Emission of sulfur-containing volatiles from *Arabidopsis thaliana* (L.) Heynh Col-0 related to diamondback moth (*Plutella xylostella* (L.)) infestation

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Abstract: Herbivore-infested plants often release a variety of volatile organic compounds (VOCs). Here, we studied the effects of feeding *Plutella xylostella* (L.) (0, 3, 9, 20 pest larvae within 0-4 h and 4-8 h infestation, respectively) on the emission of sulfur-containing VOCs in *Arabidopsis thaliana* (L.) Heynh Col-0 (*A.t.* Col-0) by headspace solid-phase micro-extraction coupled to gas chromatography - mass spectrometry (HS-SPME-GC/MS). The analytical results showed that the relative emission of sulfur-containing metabolites increased significantly in Arabidopsis plants subjected to *P. xylostella* infestation according to the density and residence duration of pest larvae on shoot organs. The main compound from infested plants was dimethyl disulfide. We suggest that the correlations between the stress level (density and time infestation) and the sulfides observed in this study provide a means to understand the changes of VOCs profile of plant under chewer infestation.

Key words: *Arabidopsis thaliana* (L.) Heynh Col-0, diamondback moth (*Plutella xylostella* (L.)), volatile organic compounds, sulfur-containing volatiles, HS-SPME-GC/MS.

Introduction

Like all Brassicaceae plants, *Arabidopsis thaliana* (L.) Heynh has developed effective chemical pathways reaction to abiotic and/or biotic stresses, e.g. terpenoids, glucosinolates and sulfur-containing organic compounds (Mewis *et al.*, 2005). Evidences indicated that Arabidopsis–herbivore interactions are complex, and that the interactions of phloem-feeders differ to those of chewers with regard to the elicitation of induced defenses (Bidart-Bouzat *et al.*, 2011; Ali *et al.*, 2012 (in review)). Contrary to the piercing-sucking insect, chewing ones can cause extensive damage to plant cells following infestation and their oral secretions are major signals that trigger volatile release from plants. Gas chromatography-mass spectrometry based analyses of *A. thaliana* plants showed that infestation by *Pieris rapae* larvae increased the emissions of dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) in the plant headspace (van Poecke *et al.*, 2001).

In the current study, we investigated the potential emission of sulfur-containing VOCs response to *P. xylostella* (L.) infestation in *A. thaliana* (L.) Heynh Col-0. We hypothesized that the emission of these metabolites is related to the density and residence duration of pests on plant leaves. The elucidation of this pest and related host plant interactions may be important in understanding the VOCs emission from plant – chewing insect interactions.

Material and methods

Plant material and Plutella damage

Arabidopsis thaliana (L.) Heynh Col-0 (Lehle company) seedlings were sown in 3x7 plastic pots with potting mix and grown for 5 weeks in growth chamber at 22 °C, 16L:8D (90 µmol m^{-2} s^{-1} photosynthetically active radiation during the light period), 65% relative humidity.

Feeding damage was caused by transferring 3, 9 and 20 third-instar *P. xylostella* (L.) larvae (reared on cabbage plants) onto one randomly selected Arabidopsis plants grown within 0-4 h and 4-8 h after infestation respectively in the laboratory conditions. Plants without *P. xylostella* were used as controls.

Collection and Analysis of Volatile compounds

Three intact control plants and *P. xylostella*-damaged plants (fresh weight ranging from 0.2-0.4 g/plant) were used for the collection of the volatile compounds. 65 µm Divinylbenzene/Polydimethylsiloxane (PDMS/DVB) fiber (Supelco; Bellefonte, PA) was exposed to the headspace of the sample for the extraction (4 h) in the laboratory conditions at 22 ^{0}C. Before collecting VOCs, the fiber was conditioned at 225 °C for 30 min.

Analytical GC/MS system (Thermo-Fisher Scientific; Waltham, MA, USA) was equipped with an apolar column (30 m; 0.25 mm i.d.; 0.25 µm film thickness, Optima-5-MS, Macherey-Nagel, Düren, Germany). The carrier gas was helium at 0.5 ml/min. The oven temperature program was from 40 °C to 220 °C (1-min hold) at 4 ^{0}C/min, then from 220 °C to 320 °C (10-min hold) at 100 °C/min. The mass spectra were obtained by using a mass selective detector with the electron impact at 70 eV; temperature source was at 230 ^{0}C, interface at 250 ^{0}C. Scanned mass range was from 39 to 400 amu at rate of 1 scan $s^{-1.}$ The components of the volatile emission were identified on the basis of retention times and by careful examination of their main spectra in comparison with existing computed databases (Wiley and NIST MS 2.0).

Statistical analysis

The percentage of compound class (sulfur-containing VOCs) and of each compound was obtained by calculating the ratio between their areas and the total area of detected VOCs. Analyses of variance (1-way and 2-way ANOVA) have been performed to evaluate differences between VOCs emitted from control and infested plants (SPSS Statistics, 16.0 Inc. Chicago, IL). Before the analysis, square root transformation of the data was performed to match the application conditions.

Results and discussion

Sulfur-containing volatiles emission in responses to P. xylostella (L.) stress

GC-MS analyses indicated differences in the proportions of VOCs released by *A. thaliana* Col-0 subjected to different treatments.

The percentage of sulfur-containing metabolites increased significantly according to the density and time infestation of *P. xylostella* larvae on *A. thaliana* shoot organs. The discriminant analysis at 0-4 h and 4-8 h significantly separated shoot organs blends of *A. thaliana*-damaged plants from those of undamaged plants ($r^2 = 0.999$, $p < 0.001$***) (Figure 1a). From 4 to 8 h after the beginning of infestation, the emitted proportions of VOCs were correlated to the density of pest larvae on plant leaves (3, 9 & 20 larvae per plant) ($p <$

0.001***). The compound that was predominantly liberated was dimethyl disulfide (Figure 1b; Table 1).

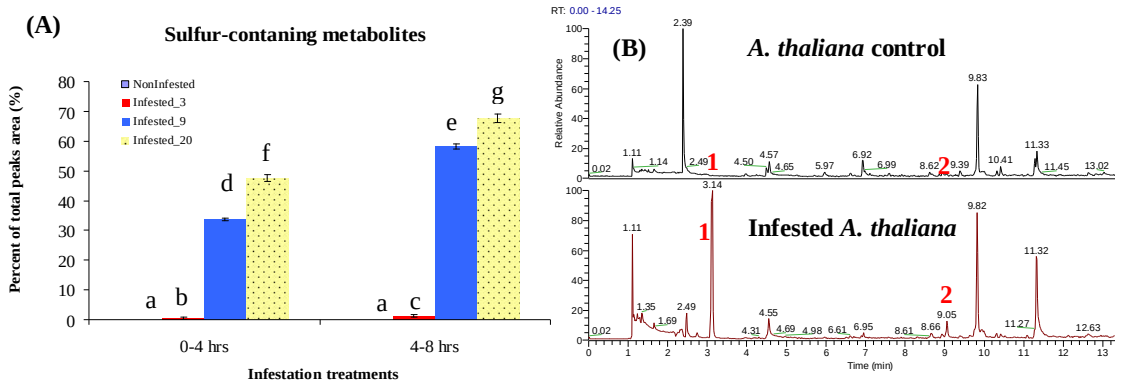

Figure 1. (A) Mean percentage ± SD (n = 8) of total sulfur-containing volatiles emitted from intact control and *P. xylostella*-infested *A. thaliana* Col-0 at different density and residence duration of pest on plant. Different letters above the bars indicate significant differences between the treatments by Tukey post hoc tests ($p < 0.05$, 2-way ANOVA). NonInfested: intact control plants; Infested_3, Infested_9, Infested_20: 3, 9, 10 pest larvae on plant leaves within 0-4 h or 4- 8 h respectively. (B) Chromatograms of VOCs extracted from *A. thaliana* control and infested by 20 *P. xylostella* larvae within 8 h. 1 = dimethyl disulfide; 2 = dimethyl trisulfide (peak 1 and 2 were detected only in infested *A. thaliana* plants).

Discussion

Our results have shown that the shoot organs damage of *A. thaliana* Col-0 by the larvae of *P. xylostella* induced the emission of sulfur-containing volatiles (dimethyl disulfide and dimethyl trisulfide) (Figure 1, Table 1). We found that (i) the proportions of sulfides inscreased according to the density of pest larvae and time infestation on shoot organs; (ii) dimethyl disulfide was the most abundant. These suggested that the sulfides only appear in *P. xylostella*-infested Arabidopsis plants, chemically similar to those infested by *P. rapae* (van Poecke *et al.*, 2001).

Dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) emissions were significantly inscreased from plants subjected to *P. xylostella* infestation. Experimental evidences indicated that larvae of diamondback moth could detoxify glucosinolates by the activity of enzyme sulfatase and its expression may only be found in the gut of the larvae (de Vos *et al.*, 2007). Some studies indicated that aliphatic glucosinolates related metabolites probably play a key role in the interaction between *A. thaliana* and lepidopteran herbivores (reviewed by Ali *et al.*, 2012). Recently, van Dam *et al.*, (2012) have just demonstrated that sulfides emission (DMDS and DMTS) correlated to the glucosinolate composition from root of *Brassica nigra* plants infested by *Delia radicum* larvae (van Dam *et al.*, 2012). The presence of two sulfides within the volatile profiles of Arabidopsis plants damaged by *P. xylostella* larvae may be related to the formation of glucosinolates breakdown products. De Vos *et al.* (2007) showed that chewing insects can overcome plant defenses and use some glucosinolates and their degradation products as oviposition and feeding stimulants (de Vos *et al.*, 2007). To the authors' knowledge, it is the first times that relationship between sulfur-containing VOCs emission and *P. xylostella* infestation on Arabidopsis plants is mentioned.

Table 1. Mean percentage ± SD (n = 8) of the different compounds in the total emission of sulfur-containing volatiles collected from *A. thaliana* Col-0 plants with infested *P. xylostella* larvae during different time intervals (0–4 h and 4-8 h). Means followed by the different letters are significantly different ($p < 0.05$, 2-way ANOVA, Tukey HSD test). Uninfested: intact control plants; Infested_3, Infested_9, Infested_20: 3, 9, 10 pest larvae on plant leaves.

RT	Compounds	**Treatments** (n =8)							
		Uninfested		**Infested_3**		**Infested_9**		**Infested_20**	
		Time intervals		Time intervals		Time intervals		Time intervals	
		0-4 h	4-8 h	0-4 h	4-8 h	0-4 h	4-8 h	0-4 h	4-8 h
3.12	Dimethyl disulfide	0 ± 0^{a}	0 ± 0^{a}	trace	0.33 ± 0.11^{b}	29.78 ± 0.52^{c}	56.07 ± 0.34^{e}	44.72 ± 1.23^{d}	62.52 ± 1.88^{f}
9.05	Dimethyl trisulfide	0 ± 0^{a}	0 ± 0^{a}	0.45 ± 0.34^{b}	0.93 ± 0.35^{b}	3.98 ± 0.20^{de}	2.15 ± 0.63^{c}	2.83 ± 0.38^{c}	5.19 ± 0.41^{e}
	Total	$\mathbf{0 \pm 0^{a}}$	$\mathbf{0 \pm 0^{a}}$	$\mathbf{0.45 \pm 0.34^{b}}$	$\mathbf{1.26 \pm 0.44^{c}}$	$\mathbf{33.76 \pm 0.42^{d}}$	$\mathbf{58.21 \pm 0.96^{f}}$	$\mathbf{47.55 \pm 1.16^{e}}$	$\mathbf{67.70 \pm 1.48^{g}}$

Acknowledgement

Hien Truong Thi Dieu is recipient of a PhD scholarship from Ministry of Education and Training of Vietnam.

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