How predatory arthropods learn to use herbivore-induced plant volatiles. Evidence from behavioural experiments and the field

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Chapter 4 shows that anthocorid predators aggregate around gauze cages containing *Psylla*-infested trees in a pear orchard. Because anthocorids responded to odour from *Psylla*-infested leaves in a laboratory test, it was hypothesized that these aggregative responses in the field were triggered by olfaction of compounds associated with *Psylla* injury. We present chemical analyses of volatiles from damaged and undamaged plants and studies on behavioral responses of anthocorid predators to compounds released by damaged plants. Leaf headspace volatiles from clean and *Psylla*-infested pear trees were collected on Tenax and identified by GC/MS after thermodesorption. Twelve volatiles were found exclusively in headspace samples from *Psylla*-infested leaves. Six were present in significantly higher quantities in samples from infested leaves: the monoterpenes, (E,E)-α-farnesene, the phenolic, methyl salicylate, and the green leaf compounds, (Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol, 1-hexyl-acetate and 1-penten-3-ol. These compounds are known to be produced by plants, and damage by pear psyllids seems to trigger their emission. Blend composition varied, and was partly correlated with tree or leaf age and degree of *Psylla*-infestation. To study whether compounds associated with leaf injury elicit olfactory responses in anthocorid predators, apple-extracted (E,E)-α-farnesene, synthetic methyl salicylate and (Z)-3-hexen-1-yl acetate were offered in a Y-tube olfactometer to field-collected adult *Anthocoris* spp. Significant positive responses were found to both the monoterpenes and the phenolic, but not to the green leaf volatile. The results lend support to the hypothesis that predator attraction to herbivore-infested pear trees is mediated by herbivory-induced plant volatiles.


A plant may defend itself against herbivores by promoting the effectiveness of the herbivores' antagonists, a phenomenon called extrinsic or indirect defense (Price *et al.*, 1980). One way to achieve this is by the production of herbivory-induced volatiles in the plant. Irrespective of their origin, these volatiles are beneficial to both predator and plant, as they enable the predator to locate its prey, which consequently alleviates herbivore pressure on the plant. Such volatiles are termed synomones (*sensu* Dicke and Sabelis, 1988) and are reported for several acarine and insect predator-prey and parasitoid-host
systems on plants (e.g., Dicke et al., 1993; Takabayashi and Dicke, 1996). In these systems, the origin of the volatiles is based on general plant biochemical pathways and the existence of systemic responses to damage. Evidence for a role of herbivory-induced plant volatiles in plant defense has been inferred from the following laboratory observations: (1) predators or parasitoids respond to odors from leaves infested with their prey or hosts (e.g., Sabelis and Van de Baan, 1983; Turlings et al., 1990; McCall et al., 1993); (2) headspace volatiles of herbivore-infested plants differ from those of clean and mechanically damaged plants (Dicke et al., 1990a; Turlings and Tumlinson, 1992; Takabayashi et al., 1994); (3) specific components elicit responses of the herbivore's antagonists (Dicke et al., 1990a; Turlings et al., 1991).

Recently, field evidence for synomone-mediated attraction of predators to herbivore-infested plants was obtained in a system consisting of pear trees (Pyrus communis L.), pear psyllids (Psylla pyri L., P. pyricola Forster), and anthocorid predators (Anthocoris nemorum L., A. nemoralis (Fabricius), and various Orius spp.) (Drukker et al., 1995; Chapter 4). In an orchard with very low density of pear *Psylla*, significantly more anthocorid predators were attracted towards cages containing trees heavily infested with pear *Psylla*, than towards cages with uninfested trees. These predators migrate into the orchard from surrounding hedgerows where they feed on other homopteran prey (Drukker et al., 1992; Scutareanu et al., 1993, 1999, Chapter 3). One of the hypotheses explaining the aggregative response to prey density is mediation by herbivory-induced plant volatiles (Drukker et al., 1995; Chapter 4).

To test this hypothesis derived from field experiments we previously performed laboratory experiments to demonstrate the *Psylla*-induced emission of pear volatiles. We provided evidence for the involvement of several specific compounds in predator-attraction. The evidence is based on Y-tube olfactometer-tests in which individual predators were offered the choice between clean air and air from *Psylla*-infested leaves, or air from uninfested leaves (Drukker and Sabelis, 1990; Drukker, unpublished results). In this paper, we focus on additional evidence: chemical analysis of headspace volatiles from damaged and undamaged pear trees, and behavioral analysis of the predator response to single herbivory-associated components.

**MATERIAL AND METHODS**

Young potted pear trees of variety 'Conference', grafted on either quince or pear root stocks and purchased from commercial nurseries, were kept uninfested or were infested with *P. pyricola* (Homoptera, Psyllidae) collected in a pear orchard (Watergraafsmeer, Amsterdam, The Netherlands).

Two experiments were carried out in successive year: in the first, variability due to leaf age (incorporating seasonal effects) was taken into account; in the second, variability due to time since *Psylla*-colonization was considered.

**Experiment 1**

In late March 1993, one 4-year old tree (tree no. 2, Fig. 1) was placed in a cage in a climate room (ca. 20°C; 60-70% RH; L:D= 17:7h). Field-collected twigs with several dozen *P. pyricola* adults and eggs were supplied to infest the tree. In July, when the *Psylla*
population had reached high density, two additional trees of similar age (not yet infested; tree no. 3 and 4, Fig. 1) were transferred from the field to the same cage (tree no. 3 in the first week of July, no. 4 in the last) which was now placed in another climate room (ca. 23°C; 65-70% RH; L:D= 16:8h). As a control, one outdoor tree of the same age was kept uninfested throughout the experiment (tree no. 1, Fig. 1). In early May, shortly after the trees had started to flush, one sample of 10 young leaves (tree 1) and two samples of 9 leaves each (tree 2) were collected (total fresh weight of leaf blades: 3.2, 0.9 and 1.1 g, respectively; mean infestation level of the samples: 0, 31.3 and 40.6 nymphs per leaf, respectively). In late August, shortly after the trees had stopped to flush, samples of 10 or 9 mature leaves were taken from tree 1, 3 and 4 (total fresh weight: 4.7, 1.9 and 2.3 g, mean infestation level: 0, 1.8 and 96.6 nymphs per leaf, respectively). All leaves were sampled randomly and used for headspace analysis of volatile compounds. Fresh weight and number of nymphs were assessed immediately after collection of volatiles.

Although control tree and treated trees were under different environmental conditions, weekly inspections showed that the control tree was free of psyllids and any other herbivores throughout the season. To what extent, if any, this tree had suffered from herbivory in previous years is unknown.

**Experiment 2**

In early January 1994, two 1-year old trees were placed in separate cages in a climate room (ca. 23°C; 65-70% RH; L:D= 16:8h). After the release – in late January – of several dozen *P. pyricola* adults one tree gradually became infested (as shown in Fig. 1, Exp. 2), the other tree was kept uninfested (not shown in Fig. 1) to serve as a control. At regular intervals (specified in Fig. 1, Exp. 2), random samples of 5 to 10 young leaves were taken from each tree for analysis of headspace volatile compounds (total leaf blade fresh weights: 1.2-4.0 g for clean leaves, 1.2-2.7 g for infested leaves). The samples from the infested tree were also used for monitoring *Psylla* infestation. The first headspace sample (day 0) was taken just before release of psyllids; the second (day 20) shortly after the first larvae and honeydew were observed on leaves (infestation level 9.3 nymphs per leaf); the 3rd (day 25) and 4th (day 50) were taken when levels of infestation had increased (20.0 and 31.6 nymphs per leaf, respectively). Weighing of leaves took place after collection of volatiles.

**Collection and identification of leaf volatiles**

Fresh leaves (blades and petioles), cut just prior to headspace sampling, were put into a 500 ml glass jar. Incoming air was purified by drawing it through silica gel and activated charcoal (both 400 ml). Volatiles were trapped on Tenax adsorbant (90 mg) packed in a 160 x 4 mm i.d. glass tube (Chrompack, The Netherlands). Airflow rate was ca. 100 ml/min., and sampling time was 120 min. (except once – in experiment 1, tree 2 – where sampling time was 30 min.). The Tenax tubes were closed and stored in the dark at room temperature until they were subjected to thermodesorption.

Adsorbents were first released from the Tenax by thermodesorption at 250°C for 10 minutes with a helium flow of 10 ml/min. Desorbed compounds were cryofocused in a cold trap at -90°C (M-16200, Chrompack, The Netherlands) and subsequently analysed on a Supelcowax-10 capillary column (60 x 0.25 mm i.d., 0.25 mm film thickness). The temperature program of the gas chromatograph was 40°C (4 min.), rising to 140°C at
Compounds were identified by comparison of mass spectra with those in the Wiley-Library (McLafferty and Staufer, 1989) and our own specialized library of natural products (M.A. Posthumus, Organic Chemistry, Wageningen), and by comparison of retention times with our home-built data-base of retention indices based on authentic samples. Quantification was based on the average response of a mixture of 10 selected natural compounds run in a separate trial (1000 counts corresponded to 70 ng in all samples, except the two samples taken on day 50, Experiment 2, where 1000 counts corresponded to 200 ng).

![Graph](image)

**Figure 1** The abundance (mean number ± standard error) of *Psylla pyricola* nymphs on leaves. First experiment; tree 1: uninfested, tree 2,3,4: infested. All samples were used for headspace sampling (GC/MS analysis). Second experiment, infested tree; day 0: before infestation, day 20: first nymphs present, day 25: 5 days after first presence of nymphs; day 50: 30 days after first presence of nymphs; samples collected on these days were used for assessment of nymph density and for headspace sampling. On day 34 only nymphs were counted. 'Sample size' refers to numbers of leaves.
VOLATILES FROM PSYLLA-INFESTED PEAR TREES ATTRACT ANTHOCORID PREDATORS

Olfactometer bioassays
Experiments were carried out to determine whether anthocorids respond to two volatiles ((E,E)-α-farnesene and methyl salicylate) found abundantly in headspace samples of infested trees, but not or only in minute quantity in samples of uninfested trees. Attractiveness of these two compounds was compared to (Z)-3-hexen-1-yl acetate, found in both infested and uninfested trees.

A Y-tube olfactometer was used (cf. Sabelis and Van de Baan, 1983; Dicke et al., 1990a). The two upper arms of the Y-tube were connected to tubes containing the stimuli. One of the tubes contained the experimental stimulus – a hexane solution of the volatile administered on filter paper. The other contained the control stimulus – the solvent on filter paper without the test chemical. Prior to testing, the hexane was allowed to evaporate for 3 minutes (a pre-test had shown that more than 99.9% of the solvent evaporated within this period). After five runs, tubes with experimental and control stimuli were reversed. The basal arm of the Y-tube was connected to an air pump with a valve insuring a constant air flow of 0.25-0.35 m/s in both upper arms. In the center of the Y-tube a Y-shaped metal wire served to guide the insects. Predators were released one at a time on the downwind end of the wire, from where they could walk to the junction where the two odor plumes met. At this point, the predator could choose to go towards either of the odor sources. A trial was ended when the predator stepped off the wire and reached the end of either of the upper arms, or five minutes after the start of a test. In the former case, the predator's behavior was interpreted as preferential for the stimulus concerned, in the latter, it was interpreted as not preferential for either of the stimuli. When the predator sat motionless for more than one minute on the glass beyond the end of the wire, its behavior was also interpreted as preferential for the stimulus.

Tests were performed with 0.24 mg of (E,E)-α-farnesene (isolated from apple extracts, provided by TNO, Delft, The Netherlands), 2.5 mg of synthetic methyl salicylate (Aldrich, The Netherlands), and 5 mg of synthetic (Z)-3-hexen-1-yl acetate (Aldrich). Odor sources were renewed every hour, except with farnesene (renewal every half hour). Hexane (100 μl) was used as solvent for (E,E)-α-farnesene and methyl salicylate. (Z)-3-Hexen-1-yl acetate was administered without solvent. The concentrations of chemicals offered in the olfactometer were rough estimates of concentrations which anthocorids could encounter in the field (see Results – Olfactometer bioassays).

Anthocoris nemorum males and females were collected in August and September 1994 and 1995 from pear trees in an experimental orchard at Lienden, and A. nemoralis males and females from pear trees in a commercial orchard at Goes, The Netherlands. All predators were kept at 4°C and were starved at 25°C for at least 2 hours prior to testing.

Statistical tests
Chemical samples were compared using Mann-Whitney-U test for equal or unequal sample sizes (Siegel, 1956). Responses of anthocorids in the olfactometer were analysed with a 2-tailed binomial test (null hypothesis: both odor sources are equally attractive).
**RESULTS**

**Composition of volatile blends**

Representative gas chromatograms (Fig. 2A, B) illustrate the differences of the volatile blends from infested and uninfested leaves. For further analysis we selected compounds based on the following criteria: (1) potential plant origin, and (2) presence in two or more samples. We subsequently focussed on compounds which were damage-related and hence...
could potentially mediate plant-predator communication. For this we used a third criterion: difference in frequency between infested and uninfested leaf samples exceeding 8 and/or difference in (mean) abundance between infested and uninfested leaf samples exceeding 25 ng. The purpose of the 3rd criterion was merely to serve as a conservative selection of compounds; the discriminators '8' and '25' are thus arbitrary. In the 14 samples, a total of 42 compounds satisfied the first and second criterion, 36 of which were found in Experiment 1 (Table 1), 29 in Experiment 2 (Table 2), whereas 10 compounds met the third criterion (summarized in Table 3). All 10 compounds were more abundant, and 8 compounds were more frequent in samples from infested leaves. For 6 the differences in abundance were statistically significant (Table 3), namely 1-penten-3-ol, 1-hexyl-acetate, (Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol, (E,E)-α-farnesene and methyl salicylate (Table 3). Two compounds, 3-hexen-1-yl-butanoate and linalool, were on average more abundant in samples from uninfested leaves, although not statistically significant. The frequency of linalool, however, was higher in infested than in uninfested leaf samples.

Considering the proportion of each compound in the blend, it appears that the "green leaf"-compounds (Z)-3-hexen-1-yl acetate and (Z)-3-hexen-1-ol are the most abundant (Table 1 and 2). They make up 73% (42.8–97.5%) of the blend in infested leaves and as much as 90.5% (63.7–98.9%) in uninfested leaves.

**Variability due to leaf age (Experiment 1)**

The number and total amount of compounds found in young leaves (May) was higher than in old leaves (August), both uninfested and infested (Table 1; normalized chromatograms in Fig. 3). Three aldehydes, consistently present in May (both in uninfested and infested leaves), were absent in August: octanal, nonanal and decanal. 3-Pentanol was present only in infested plants in May but not in August. (E)-β-ocimene was found in infested plants in August, while absent in May. Hexanal and methyl salicylate were more abundant in May, whereas (E)-4,8-dimethyl-1,5,7-nonatriene and (E,E)-α-farnesene were more abundant in August. Methyl salicylate was detected in high quantities in infested young leaves, but in much lower absolute and relative values in heavily infested, old leaves (August) (Table 1, Fig. 3).

Both in May and in August, amounts of volatiles increased with the degree of infestation (Table 1). Some compounds showed a consistent quantitative increase from uninfested to highly infested leaves, e.g. hexanal and (E,E)-α-farnesene. Methyl salicylate and (E)-β-ocimene were most abundant in August in the headspace of the mildly infested leaves and absent or scant in the samples from uninfested and highly infested trees. These results are consistent with those obtained in experiment 2, when only young leaves were analysed (Table 2).

**Variability due to time since colonization (Experiment 2)**

Both the total number and amount of volatiles found in infested plants (experiment 2, Table 2) increased over time until day 25, then slightly decreased remaining higher than before infestation. In uninfested plants, the number of compounds remained the same, but the total amounts decreased consistently (Table 2). Most compounds steadily increased after the onset of infestation. Five of them ((Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol, methyl salicylate, benzyl-alcohol and an unidentified compound (m/z 43 (100%)), 79
(75%), 80 (81%) were present before the tree was inoculated with *Psylla*. Seven compounds (butyl acetate, 2-pentanal, 5-pentanol, unknown (m/z 66 (81%), 96 (100%)), 1-penten-3-ol, 1-hexyl-acetate, and 3-hexen-1-yl-benzoate) were detected after the 1st lar-

Table 1 Headspace volatiles (ng/h/g fresh leaf) collected from clean pear leaves and leaves with various levels of *Psylla* infestation, in May (young leaves) and August (old leaves) (Experiment 1)*

<table>
<thead>
<tr>
<th>Volatiles</th>
<th>May (young leaves)</th>
<th>August (old leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nymphs/leaf:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree:</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1 2-butaneone</td>
<td>2.84</td>
<td></td>
</tr>
<tr>
<td>2 2-methylbutane</td>
<td>5.07</td>
<td></td>
</tr>
<tr>
<td>3 3-methylbutane</td>
<td>5.14</td>
<td></td>
</tr>
<tr>
<td>4 3-pentaneone</td>
<td>34.59</td>
<td></td>
</tr>
<tr>
<td>5 1-penten-3-one</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>6 butyl acetate</td>
<td>1.68</td>
<td>23.21</td>
</tr>
<tr>
<td>7 hexanal</td>
<td>7.19</td>
<td>31.34</td>
</tr>
<tr>
<td>8 2-pentanal</td>
<td>63.15</td>
<td>12.67</td>
</tr>
<tr>
<td>9 3-pentanol</td>
<td>18.82</td>
<td>22.42</td>
</tr>
<tr>
<td>10 1-butanone</td>
<td>19.76</td>
<td>5.88</td>
</tr>
<tr>
<td>11 1-penten-3-ol</td>
<td>23.21</td>
<td>46.42</td>
</tr>
<tr>
<td>12 (E)-2-hexenal</td>
<td>3.58</td>
<td>31.34</td>
</tr>
<tr>
<td>13 limonene</td>
<td>23.21</td>
<td>235.30</td>
</tr>
<tr>
<td>14 (E)-β-ocimene</td>
<td>36.30</td>
<td>36.30</td>
</tr>
<tr>
<td>15 1-pentanol</td>
<td>21.66</td>
<td>77.76</td>
</tr>
<tr>
<td>16 1-hexyl acetate</td>
<td>3.83</td>
<td>30.49</td>
</tr>
<tr>
<td>17 octanal</td>
<td>27.44</td>
<td>6.03</td>
</tr>
<tr>
<td>18 (E)-4,8-dimethyl-1,3,7-nonatriene</td>
<td>13.38</td>
<td>15.36</td>
</tr>
<tr>
<td>19 (Z)-3-hexen-1-yl</td>
<td>302.14</td>
<td>2080.04</td>
</tr>
<tr>
<td>20 1-hexanol</td>
<td>42.48</td>
<td>42.48</td>
</tr>
<tr>
<td>21 (Z)-3-hexen-1-ol</td>
<td>19.76</td>
<td>269.70</td>
</tr>
<tr>
<td>22 nonanal</td>
<td>16.42</td>
<td>87.08</td>
</tr>
<tr>
<td>23 (E)-2-hexen-1-ol</td>
<td>22.42</td>
<td>22.42</td>
</tr>
<tr>
<td>24 unknown (43,79,80)</td>
<td>8.68</td>
<td>16.08</td>
</tr>
<tr>
<td>25 3-hexen-1-yl</td>
<td>9.09</td>
<td>9.09</td>
</tr>
<tr>
<td>26 1-octen-3-ol</td>
<td>47.94</td>
<td>47.94</td>
</tr>
<tr>
<td>27 decanal</td>
<td>5.25</td>
<td>155.18</td>
</tr>
<tr>
<td>28 limonol</td>
<td>20.66</td>
<td>9.19</td>
</tr>
<tr>
<td>29 1-octanol</td>
<td>6.65</td>
<td>6.65</td>
</tr>
<tr>
<td>30 β-caryophyllene</td>
<td>3.18</td>
<td>5.70</td>
</tr>
<tr>
<td>31 α-copaene</td>
<td>3.43</td>
<td>3.43</td>
</tr>
<tr>
<td>32 α-farnesene</td>
<td>72.79</td>
<td>72.79</td>
</tr>
<tr>
<td>33 δ-cadinene</td>
<td>3.52</td>
<td>3.52</td>
</tr>
<tr>
<td>34 methyl salicylate</td>
<td>13.53</td>
<td>254.12</td>
</tr>
<tr>
<td>35 caproic acid</td>
<td>110.12</td>
<td>22.29</td>
</tr>
<tr>
<td>36 benzyl alcohol</td>
<td>3.07</td>
<td>20.30</td>
</tr>
</tbody>
</table>

| Total amounts   | 452   | 3239  | 3276  | 93    | 734   | 1392  |
| Total number of compounds | 22   | 16    | 27    | 6     | 13    | 21    |

* Comparison using Mann-Whitney-U test (n₁=n₂=3) for each compound showed significant differences between leaf samples in May and August at the P=0.05 level, indicated by a.
VOLATILE SS FROM PSYLLA-INFESTED PEAR TREES ATTRACT ANTHOCORID PREDATORS

Volatile emissions were observed on leaves, i.e. day 20. For these 5+7=12 compounds there was a significant difference between the 3 samples from the infested tree and the 4 samples from the uninfested tree. From day 25 onwards, four additional compounds were found (5-ethyl-2(SH)-furanone, (E,E)-α-farnesene, δ-cadinene and ethyl salicylate). On day 50, only one additional compound (E)-2-hexenal was detected. The relationship between the progression of *Psylla* infestation and the emission of volatile compounds from young leaves of a 1-year old pear tree is depicted in Fig. 4.

Table 2 Headspace volatiles (ng/h/g fresh leaf) collected from *Psylla*-infested pear leaves before and after 20, 25 and 50 days of infestation, compared to an uninfested tree (experiment 2)*.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Days after start (mean no. nymphs/leaf) in uninfested tree</th>
<th>Days after start (mean no. nymphs/leaf) in infested tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (0) 20 (0) 25 (0) 50 (0)</td>
<td>0 (0) 20 (9.3) 25 (20.0) 50 (32.3)</td>
</tr>
<tr>
<td>1 3-pentanone</td>
<td>0.02 trace</td>
<td>0.02 0.08 0.19</td>
</tr>
<tr>
<td>2 1-penten-3-one</td>
<td>0.05 trace</td>
<td>0.03 0.10 0.76</td>
</tr>
<tr>
<td>3 butyl acetate</td>
<td>a 0.01</td>
<td>0.02 0.03 0.06</td>
</tr>
<tr>
<td>4 hexanal</td>
<td>a 0.01 0.01</td>
<td>0.07 0.07 0.68</td>
</tr>
<tr>
<td>5 2-pentenal</td>
<td>a trace</td>
<td>0.04 0.07</td>
</tr>
<tr>
<td>6 3-pentanol</td>
<td>a 0.05 0.12</td>
<td>0.19 0.92 1.20</td>
</tr>
<tr>
<td>7 1-butanol</td>
<td>a 0.19 0.05 0.04</td>
<td>0.09 0.06 0.09</td>
</tr>
<tr>
<td>8 unknown (66,96)</td>
<td>a</td>
<td>0.01 0.14 0.09</td>
</tr>
<tr>
<td>9 1-penten-3-ol</td>
<td>a</td>
<td>0.04 0.19 0.50</td>
</tr>
<tr>
<td>10 (E)-2-hexenal</td>
<td>a</td>
<td>1.82</td>
</tr>
<tr>
<td>11 limonene</td>
<td>0.26 0.02 0.02 0.03</td>
<td>0.11 0.08 0.42</td>
</tr>
<tr>
<td>12 1-hexyl acetate</td>
<td>a 0.37</td>
<td>1.72 0.93</td>
</tr>
<tr>
<td>13 (Z)-3-hexen-1-yl acetate</td>
<td>a 61.21 62.93 51.10 30.35</td>
<td>24.82 147.41 538.16 223.59</td>
</tr>
<tr>
<td>14 anisole</td>
<td>a 0.02 0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>15 (Z)-3-hexen-1-ol</td>
<td>a 9.53 4.12 1.00 0.44</td>
<td>1.92 9.74 81.83 46.13</td>
</tr>
<tr>
<td>16 nonanal</td>
<td>0.13 0.02 0.01</td>
<td>0.01 0.01</td>
</tr>
<tr>
<td>17 unknown (43, 79, 80)</td>
<td>a 0.31 0.48 0.45 0.31</td>
<td>0.09 0.62 1.71 0.56</td>
</tr>
<tr>
<td>18 3-hexen-1-yl butanoate</td>
<td>a 0.21 0.09 0.02 0.07</td>
<td>0.07 0.30 2.16 0.10</td>
</tr>
<tr>
<td>19 3-hexen-1-yl 2- methylbutanoate</td>
<td>a 0.01</td>
<td>0.09 0.41</td>
</tr>
<tr>
<td>20 linalool</td>
<td>0.06</td>
<td>0.02 0.07</td>
</tr>
<tr>
<td>21 β-caryophyllene</td>
<td>0.10 0.08 0.02 0.13</td>
<td>0.13 0.09</td>
</tr>
<tr>
<td>22 α-copaene</td>
<td>a 0.05 0.17</td>
<td>0.14 0.80</td>
</tr>
<tr>
<td>23 5-ethyl-2(SH)-furanone</td>
<td>a 0.02 0.03 0.11</td>
<td>0.03 0.15</td>
</tr>
<tr>
<td>24 α-farnesene</td>
<td>a</td>
<td>0.28 2.03</td>
</tr>
<tr>
<td>25 δ-cadinene</td>
<td>a 0.12 0.10 0.01 0.01</td>
<td>0.05 0.09 0.15</td>
</tr>
<tr>
<td>26 methyl salicylate</td>
<td>a 1.24 0.10 0.07 0.01</td>
<td>0.51 3.92 6.51 23.93</td>
</tr>
<tr>
<td>27 ethyl salicylate</td>
<td>a 0.07 0.16 0.29</td>
<td>0.02 0.11</td>
</tr>
<tr>
<td>28 benzyl alcohol</td>
<td>a 0.07 0.16 0.29</td>
<td>0.02 0.11</td>
</tr>
<tr>
<td>29 3-hexen-1-yl benzoate</td>
<td>a 0.22 0.53 0.32</td>
<td>0.01 0.01</td>
</tr>
<tr>
<td>Total amounts (ng/h/g fresh weight)</td>
<td>73 68 53 32 27 164 636 307</td>
<td></td>
</tr>
<tr>
<td>Total number of compounds</td>
<td>13 9 17 12 6 21 25 24</td>
<td></td>
</tr>
</tbody>
</table>

* Comparison with a Mann-Whitney-U test (n1=4, n2=3) for each compound showed significant differences between infested and uninfested leaf samples at the P=0.05 level, indicated by *.
Table 3 Summary of compounds collected from clean and pear *Psylla* infested pear leaves; response by two species of anthocorid predators to some of some chemicals*

<table>
<thead>
<tr>
<th>Volatiles</th>
<th><em>Psylla pyricola</em> infested leaves</th>
<th>Uninfested leaves</th>
<th>Response by</th>
<th>Anthocoris nemoralis</th>
<th>Anthocoris nemorum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/h/g frequency</td>
<td>%GC area</td>
<td>ng/h/g frequency</td>
<td>%GC area</td>
<td></td>
</tr>
<tr>
<td>1 hexanal</td>
<td>42</td>
<td>7</td>
<td>1.5</td>
<td>42</td>
<td>1.4</td>
</tr>
<tr>
<td>2 2-pentenal</td>
<td>2.7</td>
<td>4</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 1-penten-3-ol</td>
<td>15 a</td>
<td>5</td>
<td>0.7</td>
<td>a</td>
<td>0</td>
</tr>
<tr>
<td>4 (E)-2-hexenal</td>
<td>126</td>
<td>4</td>
<td>4.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 1-hexyl-acetate</td>
<td>17 a</td>
<td>6</td>
<td>0.7</td>
<td>a</td>
<td>0.6</td>
</tr>
<tr>
<td>6 (E)-4,8-dimethyl-1,3,7-nonatriene</td>
<td>30</td>
<td>3</td>
<td>3.0</td>
<td>1.9</td>
<td>1</td>
</tr>
<tr>
<td>7 (Z)-3-hexen-l-yl acetate</td>
<td>733 b</td>
<td>7</td>
<td>62.2</td>
<td>85</td>
<td>7</td>
</tr>
<tr>
<td>8 (Z)-3-hexen-l-ol</td>
<td>132 b</td>
<td>7</td>
<td>10.1</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>9 (E,E)-a-farnesene</td>
<td>35 a</td>
<td>6</td>
<td>2.3</td>
<td>0.8</td>
<td>2</td>
</tr>
<tr>
<td>10 methyl salicylate</td>
<td>79 a</td>
<td>7</td>
<td>4.8</td>
<td>a</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* Amounts are expressed in ng/h/g fresh weight of leaves estimated from peak areas of gas chromatogrammes (means of 7 leaf samples), frequencies are per 7 leaf samples, %GC-area express the proportion of each compound in the total blend of volatiles (means of 7 leaf samples). Comparison using Mann-Whitney-U test (n1=n2=7) for each compound showed significant differences between infested and uninfested leaf samples at the P = 0.05 and 0.01 level, indicated by a and b respectively.

![Figure 3](image-url)

**Figure 3** Normalized GC peak areas of the main volatile compounds in young and old leaves of the 4-year-old pear trees related to the degree of *Psylla* infestation (first experiment). A: uninfested tree 1, B: infested trees 2, 3 and 4. (Peak numbers as in Table 1).
Figure 4 Normalized GC peak areas of the main volatile compounds in young leaves of the 1-year-old pear trees related to the development of *Psylla* infestation (2nd experiment). (a)–(d) uninfested tree, (e)–(h) infested tree. Day 0, 20, 25, 50 as in Fig. 1. (Peak numbers as in Table 2).
Table 4 Response in Y-tube olfactometer by orchard-caught *Anthocoris nemoralis* and *A. nemorum* adults to (E,E)-α-farnesene, methyl salicylate and (Z)-3-hexen-1-yl acetate, tested versus clean air (2-tailed binomial test; H0: p(+) = p(-) = 0.5)

<table>
<thead>
<tr>
<th>Experimental (+) stimulus</th>
<th>species</th>
<th>n(+)</th>
<th>n(-)</th>
<th>n(0)</th>
<th>n(+) + n(-)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.24 mg (E,E)-α-farnesene</td>
<td><em>A. nemoralis</em></td>
<td>49</td>
<td>15</td>
<td>6</td>
<td>0.77</td>
<td>0.000002</td>
</tr>
<tr>
<td></td>
<td><em>A. nemorum</em></td>
<td>50</td>
<td>20</td>
<td>8</td>
<td>0.71</td>
<td>0.00004</td>
</tr>
<tr>
<td>2.3 mg methyl salicylate</td>
<td><em>A. nemoralis</em></td>
<td>41</td>
<td>10</td>
<td>5</td>
<td>0.80</td>
<td>0.000001</td>
</tr>
<tr>
<td></td>
<td><em>A. nemorum</em></td>
<td>39</td>
<td>8</td>
<td>7</td>
<td>0.83</td>
<td>0.000006</td>
</tr>
<tr>
<td>5 mg (Z)-3-hexen-1-yl acetate</td>
<td><em>A. nemoralis</em></td>
<td>8</td>
<td>14</td>
<td>8</td>
<td>0.36</td>
<td>0.3 (ns)</td>
</tr>
</tbody>
</table>

**Interexperimental variability**

There were no consistent differences between 1-year old trees in 1994 (Table 2) and 4-year old trees in 1998 (Table 1) with respect to number of volatiles, in infested or uninfested plants. Total amounts, however, were consistently higher in 4-year old trees than 1-year old trees. Some compounds from 1-year old trees were absent in samples from 4-year old trees (isobutyl-acetate, unknown (66, 96), anisole, 3-hexen-1-yl butanoate, 3-hexen-1-yl methylbutanoate, 5-ethyl-2(5H)-furanone, ethyl salicylate and 3-hexen-1-yl benzoate). Others were exclusively present in samples from 1-year-old trees (2-butanone, 2- and 3-methylbutanal, (E)-β-ocimene, 1-pentanol, octanal, (E)-4,8-dimethyl-1,3,7-nonatriene, 1-hexanol, nonanal, (E)-2-hexen-1-ol, 1-octen-3-ol, decanal and 1-octanol). Caution, however, should be taken when attributing the observed differences to differential tree age, since trees also differed with respect to season and year of investigation and in the rootstock upon which they were grafted – quince ("kwee-C") for 4-year old trees and seedlings for 1-year old trees.

**Olfactometer bioassays**

In the Y-tube olfactometer, both *Anthocoris nemoralis* and *A. nemorum* showed positive responses to methyl salicylate (80% and 83%, respectively) and (E,E)-α-farnesene (77% and 71%, respectively) that significantly deviated from the 50% expected under the null hypothesis (Table 4). (Z)-3-hexen-1-yl acetate, a compound not indicative of *Psylla*-infestation, did not elicit a positive response (only 36% of *A. nemoralis* adults responded positively, P = 0.3).

Quantitative results of the chemical analysis can be compared with amounts of compounds administered in the olfactometer. Evaporation of compounds from the filter paper was determined in a pre-test: 63% (1.5±0.4 mg, n=5) of methyl salicylate and 76% (2.7±0.4 mg, n=5) of (Z)-3-hexen-1-yl acetate evaporated in 30 min. in the Y-tube (wind speed: 0.3 liter/s). Evaporation of (E,E)-α-farnesene was not measured, but because this compound has a boiling point in between that of the other two compounds evaporation rate is assumed to have an intermediate value. For methyl salicylate the concentration in the odor plume in the olfactometer was calculated to be 2.78 μg/l (mean value over 30 min.). In the airstream from the sampled infested leaves, the concentration was 6.58 ng/leaf (6 l sampled, on average 79 ng/h/g fresh weight (Table 3), leaves weighing ca. 0.5 gram). Therefore, ca. 420 infested leaves would be needed to cause the same concentration as 2.3 mg on filter paper (one single orchard tree will have manyfold 420 leaves). Likewise, the used amounts of (E,E)-α-farnesene and (Z)-3-hexen-1-yl acetate are equivalent to ca. 80 infested leaves.
VOLATILES FROM *Psylla*-infested pear trees attract anthocorid predators

**DISCUSSION**

Olfactometer bioassays carried out with two of the volatile compounds that are correlated with *Psylla*-infestation in pear trees elicit a response in anthocorid predators, namely methyl salicylate and (E,E)-α-farnesene. Because these compounds are of plant origin, it may be inferred that their production is induced by *Psylla* injury. These chemical signals may be plant-predator synomones because upon herbivore attack plants may benefit from attracting predators and predators profit from responding to prey-related signals (Dicke and Sabelis, 1988).

We did not make further attempts to investigate specificity with respect to the damaging agent. Previous claims (Dicke *et al.*, 1990a; Turlings *et al.*, 1990; Mattiacci *et al.*, 1994) that mechanical damage does not induce the production of volatiles in plants seem unwarranted because it is difficult, if not impossible, to mimic the way (site and process) insects damage a leaf, let alone to generate the damage as continually as the insect does. The only inference we can make from our data is that repetitive picking of leaves from uninfested trees does not trigger enhanced production of volatiles found in the headspace of infested trees (Table 2). In fact, the levels of these volatiles are diminishing.

Earlier studies described volatiles from undamaged pear leaves (Miller *et al.*, 1989) and fruits (Jennings *et al.*, 1960). Miller *et al.* (1989) analysed volatiles from pear leaves of two other pear cultivars: Bartlett and Bradford, cultivars considered to be susceptible and resistant to pear *Psylla* attack, respectively. They identified fourteen compounds, eight of which were also present in one or more of our samples from infested trees: (E,E)-α-farnesene, (Z)-8 hexen-1-yl acetate, (E)-β-ocimene, linalool, α-copaene, β-caryophyllene, δ-cadinene and limonene. The compound they tentatively identified as perillene is most probably (E)-4,8-dimethyl-1,3,7-nonatriene, a methylene terpenoid with the same molecular weight and almost the same mass spectrum as perillene and frequently present in volatile blends from arthropod-attacked plants (lima bean-mites, apple-mites, Takabayashi *et al.*, 1991; cotton-caterpillars, McCall *et al.*, 1994; corn-caterpillars, Turlings *et al.*, 1991; pear-psyllids, present study). Also the reported Kovats index corresponds well with the Kovats index on DB-1 for (E)-4,8-dimethyl-1,3,7-nonatriene (M.A. Posthumus, unpublished data; see also Dicke *et al.*, 1990a). Some other volatiles like methyl salicylate were not found by Miller *et al.* (1989). These authors did not specify the infestation level of the trees they sampled, but in August, the time of sampling, it is rare to find trees devoid of psyllids in North American orchards (Watson and Wilde, 1963; Van de Baan and Croft, 1991). Also the composition of the leaf volatiles suggest that the trees sampled by Miller *et al.* (1989) may have been infested at the time of leaf sampling.

Miller *et al.* (1989) found significant differences between the two cultivars: linalool was found only in Bradford, α-copaene only in Bartlett. We also have evidence for differences in volatile composition between two pear cultivars, *i.e.* Conference and Beurré Hardy, the latter failing to release methyl salicylate (Dukker and Posthumus, unpublished). Miller *et al.* (1989) argue that leaf volatiles are used by psyllids to locate their host plants. Thus, differences in volatile composition can be indicative of differential cultivar susceptibility. We suggest that difference in volatile composition may, in addition, reflect cultivar-related differences in indirect defense by means of predator recruitment. The frequently reported heavy *Psylla* damage on Beurré Hardy compared to Conference (Drukker and Van der Blom, unpublished) may be explained by this difference.
Variability in blend composition

Several of the compounds we found associated with *Psylla* infestation, were also found to be damage-associated in other, totally different plant-herbivore systems, such as apple-spider mite (Takabayashi *et al.*, 1991), lima bean-spider mite (Dicke *et al.*, 1990a), cucumber-spider mite (Takabayashi *et al.*, 1994), cotton-caterpillar (McCall *et al.*, 1994), corn-caterpillar (Turlings *et al.*, 1991), Brussels sprouts-caterpillar (Mattiacci *et al.*, 1994), broad bean-aphid (Hardie *et al.*, 1994), apple-sawfly larvae (Bové *et al.*, 1996), and cabbage-caterpillar (Blaakmeer *et al.*, 1994). Five compounds found exclusively in our infested-pear samples have not been mentioned before in association with herbivory: 2- and 3-methyl butanal, 5-ethyl-2(5H)-furanone, ethyl salicylate, and anisole.

Some of the variation in blend composition may be attributed to leaf age. We observed both qualitative and quantitative differences between young leaves in May and mature leaves in August, and not all of these differences could be attributed to the higher infestation level in August. For example, (E)-β-ocimene was found only in August in leaf samples that had a similar level of infestation to those in May. Alternatively, methyl salicylate was absent in highly infested and uninfested old leaves in August, whereas it was found in considerable quantities in infested young leaves in May. Age-related differences were also found by Takabayashi *et al.* (1994) in samples from spider-mite infested cucumber leaves.

Blend composition may also be affected by age, rootstock, and attack history of the trees. Our data leave these possible effects open, but we cannot assess their relative contributions. Further research is needed, especially because effects of tree age and attack history are known for direct defensive responses, as reviewed by Haukioja (1990; see also various chapters in Tallamy and Raupp, 1991). This author also makes a useful distinction between induced responses based on the time scale of the induction process; he distinguished between short-term and long-term (or delayed) induced responses. In our experiments, we used trees that originated from commercial nurseries. Hence, we cannot be absolutely sure that these trees had always been free of pests.

Another source of variation in blend composition stems from the accumulation of damage since herbivore colonization. We observed a steady increase over time for most compounds as the infestation proceeded. Some volatiles were observed already before the plants were infested (methyl salicylate in very low quantity, (Z)-3-hexen-1-yl acetate and (Z)-3-hexen-1-ol), others were only found after first appearance of *Psylla* nymphs and associated honeydew (1-hexyl acetate), 5 days later ((E,E)-α-farnesene, 5-ethyl-2(5H)-furanone) or 30 days later ((E)-2-hexenal).

**Predator response to synomones**

The responses of the two species of anthocorids to the three compounds tested are in line with the findings by Dicke *et al.* (1990a) for phytoseiid predators. They too found a significant positive response to methyl salicylate and an indifferent response to (Z)-3-hexen-1-yl acetate. As the latter green leaf compound was not indicative of spider mite damage, Dicke *et al.* (1990a) considered the indifferent response adaptive. However, in our pear trees this green leaf compound is clearly more abundantly released by infested plants and therefore, using the same line of reasoning (Dicke *et al.*, 1990a), we could have expected the bugs to respond to it, but they appeared not to. Also in cotton, (Z)-3-hexen-1-yl acetate was found to be associated with herbivory (McCall *et al.*, 1994) and here
VOLATILES FROM PSIyllA-INFESTED PEAR TREES ATTRACTION ANTHOCORID PREDATORS

parasitoids actually do respond to this and other green leaf compounds (Whitman and Eller, 1990). Thus, it is not clear why anthocorids do not respond to the green leaf compound, but do respond to the other two damage-related volatiles. However, synergistic effects among these (and any other) compounds in the headspace of the infested plants should not be ignored as a possibility. The other damage-related volatiles (Table 3) still remain to be tested, as well as the attractiveness of different concentrations of all compounds, to anthocorid predators found to be responding to the total blend in the field (Drukker et al., 1995).

The positive responses to the single compounds are surprising in that their information content can only be limited. The full blend, of course, contains much more information. Nevertheless, additional olfactometer tests showed that responses of wild-caught predators to full blends from infested pear leaves (A. nemoralis: 76%, n=73; A. nemorum: 82%, n=61; Drukker, unpublished data) were comparable to responses to (E,E)-α-farnesene and methyl salicylate (both species: 71-83%; see Table 4). Preliminary results showed that one of the damage-related compounds, methyl salicylate, elicits positive responses under field conditions as well; predatory bugs were found to be attracted towards sticky traps with dispensers of methyl salicylate (on average 9.6 bugs per trap over a period of 1-2 months, compared to 0.8 in unbaited control traps; Drukker, unpublished data). A possible explanation for positive responses to a single compound may be that the compound is a common denominator of damage inflicted by a wide range of phytophages that include prey items on the menu. This may explain that A. nemorum responds to odor blends from French bean leaves infested by prey mites (Tetranychus urticae and T. cinnabarinus, Dwumfour, 1992), because these blends are known to include methyl salicylate. Additional damage-related compounds may act in fine-tuning the information content of the signal, thereby enabling preferential responses.

One wonders to what extent predatory arthropods can smell “the tree before the forest” within the wealth of chemical information contained in the blends released by plants upon herbivore attack. Whether their responses are plastic or fixed, is a major question for future research. In some cases natural enemies have been shown to change their behavior gradually over days of exposure to a stimulus (Dicke et al., 1990b; Dwumfour, 1992), in others, associative learning was demonstrated over short-term exposure to a stimulus (Lewis and Tumlinson, 1988). These possibilities are currently being investigated with respect to anthocorid predators (Drukker, in progress).

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VOLATILES FROM PSYLLA-INFESTED PEAR TREES ATTRACT ANTHOCORID PREDATORS


