Changes in the volatile profile of *Brassica oleracea* due to feeding and oviposition by *Murgantia histrionica* (Heteroptera: Pentatomidae)

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**Abstract.** The mixture of volatile compounds emitted by *Brassica oleracea* var. sabauda changed significantly in response to feeding and/or oviposition by *Murgantia histrionica* (Heteroptera: Pentatomidae). Volatiles were collected from (1) healthy plants and those with (2) feeding punctures, (3) a combination of feeding punctures and oviposition, (4) feeding punctures and one hatched egg mass and (5) plants bearing only an egg mass. In the case of plants with feeding punctures or feeding punctures plus an egg mass, the volatiles were also collected at different time intervals after plants were subjected to these two treatments (0–24 h, 24–48 h and 48–72 h). Gas chromatographic and gas chromatograph-mass spectrometric analysis showed that the percent emission of several compounds changed significantly from plants subjected to the feeding and oviposition or just oviposition. Percentage of terpenes generally decreased after feeding and oviposition, although the percentage emission of (E)-β-caryophyllene from these plants and those with just feeding punctures significantly increased. Plants with just an egg mass emitted limonene de novo but not (E)-β-caryophyllene. The emission of jasmonates, mainly methyl jasmonate, increased from plants with feeding punctures plus an egg mass compared to those with only an egg mass. Higher percentages of the volatile glucosinolate derivatives (VGs), mainly 4-methoxy-3-indolylacetonitrile, were emitted by plants with feeding punctures and an egg mass. The percentage emission of most of these compounds increased during the first 24 h after the treatment and then decreased over the next 24 h, except for methyl jasmonate, which remained high also 48–72 h later. The possible ecological roles of such volatiles in plant interactions with the second and third trophic levels are discussed.

**INTRODUCTION**

Chemical changes in plants damaged by insects have interested plant physiologists and ecologists, ever since these changes were considered to function as possible defences against herbivores (Green & Ryan, 1972). In particular, the studies on plants’ volatile organic compounds (VOCs) provide useful information on plant interactions with other organisms, their physiological status and the level of stress they are experiencing. Plants are known to release more than 30,000 different VOCs including alkanes, alkenes, alcohols, ketones, aldehydes, ethers, esters and carboxylic acids (Niinemets et al., 2004). The most important emitted VOCs can be categorized into three basic chemical classes according to their biosynthetic pathways: the fatty acid-derivatives [green leaf volatiles (GLV) and jasmonates] produced by the lipoxygenase pathway, the terpenoids by the isoprenoid pathway (via mevalonate or deoxyxylulose) and aromatic compounds, such as methyl salicylate and indole, by the shikimic acid pathway.

Many VOCs are constitutively emitted by undamaged plants, but biotic factors and the interaction between biotic and abiotic factors can induce plants to emit much larger amounts of these compounds (Gouinguené & Turlings, 2002) and may also trigger the de novo synthesis of several VOCs (Paré & Tumlinson, 1997a, b). In particular, the induction and release of such compounds depends on the interaction of biotic factors, such as hormones (de Bruxelles & Roberts, 2001; Thaler et al., 2002; Farmer et al., 2003; Rojo et al., 2003; Ament et al., 2004; van Poecke & Dicke, 2004), herbivore-derived elicitors (Alborn et al., 1997; Halitschke et al., 2001; Schmelz et al., 2001; Mithöfer et al., 2005), O3 and CO2 concentration (Vourenen et al., 2004a, b), UV radiation (Johnson et al., 1999; Winter & Rostáš, 2008), heavy metals (Mithöfer et al., 2004), temperature (Gouinguené & Turlings, 2002) and light (Takabayashi et al., 1994; Gouinguené & Turlings, 2002).

VOCs emitted as a consequence of herbivore attack may have a role in plant indirect defences, attracting natural enemies of herbivores and helping them to find the attacked plants (induced synomones) (Dicke & Sabelis 1988; Turlings et al., 1990; Agrawal et al., 1999; Walling, 2000). These compounds are recorded for more than 15 different tritrophic systems, suggesting that herbivore induced synomones are a common phenomenon, with possible applications in biological control programs (Cortesero et al., 2000; Degenhardt et al., 2003).

Previous research has focused mainly on synomones induced by insects that damage plants by chewing, such as caterpillars (Potting et al., 1995; De Moraes et al., 1998), or mites that feed on cell contents (Takabayashi & Dicke, 1996). Leaf-eating by caterpillars activate plant
defence by means of chemical elicitors present in their oral secretions and regurgitants (Alborn et al., 1997, Arimura et al., 2005). Recently there has been an increase in the information on tri-trophic systems comprising herbivores with phloem-feeding or stylet-sheath-feeding habits, such as Homoptera and Heteroptera (Bernasconi et al., 1998; Du et al., 1998; Turlings et al., 1998b; Guerrieri et al., 1999; Rodriguez-Saona et al., 2002). These feeding habits generally are characterized by limited mechanical damage and may elicit somewhat different responses from those activated by defoliators (Walling, 2000).

Furthermore, synomones, acting over long (volatile) or very short range (volatile or through contact), are induced not only by feeding but also by oviposition by herbivores, although so far this has been shown for only a few tri-trophic systems (Meiners & Hilker, 2000; Hilker & Meiners, 2002, 2006; Hilker et al., 2002; Colazza et al., 2004a, b; Conti et al., 2006). As reported by Colazza and co-authors (2004a) bean plants react to feeding and oviposition by Nezara viridula L. (Heteroptera: Pentatomidae) by changing the profile of volatiles emitted, thus attracting the egg parasitoid Trissolcus basalis (Hymenoptera: Scelionidae).

Some VOCs are taxon specific whereas others appear to be common to many different plant families. For example the glucosinolates (GSs) are secondary metabolites typical of brassicaceous crops (Halkier & Liang-cheng, 1997; Walling, 2000; Mattiacci et al., 2001a). GSs are implicated in plant defence against various insects and pathogens, and they are important in host-plant recognition by specialized predators (Mattiacci et al., 2001a; Bukovinszky et al., 2005). The biologically active molecules are not the intact GSs but the hydrolysis products that are formed and released into the environment only when tissue is damaged. The levels of such products (i.e. isothiocyanates) can be increased in Brassica napus by wounding (Bodnaryk, 1994), insect feeding (Hopkins et al., 1998), treatment with hormones like methyl jasmonate (MeJa) or jasmonic acid (Bodnaryk, 1994; Doughty et al., 1995) and also by fungal infection (Doughty et al., 1991). Herbivore attack, particularly by chewing insects, causes tissue disruption, thereby bringing GSs into contact with myrosinase, which results in the production of a variety of toxic degradation compounds. The role of the GSs myrosinase system in plant interactions with insect herbivores was recently reviewed (Agrawal & Kurashige, 2003; Renwick et al., 2006).

In this paper we present the results of investigations on the induced VOCs emitted by Brassica oleracea L. (var. sabauda) plants attacked by the harlequin bug Margarista histrionica Hahn (Heteroptera: Pentatomidae), a pest of cabbage and other brassicaceous crops (McPherson, 1982). We show that cabbage plants with feeding punctures and/or an egg mass deposited by M. histrionica change their volatile blend and that the emission of some compounds varies in time. These results provide information for studies necessary on tri-trophic interactions involving egg parasitoids (Colazza et al., 2004b; Conti et al., 2006).

**MATERIAL AND METHODS**

**Insects**

*Margarista histrionica,* originated from individuals collected from cabbage in the Beltsville area, Maryland, was reared in quarantine conditions in a climate room (25 ± 1°C, 60 ± 5% RH, 16L : 8D) inside plastic cages (30 × 19.5 × 12.5 cm) with 5 cm diameter mesh-covered holes. All stages were fed leaves of cabbage and broccoli. The food was replaced three times per week, and egg masses transferred to other cages for nymphal development.

**Plants**

Seeds of *Brassica oleracea* var. sabauda (cv. Salto, kindly provided by Royal Sluis Brand) were individually planted in pots filled with peat and after ~ 7 days were transplanted into plastic pots filled with a mixture of inert substrates such as agriperlite (BBP Vic; Agrimport, Italy) and vermiculite. Plants were kept in a greenhouse under controlled conditions (25 ± 1°C, 12L : 12D and 50–60% RH), watered daily and fertilized with a water solution of Flory 9 Hydro (N-P-K 15-7-22) (Planta Regenstauf, distributed from Agrimport S.p.A.), sequestrene (NK 3–15 containing Fe EDDHA, produced by Syngenta) and urea (produced by Hydro Agri Italia Milano) (1 l solution: 1 g Flory 9, 0.04 g sequestrene and 0.1 g urea). All volatile captures were done using 4–5 weeks old plants.

**Treatments**

*Brassica oleracea* plants were either untreated, as control (CNT), or attacked by *M. histrionica.* Attacked plants were obtained by exposing them to two *M. histrionica* females, placed on the lower leaf surface inside a suitable “clip cage” (Ø = 3.8 cm; h = 1 cm; modified Petri dish with the rim covered by a small sponge ring), under controlled environmental conditions (25 ± 1°C, 60 ± 5% RH and photoperiod 15L : 9D) and removed after 24 h. Volatiles were collected from plants subjected to the following treatments: feeding punctures (F); combination of feeding punctures and oviposition of one egg mass (FO); feeding punctures and an hatched egg mass (FOH); just oviposition (O). Virgin females were used to obtain plants with only feeding punctures, and gravid females for plants subjected to both feeding and oviposition. The FOH plants were produced by keeping plants bearing an egg mass in a climatic chamber until the nymphs emergence (~7 d). Just after emergence the 1st instar nymphs, which do not feed until after moulting (Canerday, 1965), were removed using a fine brush. To obtain plants just oviposited on (O), gravid females with excised stylets were used. Ovipositing females were previously anaesthetized inside a glass tube with CO2; for 4–5 s in order to immobilize their labium. Afterwards their stylets were drawn from the labium with an entomological pin (no. 000) and half their length amputated. Afterwards the stylet was introduced into the plant and oviposited on a leaf. Following treatments: feeding punctures (F); combination of feeding punctures and oviposition of one egg mass (FO); feeding punctures and an hatched egg mass (FOH); just oviposition (O).

**Plant volatiles**

The collection of volatiles was carried out over a period of 24 h, randomly combining every day four of the five treatments (F, FO, FOH, O and CNT). In addition, in the case of plants with only feeding punctures (F) and those with feeding punctures and an egg mass (FO), the volatile collections were also made after intervals of 0–24 h, 24–48 h and 48–72 h.

A cylindrical glass chamber (Ø = 13.5 cm i.d; h = 19 cm), with an o-ring sealed middle joint, was used to collect head-
space chamber in all the treatments. Before use the glass chamber was washed with water and detergent, rinsed with acetone and hexane, and kept in an oven at ~180°C. Air, purified by passage through an activated charcoal filter, was pumped at a flow of ~1800 ml/min and switched in two chambers containing plants. The air was simultaneously ejected from each chamber at a rate of 600 ml/min and plant volatiles were trapped in a glass tube filled with Porapak Q (Sigma Aldrich; 60 mg, 80–100 mesh), which was pre-cleaned with hexane and then heat conditioned at least for 2 h in a stream of nitrogen (100 ml/min) at 130°C. All entrainments were carried out in a temperature-controlled room (24 ± 1°C, 60 ± 5% RH and photoperiod 12L : 12D; lights on from 8:00 to 20:00) under 8 fluorescent lamps (Philips Master TLD 58W/840) starting between ~11.00–12.00 am and ending 24 h later. In the case of entrainments collected at intervals, the Porapak traps were changed every 24 h and entrainments ended after 72 h. Traps were eluted with 700 µl of hexane, and the obtained extracts were successively concentrated to 100 µl under a gentle stream of nitrogen.

Calibration curves
A set of calibration standard curves were obtained by using solutions of each standard compound dissolved in hexane at a range of concentrations from 20 ppm to 0.2 ppm. Good linearity was achieved with correlation coefficients between 0.9987 and 0.9995. Both standard solutions and samples were analysed in the same way.

The limits of detection (LODs) (estimated to be 3 times the background noise) ranged from 0.5 to 1 ppb for GLV and from 0.2 to 0.5 ppb for the other chemicals.

Gas chromatographic analysis (GC)
Analyses of 2 µl of each extract were performed on a Varian CP-3800 gas chromatograph equipped with a FID detector and an electronic pressure control (EPC). The split/splitless injector was operated in the split mode (1 : 10). Compounds were separated on a VF-1ms fused silica capillary column (30 m, 0.32 mm i.d., 0.25 µm film thickness, Varian, Palo Alto, CA, USA). The injector and detector operated at 260°C and 280°C, respectively. The oven temperature was programmed as follows: from 40°C to 250°C (10-min hold) at 2°C/min. The EPC (10 Psi) was used to provide the desired carrier gas flow rates for the various experimental conditions. The carrier gas was ultrapure helium.

The entire system was controlled by Galaxie software (Varian, version 1.8). Calibration graphs for the compounds were constructed by measuring peak heights vs. concentrations. Individual components were identified by injection of pure standard compounds and comparison with their retention times. After analysis, the compounds were quantified as percentage peak area using integration data.

Chemicals
Standard compounds of GLV (3-methyl-2-pentanone, (E)-2-hexen-1-ol, (Z)-3-hexen-1-ol, (E)-3-hexen-1-ol, (E)-2-hexenal, (Z)-3-hexenal, (Z)-3-hexenyl acetate, hexyl alcohol, hexyl acetate, capronaldehyde), terpenes (α-thujene, α-pinene, sabine, β-pinene, myrcene, 1,4-cineole, 1,8-cineole, limonene, sabine hydrate, linalool, trans-nerolol, (E)-β-caryophyllene, (E,E)-α-farnesene and (E)-β-ocimene), glucosinolate hydrolysis products (3-indolylacetonitrile, benzyl isothiocyanate, allyl isothiocyanate, benzyl cyanide, 4-methoxy-3-indolylacetonitrile), jasmonates (cis-jasmon and methyl jasmonate) and other compounds (methyl salicylate, indole, dodocane) were purchased from Sigma-Aldrich. GC pure hexane was also obtained from Sigma-Aldrich.

Coupled gas chromatography-mass spectrometry (GC-MS)
Confirmatory analyses were performed on a Saturn II GC-MS system (Varian, Walnut Creek, CA). Saturn WS software was used to check the instrument and collect the data. The GC was equipped with a VF-1 ms fused silica capillary column (Varian, 30 m, 0.32 mm i.d., 0.25 µm film thickness). The carrier gas was ultrapure helium at a flow rate of 1 mL min⁻¹.

The temperature program was from 40°C to 280°C (10-min hold) at 2°C/min. The injection of 1 µl of selected samples was in the splitless mode. The MSD was operated in electron impact (EI) mode at 70 eV. The MSD transfer line and injector temperatures were 280°C and 250°C, respectively.

The components separated under the above conditions were identified using the NIST 98.1 mass spectral library.

Statistical analysis
The estimation of the percentage of each class of compounds (terpenes, jasmonates, glucosinolate derivatives – VGSs) and of each compound was obtained by calculating the ratio between their concentration values and the total concentration of detected VOCs of each plant. The data obtained for the volatiles collected over 24 h were analyzed by 1-way ANOVA (Statistics 6.0, Statsoft Inc., 2001). In the case of the volatiles collected at intervals the data were analyzed using a randomized block design analysis of variance (ANOVA) with plants as blocks. Before the analysis, Box-Cox transformation was used to reduce data heteroscedasticity (Sokal & Rohlf, 1998).

RESULTS
Volatile emission from healthy and attacked plants
GC analysis showed differences in the proportions of volatile compounds released by B. oleracea var. sabauda subjected to different treatments.

The percentage of terpenes significantly decreased in the emissions from plants subjected to feeding and oviposition (FO) compared to the control (CNT) and other treatments, i.e. plants with feeding punctures (F), with feeding punctures and one hatched egg mass (FOH) and with a freshly deposited egg mass (O) and with a freshly deposited egg mass (O) and with a freshly deposited egg mass (O). However, there were no significant differences in the terpene emissions from CNT, F, FOH and O plants (Table 1).

As regards jasmonates, significant differences were found in the total emission (%), which was higher in FO and O compared to the other treatments (F = 3.87; df = 4, 53; P < 0.001) (Table 1). However, there were no significant differences in the terpene emissions from CNT, F, FOH and O plants (Table 1).

The total percentage of VGSs significantly increased in FO compared to all other treatments (F = 9.63; df = 3, 44; P < 0.001) except O, where these compounds were not detected (Table 1).

Considering the specific terpenes with significant differences among treatments, the emission (%) of α-thujene (F = 5.64; df = 4, 53; P < 0.001) and sabine (F = 5.09; df = 4, 53; P = 0.001) was significantly lower from FO compared to CNT and FOH plants. Moreover only in the case of α-thujene, the emission from FO plants was lower than from O plants (Table 1). β-pinene was released at higher percentages by O compared to F and FO plants (F(2,43) = 4.13; df = 4, 53; P = 0.005) (Table 1). The amount (%) of 1,8-cineole was higher in O compared to F (F(2,96) = 2.96; df = 3, 43; P = 0.042), whereas this compound was not detected in the emission from FO plants (Table 1). (E)-β-
ocimene was not detected in O, and there were no significant differences among the other treatments (Table 1). By contrast, the oxygenated monoterpene linalool was only detected in the volatile blend from O (Table 1). Trans-nerolidol was detected only in CNT, F and FO (Table 1). The emission (%) of the sesquiterpene \( \text{trans-nerolidol} \) from \( \text{FO} \) and \( \text{F} \) significantly increased compared to \( \text{CNT} \) \( (\text{df} = 2, 12; P < 0.001) \) and was not detected from \( \text{FOH} \) and \( \text{O} \) plants (Table 1).

Considering jasmonates, \( \text{cis-jasmone} \) appeared to be induced only by FO and F and methyl jasmonate differed significantly among treatments, being highest in the case of FO and O \( (\text{df} = 2, 10; P = 0.002) \) (Table 1).

As regards the VGSs, 3-indolylacetonitrile was not detected in O, instead, 4-methoxy-3-indolylacetonitrile showed a significant increase in FO compared with F and FOH \( (\text{df} = 2, 12; P = 0.004) \) (Table 1).

Volatile emission at different time intervals from plants with feeding punctures (F)

The percentage of the four chemical classes in volatiles from \( \text{B. oleracea} \) plants did not change significantly with time \( (0–24 \text{ h}, 24–48 \text{ h} \text{ and } 48–72 \text{ h}) \) (Table 2). Considering the specific compounds, only for two terpenes were the differences significant. Trans-nerolidol was detected only during the first \( 0–24 \text{ h} \), whereas the emission (%) of \( \text{(E)-\text{b}-\text{caryophyllene}} \) increased from similarly damaged plants in the previous experiments (see previous section and Table 1), started decreasing \( 48–72 \text{ h} \) after treatment \( (\text{df} = 2, 12; P = 0.035) \) (Table 2).

Volatile emission at different time intervals from plants with a combination of feeding punctures and oviposition (FO)

During the \( 24–48 \text{ h} \) and \( 48–72 \text{ h} \) time intervals, the percentage of total terpene emissions, which decreased from FO cabbage plants (see above and Table 1), increased percentage of total terpenes, which decreased from FO cabbage plants (see above and Table 1), increased significantly among treatments, being highest in the case of FO and O \( (\text{df} = 2, 10; P = 0.002) \) (Table 2).

Considering the single terpenoids, the percentage emission of \( \text{\alpha -thujene} \) \( (\text{df} = 2, 10; P = 0.009) \), \( \text{\beta -pinene} \) \( (\text{df} = 2, 10; P = 0.012) \), \( \text{\beta -pinene} \) \( (\text{df} = 4.90; \text{df} = 2, 10; P = 0.033) \), \( \text{myrcene} \) \( (\text{df} = 4.23; \text{df} = 2, 10; P = 0.047) \) and \( \text{limonene} \) \( (\text{df} = 12.82; \text{df} = 2, 10; P = 0.002) \) generally increased over the three time intervals, although such increase was not significant at the third interval for \( \text{\beta -pinene} \) (Table 2). Trans-nerolidol was only detected during the first \( 0–24 \text{ h} \) and the second \( 24–48 \text{ h} \) time interval, and the emission (%) of the sesquiterpene \( \text{(E)-b-caryophyllene} \) decreased significantly in the last time interval compared to the first and second \( (\text{df} = 35.89; \text{df} = 2, 10; P < 0.001) \) (Table 2).

### Table 1. Mean percentage ± SE of the different compounds in the total emission of volatiles collected from untreated B. oleracea plants (CNT) and those with feeding punctures (F), a combination of feeding punctures and oviposition (FO), feeding punctures plus one hatched egg mass (FOH) and only oviposition (O). Means followed by the same letter are not significantly different \( (P > 0.05, \text{1-way ANOVA, Tukey HSD test}); – not detected.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Treatments</th>
<th>CNT n = 12</th>
<th>F n = 15</th>
<th>FO n = 11</th>
<th>FOH n = 10</th>
<th>O n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha -thujene )</td>
<td>5.55 a ± 0.12</td>
<td>4.55 ab ± 0.36</td>
<td>3.80 b ± 0.44</td>
<td>5.25 a ± 0.19</td>
<td>5.29 a ± 0.33</td>
<td></td>
</tr>
<tr>
<td>( \alpha -pinene )</td>
<td>6.60 a ± 0.18</td>
<td>7.17 a ± 0.85</td>
<td>6.45 a ± 1.09</td>
<td>5.72 a ± 0.21</td>
<td>5.68 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>( \text{sabinene} )</td>
<td>27.67 a ± 0.40</td>
<td>23.92 ab ± 1.62</td>
<td>20.75 b ± 2.08</td>
<td>27.24 a ± 0.79</td>
<td>24.83 ab ± 1.16</td>
<td></td>
</tr>
<tr>
<td>( \beta -pinene )</td>
<td>2.53 ab ± 0.06</td>
<td>2.16 b ± 0.23</td>
<td>1.95 b ± 0.16</td>
<td>2.56 ab ± 0.06</td>
<td>3.65 a ± 0.47</td>
<td></td>
</tr>
<tr>
<td>( \text{myrcene} )</td>
<td>12.16 a ± 0.34</td>
<td>12.22 a ± 0.87</td>
<td>11.72 a ± 1.32</td>
<td>12.60 a ± 0.60</td>
<td>9.00 ± 1.54</td>
<td></td>
</tr>
<tr>
<td>( 1,8\text{-cineole} )</td>
<td>5.69 ab ± 1.72</td>
<td>3.59 b ± 1.36</td>
<td>–</td>
<td>7.08 ab ± 1.94</td>
<td>10.03 ± 1.20</td>
<td></td>
</tr>
<tr>
<td>( \text{limonene} )</td>
<td>31.69 a ± 1.71</td>
<td>31.12 a ± 1.52</td>
<td>29.47 a ± 1.37</td>
<td>29.80 a ± 1.54</td>
<td>28.45 ± 1.63</td>
<td></td>
</tr>
<tr>
<td>( \text{(E)-b-ocimene} )</td>
<td>2.20 a ± 0.22</td>
<td>3.06 a ± 0.67</td>
<td>3.55 a ± 1.07</td>
<td>1.56 ± 0.43</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>( \text{sabinene hydrate} )</td>
<td>1.42 ± 0.51</td>
<td>2.67 ± 0.38</td>
<td>2.29 ± 0.45</td>
<td>2.79 ± 0.63</td>
<td>3.42 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>( \text{linalool} )</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.83 ± 1.44</td>
<td></td>
</tr>
<tr>
<td>( \text{trans-nerolidol} )</td>
<td>0.42 ± 0.14</td>
<td>1.08 ± 0.41</td>
<td>0.37 ± 0.26</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>( \text{(E)-b-caryophyllene} )</td>
<td>0.15 ± 0.07</td>
<td>2.68 ± 0.72</td>
<td>4.50 ± 0.71</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>( \text{Total terpenes} )</td>
<td>96.08 ± 0.69</td>
<td>94.23 ± 1.00</td>
<td>85.34 ± 2.54</td>
<td>94.61 ± 1.73</td>
<td>94.17 ± 0.90</td>
<td></td>
</tr>
<tr>
<td>( \text{cis-jasmone} )</td>
<td>–</td>
<td>0.30 ± 0.11</td>
<td>0.50 ± 0.15</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>( \text{methyl jasmonate} )</td>
<td>1.27 ± 0.35</td>
<td>1.07 ± 0.33</td>
<td>1.94 ± 0.33</td>
<td>1.21 ± 0.40</td>
<td>3.81 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>( \text{Total jasmonates} )</td>
<td>1.27 ± 0.35</td>
<td>1.37 ± 0.40</td>
<td>2.45 ± 0.42</td>
<td>1.21 ± 0.40</td>
<td>3.81 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>( 3\text{-indolylacetonitrile} )</td>
<td>0.87 ± 0.23</td>
<td>1.17 ± 0.29</td>
<td>1.47 ± 0.33</td>
<td>0.90 ± 0.27</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>( 4\text{-methoxy-3-indolylacetonitrile} )</td>
<td>–</td>
<td>1.97 ± 0.66</td>
<td>8.93 ± 2.22</td>
<td>2.18 ± 1.06</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>( \text{Total VGSs} )</td>
<td>0.87 ± 0.23</td>
<td>3.14 ± 0.68</td>
<td>10.39 ± 2.34</td>
<td>3.08 ± 1.20</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>( \text{dodecane} )</td>
<td>1.78 ± 0.27</td>
<td>1.26 ± 0.22</td>
<td>1.82 ± 0.18</td>
<td>1.10 ± 0.34</td>
<td>2.02 ± 0.65</td>
<td></td>
</tr>
</tbody>
</table>
Although total emission (%) of jasmonates did not differ significantly in the three intervals (Table 2), the octadecanoic-derived cis-jasmonate was detected only during the first time interval (0–24 h) (Table 2) and was not emitted by undamaged plants (see also Table 1).

The total VGSs ($F = 12.98$; $df = 2, 10$; $P = 0.002$), as well as the single compounds 3-indolylacetonitrile ($F = 6.13$; $df = 2, 10$; $P = 0.018$) and 4-methoxy-3-indolylacetonitrile ($F = 6.88$; $df = 2, 10$; $P = 0.013$), which were also higher in damaged compared to undamaged plants (see also Table 1), significantly decreased with increasing time intervals (Table 2).

### DISCUSSION

Feeding and oviposition by *Murgantia histrionica* on *Brassica oleracea*, especially when combined, induce a change in the percentage emission of several constitutive volatiles compared with those released by healthy plants, and the release de novo of linalool after oviposition (Table 1).

The most significant difference among the treatments was in terpene compounds. Specifically, the proportion of α-thujene, sabinene, β-pinene and 1,8-cineole decreased in the emissions from attacked plants, and only the sesquiterpene (E)-β-caryophyllene increased significantly, although it was not detected in the FOH and O treatments (Table 1). The changes in the emission of such compounds occurred rapidly during the first 24 h and then increased or declined during the following 48–72 h (Table 2).

Plants attacked by insects commonly emit terpenes (Paré & Tumlinson, 1997b, 1999; Kessler & Baldwin, 2001) as do plants damaged by other causes (Agelopoulos et al., 1999). A rapid increase in terpene emissions is also recorded for broad beans with egg masses and feeding punctures made by *Nezara viridula* (Colazza et al., 2004b). In contrast, there is a latency period between the initial stimulus and the resulting increase in terpenes induced by other insects (Paré & Tumlinson, 1997a; Agelopoulos et al., 1999; Wegener et al., 2001; Gouinguené & Turlings, 2002; Rodriguez-Saona et al., 2002; Colazza et al., 2004b). In the *B. oleracea*– *M. histrionica* system investigated here, oviposition per se did not induce plants to emit (E)-β-caryophyllene since this compound was not detected in the emissions of plants only subjected to oviposition (Table 1). Instead (E)-β-caryophyllene was detected in the highest amounts (%) for plants subjected

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**Table 2.** Mean percentage ± SE of the different compounds in the total emission of volatiles collected from *B. oleracea* plants with feeding punctures (F) and those with a combination of feeding punctures and oviposition (FO) at different time intervals (0–24 h, 24–48 h and 48–72 h). Means followed by the same letter are not significantly different ($P > 0.05$, 2-way ANOVA, Tukey HSD test); – not detected.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Plants with feeding punctures (F) n = 7</th>
<th>Time intervals</th>
<th>Plants with feeding punctures and egg mass (FO) n = 6</th>
<th>Time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–24 h 24–48 h 48–72 h</td>
<td></td>
<td>0–24 h 24–48 h 48–72 h</td>
<td></td>
</tr>
<tr>
<td>α-thujene</td>
<td>4.92 a ± 0.28 5.17 a ± 0.49 5.26 a ± 0.15</td>
<td></td>
<td>3.99 b ± 0.19 4.61 ab ± 0.27 4.92 a ± 0.13</td>
<td></td>
</tr>
<tr>
<td>α-pinene</td>
<td>6.24 a ± 0.38 5.80 a ± 0.61 5.79 a ± 0.19</td>
<td></td>
<td>5.29 a ± 0.18 5.70 a ± 0.30 5.32 a ± 0.28</td>
<td></td>
</tr>
<tr>
<td>sabinene</td>
<td>26.00 a ± 1.10 27.93 a ± 1.91 26.97 a ± 0.36</td>
<td></td>
<td>20.66 b ± 0.86 24.88 a ± 1.37 25.91 a ± 0.30</td>
<td></td>
</tr>
<tr>
<td>β-pinene</td>
<td>2.53 a ± 0.11 2.47 a ± 0.22 2.46 a ± 0.04</td>
<td></td>
<td>1.99 b ± 0.05 2.38 a ± 0.14 2.30 ab ± 0.09</td>
<td></td>
</tr>
<tr>
<td>myrcene</td>
<td>11.09 a ± 0.48 12.02 a ± 0.65 11.52 a ± 0.15</td>
<td></td>
<td>9.52 b ± 0.50 8.70 b ± 1.81 11.03 a ± 0.30</td>
<td></td>
</tr>
<tr>
<td>1,8-cineole</td>
<td>6.10 a ± 2.16 6.52 a ± 2.31 6.41 a ± 2.27</td>
<td></td>
<td>– – –</td>
<td></td>
</tr>
<tr>
<td>limonene</td>
<td>29.30 a ± 2.01 27.24 a ± 2.82 30.94 a ± 2.14</td>
<td></td>
<td>28.97 b ± 1.19 33.84 a ± 1.26 36.20 a ± 0.48</td>
<td></td>
</tr>
<tr>
<td>(E)-β-ocimene</td>
<td>2.15 a ± 0.37 2.27 a ± 0.41 2.42 a ± 0.12</td>
<td></td>
<td>2.26 a ± 0.47 1.83 a ± 0.59 2.44 a ± 1.19</td>
<td></td>
</tr>
<tr>
<td>sabinene hydrate</td>
<td>2.97 a ± 0.56 3.71 a ± 0.28 3.62 a ± 0.09</td>
<td></td>
<td>2.50 a ± 0.50 3.51 a ± 0.26 3.53 a ± 0.17</td>
<td></td>
</tr>
<tr>
<td>linalool</td>
<td>– – –</td>
<td></td>
<td>– – –</td>
<td></td>
</tr>
<tr>
<td>(E)-β-caryophyllene</td>
<td>1.67 ab ± 0.88 2.05 a ± 0.87 0.86 b ± 0.27</td>
<td></td>
<td>4.54 a ± 0.69 3.64 a ± 0.48 1.39 b ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Total terpenes</td>
<td>93.50 a ± 1.64 95.16 a ± 0.85 96.24 a ± 0.71</td>
<td></td>
<td>80.29 b ± 2.94 89.52 a ± 2.98 93.04 a ± 0.67</td>
<td></td>
</tr>
<tr>
<td>cis-jasmonate</td>
<td>– – –</td>
<td></td>
<td>– – –</td>
<td></td>
</tr>
<tr>
<td>methyl jasmonate</td>
<td>0.99 a ± 0.39 1.69 a ± 0.19 0.85 a ± 0.24</td>
<td></td>
<td>2.30 a ± 0.16 2.27 a ± 0.16 2.34 a ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Total jasmonates</td>
<td>0.99 a ± 0.39 1.69 a ± 0.19 0.85 a ± 0.24</td>
<td></td>
<td>2.74 a ± 0.22 2.27 a ± 0.16 2.34 a ± 0.40</td>
<td></td>
</tr>
<tr>
<td>3-indolylacetonitrile</td>
<td>0.76 a ± 0.26 0.38 a ± 0.30 0.80 a ± 0.25</td>
<td></td>
<td>1.90 a ± 0.43 1.11 ab ± 0.43 0.23 b ± 0.15</td>
<td></td>
</tr>
<tr>
<td>4-methoxy-3-indolylacetonitrile</td>
<td>3.51 a ± 1.10 1.35 a ± 0.66 0.78 a ± 0.58</td>
<td></td>
<td>12.96 a ± 2.82 5.08 b ± 3.08 2.35 b ± 0.32</td>
<td></td>
</tr>
<tr>
<td>Total VGSs</td>
<td>4.29 a ± 1.32 1.35 a ± 0.66 1.59 a ± 0.49</td>
<td></td>
<td>14.87 a ± 2.88 6.19 b ± 2.85 2.58 b ± 0.32</td>
<td></td>
</tr>
<tr>
<td>dodecane</td>
<td>1.22 a ± 0.39 1.80 a ± 0.23 1.32 a ± 0.12</td>
<td></td>
<td>2.10 a ± 0.19 2.02 a ± 0.16 2.05 a ± 0.26</td>
<td></td>
</tr>
</tbody>
</table>
to feeding and oviposition (Table 1), suggesting a synergistic action, similar to that recorded in the *Vicia faba / Phaseolus vulgaris* – *N. viridula* system (Colazza et al., 2004a, b). The effect of substances present on egg surfaces and/or in the follicular secretion combined with the effect of the saliva or just feeding damage might enhance the release of this compound.

Unlike the terpenes reported above, linalool appeared to be released de novo by plants with only oviposition (Table 1). This monoterpenic is not detected in the emission from healthy cabbage and Brussels sprouts, but it is emitted by these plants when damaged by *Plutella xylostella* (Lepidoptera: Plutellidae) (Bukovinszky et al., 2005; Pinto et al., 2007).

The emission of (E)-α-ocimene, present in healthy and damaged cabbage plants, was not detected from plants with only oviposition (Table 1), confirming that oviposition by this bug, in the absence of feeding, is able to cause changes in the emission of volatile compounds by cabbage plants.

The percentage emission of the two jasmonate compounds increased when the plants were infested by *M. histrionica* (Table 1). These endogenous chemical signals are produced by many plants (Preston et al., 2001) and their volatile derivatives are involved in plant responses following mechanical, herbivore or pathogen stress (Wallig, 2000). In the system *B. oleracea* – *M. histrionica*, cis-jasmone was induced by a combination of feeding and oviposition, whereas methyl jasmonate was induced just by oviposition. These results suggest that the lipoxygenase pathway may be activated by the bug’s activity on the host plant, whereas the defence signal methyl salicylate was not detected. Plant responses to insects are generally distinctive and dependent on their different feeding habits. Phloem feeders such as aphids and whiteflies induce the SA pathway, commonly activated also by bacterial, fungal and viral pathogens, whilst cell-content feeders such as thrips induce the JA pathway (Wallig, 2000). Studies on piercing-sucking insects like *Lygus hesperus* Knight (Heteroptera: Miridae) and *N. viridula* suggest that the mechanisms of volatile induction are similar to those induced by chewing insects (Williams et al., 2005). Consequently in the case of cabbage attacked by *M. histrionica* it is possible to hypothesize the activation of a JA-dependent wound-signal transduction pathway. In fact, the increased emission of methyl jasmonate does not change over the 72 h period, and cis-jasmone is not detected after 24 h (Table 2). This result confirms that in plants stressed by biotic or abiotic factors, the maximum emission of this hormone-signalling molecule occurs after 12–24 h (Lee et al., 2004; Zadra et al., 2006).

An interesting result concerns the volatile compounds derived from glucosinolates (VGSs). In general the glucosinolates are considered to be the major chemical defence of crucifers and they normally occur in low concentrations (Mattiacci et al., 2001a). Previous studies show that their production is enhanced by insect damage (Koristas et al., 1991; Bodnaryk, 1994; Mattiacci et al., 1994, 2001b; Agrawal et al., 1999; Walling, 2000). In the *B. oleracea* – *M. histrionica* system two glucosinolate hydrolysis products (VGSs) were identified, 3-indolylacetonitrile and 4-methoxy-3-indolylacetonitrile. These compounds are formed by the hydrolysis of glucorbrasicin and 4-methoxy-glucorbrasicin, respectively. The latter especially was emitted in large amount by plants with feeding punctures plus oviposition (Table 1), suggesting a possible synergistic action. The emission of 4-methoxy-3-indolylacetonitrile decreased after 24 h (Table 2). Indole-derivatives are the most potent class of glucosinolates for attracting several crucifer-feeding insects (Bartlet et al., 1999) and even if these chemicals can be used as plant defense, they are supposed to be important cues used by specialist herbivores for recognition, oviposition, and feeding (Agrawal & Kurashige, 2003).

In the analyses of *B. oleracea* volatiles, GLVs were never detected. This contrasts with other systems involving the same plant species attacked by chewing insects (Mattiacci et al., 2001b; Fatouros et al., 2005; Bukovinszky et al., 2005; Geervliet et al., 1997; Pinto et al., 2007) or other plant species attacked by piercing-sucking insects (Rodriguez-Saona et al., 2002; Colazza et al., 2004b). This inconsistency might be due to the infestation level (Dicke et al., 1993; Tumlinson et al., 1993), which was very low in the case of air entrainments from *B. oleracea* attacked by *M. histrionica* (2 adults per plant), and/or to the fact that, in our experiments, the air entrainments were not conducted contemporarily with the insect attack, i.e., the insects were removed before the capture of volatiles began.

Interestingly the change in the emission of terpenes, jasmonates and VGSs observed after an egg mass was deposited on the host plant, either with or without feeding, returned to levels similar to those from the control when the eggs hatched. A similar response was observed in the *V. faba – N. viridula* system, where the emission of several compounds decreases after egg hatch (Colazza et al., 2004b). These results can be interpreted as a plant adaptation to the decrease in time of host egg suitability to parasitoid attack. Many plants have developed indirect induced defences against herbivores, thus attracting or activating the searching behaviour of natural enemies associated with the herbivore (Dicke et al., 1993; De Moraes et al., 1998). In the specific case of pentatomid bugs, indirect defences are induced by oviposition combined with feeding, which result in the emission of volatile compounds that attract egg parasitoids (Colazza et al., 2004a) or through changes in the chemical composition of the plant surface that stimulates parasitoid searching behaviour on the substrate (Conti et al., 2006).

These responses, however, disappear when the bug eggs are no longer suitable for parasitoid attack, thus avoiding useless metabolic costs (Cipollini et al., 2003) by the plant and waste of time by the parasitoid (Colazza et al., 2004a). The changes in the emission of plant volatiles that are induced in *B. oleracea* as a consequence of oviposition and feeding by *M. histrionica*, therefore, may be
involved in the recruitment of egg parasitoids, such as Trissolcus brochymenae (Ashmead) (Hymenoptera: Scelionidae), thus providing additional, highly reliable and detectable cues to those originating from the host bug (Conti et al., 2003).

In conclusion, this research reveals the effects of the feeding and reproductive activity of herbivorous bugs on the induction of volatile compounds in cabbage plants. Future research should focus on the role of such volatiles in the plant-herbivore-parasitoid interactions.

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