

## Effects of Cyclamen Mite (*Phytonemus pallidus*) and Leaf Beetle (*Galerucella tenella*) Damage on Volatile Emission from Strawberry (*Fragaria* × *ananassa* Duch.) Plants and Orientation of Predatory Mites (*Neoseiulus cucumeris*, *N. californicus*, and *Euseius finlandicus*)

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Volatile emission profile of strawberry (*Fragaria* × *ananassa* Duch.) plants (cvs. Polka and Honeoye) damaged by cyclamen mite (*Phytonemus pallidus* Banks) or leaf beetle *Galerucella tenella* (L.) (cv. Polka) was analyzed to determine the potential of these strawberry plants to emit herbivore-induced volatiles. The total volatile emissions as well as emissions of many green leaf volatiles (e.g., (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate) and methyl salicylate were greater from cyclamen mite-damaged strawberry plants than from intact plants. Leaf beetle feeding increased emissions of monoterpenes (*Z*-ocimene and (*E*)- $\beta$ -ocimene, sesquiterpenes (*E*)- $\beta$ -caryophyllene, (*E,E*)- $\alpha$ -farnesene, and germacrene-D, and a homoterpene (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) significantly. Nevertheless, the naïve generalist predatory mites, *Neoseiulus cucumeris*, *Neoseiulus californicus*, and *Euseius finlandicus* did not prefer *P. pallidus*- or *G. tenella*-damaged plants over intact plants in a Y-tube olfactometer, suggesting that these predatory mite species are not attracted by the herbivore-induced volatiles being released from young strawberry plants.

**KEYWORDS:** *Fragaria* × *ananassa* Duch.; *Phytonemus pallidus* (Acari: Tarsonemidae); *Galerucella tenella* (Coleoptera: Chrysomelidae); *Neoseiulus cucumeris* (Acari: Phytoseiidae); *Neoseiulus californicus* (Acari: Phytoseiidae); *Euseius finlandicus* (Acari: Phytoseiidae); strawberry; herbivore-induced VOCs; tritrophic interactions

### INTRODUCTION

Strawberry plants emit a wide range of volatile organic compounds (VOCs) from leaves, flowers, and fruits. Over 360 compounds have been identified in these fruits (1–4), and these volatiles account for a large proportion of the aromatic properties, scent, and taste, of individual cultivars. Strawberry leaves can emit many alcohols, aldehydes, esters, aromatic methyl salicylate (MeSA), and terpenes (*E*)- $\beta$ -ocimene, (*Z*)-ocimene, linalool,  $\alpha$ -farnesene, and germacrene-D (5, 6). VOC emission is dependent on abiotic factors such as temperature, light, water, and nutrient availability and biotic factors such as the presence of herbivores and pathogens (7, 8).

When herbivores damage a plant, shortly after injury this leads to the emission of C<sub>6</sub> green leaf volatiles (GLVs) derived via lipoxygenase cleavage of fatty acids followed later by the release of specific terpene volatiles (9, 10). Herbivore-induced

plant volatiles and their attractiveness to the third trophic level (predators and parasitoids) have been examined for several tritrophic systems and plant species (11–13). One of the best-known tritrophic interactions concerns lima bean (*Phaseolus lunatus* L.), the two-spotted spider mite (*Tetranychus urticae* Koch, Acari: Tetranychidae) and a predatory mite (*Phytoseiulus persimilis* Athias-Henriot, Acari: Phytoseiidae) (11). Predatory mites use herbivore-induced VOCs to locate their prey on other plant species, e.g., lima bean, apple, and gerbera (11, 14–16), but it is not known whether similar behavior occurs in strawberry plants.

The cyclamen mite, *Phytonemus pallidus* (Banks) (Acari: Tarsonemidae), is a serious pest of strawberry (17). It feeds on strawberry leaf fluids, preferring to attack young folded leaves. It spreads rapidly if appropriate pest control measures are not taken. Chemical control of *P. pallidus* is difficult because of the lack of effective and environmentally acceptable acaricides. Also, the location of the mite in the folded leaves and crown of strawberry plants helps the mite avoid contact with the chemical (18). Biological control of phytophagous mites by predatory

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mites is becoming more popular in both open-field and greenhouse strawberry production (19, 20). If predators respond to attractant VOCs and seek out their prey populations by following the volatile cues emitted by damaged plants, they could avoid undamaged plants and concentrate on actual pest problem sites, and this could lead to improved biological control of pests.

We examined the VOC spectrum of Polka and Honeoye, the most common strawberry cultivars in open-field cultivation in Finland. Both cultivars have favorable cultivation and taste properties, and the VOCs emitted from the fruits have been studied previously (4). New means to increase the efficacy of biological control of the cyclamen mite are needed, and thus, this species was chosen as the mite herbivore species in this study. Two commercial mass-reared *Neoseiulus* predatory mite species mostly used for biological control of cyclamen mite in Finland (19) and one native species, familiar to strawberry VOCs, were chosen for behavioral testing. To investigate in greater detail the potential of herbivore-induced VOC emission from strawberry plants, a leaf-chewing herbivore, *Galerucella tenella* (L.) (Coleoptera: Chrysomelidae), was introduced and the nonprey response of the predatory mites was also tested. In this way we could detect repellency or attraction by the plant volatiles induced by nonprey or if the predators were encouraged to orientate toward higher concentrations of herbivore-induced terpenes.

The aim of this study was to examine if cyclamen mite and leaf beetle damage would change the volatile emission spectrum of strawberry plants. We also wanted to discover if there was a tritrophic interaction between the strawberry plant, the cyclamen mite, and a predatory mite and whether nonprey herbivore feeding could affect the behavior of the predatory mite species tested. We hypothesized that cyclamen mite feeding could change the VOC profile of strawberry plants to such a degree that predatory mites would orientate toward prey-damaged plants by following the induced volatile cues.

## MATERIALS AND METHODS

**Plant Material, Herbivores, and Predators.** Micropropagated strawberry seedlings on agar, produced by the Agrifood Research Finland (MTT), Quality Plant Station Laukaa, cultivars Polka and Honeoye, were planted in 5 × 5.5 × 5 cm pots filled with peat and sand (3:1) (Kekkilä Y2 growing peat) 1.5 months after micropropagation. The plants were grown in computer-controlled growth chambers (Bioklim 2600T, Kryo-Service Oy, Helsinki, Finland) at +19/12 °C, 52/80% rh, 16 h:8 h light:dark period, and approximately 450 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation (PAR) prior to the experiments. *P. pallidus* originated from mass rearing at Agrifood Research Centre Finland, Plant Protection, and were reared on cv. Polka enclosed in an insect propagation cage (33 × 60 × 33 cm, two sides covered with fine mesh) at 24–28 °C approximately 400 μmol m<sup>-2</sup> s<sup>-1</sup> PAR and 16 h:8 h light:dark period.

The cyclamen mites were transferred with a fine brush to the youngest leaves of the seedlings using a stereomicroscope to ensure that the tiny mites were not damaged during the transfer process. The first experiment with 48 h mite feeding (describing the immediate induced response of the plant to cyclamen mite damage) was conducted 75–90 days after the micropropagation of the plants. The plants had 6–11 leaves and approximately two runners per plant at that time. The experiment with approximately 3 weeks mite feeding damage was performed twice. The first experiment started 45–60 days after micropropagation, 1 week from planting (plants had 4–7 leaves), and the second 75–90 days after micropropagation, 45 days from planting (5–11 leaves, 2 runners). The mites were left to colonize the plants for 21–28 days. Fifteen and ten adult female mites were transferred onto each plant in the 48 h (*n* = 5) and 3-week feeding experiments (*n* = 10), respectively. The mite-damaged plants and intact control plants

were put in separate growth chambers, where the conditions were the same as described before. The cyclamen mites were not removed from foliage before VOC collection and behavioral assay.

*Galerucella tenella* was collected from a strawberry field southeast Finland. Third-instar larvae were used in the experiment. Three larvae were placed on each cv. Polka strawberry plant (*n* = 15), 90 days from micropropagation, and the plants were put inside an insect propagation cage. Larvae were allowed to feed for 48 h. The leaf beetle-damaged plants and the intact control plants were placed in separate growth chambers with the same growing conditions as described before. *G. tenella* larvae were removed from foliage right before collection of VOCs.

The predatory mites *Neoseiulus cucumeris* (Oudemans) and *Neoseiulus californicus* (McGregor) were obtained from a commercial supplier (Koppert BV Netherlands). The mites were kept in the delivery packages with grain mites used as food at 4 °C in the dark. For one of the three separate tests *N. californicus* and *N. cucumeris* were starved for 24 h in eppendorf tubes at 4 °C before behavioral assay. *Euseius finlandicus* (Oudemans) was produced at Agrifood Research Finland, Plant Protection. They were reared on strawberry with spider mites, cyclamen mites, and pollen for 4 days prior to testing (24–28 °C, PAR approximately 400 μmol m<sup>-2</sup> s<sup>-1</sup>, and 16:8 light:dark period).

**VOC Collection and Analysis.** Prior to collection of volatiles, the peat and sand mixture from plant roots was washed with water without damaging the plant. The plant root system was then enclosed in a water-filled glass tube, which was put in a 20 mL Erlenmeyer flask. The whole plant was then carefully placed in a 1 L glass vessel that was closed with a Teflon-sealed lid, avoiding touching the leaves. Intact and herbivore-damaged plants were handled separately to avoid any volatile contamination.

All the glassware had been cleaned before use by placing it in an oven at 120 °C for 1 h. The Teflon-sealed lids had an inlet for ingoing purified air and an outlet carrying the outgoing sampling air. The airflow was set to 0.220 L min<sup>-1</sup> for the ingoing air and 0.2 l min<sup>-1</sup> for the outgoing air, and the airflow was calibrated with a mini-Buck calibrator (model M-5, A.P. Buck, Inc., Orlando, FL). Sampling air was led through Teflon tubing to a stainless steel tube containing approximately 150 mg of Tenax TA-adsorbent (Supelco, mesh 60/80). VOCs were collected at 24 °C (laboratory temperature) and 250 μmol m<sup>-2</sup> s<sup>-1</sup> PAR for 1 h. VOCs were collected from five (cyclamen mite) or six (leaf beetle) plants in the 48 h feedings and from a total of 10 plants in the 3-week feeding (repeated twice with 5 plants on both testing times) per treatment.

The samples were analyzed with a gas chromatograph–mass spectrometer (Hewlett-Packard GC 6890, MSD 5973). Trapped compounds were desorbed with a thermal desorption unit (Perkin-Elmer ATD400 Automatic Thermal Desorption system) at 250 °C for 10 min, cryofocused at –30 °C, and injected onto a HP-5 capillary column (50 m × 0.2 mm i.d. × 0.5-μm film thickness, Hewlett-Packard) with helium as a carrier gas. The temperature program was as follows: 40 °C 1 min, 5 °C/min to 210 °C, 20 °C/min to 250 °C, 250 °C 8 min. Compounds were identified by comparing their mass spectra with Wiley library and pure standards. Also, comparing the retention times to literature values (21, 22) verified identification. The volatiles were quantified according to commercial reference substances (see Chemicals). When a compound whose standard was not available appeared, the structurally most similar compound was used as a reference substance. This was the case for (*E*)-β-ocimene (*Z*)-ocimene as standard), (*E,E*)-α-farnesene (*E*)-β-farnesene as standard), and (*Z*)-3-hexenyl-2-methyl butanoate and (*Z*)-3-hexenyl tiglate ((*Z*)-3-hexenyl butyrate as standard). Emissions of (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), acetic acid, 1-penten-3-ol, 6-methyl-5-hepten-2-one, and germacrene-D were quantified by the amount of internal standard 1-chloro-octane (injected 1 μL/tube, concentration 87.6 ng/μL). Emissions were presented as ng g<sup>-1</sup> dry wt h<sup>-1</sup>.

**Behavioral Assay.** Orientation behavior of individual female adult *E. finlandicus* and both sexes of adult *Neoseiulus* species predatory mites toward herbivore-damaged or intact plants was tested in a two-arm Y-tube olfactometer. The Y-tube was made of transparent glass, with dimensions 5.5 cm × 5.5 cm × 5.5 cm for stem and arms, i.d. 0.5 cm, stem-arms angle 125°. Filtered air was directed to both arms

of the Y-tube at a flow of 0.5 L min<sup>-1</sup> through a 1 L glass vessel containing either the herbivore-damaged or intact strawberry plant. The predator was placed at the end of the Y-tube and observed for 5 min or until it made the final choice. The choice was recorded when the predator had reached the end of one of the arms. The Y-tube was cleaned with water and 70% ethanol after each predator to avoid the occurrence of intraspecific signaling. Also, the tube was rotated 180° after each predator, and the places of plants were switched after three tests. Six predatory mites were tested with the same plant pair.

After 48 h cyclamen mite feeding 30 *N. cucumeris* mites were tested on both strawberry cultivars. In the 3-week mite-feeding test 60 *N. cucumeris* mites were tested on both cultivars in two separate testing periods. On the second testing time 60 *N. californicus* mites were also tested on cv. Honeoye and 60 *E. finlandicus* mites on cv. Polka, and the effect of starvation level in the response to volatiles was also studied with 60 *N. cucumeris* and 60 *N. californicus*. In the test with 48 h leaf beetle damage, 90 *N. cucumeris* were studied on cv. Polka.

**Chemicals.** The GLV standard used as a reference for VOCs included 2-butanol, *n*-hexanal, (*Z*)-3-hexenol, *n*-heptanal, (*Z*)-3-hexenyl acetate, octanal, nonanal, (*Z*)-3-hexenyl butyrate, decanal, methyl salicylate, and 1-chloro-octane (Aldrich). The terpene standard included  $\alpha$ -pinene (Fluka),  $\beta$ -pinene (Aldrich),  $\beta$ -myrcene (Sigma), (*Z*)-ocimene (Fluka), limonene (Sigma), linalool (Fluka), caryophyllene oxide (Aldrich), and (*E*)- $\beta$ -farnesene (Bedoukian Research).

**Statistical Methods.** Statistical analyses were performed using SPSS 11.5 for Windows statistical package. Depending on the homogeneity of variances and normality of data, independent samples *t* test or in a few cases Mann–Whitney *U* for log(*x*+1) transformed VOC results was used for *P. pallidus* results. ARCSIN-transformed relative proportions of compounds were tested by independent samples *t*-test. Independent samples *t* test or Mann–Whitney *U* was used for *G. tenella* results. Nonparametric  $\chi^2$  test was used for results of the Y-tube olfactometer behavioral tests (intact vs damaged, equal orientation assumed).

## RESULTS

**VOCs after 48 h of Cyclamen Mite Feeding.** Cyclamen mite-damaged strawberry plants emitted greater total VOC and GLV emissions than intact plants from both cultivars (**Table 1**). Cv. Polka also emitted more MeSA (*P* = 0.016) from herbivore-damaged plants than from intact plants. Emissions of individual mono- and sesquiterpenes showed an increasing trend in response to damage, but statistical significances were found only with  $\beta$ -pinene + myrcene and (*E*)- $\beta$ -caryophyllene on cv. Honeoye due to the large variability in amounts emitted from individual plants. Polka emitted more total volatiles from both intact (*P* = 0.032) and *P. pallidus*-damaged (*P* = 0.008) plants than Honeoye.

In a comparison of the relative amounts of individual VOCs present in the overall profile, the GLV (*Z*)-3-hexenyl acetate dominated the emission profile. It accounted for 79 ± 1% and 75 ± 2% and 68 ± 4% and 70 ± 3% of the total emissions on intact and on *P. pallidus*-damaged Polka and Honeoye plants, respectively.

**VOCs after 3 Weeks of Cyclamen Mite Feeding.** After 3 weeks of mite feeding the variability in the emission of volatiles between damaged plants tested appeared to be quite large (Supporting Information, **Table S1**). There were only a few significant differences in the emissions of the individual compounds. For cv. Polka, GLVs were induced by cyclamen mite damage (*P* = 0.029). Also, total VOC emissions were over 4-fold greater from damaged than from intact Polka (*P* = 0.019). With Honeoye the total VOC and GLV emissions from cyclamen mite-damaged plants were about 2-fold greater than from intact plants, but no statistically significant differences were detected. Only cyclamen mite-damaged plants emitted MeSA, but the emission was quite minimal.

When comparing the relative proportions of individual compounds as a percentage of the total emission, (*E,E*)- $\alpha$ -farnesene dominated the VOC profile of cv. Honeoye (Supporting Information, **Table S1**). GLV emission was high from both intact and damaged strawberry plants of both cultivars, and no significant differences were observed between treatments.

***Galerucella tenella*-Induced VOCs.** The VOC profile of *G. tenella*-damaged plants differed from the profile of intact Polka plants by many specific compounds (**Table 1**). Leaf beetle damage induced emissions of several mono- and sesquiterpenes: (*Z*)-ocimene, (*E*)- $\beta$ -ocimene, DMNT, (*E,E*)- $\alpha$ -farnesene, (*E*)- $\beta$ -caryophyllene, and germacrene-D. The emissions of GLVs were not significantly altered by *G. tenella* feeding. The proportions of induced terpenes of the total emission were higher with damaged plants than with intact plants.

**Behavioral Assay.** The predatory mite species *N. cucumeris*, *N. californicus*, and *E. finlandicus* did not significantly prefer cyclamen mite-damaged strawberry plants over intact plants or vice versa on either cultivar after 48 h or 3 weeks of mite feeding (**Figure 1**). Also, with nonprey leaf beetle damage, *N. cucumeris* showed no preference between damaged or intact plants. Furthermore, starving the predators before testing did not affect the response of any species (**Figure 1E, F, H, and I**).

## DISCUSSION

**Herbivore-Induced VOCs.** These results indicate that cv. Polka strawberry plants respond to herbivore feeding by emitting a different profile of VOCs depending on the damaging herbivore species. Leaf beetle damage elicited de novo synthesis of specific terpenoids. In particular, the emissions of (*E*)- $\beta$ -ocimene and DMNT increased markedly. In contrast, cyclamen mite damage, which mostly occurs on folded strawberry leaves with little photosynthetically active leaf area, led to increased release of GLVs, formed via the octodecanoid pathway from the damaged plant surface (23). Different herbivores are known to trigger the release of different odor blends in other plant species (24–26). These can be both qualitative, i.e., novel compounds being emitted after herbivore damage, and quantitative, i.e., the ratio of blend components changing (11, 25–26). All compounds detected have been identified previously from a VOC profile of strawberry leaves (5, 6) and were emitted also from intact plants. This indicates that strawberry plants respond mostly by changing the quantities of VOCs they emit in response to cyclamen mite and leaf beetle feeding, not by synthesizing novel compounds.

Cv. Polka emitted a greater total amount of VOCs than cv. Honeoye, and this was correlated to its greater GLV emissions. Both cultivars emitted qualitatively rather similar blends. The extensive GLV emission from intact plants shows that strawberry plants emit these C<sub>6</sub> compounds as a characteristic feature of their VOC profile (5, 6). The handling of plants during preparation for VOC collection may also have resulted in some mechanical damage to foliage, which could have slightly added to the observed GLV emissions. The amount of total GLV emission varied quantitatively greatly between control treatments of the individual experiments also, but cyclamen mite feeding clearly increased the amounts of emissions.

The differential VOC emission noted in the *P. pallidus* and *G. tenella* experiments is interesting in illustrating the differences between induced GLVs versus terpenes. Cyclamen mites damaged the plant structure with their sucking, which resulted in enhanced emission of GLVs, volatiles typically released in response to both mechanical injury and herbivore feeding. Since feeding by *G. tenella* damages the leaf structure more than

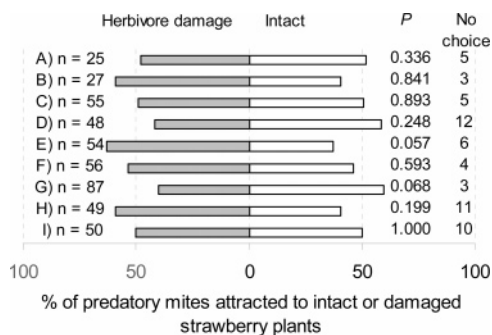
**Table 1.** VOCs Emitted (mean  $\pm$  SE, ng g<sup>-1</sup> dry weight h<sup>-1</sup>) from Intact and Cyclamen Mite-Damaged (48 h feeding) cv. Polka and Honeoye ( $N = 5$ ) and Intact and Leaf Beetle-Damaged (48 h feeding) cv. Polka ( $N = 6$ ) Strawberry Plants, and Contributions of Individual Compounds to Total Emission (the compound emitted at the highest concentration set to 100)<sup>a</sup>

compound	RT, <sup>b</sup> min	RI <sup>c</sup>	Polka		Honeoye		Polka	
			intact	cyclamen mite damage	intact	cyclamen mite damage	intact	leaf beetle damage
acids, alcohols, aldehydes, and ketones								
acetic acid	6.50	622 <sup>d</sup>	59 $\pm$ 25 0.6	48 $\pm$ 26 0.3	nd <sup>f</sup>	nd	92 $\pm$ 19 14.1	91 $\pm$ 27 4.6
1-butanol	8.20	653 <sup>d</sup>	6.0 $\pm$ 1.0 0.1	7.1 $\pm$ 1.8 <0.1	2.5 $\pm$ 1.1 0.2	5.9 $\pm$ 1.7 0.1	18 $\pm$ 3.4 2.2	18 $\pm$ 4.0 1.1
1-penten-3-ol	8.68	683 <sup>d</sup>	15 $\pm$ 5.4 0.1	20 $\pm$ 4.6 0.1	nd	nd	nd	16 $\pm$ 9.4 0.3
heptanal	15.90	903 <sup>d</sup>	7.2 $\pm$ 2.0 0.1	11 $\pm$ 1.2 0.1	8.2 $\pm$ 2.3 0.5	17 $\pm$ 4.0 0.2	16 $\pm$ 4.5 2.7	17 $\pm$ 1.9 1.4
6-methyl-5-hepten-2-one	19.02	987 <sup>d</sup>	4.4 $\pm$ 0.8 0.1	5.1 $\pm$ 0.6 <0.1	2.4 $\pm$ 0.8 0.1	4.8 $\pm$ 1.5 0.1	3.4 $\pm$ 2.2 0.6	4.4 $\pm$ 1.4 0.4
nonanal	23.27	1113 <sup>d</sup>	39 $\pm$ 3.4 0.5	46 $\pm$ 4.3 0.3	31 $\pm$ 9.4 1.8	68 $\pm$ 27 0.8	65 $\pm$ 13 9.6	66 $\pm$ 5.4 5.7
decanal	26.70	1204 <sup>d</sup>	34 $\pm$ 4.7 0.4	42 $\pm$ 5.7 0.3	34 $\pm$ 15 1.6	74 $\pm$ 43 0.7	72 $\pm$ 13 11.0	64 $\pm$ 8.3 5.7
green leaf volatiles								
(Z)-3-hexenal	12.20	803 <sup>d</sup>	111 $\pm$ 66 1.0	102 $\pm$ 24 0.6	7.7 $\pm$ 1.5 0.5	68 $\pm$ 28*** 0.8	191 $\pm$ 138 5.7	470 $\pm$ 251 9.3
(Z)-3-hexen-1-ol	14.20	855 <sup>d</sup>	2067 $\pm$ 275 22.9	5402 $\pm$ 703*** 31.0	861 $\pm$ 315 40.6	3006 $\pm$ 625* 37.0	2157 $\pm$ 1160 36.7	3184 $\pm$ 1947 50.0
(Z)-3-hexenyl acetate	19.69	991 <sup>d</sup>	9256 $\pm$ 1446 100.0	17102 $\pm$ 546* 100.0	2543 $\pm$ 1284 100.0	8010 $\pm$ 1500* 100.0	5006 $\pm$ 2968 100.0	5588 $\pm$ 2913 100.0
(Z)-3-hexenyl butyrate	25.90	1187 <sup>d</sup>	2.8 $\pm$ 0.3 <0.1	6.7 $\pm$ 0.6*** <0.1	0.4 $\pm$ 0.4 <0.1	4.3 $\pm$ 1.4 0.1	nd	17 $\pm$ 8.8 0.3
(Z)-3-hexenyl-2-methylbutanoate	27.50	1217 <sup>d</sup>	5.6 $\pm$ 1.3 0.1	11 $\pm$ 0.9* 0.1	1.9 $\pm$ 0.6 0.1	4.6 $\pm$ 1.9 0.1	2.1 $\pm$ 1.3 <0.1	13 $\pm$ 5.4 0.4
(Z)-3-hexenyl tiglate	30.48	1322 <sup>d</sup>	9.7 $\pm$ 1.4 0.1	16 $\pm$ 0.4* 0.1	7.2 $\pm$ 2.9 0.4	14 $\pm$ 4.2 0.2	nd	nd
terpenoids								
$\alpha$ -pinene	17.50	942 <sup>e</sup>	0.6 $\pm$ 0.3 <0.1	1.2 $\pm$ 0.2 <0.1	0.9 $\pm$ 0.4 <0.1	1.7 $\pm$ 0.7 <0.1	nd	nd
$\beta$ -pinene + myrcene	19.20	981 <sup>e</sup> /986 <sup>e</sup>	2.8 $\pm$ 0.6 <0.1	3.2 $\pm$ 0.3 <0.1	0.2 $\pm$ 0.2 <0.1	2.8 $\pm$ 0.4** <0.1	4.1 $\pm$ 1.6 0.4	7.3 $\pm$ 1.2 0.6
(Z)-ocimene	20.86	1025 <sup>e</sup>	0.4 $\pm$ 0.4 <0.1	3.3 $\pm$ 2.1 <0.1	2.1 $\pm$ 1.1 0.2	8.9 $\pm$ 5.7 0.1	nd	31 $\pm$ 5.4*** 2.1**
limonene	20.92	1030 <sup>e</sup>	3.2 $\pm$ 0.6 <0.1	5.2 $\pm$ 0.8 <0.1	2.0 $\pm$ 0.9 0.1	3.5 $\pm$ 1.0 0.1	0.5 $\pm$ 0.5	2.4 $\pm$ 1.3 0.2
(E)- $\beta$ -ocimene	21.28	1038 <sup>e</sup>	8.1 $\pm$ 5.3 0.1	40 $\pm$ 20 0.2	10 $\pm$ 2.3 0.7	151 $\pm$ 136 1.7	28 $\pm$ 11	1140 $\pm$ 189*** 85.4*
linalool	23.12	1092 <sup>e</sup>	nd	nd	nd	nd	nd	454 $\pm$ 225 28.2
(3E)-4,8-dimethyl-1,3,7-nonatriene	23.68	1097 <sup>d</sup>	nd	nd	nd	nd	nd	76 $\pm$ 7.3*** 6.4*
(E)- $\beta$ -caryophyllene	34.22	1428 <sup>e</sup>	5.9 $\pm$ 2.0 0.1	7.5 $\pm$ 2.5 <0.1	nd	7.8 $\pm$ 2.4** 0.1	nd	47 $\pm$ 8*** 3.9*
(E,E)- $\alpha$ -farnesene	35.39	1485 <sup>e</sup>	12 $\pm$ 2.2 0.1	18 $\pm$ 3.6 0.1	12 $\pm$ 1.3 1.1	11 $\pm$ 3.6 0.2	30 $\pm$ 10	240 $\pm$ 52** 26.1
germacrene-D	35.55	1493 <sup>d</sup>	2.0 $\pm$ 2.0 <0.1	9.4 $\pm$ 3.7 0.1	1.3 $\pm$ 0.7 0.1	26 $\pm$ 22 0.3	5.3 $\pm$ 2.5	294 $\pm$ 66** 24.3*
aromatic compounds								
MeSA	26.95	1192 <sup>d</sup>	0.6 $\pm$ 0.4 <0.1	4.6 $\pm$ 0.9* <0.1	nd	nd	nd	0.8 $\pm$ 0.8 0.2
total GLVs			11451 $\pm$ 1745	22639 $\pm$ 1132***	3420 $\pm$ 1590	11107 $\pm$ 2115*	7356 $\pm$ 4090	9272 $\pm$ 5076
total monoterpenes			15 $\pm$ 5.9	53 $\pm$ 23	16 $\pm$ 4.0	168 $\pm$ 142	33 $\pm$ 12	1636 $\pm$ 376**
total sesquiterpenes			20 $\pm$ 5.9	35 $\pm$ 7.5	13 $\pm$ 1.8	45 $\pm$ 22	36 $\pm$ 12	581 $\pm$ 105***
total emissions			11660 $\pm$ 1769	22922 $\pm$ 1093***	3527 $\pm$ 1617	11495 $\pm$ 2213*	7748 $\pm$ 4120	11890 $\pm$ 5192

<sup>a</sup> Asterisks indicate significant differences between control (intact) and herbivore-damage treatments (independent samples *t*-test or Mann–Whitney *U* test): \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ . <sup>b</sup> Retention time. <sup>c</sup> Retention index. <sup>d</sup> Reference (24). <sup>e</sup> Reference (23) <sup>f</sup> Not detected.

sucking by the cyclamen mite, a considerable increase in GLV emissions would also be expected after *G. tenella* attack. Thus, it is surprising that the anticipated increase in GLV emission from *G. tenella*-damaged plants did not occur. The leaf beetle does not break the leaf structure totally since gnawing of leaf mesophyll does not damage the upper epidermis of the leaf.

Thus, the GLV release from the leaf surface might be less than that emitted in response to other leaf-chewing herbivores. Terpene emissions, on the other hand, were greatly induced by *G. tenella* damage. Monoterpene emissions were elevated 50-fold and sesquiterpenes 16-fold. This is assumed to be attributable to changes in the plant's metabolic pathways. GLVs



**Figure 1.** Orientation of predatory mite individuals tested in the Y-tube olfactometer to intact or herbivore-damaged strawberry plants, number of no choices detected, and  $P$  values of a  $\chi^2$ -test (equal orientation to treatments assumed). (A–G) *N. cucumeris*: 48 h cyclamen mite (CM) damage on (A) Polka and (B) Honeoye; 3-week CM damage on (C) Polka and (D) Honeoye; 3-week CM damage and 24 h starvation of predators before testing on (E) Polka and (F) Honeoye; (G) 48 h leaf beetle damage on Polka. (H) *N. californicus*: 3-week CM damage on Honeoye and 24 h starvation of predators before testing. (I) *E. finlandicus*: 3-week CM damage on Polka and 24 h starvation of the predators before testing.

released from cell membrane are known to be emitted rapidly after injury, but terpene synthesis and release is slower and more prolonged (9, 10). The herbivore damage had occurred for 48 h before we analyzed the emissions, allowing time for terpene synthesis to be activated (10, 23). Since the collections of VOCs were performed in the morning, a time when the herbivores were not very actively feeding, GLV emissions were not induced to any massive extent but the more stable terpene emission was detected.

With the spider mite *T. urticae* damage, emission of induced VOCs is known to increase as the density of spider mites and the damaged leaf area increases (27–29). Compared to the spider mite damage on full-grown leaves, the leaves attacked by the cyclamen mite are the youngest and smallest and represent only a small proportion of the total leaf area. The initial number of cyclamen mites in the 48 h experiment, 15 per plant, triggered higher VOC emission than normally occurs in intact plants, though this level of infestation is not thought to be harmful to either plant vigor or yield (17). If greater cyclamen mite densities were needed to enhance the VOC emission of strawberry plants also, then the VOCs emitted after 3 weeks of cyclamen mite attack, i.e., where progeny of transferred adult females increase the mite density, should have exceeded the amount of VOCs at 48 h damage, but this was not the case. This could be explained by the fact that though cyclamen mite density is higher, the feeding only damages a small proportion of total leaf area, in contrast to spider mite damage, which typically spreads over the whole vegetative biomass.

In the 3-week cyclamen mite feeding experiment, cv. Honeoye emitted major amounts of the sesquiterpene (*E,E*)- $\alpha$ -farnesene from both intact and damaged plants. Thus, emission of (*E,E*)- $\alpha$ -farnesene seems to be connected to factors other than continuous *P. pallidus* feeding. This was also a cultivar-specific response since no increase in the (*E,E*)- $\alpha$ -farnesene emission was observed from cv. Polka. (*E,E*)- $\alpha$ -Farnesene has been identified as a compound commonly emitted from strawberry leaves (6). In previous studies the same type of metabolic contribution has been observed with MeSA. Emission of MeSA increased 10-fold from flowering to the time after removing the fruits, and at the same time an increase in resistance to spider mite *T. urticae* was detected (6). The function of this elevated (*E,E*)- $\alpha$ -farnesene release in this context has remained puzzling

since no further studies on metabolomics have been conducted. However, it indicates that strawberry does have the ability to emit specific terpene volatiles in high amounts, similar to the degree of GLV emissions.

**Behavioral Assay.** The predatory mite species tested did not respond to the odor of *P. pallidus*-damaged strawberry plants, so we could not establish any tritrophic interaction between a strawberry plant, the cyclamen mite, and three of the mite's predators. The tested predatory mite species are all generalists and all prey on cyclamen mite (30–32). *E. finlandicus* has previously been reported to use plant volatiles in detection of its prey mite *Oligonychus ununguis* on *Quercus* species (33). It has also been able to target its preferred prey species, the European red spider mite (*Panonychus ulmi*), in preference to *T. urticae* in response to herbivore-induced VOCs on apple leaves. For this behavior to occur it was essential that there had to be a sufficiently large number of prey-infested apple leaves emitting attractant VOCs (24). Also, *N. californicus* has the capacity to respond to prey-associated volatiles (15), and it has been observed to use volatiles to orientate toward a complementary food source, the pollen nectar (34). *N. cucumeris* has been significantly more attracted to chrysanthemum (*Denranthema morifolium*) plants damaged by western flower thrips *Frankliniella occidentalis* compared to intact plants in an olfactometer (35). The response was attributed to the greater emissions of germacrene-D from damaged plants. In our study with strawberry plants the differences in emissions of single compounds were either too small or not specific enough to attract these generalist predators. The total VOC emission increased significantly in response to *P. pallidus* damage; this was attributable to the increase in the GLV emission. GLVs do not attract nor repel *P. persimilis* (11), and our results suggest they do not alter the behavior of our predator species either.

The feeding of a nonprey species *G. tenella* on strawberry plants caused no significant orientation of *N. cucumeris* predatory mites toward damaged or intact strawberry plants, although a slight repellent effect toward damage was detected (ratio damaged:intact 40:60,  $P = 0.068$ ). This demonstrates that higher emission of (*E*)- $\beta$ -ocimene or DMNT that could have acted as olfactory cues, as has been reported for *P. persimilis*,<sup>11</sup> did not attract the species studied. Volatiles emitted from lima bean leaves infested by a nonprey herbivore (*Spodoptera exigua*) have previously repelled *P. persimilis* at high nonprey density but attracted at a lower density (36–37). Also, no response has been recorded (29). Therefore, it might well be the blend, the various amounts of compounds present at a certain ratio, rather than the maximal concentration of a single compound to which the predator responds (29, 38). Our results indicate that low density of nonprey leaf beetles did not alter the VOC profile of cv. Polka strawberry plants to such a degree to alter the behavior of *N. cucumeris*.

Associative learning of prey-finding cues typically is a trait of specialist predators, whose prey species live on many different host plants, e.g., to *P. persimilis* (16). *P. persimilis* has been reported to use monoterpenes (*E*)- $\beta$ -ocimene, linalool, DMNT, and MeSA to locate its host (11). For generalist predators, impact of learning can be differential. No previous experience with a particular plant and prey species was required for host finding by *E. finlandicus* (24). Also, *N. cucumeris* was able to orientate to volatiles from rust mite (*Aceria tulipae*) infested tulip bulbs without any previous experience of that volatile spectrum (39). However, e.g., for the generalist *Cotesia glomerata*, previous experiences were obligatory for attraction toward herbivore-damaged plants (40). One of the reasons for weak

prey locating in this study could be the predators' unfamiliarity with the VOC profile of young strawberry plants, since the mass-reared predators never encountered the typical VOCs emitted from strawberry plants. Our results indicate that inexperienced *N. cucumeris* and *N. californicus* predatory mites are not able to orientate toward their prey, the cyclamen mite, on young strawberry plants by following induced volatile cues. This emphasizes the need for testing the influence of previous experience on the prey-finding ability of the *Neoseiulus* species. Our tested *E. finlandicus* were experienced predators, but they still were not able to orientate toward cyclamen mite damage on strawberry plants. This indicates that the tritrophic interaction to cyclamen mite damage does not occur even though this species had undergone a learning period with strawberry's VOC environment. Since strawberry does possess the potential for emitting induced VOCs, it would be advisable to do similar tests using more specialized predatory mites, e.g., *Anthoseius rhenanus* (Oudemans) (41) and *P. persimilis*. Our current results suggest that manipulation of strawberry volatile profiles and emissions, e.g., with elicitors (42), would not be likely to increase the efficiency of the tested predatory mites in the control of cyclamen mites.

**Supporting Information Available:** Table S1. VOCs Emitted (mean  $\pm$  SE, ng g<sup>-1</sup> dry weight h<sup>-1</sup>) from Intact and Cyclamen Mite-Damaged (21–28 days feeding) cv. Polka and Honeoye (*N* = 10) Strawberry Plants, and Contributions of Individual Compounds to Total Emission (the compound emitted at the highest concentration set to 100). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Received for review March 25, 2005. Revised manuscript received August 29, 2005. Accepted September 4, 2005. This work was supported by the Research Council for Biosciences and Environment, the Academy of Finland (T.V. and J.K.H., decision no. 202300), and Berry- & Garden Know-Howcentre, Suonenjoki, Finland (<http://www.berryknow-how-centre.fi/intro.asp>) (S.H).

JF050676J