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Influence of Carrot Psyllid (*Trioza apicalis*) Feeding or Exogenous Limonene or Methyl Jasmonate Treatment on Composition of Carrot (*Daucus carota*) Leaf Essential Oil and Headspace Volatiles

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The effect of carrot psyllid (*Trioza apicalis* Förster) feeding and limonene and methyl jasmonate (MeJA) treatments on the essential oil composition and headspace volatiles of carrot (*Daucus carota* ssp. *sativus*), cvs. Parano and Splendid, leaves was studied. Carrot psyllid feeding significantly increased the concentrations of sabinene, β-pinene, and limonene, whereas limonene treatment increased the concentration of (*Z*)-β-ocimene in the leaves of both cultivars. The limonene treatment significantly increased the concentration of total phenolics in the leaves of both cultivars, and MeJA treatment increased phenolic concentration in the leaves of Parano. Exogenous limonene spray did not decrease the number of carrot psyllid eggs laid either 2 or 24 h after treatment. The results suggest that carrot psyllid feeding induces changes in the endogenous monoterpene pool in the carrot leaves. Limonene and MeJA treatments affect some induced defenses of the carrot, but the exogenous limonene spray is not an effective oviposition deterrent against carrot psyllid.

KEYWORDS: ***Trioza apicalis*; *Daucus carota*; damage; limonene; methyl jasmonate; monoterpenes; sesquiterpenes; biomass; total phenolics**

INTRODUCTION

The carrot psyllid (*Trioza apicalis* Förster, Homoptera: Psylloidea) is an economically important carrot pest in the Nordic countries (*1*). Damage by overwintered females may cause 100% yield loss if control methods are not used (*2*, *3*). Pyrethroids have been used as chemical control methods against carrot psyllid (*1*) for two decades. Farmers have found the field monitoring of carrot psyllid with sticky traps difficult, because several *Trioza* and other psyllid species are found in the monitoring traps simultaneously (*4*). Therefore, farmers in Finland tend to use calendar-based spraying programs instead of supervised programs (*1*). However, there have recently been some suspicions that the effectiveness of pyrethroid treatments has decreased in Nordic countries. Thus, the need for new control methods is increasing.

The symptoms of carrot psyllid feeding damage are leaf-curling and stunted growth of the carrot seedling (*2*). The systemic nature of psyllid damage, also demonstrated by Markkula et al. (*2*), indicates that the feeding damage may have an effect on the metabolism of the carrot. Accumulation of amino acids has been found in carrot leaves infested with carrot psyllid (*5*); however, there have been no attempts to analyze the secondary metabolites of damaged leaves. Herbivore-induced defenses are well-studied phenomena of different herbivore−plant systems (e.g., ref *6*). In addition, some terpenoids detected in the headspace volatiles of herbivore-damaged plants have been found to be responsible for the activation of defense genes in undamaged neighboring plants (e.g., ref *7*). However, these phenomena have not been studied on carrot.

Mono- and sesquiterpenes together with propenyl benzenes have been found to be the main substances in the essential oil composition of different carrot cultivars (*8*). The oil composition significantly differs between the leaves of different carrot cultivars (*8*−*10*). The proportion of propenyl benzenes decreases with the aging of the seedling (*8*). Limonene, one of the monoterpenes in carrot essential oil, in high concentrations has previously been shown to have a repellent effect on carrot psyllids (*3*, *11*). Furthermore, limonene mixed with other monoterpenes has a deterrent effect on several insects, for example, cabbage maggot (*Delia radicum*) (*12*), strawberry leaf beetle (*Galerucella sagittariae*) (*13*), and diamondback moth (*Plutella xylostella*) (*14*). Previously, exogenous fumigation with monoterpenes has been shown to affect the emission of monoterpenes and the endogenous monoterpene pool in *Quercus*

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leaves (*15*, *16*). Similarly, exogenous applications of limonene have been found to confer a limited thermotolerance on carrot (*17*) or *Quercus ilex* (*18*).

On the basis of previous studies we hypothesize that limonene could be a repellent against carrot psyllid when exogenously sprayed on carrot leaves, but it may also affect the secondary chemistry of carrot. Therefore, we studied the role of exogenous limonene spray in the control of carrot psyllid and its effects on the emission and the concentration of volatile compounds in the leaves. The specific aims of the present study were to investigate (1) if carrot psyllid feeding changes the concentrations of volatile compounds in carrot leaf tissue and plant headspace, (2) if limonene can be used as a deterrent for carrot psyllids, and (3) if limonene and methyl jasmonate (MeJA) can be used as elicitors that induce direct and indirect chemical defense in carrot plants. MeJA treatment was included in the experiment because it has been shown to have a similar kind of effect on plant volatiles as insect damage, for example, in cotton and in Sitka spruce (*19*, *20*).

MATERIALS AND METHODS

Plant Material and Growing Conditions. Three separate experiments were conducted using carrot cultivars Splendid and Parano, which are known to have different proportions of limonene (*10*). For experiments 1 and 3, seeds were sown in plastic pots (vol of 0.75 L) and grown in a mixture of peat and sand (2:1 v/v), one and three seedlings per pot, respectively. The seedlings were grown in growth chambers (Bioklim 2600T, Kryo-Service Oy, Helsinki, Finland) programmed to simulate the light and temperature conditions in June in central Finland (19/12 °C, 50/80 relative humidity, and 22/2 h light/dark photoperiod). For experiment 1, the seedlings were fertilized once with 0.1% Superex-9 solution (19:5:20 N/P/K, Kekkilä Oyj, Tuusula, Finland). For experiment 2 and for the biomass test of experiment 1, carrot seeds were sown in fertilized peat−sand mixture (Kekkilä TH1) in 1.1-L pots. For experiment 2, three seedlings were grown in each pot in conditions similar to those used for the carrot psyllid rearing. Extra light was given to the plants from 4:00 a.m. to 12:00 p.m. with 10 400-W metal halide lamps (Philips HP1-T, Philips, Eindhoven, The Netherlands) (one lamp/two cages). For the biomass test of experiment 1, one seedling in each pot was grown in a greenhouse at temperatures of 19 and 12 °C. The natural light period (14 h) was extended to 22 h with 12 400-W metal halide lamps (one lamp/two cages).

Insect Material. The carrot psyllids used in experiment 1 were collected from an organic carrot field at Haukivuori (61° 58′ 28″ N, 27° 11′ 00″ E), central Finland, 1 day before the experiment and allowed to feed on carrot cv. Nantura until the experiment was started.

In the biomass test of experiment 1 and and in experiment 2, we used carrot psyllids from our own continuous rearing, originally collected in June 2000 from the same farm mentioned earlier and maintained for several generations in cages (33 × 33 × 60 cm) on carrot cv. Nantura in a greenhouse (20/17 °C, on average 50% RH, 20/4 h light/dark). Each new carrot psyllid generation developed on average in 7−8 weeks. The adults of a new generation were allowed to copulate freely in the original cage for approximately 2 weeks. Thereafter, the gravid females were transferred to a new cage (50−80 individuals, 1:1 sex ratio).

Experiment 1. In experiment 1, the effect of carrot psyllid feeding on VOC emissions, leaf biomass, and terpenoid concentration was studied. Two 3.5-week-old carrot seedlings were placed in one plastic (polypropene) cylinder cage (volume of 10 L) sealed at the top with a thin cloth. Two female and two male carrot psyllids were released in each cage, except the control cages, and allowed to feed on the carrot seedlings for 7 days before they were removed. Each treatment (cages with and without psyllids) was replicated randomly four times in the growth chamber. The volatile organic compounds (VOCs) were collected with the equipment described in Loivamäki et al. (*21*) from six plants per treatment on the same day or 1 day after the removal of the psyllids. The foliage of carrots growing in pots was sealed in 1.5-L glass containers with aluminum foil at the base of the container. Collection was done in the laboratory under natural light (100−150 μmol/m/s) at room temperature. Charcoal-filtered air was led into the container through 6-mm Teflon tubing at a flow rate of 100 mL/min, and volatile samples were collected into Tenax TA adsorbent (mesh 60/80, Supelco, Bellefonte, PA), which was packed in stainless steel tubes (dimensions of 89 mm, ∅ 6 mm, Perkin-Elmer, Norwalk, CT) (150 mg/tube) for 2 h. Volatiles were analyzed with the method described below. The two youngest leaves (third and fourth leaves) of the same plant individuals as used in VOC collections were extracted with *n*-hexane 5 days after the collection of headspace volatiles to obtain information on the long-term effect of carrot psyllid feeding. Due to the considerably higher fresh weight of undamaged leaves, only half of the blades (cut along the petiole) were extracted, whereas from the damaged plants the whole blades were extracted. The leaf extracts analysis method is described below.

For the determination of psyllid feeding effect on leaf biomass, two 4-week-old seedlings were transferred into insect cages (33 × 33 × 60 cm) and two female and two male carrot psyllids were released into each cage except for control treatment. The insects were allowed to feed on the seedlings for 7 days before being removed. There were six replicate seedlings of each treatment per cultivar. For biomass measurements, the blades and petioles were cut separately 24 h after the psyllids were removed, weighed, dried in an oven at 60 °C for 2 days, and reweighed.

Experiment 2. Egg-laying preference of the carrot psyllid was tested on limonene-treated (97%, Aldrich Chemical Co. Ltd., Milwaukee, WI) plants. Four-week-old carrot seedlings were sprayed using a 0.65-L spray bottle (Plastex Oy, Lohja, Finland) with tap water, 5% ethanol in aqueous solution, 3% limonene in 5% ethanol in aqueous solution, or 6% limonene in 5% ethanol in aqueous solution. The control plants and limonene-treated plants were kept in separate small greenhouses until each treatment was randomly placed in each corner of an insect cage. One gravid carrot psyllid female was released 2 or 24 h after the treatments into the middle of the cage. The experiment was conducted twice with different randomization, and each cultivar (with all of the treatments) was replicated in 10 cages for each release time (2 or 24 h), except the second randomization for 24 h with nine cages/cultivar. Thus, there were altogether 20 and 19 replicates per each cultivar for the release 2 and 24 h after the treatment, respectively. Each female was allowed to lay eggs for 48 h. After females were removed, the eggs were counted under a microscope.

Experiment 3. The effect of limonene and MeJA (96% purity, Bedoukian Research Inc., Danbury, CT) treatments on emission of volatiles and concentrations of essential oil and total phenolics was studied. Four-week-old seedlings, with three to four true leaves, were sprayed with 5% ethanol (control), 3% limonene in 5% ethanol in aqueous solution, or 13.4 mM (3 mg/mL) MeJA in 5% ethanol in aqueous solution (5 mg/plant) using a 0.65-L spray bottle. Headspace volatiles were collected 24 h after the treatment from five seedlings per treatment. The roots of each seedling were washed and pruned slightly before being introduced into a small glass vial filled with water. Thereafter, the whole plant was sealed in a 1.5-L glass container sealed with a glass lid. The charcoal-filtered air was led through the container at a flow rate 200 mL/min, and the headspace volatiles were collected for 1 h. During the collection, additional light was given to the plants (one lamp/container) at a light intensity of 250 μmol/m/s. The volatiles were analyzed using the method described below. Immediately after the collection of volatiles, the youngest fully developed (third) leaf of each seedling was extracted with hexane. The rest of the plant was dried in the oven (40 °C, 5 days) to obtain the dry weight and the total phenolics of the plant. To determine the long-term effects of the treatments, the second extraction (nine seedlings/teatment) was performed 6 days after the treatment.

VOC Analysis. Plant volatiles were analyzed by GC-MS (Hewlett-Packard GC 6890, MSD 5973) equipped with a HP-5 capillary column (50.0 m × 0.2 mm i.d. × 0.50-μm film thickness). Compounds adsorbed to Tenax TA were desorbed using a thermal desorption unit (ATD 400, Perkin-Elmer) at 250 °C for 10 min and cryofocused in a cold trap at −30 °C. First, the column temperature was maintained at 40 °C for 1 min and thereafter increased to 210 °C at 5 °C/min and finally to 250

Table 1. Concentrations (Mean ± Standard Error, Micrograms per Gram of Fresh Weight) of Some Mono- and Sesquiterpenes and Propenyl Benzenes in the Leaves of Undamaged and Carrot Psyllid (*T. apicalis*) Damaged Leaves of Two Carrot Cultivars, Parano and Splendid ($n = 6$)[a]

		Parano		Splendid		main effects	
compound	KI[b]	undamaged	damaged	undamaged	damaged	cultivar	treatment
α-pinene	939	4.5 ± 1.0	20.3 ± 6.0	9.7 ± 4.9	16.4 ± 6.2	ns	ns
sabinene	976	66.9 ± 14.9	102.2 ± 10.1	0.9 ± 0.1	1.5 ± 0.2	0.000	0.027
β-pinene	978	nd	4.9 ± 2.4	0.6 ± 0.2	2.7 ± 1.3	ns	0.008
myrcene	984	61.6 ± 8.1	92.2 ± 13.0	47.9 ± 10.7	53.9 ± 10.0	0.045	ns
limonene	1025	9.7 ± 0.8	14.3 ± 1.2	17.7 ± 4.2	28.7 ± 5.4	0.004	0.054
(*Z*)-β-ocimene	1025	2.1 ± 0.4	5.4 ± 1.5	5.7 ± 1.8	2.3 ± 0.9	ns	ns
terpinolene	1081	0.6 ± 0.4	4.8 ± 3.0	0.3 ± 0.2	0.8 ± 0.4	ns	ns
bornyl acetate	1278	0.5 ± 0.2	1.4 ± 0.5	0.9 ± 0.2	3.0 ± 0.8	0.012	ns
β-caryophyllene	1418	9.3 ± 1.7	15.6 ± 5.6	19.8 ± 6.6	26.6 ± 5.9	ns	ns
methylisoeugenol		27.5 ± 18.2	42.3 ± 10.2	88.9 ± 18.7	99.4 ± 12.4	0.007	ns
α-asarone	1587	0.4 ± 0.2	1.4 ± 0.6	2.3 ± 1.1	1.1 ± 0.4	ns	ns

[a] Carrot psyllids were allowed to feed for 7 days on the carrots before they were removed. Terpenoids were extracted 5 or 6 days after the removal of the carrot psyllids. Main effects were tested with GLM procedure using lg ($x + 1$) transformed data. ns indicates nonsignificant main effect, nd indicates not detected compound, and tr indicates a compound with concentration under 0.1 μg/g of fw. [b] Kovats indices, which are obtained from refs *37–39.*

°C at 20 °C/min. Mass numbers from *m*/*z* 30 to 350 were recorded. The absolute amounts of terpenoids were calculated on the basis of the peak area of the pure compounds in external standard consisting of known amounts of several mono- and sesquiterpenes, propenyl benzenes, and green leaf volatiles. Compound identification was based on retention times and mass spectra of known standards.

Analysis of Leaf Extracts. Leaf samples were extracted with *n*-hexane for 2 h at room temperature, and the plant residues were washed two times. In experiment 1, the plant extracts were analyzed by GC-MS (Hewlett-Packard GC 6890, MSD 5973) and in experiment 3 by GC-MS (Hewlett-Packard GC type 5890, MSD 5970). Both devices used similar 30-m-long HP-5MS (0.25 mm i.d., 0.25-μm film thickness) capillary columns and helium as carrier gas. In experiment 1, the column temperature was held at 50 °C for 2 min, increased to 110 °C at 10 °C/min, increased to 150 °C at 5 °C/min, and finally increased to 270 °C at 30 °C/min and then held at 270 °C for 5 min. In experiment 3, column temperature was held at 50 °C for 2 min, followed by increases of 10 °C/min to 110 °C and 5 °C/min to 200 °C. SCAN technique, recording mass numbers between *m*/*z* 30 and 300, was used for the samples. Single ions 69, 93, 95, 121, 133, 136, 154, 161, 196, and 204 were monitored for quantification of terpenes and 163, 178, 193, and 208 for quantification propenyl benzenes. Quantification of the compounds was accomplished using known amounts of available pure terpenes and propenyl benzenes relative to known amount of internal standard (1-chloro-octane).

Total Phenolics. In experiment 3, for total phenolic analysis, all of the blades from each carrot seedling were dried at 40 °C and powdered. Approximately 40 mg of powdered leaf material was extracted with 5 mL of 80% aqueous acetone. The suspension was centrifuged at room temperature at 2200*g* for 2 min, and the supernatant was saved. The residue was re-extracted twice with 80% acetone, and supernatants were pooled and made up to 10 mL with 80% acetone. One hundred microliters of this extract was mixed with 1 mL of Folin−Ciocalteu reagent and 5 mL of 20% sodium carbonate, diluted to 10 mL with water, and mixed thoroughly. The absorbance was measured from two parallel samples after 20 min with a spectrophotometer (Shimadzu UV-1201) at a wavelength of 735 nm using tannic acid as standard. Results are expressed as tannic acid equivalents.

Chemicals. The standard compounds used in headspace volatile and foliar essential oil analyses were as follows: α-pinene (Fluka), camphene (Aldrich), β-pinene (Aldrich), sabinene (Carl Roth), myrcene (Sigma), limonene (Sigma), (*Z*)-β-ocimene (Fluka), terpinolene (Fluka), bornyl acetate (Sigma), α-copaene (Fluka), linalool (Fluka), longifolene (Fluka), β-caryophyllene (Sigma), α-humulene (Fluka), methylisoeugenol (Aldrich), α-asarone (Aldrich), (*Z*)-3-hexenyl acetate (Aldrich), nonanal (Aldrich), and methyl salicylate (Aldrich).

Statistical Analyses. In experiment 1, two control plants of cv. Parano, one mechanically damaged and one showing abnormal growth, had to be omitted from the VOC data before statistical analyses because they emitted extremely high concentrations of terpenoids and propenyl benzenes. In cv. Parano, slight aphid infestation was observed together with the psyllid damage on three seedlings. The differences in the volatile emissions and terpenoid concentrations of carrot psyllid damaged and undamaged carrot leaves were tested by GLM procedure using the lg ($x + 1$) transformed concentrations as dependent variables, cultivar and treatment as fixed factors, and sampling day and aphid infestation as covariate. The fresh weight of insect-damaged plants was tested with independent samples *t* test, whereas the fresh and dry weights of elicitor-treated plant were tested with ANOVA. The biomass of the carrot psyllid damaged carrots was analyzed with the GLM procedure using fresh and dry weight of blades and petioles as dependent factors, cultivar and damage as fixed factors, and number of true leaves as a covariate. In experiment 3, the differences in VOC emissions and the essential oil composition of elicitor-treated carrots were tested by GLM procedure using the lg ($x + 1$) transformed terpenoid concentrations as dependent variables and cultivar, day of extraction, and treatment as fixed factors. In experiment 2, the choices of carrot psyllid females between control and limonene treatments and differences in egg-laying preference were tested with nonparametric Kruskall−Wallis test followed by Dunnett T3 as post hoc.

RESULTS

Experiment 1: Psyllid Feeding Effects. *VOCs and Foliar Essential Oils.* Carrot psyllid feeding significantly increased the concentrations of sabinene, β-pinene, and limonene (**Table 1**) in the leaves of both cultivars. The only significant volatile induction was of emission of (*Z*)-β-ocimene from Parano leaves (**Table 2**). A significant cultivar × feeding interactive effect was found both in foliar concentration and in emission of (Z)-β-ocimene ($P = 0.005$ and 0.013, respectively). Foliar concentrations of sabinene, myrcene, limonene, bornyl acetate, and methylisoeugenol and emissions of sabinene, (*Z*)-β-ocimene, limonene, methyl salicylate, bornyl acetate, and β-caryophyllene differed significantly between cultivars.

Biomass. The visible carrot psyllid feeding damage caused over 7 days had no significant effect on the fresh or dry weight of the blades, but it significantly reduced the fresh and dry weight of petioles (**Table 3**). When the leaves were extracted (5 days after psyllid removal), the fresh weight of undamaged seedlings was significantly higher than that of damaged seedlings in both cultivars (Parano and Splendid: $P = 0.031$ and 0.002, respectively).

Experiment 2: Egg-Laying Preference of Carrot Psyllid on Limonene-Treated Plants. The limonene treatment did not influence the number of eggs laid by psyllids released in cages 2 h after the limonene treatment (data not shown). However, in

Table 2. Concentrations (Mean ± Standard Error, Nanograms per Plant per Hour) of Some Mono- and Sesquiterpenes and Propenyl Benzenes in the Headspace Emission of Undamaged and Carrot Psyllid (*T. apicalis*) Damaged Leaves of Two Carrot Cultivars, Parano (Control, $n = 4$; Damaged, $n = 6$) and Splendid ($n = 6$)[a]

		Parano		Splendid		main effects	
compound	KI[b]	undamaged	damaged	undamaged	damaged	cultivar	treatment
α-pinene	939	1.3 ± 0.6	26.2 ± 21.4	7.6 ± 2.8	17.2 ± 14.2	ns	ns
sabinene	976	12.6 ± 4.0	100.6 ± 73.1	1.8 ± 0.7	2.2 ± 2.0	0.003	ns
β-pinene + myrcene	978	21.5 ± 9.7	82.1 ± 31.5	52.0 ± 25.2	98.5 ± 88.6	ns	ns
(*Z*)-β-ocimene	1025	0.4 ± 0.4	1.5 ± 0.5	nd	nd	0.000	0.013
limonene	1025	2.5 ± 0.4	9.5 ± 4.6	105.4 ± 62.6	63.8 ± 47.6	0.008	ns
terpinolene	1081	nd	10.0 ± 8.3	4.0 ± 4.0	1.7 ± 1.3	ns	ns
nonanal	1104	nd	1.4 ± 1.4	nd	1.6 ± 1.6	ns	ns
methyl salicylate	1234	0.4 ± 0.2	0.3 ± 0.2	1.7 ± 0.6	1.3 ± 0.6	0.038	ns
bornyl acetate	1278	nd	2.0 ± 1.3	0.5 ± 0.2	0.6 ± 0.2	0.007	ns
β-caryophyllene	1418	0.1 ± 0.1	19.2 ± 15.9	6.3 ± 2.6	19.0 ± 15.4	0.008	ns
methylisoeugenol		nd	8.1 ± 6.7	17.8 ± 12.7	10.4 ± 5.0	0.051	ns

[a] Carrot psyllids were allowed to feed for 7 days on the carrots before they were removed. Terpenes were collected within 32 h after the removal of the carrot psyllids. Main effects were tested with GLM procedure using lg ($x + 1$) transformed data. ns indicates nonsignificant main effect, and nd indicates not detected compound. [b] Kovats indices, which are obtained from refs *37*–*39*.

Table 3. Fresh and Dry Weights (Mean ± Standard Error, Grams) of Undamaged and Damaged Leaves of Two Carrot Cultivars [Parano and Splendid ($n = 6$)] after a 7-Day Carrot Psyllids Feeding Period[a]

	Parano		Splendid		main effects	
	undamaged	damaged	undamaged	damaged	cultivar	treatment
fresh wt, blades	0.570 ± 0.036	0.517 ± 0.029	0.349 ± 0.053	0.414 ± 0.046	0.002	ns
dry wt, blades	0.088 ± 0.005	0.081 ± 0.005	0.047 ± 0.007	0.060 ± 0.007	0.000	ns
fresh wt, petioles	0.585 ± 0.038	0.376 ± 0.051	0.352 ± 0.070	0.247 ± 0.028	0.009	0.000
dry wt, petioles	0.052 ± 0.003	0.037 ± 0.005	0.028 ± 0.005	0.023 ± 0.002	0.000	0.001

[a] Main effects were tested with GLM procedure, and ns indicates nonsignificant main effect.

cv. Splendid, 3% limonene treatment marginally significantly ($P = 0.089$) increased the number of eggs laid by psyllids released into cages 24 h after treatment compared to water treatment (**Figure 1**).

Experiment 3: Effects of Limonene and MeJA. *VOCs.* The emissions of several mono- and sesquiterpenes and methylisoeugenol were significantly different between cultivars. In contrast, no significant differences in emission of terpenes after limonene and MeJA treatments were found. However, emission of (*Z*)-3-hexenyl acetate was significantly different among the treatments (**Table 4**). There was a significant cultivar × treatment interaction on emission of (*Z*)-3-hexenyl acetate ($P = 0.012$) and methyl salicylate ($P = 0.040$). No significant differences in the fresh or dry weight of the blades or petioles between limonene- and MeJA-treated carrots were found (data not shown).

Foliar Essential Oil. Elicitor treatment had a significant effect only on (*Z*)-β-ocimene concentration of the leaves 24 h after the treatment. We found a significant cultivar effect on the concentrations of sabinene, myrcene, limonene, bornyl acetate, β-caryophyllene, and methylisoeugenol (**Table 5**). None of the compounds was affected 6 days after the treatment; thus, no long-term effects of the treatment were detectable (data not shown). There was a significant effect of cultivar × treatment interaction on limonene ($P = 0.005$), whereas none of the other interactive effects was significant.

Total Phenolics. The concentration of total phenolics in the leaves of Parano was significantly higher in limonene and MeJA treatments than in control treatment, whereas in Splendid only the total phenolic concentration of limonene-treated leaves was significantly higher than that of the control (**Table 6**).

DISCUSSION

Psyllid Effect on Leaf Chemistry and VOCs. The carrot psyllid feeding increased the concentrations of sabinene, β-pinene, and limonene in the leaves of both cultivars. The foliar concentration of (*Z*)-β-ocimene decreased with the feeding damage in the leaves of cv. Splendid, whereas it increased in the leaves of cv. Parano, which may indicate a cultivar-specific response to carrot psyllid feeding. However, the feeding did not markedly affect the emissions of carrot leaves: only the emission of (*Z*)-β-ocimene was increased from leaves of Parano. Similarly, no clear correlation was found between the foliar concentration and the emission of terpenes in some terpene-storing species, such as conifers, whereas some nonstoring species, such as oaks, emit a large amount of terpenes (*22*). Previously, the feeding of pear psylla on pear trees was found to increase the emission of (*E*,*E*)-α-farnesene and methyl salicylate (*23*), and quantitative and qualitative differences were found in the emission of pear cultivars infested with *Cacopsylla* spp. (*24*). However, it has been reported that some other phloem feeders, such as aphids or whiteflies, do not induce volatile emission (*25*−*27*), probably because they avoid damaging cells during stylet injection (*25*). Similarly, psyllids living on *Eucalyptus* have been shown to avoid oil glands during stylet penetration (*28*), which may explain the relatively low induction of volatiles caused by psyllid feeding.

There is some evidence that Homoptera benefit from group feeding (*29*). Previously, a higher concentration of free amino acids was found in carrot psyllid damaged leaves than in undamaged leaves (*5*), which may predict that carrot psyllids benefit from conspecific feeding. However, pear psylla females

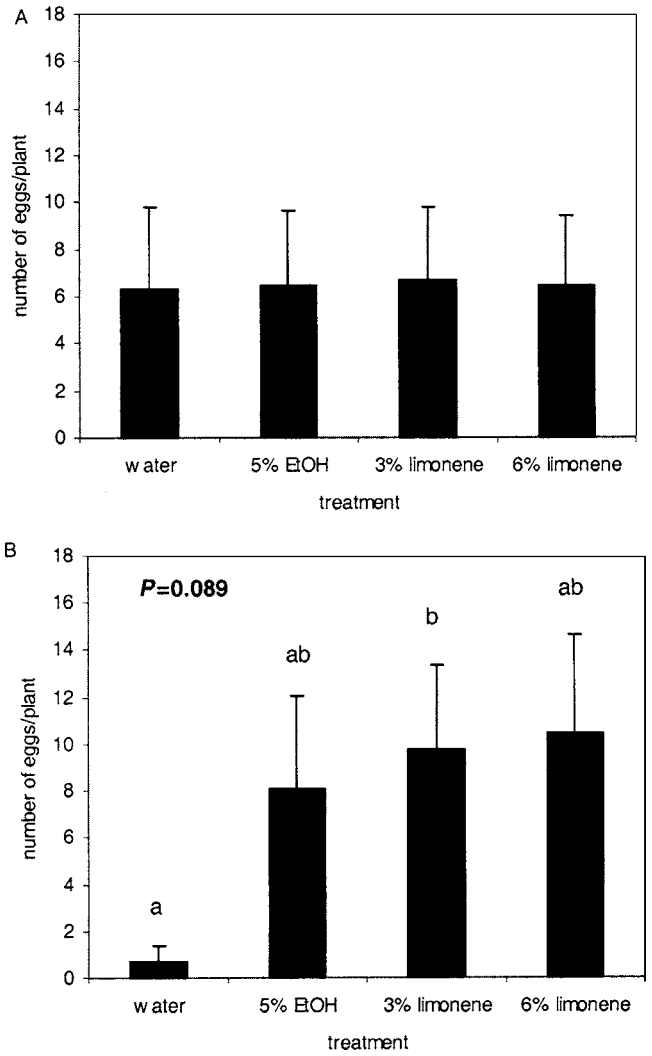

Figure 1. Mean number of eggs (± standard error) laid by carrot psyllids released 24 h after the limonene treatment: (**A**) Parano ($n = 20$); (**B**) Splendid ($n = 19$).

seemed to avoid pear branches with conspecific infestation (*30*). Thus, the ecological function of psyllid-induced defenses and other chemical changes remains to be determined.

Psyllid Effect on Plant Growth. Carrot psyllid feeding did not reduce foliar fresh weight at the end of a 7-day feeding period, but differences were observed 5−6 days after the removal of the psyllids. This delayed reduction of blade fresh weight may be due to malformation of plant cells caused by carrot psyllid feeding. Nymphal feeding of another gall-forming species, *Diaphorina truncata* (Homoptera: Psylloidea), caused hyperplasia, hypertrophy, and nuclear gigantism in the mesophyll cells (*31*). When psyllids are removed, undamaged leaves continue to grow, but damaged leaves remain stunted, which may explain the difference of the fresh weight observed later between the treatments.

Limonene Treatment and Egg Laying. Carrot psyllid females released into cages 2 h after the treatment showed no preference for any treatment, which suggests that increased exposure of limonene does not affect oviposition behavior of carrot psyllids. In cv. Parano, there was no difference in egg-laying preference of carrot psyllids 24 h after the treatment, whereas in cv. Splendid there were marginally more eggs laid on plants treated with 3% limonene than on water-treated plants. Although the difference was only marginally significant, it was interesting because limonene has previously been regarded as possible repellent against carrot psyllid (*3*, *11*). Previously, Ibrahim et al. (*17*) found increased limonene emission from carrot leaves 24 h after limonene treatment. However, in this study the emission of limonene was not significantly increased 24 h after the treatment, but the concentration of (*Z*)-β-ocimene was increased in the carrot leaves, which may indicate that limonene spray may make the total volatile emission spectrum of carrot seedlings even more attractive to the pest in 24 h. Thus, spraying limonene on carrot leaves to repell carrot psyllids is not recommended.

Limonene and MeJA Effect on Leaf Chemistry and VOC Emissions. Limonene treatment increased the concentration of (*Z*)-β-ocimene in the leaves, which differs from the earlier observation of Ibrahim et al. (*17*). We found that limonene treatment increased the total phenolic concentration of both cultivars. These results may indicate that exogenous limonene treatment can affect the induced defense of carrot. Limonene treatment could have some value as a control method against carrot pathogens because high phenolic concentration has been shown to increase the resistance of carrot roots to fungal pathogens (*32*, *33*). The phenolic content of carrot leaves should

Table 4. Concentrations (Mean ± Standard Error, Nanograms per Gram of Dry Weight per Hour) of Some Mono- and Sesquiterpenes, Green Leaf Volatiles, and Propenyl Benzenes in the Headspace Emissions of Two Carrot Cultivars [Parano and Splendid ($n = 5$)] 24 h after Treatments[a]

	Parano			Splendid			main effects	
compound	control	limonene	MeJA	control	limonene	MeJA	cultivar	treatment
(*Z*)-3-hexen-ol	2.7 ± 2.7	nd	nd	12.6 ± 9.7	12.0 ± 12.0	nd	ns	ns
α-pinene	54.1 ± 19.6	116.1 ± 61.4	35.2 ± 11.9	72.9 ± 14.1	87.6 ± 29.9	53.6 ± 25.8	ns	ns
camphene	1.0 ± 1.0	1.4 ± 0.9	0.8 ± 0.8	6.1 ± 1.9	5.7 ± 1.8	2.6 ± 2.6	0.017	ns
sabinene	95.5 ± 42.3	62.0 ± 16.3	63.9 ± 9.3	10.3 ± 3.5	27.9 ± 14.4	8.1 ± 2.7	0.000	ns
myrcene	532.9 ± 131.0	308.0 ± 168.5	400.2 ± 94.2	671.4 ± 137.0	1669.6 ± 873.1	959.7 ± 285.2	0.013	ns
(*Z*)-3-hexenyl acetate	105.9 ± 30.5	nd	nd	251.3 ± 242.9	167.1 ± 141.0	nd	ns	0.002
(*Z*)-β-ocimene	7.6 ± 3.6	3.5 ± 3.5	6.0 ± 4.7	37.6 ± 23.2	157.1 ± 105.2	76.8 ± 45.0	0.005	ns
limonene	94.6 ± 21.0	63.5 ± 19.5	50.0 ± 2.9	180.8 ± 52.3	522.5 ± 236.0	226.9 ± 61.2	0.001	ns
terpinolene	149.3 ± 131.2	90.0 ± 80.0	96.0 ± 15.3	119.7 ± 50.3	854.1 ± 462.3	123.8 ± 29.1	0.020	ns
nonanal	36.1 ± 4.3	30.7 ± 2.7	33.0 ± 2.8	34.0 ± 6.5	36.3 ± 5.3	30.7 ± 7.4	ns	ns
methyl salicylate	2.0 ± 1.2	0.7 ± 0.7	nd	1.0 ± 1.0	11.0 ± 3.9	1.7 ± 1.7	ns	ns
bornyl acetate	14.8 ± 4.6	16.5 ± 13.8	21.0 ± 5.8	25.6 ± 12.1	33.8 ± 10.0	30.1 ± 12.8	ns	ns
β-caryophyllene + (*E*)-β-farnesene	16.3 ± 8.0	106.1 ± 85.7	41.0 ± 26.3	211.7 ± 75.2	608.1 ± 307.4	126.7 ± 23.4	0.000	ns
α-humulene	nd	4.4 ± 4.4	1.6 ± 1.6	8.7 ± 4.3	31.0 ± 15.3	4.0 ± 1.7	0.004	ns
methylisoeugenol	nd	nd	nd	3.3 ± 2.1	16.0 ± 14.7	12.6 ± 6.8	0.005	ns

[a] Control = 5% ethanol in aqueous solution. Limonene = 3% of limonene in 5% ethanol in aqueous solution. MeJA = methyl jasmonate at a concentration of 13.4 mM in 5% ethanol in aqueous solution. Main effects were tested with GLM procedure using lg ($x + 1$) transformed data. ns indicates nonsignificant main effect, and nd indicates a nondetected compound.

Table 5. Concentrations (Mean ± Standard Error, Micrograms per Gram of Fresh Weight) of Some Mono- and Sesquiterpenes and Propenyl Benzenes in the Leaves of Carrot [Cvs. Parano and Splendid ($n = 5$)] 24 h after Treatment[a]

	Parano			Splendid			main effects	
compound	control	limonene	MeJA	control	limonene	MeJA	cultivar	treatment
α-pinene	4.4 ± 2.8	3.4 ± 2.5	2.3 ± 1.4	4.3 ± 2.7	5.7 ± 3.1	nd	ns	ns
sabinene	50.1 ± 25.4	70.8 ± 12.7	44.7 ± 11.7	nd	nd	nd	0.000	ns
myrcene	98.5 ± 14.6	71.7 ± 2.1	75.3 ± 10.8	42.4 ± 10.6	30.5 ± 3.8	37.8 ± 8.2	0.000	ns
limonene	18.9 ± 3.3	12.1 ± 0.6	12.2 ± 2.0	14.5 ± 1.1	20.3 ± 4.0	13.8 ± 2.1	0.033	ns
(*Z*)-β-ocimene	2.1 ± 2.1 a	9.2 ± 1.3 b	4.1 ± 2.8 ab	2.8 ± 1.8 a	11.3 ± 2.2 b	4.3 ± 2.7 ab	ns	0.002
linalool	0.9 ± 0.9	nd	nd	0.7 ± 0.7	nd	0.4 ± 0.4	ns	ns
bornyl acetate	nd	nd	nd	3.0 ± 1.1	1.2 ± 0.8	0.4 ± 0.4	0.000	ns
β-caryophyllene	8.6 ± 2.7	17.8 ± 4.7	14.8 ± 8.5	19.4 ± 6.1	22.2 ± 6.2	18.2 ± 4.2	0.002	ns
methylisoeugenol	121.1 ± 39.3	98.9 ± 26.0	75.7 ± 19.5	244.0 ± 36.5	201.9 ± 27.0	135.6 ± 25.6	0.000	ns
α-asarone	3.5 ± 2.2	2.4 ± 1.7	2.5 ± 2.0	9.0 ± 5.6	36.3 ± 23.3	13.5 ± 8.4	ns	ns

[a] Control = 5% ethanol in aqueous solution. Limonene = 3% of limonene in 5% ethanol in aqueous solution. MeJA = 13.4mM methyl jasmonate in 5% ethanol in aqueous solution. Main effects were tested with GLM procedure using lg ($x + 1$) transformed data followed by Dunnett T3 test. Different letters within a row indicate significant differences between treatments. ns indicates nonsignificant main effect, and nd indicates nondetected compound.

Table 6. Concentration (Mean ± Standard Error, Milligrams per Gram of Dry Weight) of Total Phenolics in the Blades of Two Carrot Cultivars ($n = 5$) Treated with Limonene or Methyl Jasmonate[a]

	cv. Parano	cv. Splendid
control	28.3 ± 1.7 a	28.2 ± 1.9 a
limonene	37.2 ± 2.2 b	38.8 ± 1.9 b
MeJA	38.9 ± 1.8 b	29.6 ± 3.8 ab

[a] Differences between treatments were tested with one-way ANOVA followed by Dunnett T3 test. Different letters within a column indicate significant differences between treatments.

be more carefully studied with regard to carrot psyllid behavior, because high phenolic content of the olive buds was associated with a certain degree of resistance to olive psylla (*Euphyrulla olivina*) (*34*) and the feeding of pear psyllids increased the phenolic concentration of the pear leaves (*35*).

In contrast to carrot psyllid feeding, mono- and sesquiterpene concentrations were not significantly influenced by the MeJA either 1 or 7 days after the treatment, suggesting that the 13.4 mM concentration may be too low for carrot in the manipulation of indirect chemical defense based on inducible volatile compounds. The result suggests that MeJA in low concentrations (13.4 mM) does not influence foliar monoterpene concentrations of monoterpene-storing species such as carrot. In conifers, Heijari et al. (*36*) found that spraying with low (10 mM) or high (100 mM) concentrations of MeJA did not affect the concentrations of foliar monoterpenes in Scots pine, although resin acids were affected. Similarly, Miller et al. (*20*) found no increased accumulation of total mono- and sesquiterpenoids in mature needles of MeJA-treated Sitka spruce.

Although the foliar concentration of (*Z*)-β-ocimene was increased after limonene treatment, it did not affect the emission of this compound. Emission of (*Z*)-3-hexenyl acetate was higher in the control than in MeJA treatment, and significant cultivar × treatment interaction for (*Z*)-3-hexenyl acetate and methyl salicylate indicates that these compounds reacted differently to the elicitor treatments in different cultivars. The period from MeJA treatment to VOC collection (24 h) may have been too short to detect MeJA-induced volatiles. Recently, the emissions of the homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and the sesquiterpene (*E*,*E*)-α-farnesene and (*Z*)-3-hexenyl acetate were found to increase significantly in the headspace of oilseed rape (non-terpene-storing species) 3 days after MeJA treatment (*21*), and the emission of the monoterpene linalool from Sitka spruce needles increased 60−66 h after MeJA treatment (*20*). However, terpene-storing species, such as carrot, and non-terpene-storing species, such as oilseed rape, may respond differently to MeJA treatment. Previously, it was found that MeJA did not induce the emission of stored terpenes in cotton (*19*). Similarly, linalool is de novo formed in Sitka spruce needles (*20*).

We observed a higher level of volatile emission in experiment 3 with limonene and MeJA treatment than in the carrot psyllid feeding experiment. The difference may be due to the higher light intensity used during the volatile collection in experiment 3 or due to the different collection method. However, the emission quantity and quality of the main compounds in the VOC collections of experiment 3 were on a level quite similar to that reported by Ibrahim et al. (*17*), who used a similar collection method, that is, the whole plant was enclosed inside a cuvette and the same light intensity was used. The emissions of sabinene, (*Z*)-β-ocimene, limonene, methyl salicylate, bornyl acetate, and β-caryophyllene differed between the cultivars Parano and Spendid, which is also in agreement with Ibrahim et al. (*17*). However, these results are difficult to compare because the carrots used in Ibrahim et al. (*17*) were older (7 weeks) and grown at higher temperatures than in this study. The lower concentration of methylisoeugenol in the leaf extractions of experiment 1 than in experiment 3 is likely due to the aging of the carrots (*8*). Even though the carrots were a few days younger in experiment 1, the average fresh weight of the carrot leaves was considerably higher (71−100%) than in experiment 3.

In summary, our results indicate that (*Z*)-β-ocimene may be the most easily induced compound in these carrot cultivars, because its leaf concentration was affected by both insect feeding and limonene treatment. However, in most cases no significant treatment effects on volatile emissions were detectable, perhaps because they were masked by high variation among individual plants. Although limonene spraying was not an effective deterrent against carrot psyllid oviposition, it significantly increased phenolic concentration in the carrot leaves, which may have consequences for the performance of the psyllid nymphs. The use of methyl jasmonate as an elicitor to manipulate the direct and indirect chemical defense of carrot plants did not yield very encouraging results. Total phenolics were increased by MeJA treatment only in Parano; however, the phenolic methylisoeugenol in leaf tissue or in headspace was not affected. Similarly, the leaf concentration of mono- and sesquiterpenes was not affected by MeJA treatment. Obviously, volatile concentrations and emissions of monoterpene-storing plant

species, such as carrot, are not as easily influenced as those of non-monoterpene-storing plant species.

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