DB-WAX column (60 m×0.25 mm ID, 0.15-μm film thickness, Agilent Technologies). Peak number: *1* (*syn*)-2-methylpropanal oxime, *2* (*anti*)-2-methylpropanal oxime, *3* (*syn*)-2-methylbutanal oxime, *4* (*anti*)-2-methylbutanal oxime, *5* (*syn*)-3-methylbutanal oxime, *6* (*anti*)-3-methylbutanal oxime

key's honestly significant difference (HSD) test was used for comparisons of differences in absolute quantities of volatile emissions among Lh-LP, Ls-LP, and CLP, or among Lh-FOP, Ls-FOP, and NPP. Each experiment was replicated 3–7 times. Relative percentage of volatile compounds was arcsine ($x^{-1/2}$) transformed, whereas absolute quantities were log ($x + 1$) transformed to correct for heterogeneity of variances before data analysis. We assume that the degree of damage of 300 cuts (each 1 cm in length by a razor blade) on leaves is equivalent to damage by 100 larvae, because second instars can cause an average of 3-cm length mines on leaves (personal observation). Similarly, the degree of damage of one needle puncture is equivalent to one oviposition puncture of the leafminers.

Results

Volatile emissions from artificial damaged plants and healthy plants

The volatile blends emitted from healthy plants (UP), leafminer-infested, and artificially damaged bean plants showed that there is considerable variation in numbers of compounds released (Table 1). Only two volatile compounds were detected from UP emissions: 2.82 ± 1.03 ng of (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and 1.20 ± 0.37 ng of (Z)-3-hexenyl acetate (per hour every 10 g of fresh weight, $n = 5$). In total, 14 volatile compounds were emitted from artificially damaged bean plants. No qualitative differences in numbers

of volatiles emitted from artificially damaged plants were observed [needle-punctured plant (NPP), and razor cut plant, (CLP)]. However, more (E)-2-hexen-1-al and DMNT were produced from NPP, and significantly higher amounts of (Z)-3-hexen-1-ol, (*syn*)-2-methyl-propanal oxime, and (*syn*)-2-methylbutanal oxime were emitted from CLP (Table 1). Other volatile compounds, including (Z)-3-hexenyl acetate, (Z)-3-hexen-1-ol, (*anti*)-2-methylpropanal oxime, linalool, and (E,E)-α-farne-sene, also were consistently released from artificially damaged bean plants. These compounds made up more than 70% of the total volatiles emitted from each treatment (Table 1). The structures of the six identified oximes were further confirmed by the peak enhancement method using the coinjection of volatile collections with authentic compounds (Fig. 1). Their structures are shown in Fig. 2.

Volatiles released from plants damaged by adult *L. huidobrensis* (Lh-FOP) and *L. sativae* (Ls-FOP)

The compositions and absolute amounts of volatiles released from plants damaged by adult leafminers are shown in Table 1 and Fig. 3. A large quantity of (Z)-3-hexenyl acetate was emitted from Lh-FOP but not from Ls-FOP. Both *syn*- and *anti*-forms of oximes were significantly induced by Lh-FOP and Ls-FOP. Several terpenoid compounds, such as linalool, (E,E)-α-farne-sene, β-caryophellene, and (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), also were produced significantly more than those of artificially damaged plants (Fig. 3). 3-Octanone and 1-octen-3-ol were not detected in volatile blends of Lh-FOP and Ls-FOP, but they were present in blends of mechanically damaged plants. Among volatile compounds released from Lh-FOP and Ls-FOP, four compounds [(Z)-3-hexenyl ace-tate, (*syn*)- and (*anti*)-2-methylpropanal oxime, and (*anti*)-2-methylbutananal oxime] were present in significantly higher amounts from Lh-FOP (Fig. 3). The amounts of (E)-2-hexen-1-al and (Z)-3-hexen-1-ol re-leased from Lh-FOP and Ls-FOP were only 10% of those emitted from NPP (Fig. 3).

Volatiles released from plants damaged by second instars of *L. huidobrensis* (Lh-LP) and *L. sativae* (Ls-LP)

Significant differences in emissions of green leaf vola-tiles, terpenoids, and oximes were found between vola-tile blends released from plants damaged by second instars of *L. huidobrensis* and *L. sativae* (Table 1; Figs. 2, 4). In total, 25 volatile compounds were released from the larval-damaged plants, compared with 14 compounds from CLP. Of these 25 volatiles, 14 were produced in significantly higher quantities from leaf-miner-damaged plants relative to CLP (Fig. 4). CLP released higher proportions of green leaf volatiles, whereas Lh-LP or Ls-LP released higher percentages of

Table 1 Composition of volatile blends emitted by healthy bean plants (UP), mechanically damaged plants with a needle (NPP) or a razor blade (CLP), plants with *L. huidobrensis* adult feeding and oviposition punctures (Lh-FOP), plants with *L. sativae* adult feeding and oviposition punctures (Ls-FOP), plants that were fed upon by *L. hudobrensis* second instars (Lh-LP), plants that were fed upon by *L. sativae* second instars (Ls-LP), and leaves with *L. huidobrensis* empty mines (Lh-EMP), or leaves with *L. sativae* empty mines (Ls-EMP), and a comparison of relative percentage of compounds in the blends between NPP and CLP, or Lh-LP and Ls-LP, or Lh-EMP and Ls-EMP

Chemical compound	Relative % of compounds in different treatments (mean ± SE)[a]								
	UP	NPP[b]	CLP[b]	Lh-FOP[c]	Ls-FOP[c]	Lh-LP[d]	Ls-LP[d]	Lh-EMP[e]	Ls-EMP[e]
Green leaf volatile									
Hexanal	–[f]	5.9 ± 2.2a	4.7 ± 1.2a	1.9 ± 0.6	4.0 ± 0.6	1.3 ± 0.5a	1.6 ± 0.5a	–	–
(E)-2-Hexen-1-al	–	8.7 ± 2.1a	5.8 ± 0.4b	0.7 ± 0.3	1.8 ± 0.7	0.2 ± 0.1a	0.3 ± 0.1a	6.5 ± 1.2a	1.4 ± 0.4b
(Z)-3-Hexenyl acetate	37.3 ± 8.5	34.6 ± 9.2a	34.0 ± 8.5a	30.4 ± 8.4	24.4 ± 7.2	21.5 ± 5.4a	15.8 ± 3.7a	14.7 ± 1.4a	7.3 ± 0.7b
(Z)-3-Hexen-1-ol	–	13.2 ± 3.5b	20.3 ± 3.1a	3.6 ± 0.9	4.8 ± 2.2	7.8 ± 4.6a	2.1 ± 0.6a	17.9 ± 3.2a	3.5 ± 0.9b
Terpenoid									
(E)-β-Ocimene	–	2.4 ± 1.2a	2.1 ± 0.5a	1.3 ± 0.3	4.3 ± 0.6	1.0 ± 0.2b	2.2 ± 0.5a	–	–
DMNT	62.7 ± 8.5	18.5 ± 5.8a	5.1 ± 0.8b	15.2 ± 2.7	17.7 ± 2.4	23.9 ± 3.8a	23.2 ± 1.6a	19.9 ± 4.3a	25.9 ± 7.2a
Linalool	–	0.9 ± 0.4a	4.6 ± 3.2a	0.5 ± 0.1	3.7 ± 0.6	1.0 ± 0.2a	1.1 ± 0.2a	1.9 ± 0.2a	3.0 ± 0.9a
β-Caryophellene	–	–	–	2.3 ± 0.8	–	3.2 ± 0.4a	4.0 ± 0.3a	2.1 ± 0.7b	5.1 ± 0.8a
α-Humulene	–	–	–	–	–	0.3 ± 0.03a	0.4 ± 0.1a	–	–
(E,E)-α-Farnesene	–	1.4 ± 0.6a	0.9 ± 0.3a	1.9 ± 0.4	4.5 ± 0.2	2.4 ± 0.8a	2.6 ± 0.7a	2.1 ± 0.6a	3.4 ± 0.9a
TMTT	–	–	–	1.5 ± 0.8	2.4 ± 0.3	5.3 ± 1.2a	4.4 ± 1.3a	4.8 ± 1.6b	12.3 ± 2.5a
Oximes									
(syn)-2-Methylpropanal oxime	–	1.6 ± 1.1b	4.9 ± 1.5a	16.7 ± 4.2	6.1 ± 1.1	8.2 ± 1.7a	8.4 ± 0.9a	10.4 ± 0.9a	12.0 ± 2.2a
(anti)-2-Methylpropanal oxime	–	0.8 ± 0.3a	2.1 ± 0.7a	3.2 ± 1.1	2.9 ± 0.3	2.0 ± 0.3b	4.3 ± 1.0a	7.2 ± 2.1a	6.4 ± 2.4a
(syn)-2-Methylbutanal oxime	–	1.5 ± 1.1b	4.2 ± 1.3a	6.1 ± 2.6	9.1 ± 2.9	9.3 ± 0.9b	14.7 ± 1.3a	2.4 ± 0.8b	13.8 ± 2.8a
(anti)-2-Methylbutanal oxime	–	–	–	5.0 ± 0.6	3.0 ± 1.1	2.8 ± 0.3b	5.4 ± 0.3a	1.0 ± 0.3b	3.7 ± 0.8a
(syn)-3-Methylbutanal oxime	–	–	–	–	–	1.0 ± 0.2a	0.8 ± 0.1a	–	–
(anti)-3-Methylbutanal oxime	–	–	–	–	–	0.6 ± 0.3a	0.5 ± 0.1a	–	–
Other compounds									
(Z)-3-Hexenyl butyrate	–	–	–	–	–	1.3 ± 0.5a	0.7 ± 0.1a	–	–
(Z)-3-Hexenyl iso-valerate	–	–	–	–	–	0.8 ± 0.1a	0.6 ± 0.1a	–	–
Methyl salicylate	–	–	–	–	–	0.5 ± 0.1a	0.7 ± 0.1a	–	–
Dodecane	–	6.5 ± 2.0a	7.8 ± 3.1a	8.7 ± 3.1	10.0 ± 1.6	3.2 ± 0.7a	2.6 ± 0.8a	–	–
1-Pentanol	–	–	–	1.2 ± 0.8	–	0.1 ± 0.02a	0.1 ± 0.02a	3.8 ± 1.1	–
1-Octen-3-ol	–	3.1 ± 0.9a	2.3 ± 0.2a	–	–	0.3 ± 0.1a	0.7 ± 0.3a	5.3 ± 1.5a	2.1 ± 0.6a
3-Octanone	–	0.9 ± 0.7a	1.3 ± 0.4a	–	–	0.1 ± 0.03a	0.1 ± 0.02a	–	–
2-Ethyl-cyclopentanone	–	–	–	–	–	1.9 ± 0.6a	2.7 ± 0.6a	–	–
Total number of chemical compounds	2	14	14	16	14	25	25	14	13

[a]Each mean value represents data from three to seven replicates followed by standard error (means ± SE)

[b]Mechanical damage. Means in the same row that are followed by the same letter are not significantly different (Student's *t*-test, $P > 0.05$)

[c]Damage of adult flies. Relative amount of compounds of these two treatments are not compared with each other because different volatile blends are emitted

[d]Second instar larvae damage. Means in the same row that are followed by the same letter are not significantly different (Student's *t*-test, $P > 0.05$)

[e]Empty mines of leafminers. Means in the same row that are followed by the same letter are not significantly different (Student's *t*-test, $P > 0.05$)
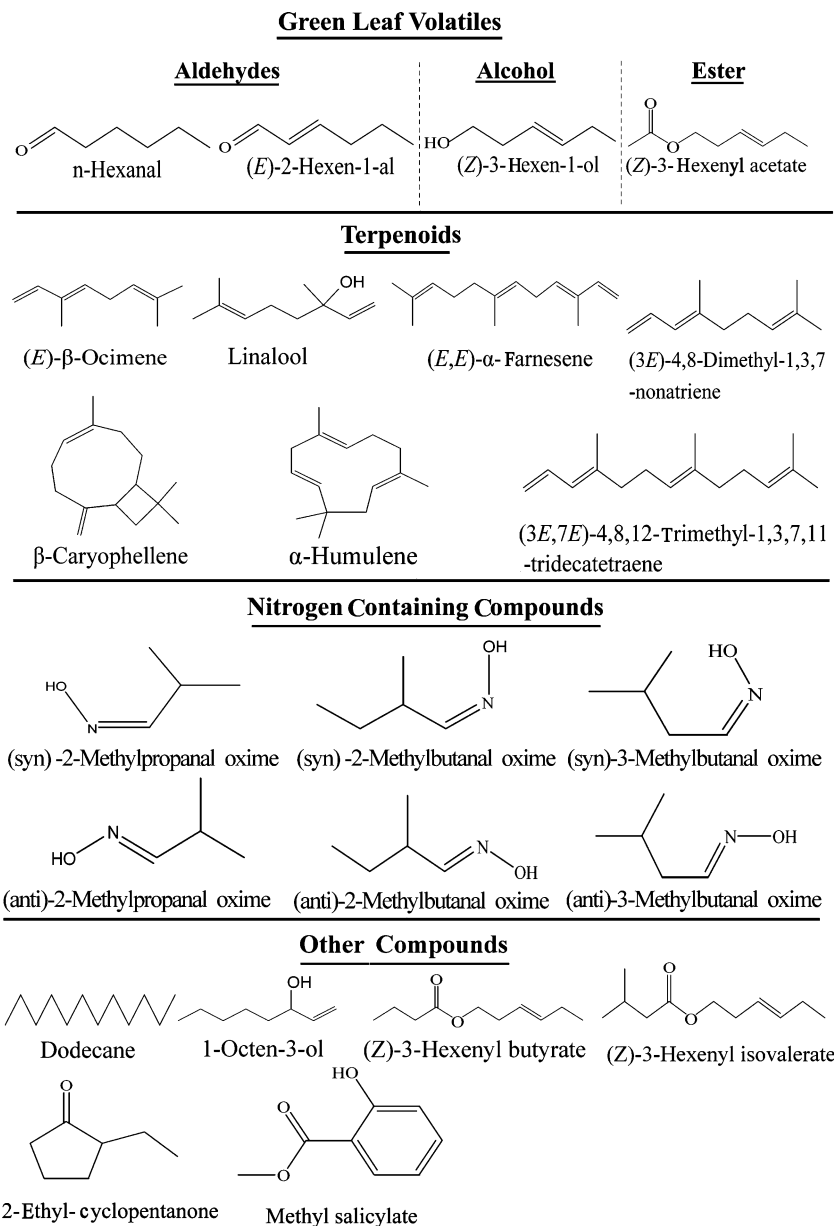
[f]Compounds marked with "–" means under detectable level

terpenoids and oximes (Table 1; Fig. 4). In addition, other volatile compounds, such as short chain alcohols, ketones, and methyl salicylate (Fig. 2), also were emitted in significant amounts from leafminer-damaged plants. Although the number of volatiles induced by the two leafminer species were the same (25 vs. 25), amounts of 13 compounds induced by larvae of *L. huidobrensis* were significantly higher than those by *L. sativae* (Fig. 4). Furthermore, relatively higher percentages of minor compounds, including (E)-β-ocimene, (anti)-2-methyl-propanal oxime, and 2-methylbutanal oxime were observed from Ls-LP compared with Lh-LP (Table 1).

Volatiles released from plants with empty mines previously infested by *L. huidobrensis* (Lh-EMP) and *L. sativae* (Ls-EMP)

In total, 14 volatile compounds were emitted from plants with empty mines (Fig. 5). 1-Pentanol was the only compound that was different in volatile blends emitted from Lh-EMP and Ls-EMP. Significant amounts of (E)-2-hexen-1-al, (Z)-3-hexenyl acetate, (Z)-3-hexen-1-ol, DMNT, linalool, and 1-octen-3-ol were released from Lh-EMP compared with Ls-EMP (Fig. 5). In contrast, (syn)- and (anti)-2-methylbutanal oxime, TMTT, and β-

Fig. 2 Chemical structures of the major volatile compounds collected from headspace samples of bean plants with different treatments. Volatiles present at 0.1% or higher proportions in the headspace of *Liriomyza* larvae-infested leaves are listed


Green Leaf Volatiles

Aldehydes | **Alcohol** | **Ester**

n-Hexanal   (E)-2-Hexen-1-al   (Z)-3-Hexen-1-ol   (Z)-3-Hexenyl acetate

Terpenoids

(E)-β-Ocimene   Linalool   (E,E)-α-Farnesene   (3E)-4,8-Dimethyl-1,3,7-nonatriene

β-Caryophellene   α-Humulene   (3E,7E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene

Nitrogen Containing Compounds

(syn)-2-Methylpropanal oxime   (syn)-2-Methylbutanal oxime   (syn)-3-Methylbutanal oxime

(anti)-2-Methylpropanal oxime   (anti)-2-Methylbutanal oxime   (anti)-3-Methylbutanal oxime

Other Compounds

Dodecane   1-Octen-3-ol   (Z)-3-Hexenyl butyrate   (Z)-3-Hexenyl isovalerate

2-Ethyl-cyclopentanone   Methyl salicylate

caryophellene were released dominantly from Ls-EMP (Table 1). Extremely low amounts or almost no green leaf volatiles were present in Ls-EMP emissions.
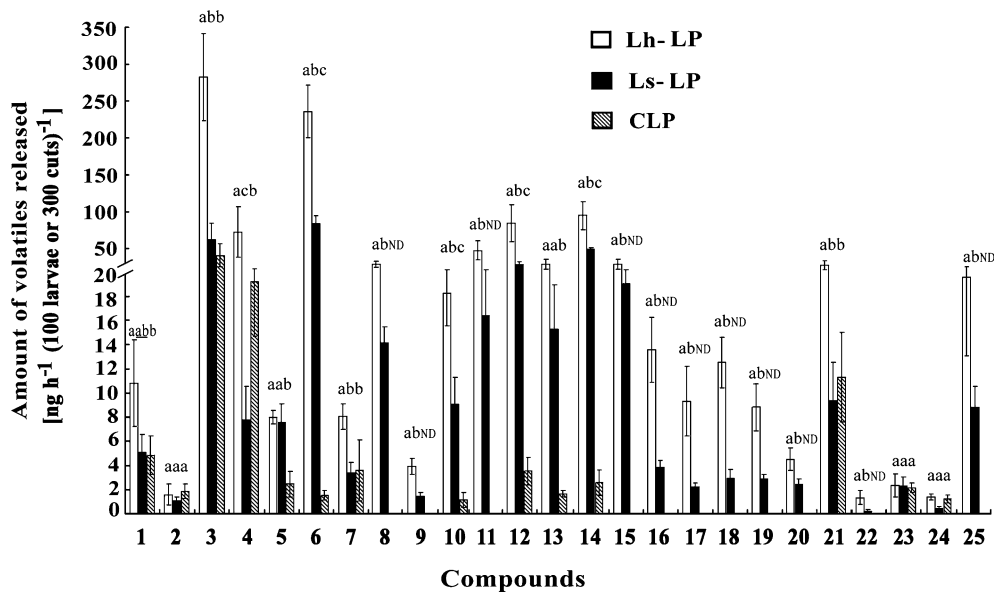
Discussion

In the present study, we have shown that there are no qualitative differences between volatile emissions from bean plants induced by adults of two *Liriomyza* species, but significant quantitative differences are found in the blends of volatiles released. *L. huidobrensis*-damaged plants produce larger amounts of volatiles than those of *L. sativae*-damaged plants. Qualitative differences also are evidenced among bean plants damaged by different developmental stages of the two leafminer species studied. The differences in volatile emissions of bean plants

in response to the two agromyzid species are probably related to differences in the feeding habits of the larvae. An individual puncture caused by *L. huidobrensis* is larger than that caused by *L. sativae*, and its mines extend toward the base of the leaf, which tend to sieve leaf segments between the veins. Furthermore, *L. huidobrensis* larvae consume mesophyll both in palisade and spongy tissues (Parrella and Bethke 1984; Wei et al. 2000), whereas *L. sativae* larvae only feed in palisade tissue, and their mines are blotched with conspicuous black thread-like strips (Kang 1996; Salvo and Valladares 2004). Apparently, these differential feeding habits may produce emissions of different volatile composition, as demonstrated by this study. The volatile profiles emitted from plants damaged by *L. huidobrensis* and *L. sativae* differ from those by leaf-feeding caterpillars and spider mites, stem-boring caterpillars, and sucking in-
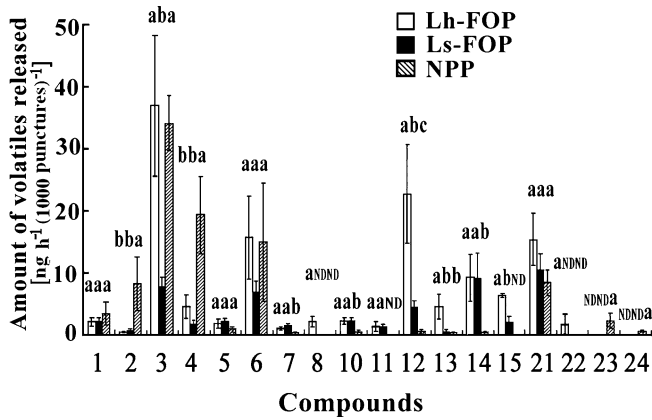
Fig. 3 Volatile compounds present in the headspace of feeding and oviposition punctures of adult *L. huidobrensis*-damaged bean leaves (Lh-FOP), feeding and oviposition punctures of adult *L. sativae*-damaged leaves (Ls-FOP), and mechanically damaged leaves by needle (NPP). Abscissa is: *1* hexanal, *2* (*E*)-2-hexen-1-al, *3* (*Z*)-3-hexenyl acetate, *4* (*Z*)-3-hexen-1-ol, *5* (*E*)-β-ocimene, *6* DMNT, *7* linalool, *8* β-caryophellene, *10* (*E,E*)-α-farnesene, *11* TMTT, *12* (*syn*)-2-methylpropanal oxime, *13* (*anti*)-2-methylpropanal oxime, *14* (*syn*)-2-methylbutanal oxime, *15* (*anti*)-2-methylbutanal oxime, *21* dodecane, *22* 1-pentanol, *23* 1-octen-3-ol, *24* 3-octanone. *Bars* for the same compound that are labeled with the *different letters* are statistically significant in average amounts for different treatments (ANOVA followed by Tukey's HSD test, *P* < 0.05). *ND* undetected compounds in corresponding volatile blends under a compound. The mean volatile amounts are presented with the standard error (±SE)

sects (aphids) (Turlings et al. 1998a), which indicate that plants respond differently to herbivore species with different feeding habits and to different herbivore developmental stages (Takabayashi et al. 1995; Gouinguené et al. 2003).

Many volatiles are produced when bean plants are wounded by leafminers or by artificial means. These volatiles can be divided into three major categories: green leaf volatiles, terpenoids, and oximes. Similar compounds have been released from plants damaged by several herbivorous species (Dicke et al. 1990; Turlings et al. 1990, 1995, 1998a, b; Loughrin et al. 1994; Takabayashi et al. 1995; Fritzsche Hoballah et al. 2002; Gouinguené et al. 2003; De Boer et al. 2004; Ven Den Boom et al. 2004). Green leaf volatiles, terpenoids, and methyl salicylate are among the most common volatile compounds from many plant species induced by different herbivores and treatments. Production of these volatile compounds by plants involves three biosynthetic pathways: the fatty acid/lipoxygenase pathway for green leaf volatiles, the isoprenoid pathway for terpenoids, and the shikimic acid pathway for methyl salicylate (Paré and Tumlinson 1997a, b, 1999).

In addition to the green leaf volatiles and terpenoids released from leafminer-damaged bean plants, we determined that these plants also release several nitrogen-containing compounds, such as oximes. Oximes have been reported to be released from *Spodoptera*
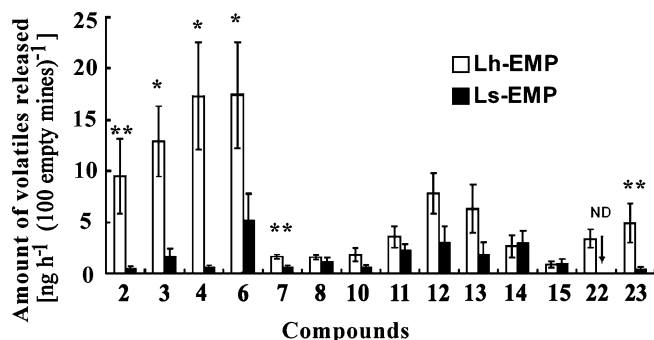

Fig. 4 Volatile compounds collected from the second instars of *L. huidobrensis*-infested bean leaves (Lh-LP), second instars of *L. sativae*-infested bean leaves (Ls-LP), and mechanically damaged bean leaves by blade (CLP). Abscissa is: *1* hexanal, *2* (*E*)-2-hexen-1-al, *3* (*Z*)-3-hexenyl acetate, *4* (*Z*)-3-hexen-1-ol, *5* (*E*)-β-ocimene, *6* DMNT, *7* linalool, *8* β-caryophellene, *9* α-humulene, *10* (*E,E*)-α-farnesene, *11* TMTT, *12* (*syn*)-2-methylpropanal oxime, *13* (*anti*)-2-methylpropanal oxime, *14* (*syn*)-2-methylbutanal oxime, *15* (*anti*)-2-methylbutanal oxime, *16* (*syn*)-3-methylbutanal oxime, *17* (*anti*)-3-methylbutanal oxime, *18* (*Z*)-3-hexenyl butyrate, *19* (*Z*)-3-hexenyl iso-valerate, *20* methyl salicylate, *21* dodecane, *22* 1-pentanol, *23* 1-octen-3-ol, *24* 3-octanone, *25* 2-ethyl-cyclopentanone. *Bars* for the same compound that is labeled with the *different letters* are statistically significant differences in average amounts for different treatments (ANOVA followed by Tukey's HSD test or student's *t*-test, *P* < 0.05). *ND* undetected compounds in corresponding volatile blends under a compound. The mean volatile amounts are presented with the standard error (±SE)

Fig. 5 Volatile compounds released from bean plant after pupation by larvae of *L. huidobrensis* (Lh-EMP) and larvae of *L. sativae* (Ls-EMP). Abscissa is: *2* (*E*)-2-hexen-1-al, *3* (*Z*)-3-hexenyl acetate, *4* (*Z*)-3-hexen-1-ol, *6* DMNT, *7* linalool, *8* β-caryophellene, *10* (*E,E*)-α-farnesene, *11* TMTT, *12* (*syn*)-2-methylpropanol oxime, *13* (*anti*)-2-methylpropanal oxime, *14* (*syn*)-2-methylbutanal oxime, *15* (*anti*)-2-methylbutanal oxime, *22* 1-pentanol, *23* 1-octen-3-ol. *Bars* for the same compound that is labeled with *asterisks* are statistically significant differences in average amounts for two treatments (Student's *t*-test, *$P < 0.05$; **$P < 0.01$). *ND* undetected compounds in corresponding volatile blends under a compound. The mean volatile amounts are presented with the standard error ($\pm$SE)

(armyworm)-infested maize, *Zea mays* L., leaves and maize plants damaged by aphids and caterpillars (Takabayashi et al. 1995; Turlings et al. 1998a). The oximes identified from the current study have similar structures to those reported by Ven Den Boom et al. (2004) from several plant species in the Fabaceae and Solanaceae families that are induced by two spotted spider mite, *Tetranychus urticae* Koch. However, the identities of these oximes were only based on comparisons of mass spectra available from an MS library. Therefore, some of the identifications may be tentative, especially for oximes with *trans–cis* isomers. In the current study, we confirmed these isomers by the peak enhancement method of coinjection of headspace samples with authentic standards. Similar oxime compounds, such as 2-methyl, butyraldoxime, and isobutyraldoxime, are suggested to be synthesized by degeneration of isoleucine and valine (Kaiser, Givaudan Schweiz AG, Vernier, Switzerland; personal communication).

Although damage ceases when *Liriomyza* larvae pupate, plants with empty mines still consistently released 13 volatiles, but their amounts were significantly lower compared with those emitted from plants damaged with larvae present. Damaged plants can continuously emit induced volatiles from hours or days after damage stops (Turlings et al. 1990, 1995, 1998a, b; Loughrin et al. 1994; Röse and Tumlinson 2004, 2005). Interestingly, emissions of several green leaf volatiles and terpenoid compounds are significantly higher in plants with empty mines that were previously damaged by *L. huidobrensis* compared with *L. sativae*. This difference may be because plants damaged by *L. huidobrensis* release significantly higher amounts of these volatiles during the period of larval damage, or the volatiles may further be released after the initial damage

by the larvae. Similar examples have been reported in cotton, *Gossypium* spp., and maize plants, from which some herbivore-induced volatiles begin to be released at least 3 days after damage occurs (Loughrin et al. 1994; Turlings et al. 1998a, b).

Natural enemies, such as parasitoids, attack a wide range of herbivorous insects. The specific volatile blend emitted from the damaged plants plays a very important role for natural enemies to locate their animal or plant hosts. De Moraes et al. (1998) have further demonstrated that a specialist parasitoid could distinguish its host caterpillar from a nonhost caterpillar based on specific volatile blends of host plants induced by two caterpillars. We have shown that different blend ratios of volatiles are induced by *Liriomyza* larvae-infested bean plants and artificially damaged plants. Furthermore, *L. huidobrensis*-infested plants release significantly higher amounts of major terpenoids and oximes than *L. sativae*-infested plants. Two species of parasitic wasp, *Opius dissitus* and *Diglyphus isaea*, parasitize larvae of *Liriomyza* leafminers (Kang 1996; Zhao and Kang 2002a). Zhao and Kang (2002a) have observed that adults of *D. isaea* orient toward plant odors associated with *L. sativae*-infested plants. Specific compounds from complex herbivore-induced volatile blends are known to play an important role in the selective foraging behavior of their natural enemies (De Boer et al. 2004). Du et al. (1998) and Guerrieri et al. (1999) have reported that the aphid parasitoid *Aphidius ervi* can distinguish plants infested with its host *Acyrthosiphon pisum* (Harris) from those with the nonhost *Aphis fabae* Scopoli by different blend ratios of the induced volatiles released from damaged plants. Takabayashi et al. (1995) further found that the parasitic wasp *Cotesia kariyai* can differentiate between maize plants under attack by different ages of larvae of *Pseudaletia separata*. Whether *O. dissitus* or *D. isaea* can distinguish volatile blends induced by plants damaged by the two different species, and different developing stages within the same species, further studies on behavioral bioassays and electrophysiological tests of these two parasitoids in response to individual volatile compounds and different volatile blends have to be conducted to be able to understand the underlying mechanism.

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