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VOLATILES FROM *Psylla*-INFESTED PEAR TREES AND THEIR POSSIBLE INVOLVEMENT IN ATTRACTION OF ANTHOCORID PREDATORS

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Abstract—Previous work showed that anthocorid predators aggregate around gauze cages containing *Psylla*-infested trees in a pear orchard. Because anthocorids responded to odor 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 monoterpene, (*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

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monoterpene 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.

Key Words—Leaf volatiles, synomones, pear tree, *Psylla*, *Anthocoris*, induced response, tritrophic interaction, attraction, GC-MS, olfactometer.

INTRODUCTION

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 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. and *P. pyricola* Forster), and anthocorid predators [*Anthocoris nemorum* L., *A. nemoralis* (Fabricius), and various *Orius* spp.] (Drukker et al., 1995). In an orchard with a 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; Scutareanu, Lingeman, and Sabelis, unpublished data). One of the hypotheses explaining the aggregative response to prey density is mediation by herbivory-induced plant volatiles (Drukker et al., 1995).

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.

METHODS AND MATERIALS

Young potted pear trees of the 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 years: 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 2, Figure 1) was placed in a cage in a climate room (ca. 20°C; 60–70% relative humidity; 17L:7D). 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; trees 3 and 4, Figure 1) were transferred from the field to the same cage (tree 3 in the first week of July, tree 4 in the last), which was now placed in another climate room (ca. 23°C; 65–70% relative humidity; 16L:8D). As a control, one outdoor tree of the same age was kept uninfested throughout the experiment (tree 1, Figure 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 flush had stopped, samples of 10 or 9 mature leaves were taken from trees 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.

FIG. 1. The abundance (mean number $\pm$ standard error) of *Psylla pyricola* nymphs on leaves. First experiment: tree 1 is uninfested; trees 2, 3, and 4 are infested. All samples were used for headspace sampling (GC-MS analysis). Second experiment: 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.

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% relative humidity; 16L:8D). After the release in late January of several dozen *P. pyricola* adults, one tree gradually became infested (as shown in Figure 1, experiment 2); the other tree was kept uninfested (not shown in Figure 1) to serve as a control. At regular intervals (specified in Figure 1, experiment 2), random samples of 5–10 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 third (day 25) and fourth (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-mm × 4-mm-ID glass tube (Chrompack). Airflow 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) and subsequently analyzed on a Supelcowax-10 capillary column (60 × 0.25 mm-ID, 0.25-mm film thickness). The temperature program of the gas chromatograph was 40°C (4 min), rising to 140°C at 3°C/min and then to 270°C at 6°C/min. The column was connected to a Finnigan MAT95 mass spectrometer, operated in the 70 eV EI ionization mode with scanning from mass 24 to 400 at 1.05 sec cycle time.

Compounds were identified by comparison of mass spectra with those in the Wiley Library (McLafferty and Staufner, 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).

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 at all 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 min (a pretest 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 ensuring a constant air flow of 0.25–0.35 m/sec 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 5 min 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 1 min 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), 2.3 mg of synthetic methyl salicylate (Aldrich), and 5 mg of synthetic (*Z*)-3-hexen-1-yl acetate (Aldrich). Odor sources were renewed every hour, except farnesene which was renewed every half hour. Hexane (100 μ l) 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 that 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 hr prior to testing.

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

RESULTS

Composition of Volatile Blends. Representative gas chromatograms (Figure 2A and B) illustrate the differences of the volatile blends from uninfested and infested leaves. For further analysis we selected compounds based on potential

FIG. 2. Representative gas chromatograms of headspace volatiles sampled from pear tree leaves (first experiment). Temperature program of the gas chromatograph was 40°C for 4 min, then rising to 140°C at 3°C/min, then to 270°C at 6°C/min. A: uninfested young leaves (May); B: heavily infested young leaves (May). Peak numbers correspond to the compounds listed in Table 1.

plant origin and presence in two or more samples. We subsequently focused on compounds that 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 three and/or difference in (mean) abundance between infested and uninfested leaf samples exceeding 25 ng. The purpose of the third criterion was merely to serve as a

TABLE 1. HEADSPACE VOLATILES COLLECTED FROM CLEAN PEAR LEAVES AND LEAVES WITH VARIOUS LEVELS OF *Psylla*-INFESTATION IN MAY AND AUGUST (EXPERIMENT 1)^a

Volatile	Nymphs/leaf: Tree:	Volatile amount (ng/hr/g fresh leaf)					
		May (young leaves)			August (old leaves)		
		0 1	31 2	41 2	0 1	1.8 3	97 4
1. 2-butanone		2.84		16.73			
2. 2-methylbutanal				5.07			9.43
3. 3-methylbutanal				5.14			9.43
4. 3-pentanone				34.59			
5. 1-penten-3-one				46.42			
6. butyl acetate		1.46					
7. hexanal		7.19	23.21	235.30	2.21	3.58	32.21
8. 2-pentenal				18.62			
9. 3-pentanol			13.21	19.82			
10. 1-butanol		2.38	64.88	27.36			10.60
11. 1-penten-3-ol				63.63			41.03
12. (<i>E</i>)-2-hexenal			31.34	691.03			155.48
13. limonene		1.68	trace		0.48	3.47	
14. (<i>E</i>)- β -ocimene						36.30	5.88
15. 1-pentanol		2.02		8.16			21.66
16. 1-hexyl acetate		3.83	30.49	77.76			9.76
17. octanal*		3.18	27.44	6.03			
18. (<i>E</i>)-4,8-dimethyl- 1,3,7-nonatriene		13.38		15.36		107.05	88.92
19. (<i>Z</i>)-3-hexen-1-yl acetate		302.14	2080.04	1289.35	63.15	417.03	436.67
20. 1-hexanol		1.86	24.23	42.48			49.28
21. (<i>Z</i>)-3-hexen-1-ol		19.76	269.70	266.98	12.67	52.38	197.87
22. nonanal*		16.42	87.08	23.31			
23. (<i>E</i>)-2-hexen-1-ol				22.42			78.81
24. unknown (43, 79, 80)		8.68		16.08			14.40
25. 3-hexen-1-yl butanoate		9.09					
26. 1-octen-3-ol			47.94			9.52	81.95
27. decanal*		5.25	155.18	17.18			
28. linalool		20.66		9.19		2.57	4.17
29. 1-octanol		6.65					4.35
30. β -caryophyllene		3.18				5.70	
31. α -copaene						3.43	
32. α -farnesene		3.41	19.65	72.79	1.86	37.01	113.63
33. δ -cadinene						3.52	
34. methyl salicylate		13.53	254.12	202.73		51.75	7.47
35. caproic acid			110.12	22.29	12.28		
36. benzyl alcohol		3.07		20.30			18.52
Total amounts (ng/hr/g fresh weight)		452	3239	3276	93	734	1392
Total number of compounds		22	16	27	6	13	21

^aComparison using Mann-Whitney U test ($N_1 = N_2 = 3$) for each compound showed significant differences between leaf samples in May and August at the $P = 0.05$ level, indicated by an asterisk.

conservative selection of compounds; the discriminators '3' and '25' are thus arbitrary. In the 14 samples, a total of 42 compounds satisfied the first and second criteria, 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 eight were more frequent in samples from infested leaves. For six, 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 (Tables 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 Figure 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 in infested plants in May but not in August. (E)- β -Ocimene was found in infested plants in August, but was absent in May. Hexanal and methyl salicylate were more abundant in May, whereas (E)-4,8-dimethyl-1,3,7-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, Figure 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 analyzed (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 but 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

TABLE 2. HEADSPACE VOLATILES COLLECTED FROM *Psylla*-INFESTED PEAR LEAVES BEFORE AND AFTER 20, 25, AND 50 DAYS OF INFESTATION, COMPARED TO UNINFESTED TREE (EXPERIMENT 2)^a

Volatile	Volatile amount (ng/hr/g fresh leaf)							
	Days after start (mean no. nymphs/leaf) in uninfested tree				Days after start (mean no. nymphs/leaf) in infested tree			
	0 (0)	20 (0)	25 (0)	50 (0)	0 (0)	20 (9.3)	25 (20.0)	50 (32.3)
1. 3-pentanone	0.02		trace			0.02	0.08	0.19
2. 1-penten-3-one	0.05		trace	0.01		0.03	0.10	0.76
3. butyl acetate*			0.01			0.02	0.03	0.06
4. hexanal	0.09		0.01	0.01		0.07	0.07	0.68
5. 2-pentenal*						trace	0.04	0.07
6. 3-pentanol*			0.05	0.12		0.19	0.92	1.20
7. 1-butanol	0.19	0.05	0.04			0.09	0.06	0.09
8. unknown (66, 96)*						0.01	0.14	0.09
9. 1-penten-3-ol*						0.04	0.19	0.50
10. (<i>E</i>)-2-hexenal								1.82
11. limonene	0.26	0.02	0.02	0.03		0.11	0.08	0.42
12. 1-hexyl acetate*						0.37	1.72	0.93
13. (<i>Z</i>)-3-hexen-1-yl acetate*	61.21	62.93	51.10	30.35	24.82	147.41	538.16	223.59
14. anisole			0.02	0.17		0.01		0.20
15. (<i>Z</i>)-3-hexen-1-ol*	9.53	4.12	1.00	0.44	1.92	9.74	81.83	46.13
16. nonanal	0.13		0.02	0.01				
17. unknown (43, 79, 80)*	0.31	0.48	0.45	0.31	0.09	0.62	1.71	0.56
18. 3-hexen-1-yl butanoate	0.21	0.09	0.02		0.07	0.30	2.16	0.10
19. 3-hexen-1-yl 2-methylbutanoate		0.01				0.09	0.41	
20. linalool	0.06					0.02	0.07	
21. β -caryophyllene	0.10		0.08	0.02		0.13	0.09	
22. α -copaene			0.05	0.17				
23. 5-ethyl-2(5H)-furanone							0.14	0.80
24. α -farnesene							0.28	2.03
25. δ -cadinene		0.02	0.03	0.11			0.09	0.15
26. methyl salicylate*	1.24	0.10	0.01		0.51	3.92	6.51	25.93
27. ethyl salicylate							0.02	0.11
28. benzyl alcohol*					0.07	0.16	0.29	0.31
29. 3-hexen-1-yl benzoate*						0.22	0.53	0.32
Total amounts (ng/hr/g fresh weight)	73	68	53	32	27	164	636	307
Total number of compounds	13	9	17	12	6	21	25	24

^aComparison with a Mann-Whitney-U test ($N_1 = 4$, $N_2 = 3$) for each compound showed significant differences between infested and uninfested leaf samples at the $P = 0.05$ level, indicated by an asterisk.

TABLE 3. SUMMARY OF COMPOUNDS COLLECTED FROM CLEAN AND *Psylla*-INFESTED PEAR LEAVES; RESPONSE BY TWO SPECIES OF ANTHOCORID PREDATORS TO SOME CHEMICALS^a

Volatile	Identified in						Response by	
	<i>Psylla pyricola</i> infested leaves			Uninfested leaves			<i>Anthocoris nemoralis</i>	<i>Anthocoris nemorum</i>
	ng/hr/g	frequency	%GCarea	ng/hr/g	frequency	%GCarea		
1. hexanal	42	7	1.5	1.4	5	0.6		
2. 2-pentenal	2.7	4	0.1	0	0	0		
3. 1-penten-3-ol	15*	5	0.7*	0	0	0.0		
4. (<i>E</i>)-2-hexenal	126	4	4.7	0	0	0.0		
5. 1-hexyl-acetate	17*	6	0.7*	0.6	1	0.1		
6. (<i>E</i>)-4,8-dimethyl-1,3,7-nonatriene	30	3	3.0	1.9	1	0.4		
7. (<i>Z</i>)-3-hexen-1-yl acetate	733†	7	62.2	85	7	83.8	0	
8. (<i>Z</i>)-3-hexen-ol	132†	7	10.1	7	7	6.7		
9. (<i>E,E</i>)- α -farnesene	35*	6	2.3	0.8	2	0.4	+	+
10. methyl salicylate	79*	7	4.8*	2.2	5	0.9	+	+

^a Amounts are expressed in ng/hr/g fresh weight of leaves estimated from peak areas of gas chromatograms (means of seven leaf samples), frequencies are per seven leaf samples, %GCarea express the proportion of each compound in the total blend of volatiles (means of seven leaf samples). Comparison using Mann-Whitney U test ($N_1 = N_2 = 7$) for each compound showed significant differences between infested and uninfested leaf samples at the $P = 0.05$ and 0.01 level, indicated by an asterisk and a dagger, respectively.

FIG. 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: infested tree 1; B: infested trees 2, 3, and 4. (Peak numbers as in Table 1.)

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, 3-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 first larvae and honeydew were observed on leaves, i.e., day 20. For these 5 + 7 = 12 compounds there was a significant difference between the three samples from the infested tree and the four samples from the uninfested tree. From day 25 onwards, four additional compounds were found [5-ethyl-2(5H)-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 Figure 4.

Interexperimental Variability. There were no consistent differences between 1-year-old trees in 1994 (Table 2) and 4-year-old trees in 1993 (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 in 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 pretest: 65% (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/sec). Evaporation of (*E,E*)- α -farnesene was not measured, but because this compound has a boiling point between that of the other two compounds, evaporation rate is assumed to have an intermediate value. For methyl salicylate, the concentration in the odor

FIG. 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 (second experiment). a-d: uninfested tree, e-h: infested tree. Days 0, 20, 25, and 50 as in Figure 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 (TWO-TAILED BINOMIAL TEST; $H_0: P(+) = P(-) = 0.5$)

Experimental (+) stimulus	Species	<i>n</i> (+)	<i>n</i> (-)	<i>n</i> (0)	<i>n</i> (+)	<i>P</i>
					<i>n</i> (+) + <i>n</i> (-)	
0.24 mg (<i>E,E</i>)- α -farnesene	<i>A. nemoralis</i>	49	15	6	0.77	0.00002
	<i>A. nemorum</i>	50	20	8	0.71	0.0004
2.3 mg methyl salicylate	<i>A. nemoralis</i>	41	10	5	0.80	0.00001
	<i>A. nemorum</i>	39	8	7	0.83	0.000006
5 mg (<i>Z</i>)-3-hexen-1-yl acetate	<i>A. nemoralis</i>	8	14	8	0.36	0.3 (ns)

plume in the olfactometer was calculated to be 2.78 $\mu\text{g/liter}$ (mean value over 30 min). In the airstream from the sampled infested leaves, the concentration was 6.58 ng/liter/leaf (6 liters sampled, on average 79 ng/hr/g fresh weight (Table 3), leaves weighing ca. 0.5 g). 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 many more than 420 leaves!). Likewise, the amounts of (*E,E*)- α -farnesene and (*Z*)-3-hexen-1-yl acetate used are equivalent to ca. 80 infested leaves.

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 diminished.

Earlier studies described volatiles from undamaged pear leaves (Miller et al., 1989) and fruits (Jennings et al., 1960). Miller et al. (1989) analyzed 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 14 compounds, eight of which were also present in one or more of our samples from infested trees: (*E,E*)- α -farnesene, (*Z*)-3-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)]. The reported Kovats index also 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, such as 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). Furthermore the composition of the leaf volatiles suggests 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 (Drukker 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 the 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 associated with damage 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 sprout caterpillar (Mattiacci et al., 1994), broad bean aphid (Hardie et al., 1994), apple sawfly larvae (Boevé 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 even 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 the first appearance of *Psylla* nymphs and associated honeydew (1-hexyl acetate), five 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 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 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 does 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 to sticky traps with dispensers of methyl salicylate (on average 9.6 bugs per trap over a period of one to two 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 within the forest” among 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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