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Influence of Field Attack by Carrot Psyllid (*Trioza apicalis* Förster) on Sensory Quality, Antioxidant Capacity and Content of Terpenes, Falcarindiol and 6-Methoxymellein of Carrots (*Daucus carota* L.)

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ABSTRACT: The effect of different degrees of attack by carrot psyllid (*Trioza apicalis*) on quality parameters of carrots was studied in field experiments for two years. Treatments were different degrees of physical insect protection by floating row cover. An increasing attack level of psyllids showed an enhancement effect on the antioxidant capacity (ORAC), content of falcarindiol, 6-methoxymellein, and terpenes, and scores for bitter taste, chemical flavor, terpene flavor, and toughness. Carrot psyllid attack decreased the yield, total sugar, fructose, glucose, and sensory attributes sweet taste, color hue, color strength, crispiness, and juiciness. Carrot plants at 8−10 weeks of age tolerated attack by psyllids at low levels (2% leaves with curling or discoloration).

KEYWORDS: *carrots, Daucus carota, carrot psyllid, Trioza apicalis, Homoptera, Psylloidea, sensory quality, terpenoids, falcarindiol, 6-methoxymellein, antioxidant capacity*

INTRODUCTION

The carrot psyllid (*Trioza apicalis* Förster, Homoptera, Psylloidea) is an economically important carrot pest in northern Europe.[1−3] Females overwinter on conifers (preferably Norway spruce, *Picea abies* L. H. Karst.), and carrot plants are attacked by both the adults and nymphs during spring and summer.[2,3] The insect feeds on carrot leaves by inserting a stylet[4] and sucking nutrients from the phloem, causing leaf curling, yellow and purple discoloration of leaves, stunted root growth, and proliferation of secondary roots.[5] Attack on young plants may cause 100% yield loss if plant protection methods are not used.[1] Mechanisms by which *T. apicalis* induces symptoms in plants are not understood, but since feeding causes curling of the youngest leaves and not necessarily at the feeding site, it has been assumed there can be a toxin involved that is systemically transported in the plant.[5] This hypothetical toxin has never been isolated, but recent studies have shown an association between the carrot psyllid and the plant pathogenic bacterium *Candidatus* Liberibacter solanacearum.[6,7]

The research on *T. apicalis* in carrots is mainly focused on physiological damage and yield loss, pest control, and studies of the biology of the pest. Less is known about how damage from this pest affects the sensory quality of carrots and contents of sensory- or health-related compounds. In one study, Nissinen et al.[8] found that carrot psyllid feeding induced changes in the endogenous monoterpene pool of the carrot leaves. A recent study found reduction in total sugars and production of some phenolic components in taproots of carrot plants attacked by *T. apicalis*.[9] The effects of the psyllid on sensory quality and production of sensory-related and secondary compounds are of interest for further studies. It is known that in carrots such compounds can easily be influenced by various kinds of stress, such as hail damage[10] or wounding of tissue.[11,12]

Psyllids show resistance to insecticides in southern Norway, and farmers need to protect their carrots by covering the entire field with nonwoven synthetic fabric described as "floating row cover". The fabric is light, translucent, and very open for gas transmission, but is not penetrable for adult egg-laying psyllids. Floating row cover may cause some increase in growing temperature and air humidity. Thus, this protection method is normally used by the farmers from sowing until the end of July. By removing the cover at this time, they avoid the adverse effects of higher temperatures in the final period of growth that can cause larger leaf mass and increased risk of pest infestation. A low attack in the uncovered period does not normally reduce yield level, but possible negative effects on sensory quality could not be ruled out. This was an important component of our study, to provide better guidelines in control of the quality of carrots.

The aim of the present study was to investigate how carrot psyllid attack in the field affects the sensory quality of carrot tap roots, as well as sensory- and health-related parameters, and to clarify whether removal of insect protection at the end of July is possible without quality reduction. This work is one of the first field studies performed on this aspect.

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Table 1. Effect of the Level of Carrot Psyllid Attack on Leaf Damage and Yield of Grade 1 and Discarded Carrots[a]

exposure period to natural pest attack[b]			root yield (kg m^{-2})	grade 1[c] root yield (kg m^{-2})	portion of discarded roots (%)	portion of plants with leaf curling or discoloration (%)
experiment A	A1	from germination	0.10 c	0.00 b	100 a	100 a
	A2	from July 5	3.11 b	0.18 b	94 a	80 b
	A3	from July 28	7.45 a	6.26 a	16 b	2.0 c
		p(ANOVA)[d]	0.001	0.001	0.001	<0.001
experiment B	B1	from germination	0.87 b	0.20 b	79 a	98 a
	B2	from July 4	4.87 a	4.43 a	9 b	1.5 b
	B3	from July 19	5.46 a	4.40 a	19 b	0 b
		p(ANOVA)[d]	0.007	0.001	0.008	<0.001

[a]Values are means of three field replicates. Values within each experiment and variable labeled with the same letter are not significantly different by Tukey's multiple-comparison test at a significance level of 0.05. [b]Actual attacks by carrot psyllids in the exposed periods are shown in Figure 1. [c]Damage-free roots with a diameter of 17−35 mm. [d]p-value from the analysis of variance.

MATERIALS AND METHODS

Field Studies of Carrot Attack by *T. apicalis*. Our study is based on registrations from two pest control experiments on neighboring farms during two years and with different carrot varieties (experiment A and experiment B). The experiments were designed as two separate field trials. The treatments tested were different ranges of physical protection by floating row cover to save from attack by the carrot psyllid. Diverging lengths of unprotected periods, and thereby differing levels of psyllid attack, were compared in terms of sensory quality and content of chemical constituents. The experiments were a randomized block design with three replicates (blocks). The fields were exposed to natural infection by *T. apicalis* in a valley with alluvial sandy soil, which has been used for intensive carrot production for several decades (Lågendalen, Vestfold, Norway, 59.3° N, 9.9° E). This location is known for annual, heavy attacks by *T. apicalis*.

The study was designed as two separate field trials (experiment A and experiment B). In experiment A (2004), carrots of cv. 'Newburg' were sown on May 17 with 1 600 000 seeds/ha. The field was fertilized as follows (ha^{-1}): 400 kg of PK fertilizer (OPTI-PK 0-5-17), 600 kg of NPK (Fullgjødsel 11-5-18), and 300 kg of N Nitrabor (calcium nitrate containing boron), all from Yara International, Oslo, Norway. In experiment B (2005), carrots of cv. 'Merida' were sown on May 6 with 1 500 000 seeds/ha. The field was fertilized as follows (ha^{-1}): before sowing with 450 kg of NPK (Fullgjødsel 11-5-18), after 6 weeks with 400 kg of PK fertilizer (OPTI-PK 0-5-17), and after 8 weeks with 450 kg of NPK (Fullgjødsel 11-5-18). Thereafter, the field was top-dressed three times, every second week, with 250 kg of Nitrabor.

The herbicide program was Fenix and Finale (both 1 L ha^{-1}, Bayer, Mannheim, Germany) prior to germination and Sencor WG (50 g ha^{-1}, Bayer) and Linuron Afalon (250 mL ha^{-1}, Agronica, Stoke, New Zealand) after germination, the latter repeated after one week. A final treatment with Fenix (0.5 L ha^{-1}) and Sencor WG (50 g ha^{-1}) was applied at the three- to four-leaf stage. Carrots were harvested after 15 and 16 weeks (Sept 5 and 8) for experiments A and B, respectively. No fungicides or insecticides were used in the experimental plots.

Yellow, sticky traps (20 × 15 cm, Rebell, Andermatt Biocontrol AG, Grossdietwil, Switzerland) were used to monitor adult *T. apicalis* attacks in the field. The traps were oriented 90° against the predominant wind direction and placed 3 cm above the leaves of the carrots (raised during growth of the plants). Five traps were placed in the field and registered two times or more per week from May 18 to Aug 15 both years, which was the actual period for adult psyllids attacking the fields. Experiment A was followed by additional weekly registrations until harvest. The experimental fields were located 8 m from the commercial carrot fields. Each plot was 1.65 m × 2.30 m, arranged as one bed with three carrot rows equally distributed on each bed.

The treatment level against *T. apicalis* was regulated by using nonwoven floating row covers (Agryl, 17 g m^2, single layer, polypropylene fleece, Crop Solutions Limited, Perth, UK) applied during the limited protection periods. Exposure periods for the different treatments (A1−A3 and B1−B3) are shown in Table 1, and the real insect attack in these periods is shown in Figure 1. An untreated control, A4, was included in experiment A, but not in experiment B. However, due to the very low attack occurring in the exposure period for treatment B3, this treatment was almost unexposed to attack (below one psyllid per trap per day; see Figure 1).

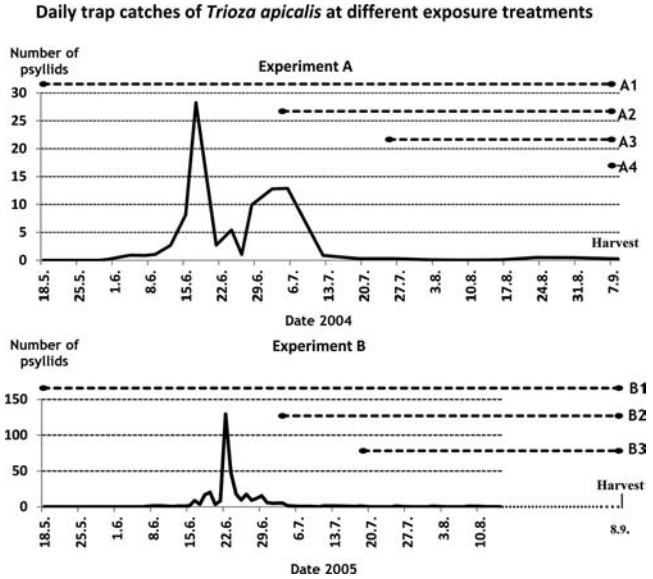

Figure 1. Number of carrot psyllids (*T. apicalis*) found in traps in the carrot fields for 2004 (experiment A) and 2005 (experiment B). Daily numbers of psyllids are given by the mean of five traps. Dotted lines indicate the exposure time (period without insect net protection) for the different treatments used in experiments A and B. Treatment A4 consisted of unexposed carrots (protected until harvest). In experiment B catches were only measured until Aug 10.

The study of naturally infected carrots from an existing field trial was only possible by use of floating row cover to manage infection levels. It was not possible to plan exact levels of damage for the treatments as in standardized infection studies.

Sampling of Carrots and Sample Preparation. Fifty plants were harvested randomly from each plot. For all treatments, the total fresh weight and yield of grade 1 (damage-free roots, 17−35 mm) were recorded and the percentage of discarded roots was calculated. The fraction of plants with leaf damage (curling, yellow and purple coloring) was visually evaluated on each plot before harvest.

After harvest, the tap roots were stored for 14 days at 0.5 °C in perforated polyethylene (PE) bags (close to saturated humidity) before sensory and chemical analyses. The carrots were hand washed by brushing (not peeling), and 20 mm of the tip and at least 20 mm of the top below any green zone were discarded. The rest of the carrots were cut into 10 mm cubes by a vegetable dicing machine (Eillert Bl1000A, Machinefabriek Eillert B.V., Ulft, The Netherlands), blended thoroughly, and stored in open polyetylene bags at 2 °C overnight.

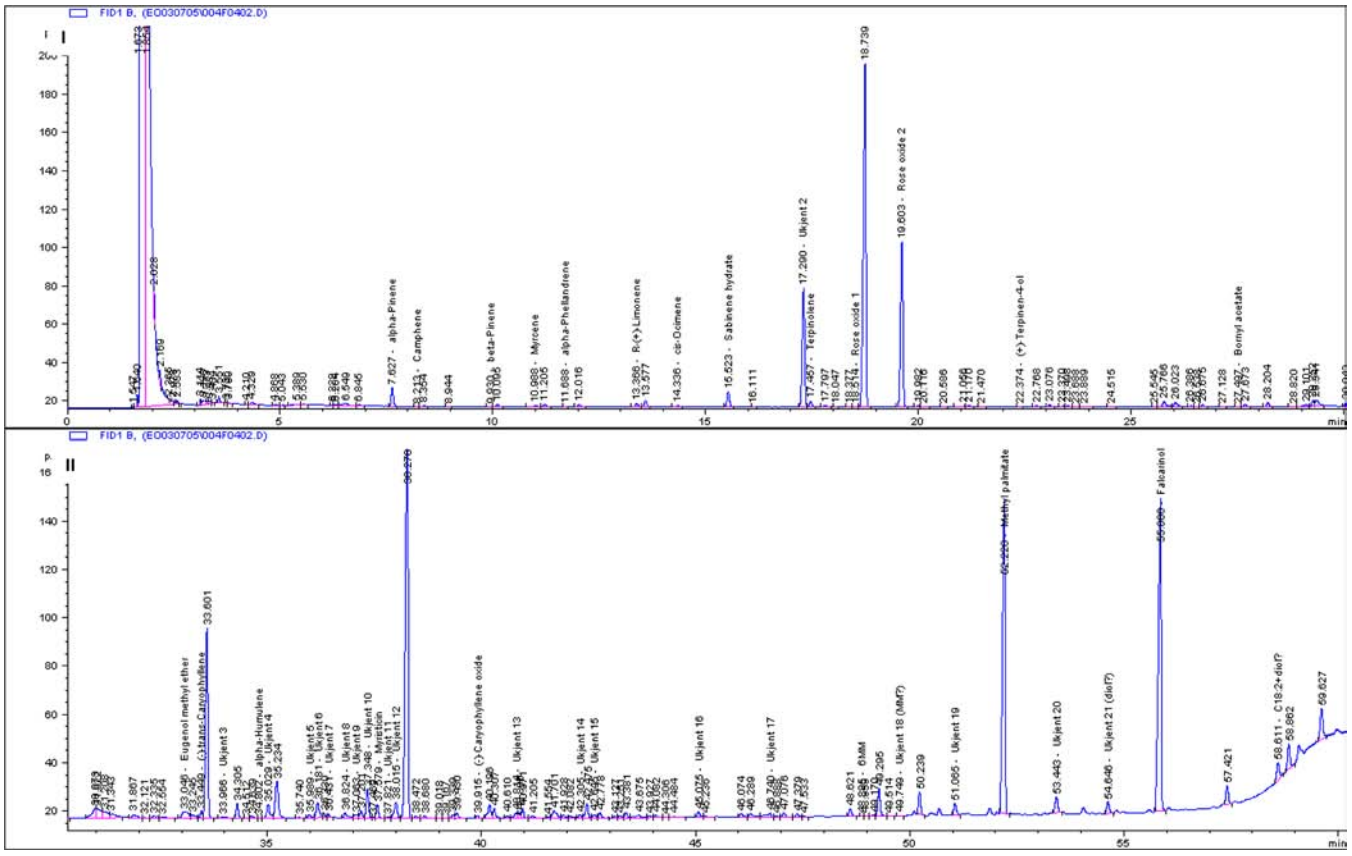

Figure 2. GC chromatogram of a characteristic carrot sample from the experiment. Details are shown separately for compounds with retention times of 0−30 and 30−60 min. *trans*-Rose oxide (isomer 2, which was the main component of the standard) was used as an internal standard for the compounds with a retention time of 0−30 min (I) and methyl palmitate for compounds with a retention time of 30−60 min (II). Ukjent = unknown compounds.

Samples of mixed cubes for chemical analysis (100 g) were frozen in liquid nitrogen, vacuum packed, stored at −80 °C, and then ground to a powder in a pre-frozen food processor, vacuum packaged, and stored at −80 °C until analysis. For sensory analysis, ca. 1 kg of cubes per treatment was used. These carrot cubes were stored as a thin layer in open polymer bags at 2 °C overnight prior to analysis to avoid drying and to allow aerobic respiration.

Chemicals. The compounds tested in this study were chosen for their importance to sensory quality and possible health effects in humans. The terpenes contribute to the aroma and a harsh, burning taste in carrots, and the sugars contribute to the sweet taste and masking of bitter or harsh flavor.[13,14] The polyacetylenes falcarinol and falcarindiol have attracted attention concerning health aspects[15,16] and bitter taste,[17] respectively. 6-Methoxymellein was chosen due to its importance for bitter taste and increase in stress situations such as ethylene exposure.[14,18] The reference compounds (+)-β-pinene, (*R*)-(+)-limonene, (−)-bornyl acetate, (−)-*trans*-caryophyllene (purity 99%), (+)-α-pinene (purity 99.5%), (*R*)-(−)-α-phellandrene, *p*-cymene (purity 95%), (+)-camphene (purity 94%), myrcene, and terpinolene (purity 90%) were all purchased from Fluka Chemie AG (Buchs, Switzerland). γ-Terpinene (purity 97%) was from Aldrich (Darmstadt, Germany). 6-Methoxymellein reference compound was isolated from carrots by the authors as described previously.[19] Standard compounds used for identification of sugars were sucrose, D-glucose, and D-fructose purchased from Chem Service (West Chester, PA, USA). The internal standards *trans*-rose oxide (purity 97%, Fluka Chemie AG, Buchs, Switzerland) and methyl palmitate (purity 99%, Sigma, United States) were used for analysis of terpenes and polyacetylenes, respectively.

Chemical Analyses. Chemical analyses were performed only for experiment A. Terpenes, 6-methoxymellein, and polyacetylenes were analyzed semiquantitatively by use of gas chromatography of dichloromethane extracts. Hydrophilic antioxidant capacity and sugars were analyzed in methanol extracts by means of the oxygen radical absorbance capacity assay (ORAC) and HPLC, respectively.

Gas Chromatography Analysis of Hydrophobic Compounds. Frozen carrot powder (15 g) was weighed into 50 mL glass tubes, and 200 μL of methyl palmitate and 200 μL of rose oxide (internal standards) and 30 mL of cold (−18 °C) dichloromethane were quickly added. The tubes were gently flushed with argon, sealed, and shaken vigorously. The mixture was then rapidly stirred in the dark for 15 min at 4 °C, followed by 15 min at room temperature. During stirring, the carrot powder slowly thawed. The liquid phase was decanted into a new tube through a filter paper (Whatman no. 1). The extraction was repeated at room temperature with 30 mL of dichloromethane and stirring for 10 min. The two extracts were placed on ice, very gently evaporated to half-volume by a stream of nitrogen, and then combined and evaporated to 1 mL. The samples were stored in amber GC vials under argon at −80 °C. Before GC analysis, the extracts were further evaporated to 200 μL. The extraction procedure was checked with regard to recovery by spiking tests prior to analysis. Recovery was checked for the internal standards and for the compounds for which we had standards. Initially, two tests with consecutive dichloromethane extractions were carried out. Only trace amounts of the compounds of interest could be found in the third and so forth extracts. Thus, extraction twice with dichloromethane was considered sufficient for a semiquantitative method.

The extracts were analyzed on a gas chromatograph (Agilent HP 6890, Agilent, Palo Alto, CA) equipped with an HP-5MS column (25m × 0.25 mm i.d., 0.25 μm film) coupled to a flame ionization detector (FID). A 1 μL sample was injected with an autosampler (Agilent 6890) at 280 °C. The oven temperature program started at 60 °C for 10 min and increased by 3 °C min^{-1} to 230 °C and then 10 °C min^{-1} to 270 °C, with a final hold time of 25 min. The FID temperature was 280 °C. The long hold time at high temperature was necessary to elute hydrophobic compounds such as falcarindiol. Peaks

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were integrated with HP GC ChemStation software (revision A.05.02), identified by use of external standards, and verified by analysis on a GC−MS system (Agilent 6890 gas chromatograph/Agilent 5973 mass spectrometer) at similar chromatographic conditions with further identification of the compounds with the NIST 90 Mass Spectral Library, John Wiley & Sons, Hoboken, NJ (match >95%). The sample contents of the individual components were calculated on the basis of rose oxide and methyl palmitate as internal standards for terpenes and the other compounds, respectively. Two injection replicates were made from each sample. The average precision varied from 0.91% to 8.3% for the identified compounds, calculated as 2 × 100(value of injection 1 − value of injection 2)/(value of injection 1 + value of injection 2), where the values are the ratio peak area of compound/peak area of internal standard. The chromatogram of a representative carrot extract is shown in Figure 2.

ORAC Assay and Sugar Analysis. All samples from experiment A were analyzed except the third replicate for sugar in sample A1, which was lost.

Frozen carrot powder (7 g) was homogenized with 10 mL of ice-cold methanol for 2 min at 23 000 rpm (Polytron, PT 3000, Kinematica AG, Littau, Luzern, Switzerland), kept for 10 min on ice, centrifuged for 10 min at 35000g_{max} and 4 °C, and decanted. The pellet was re-extracted in 10 mL of methanol. The combined supernatants were filtered. Part of the methanol extract was diluted to 4 concentrations and analyzed by the ORAC assay as applied by Aaby et al.[20] Another part of the methanol extract (1.00 g) was evaporated at 37 °C until about 100 mg remained, which was used for analysis of sugars.

The residue was dissolved in 2 mL of distilled water and filtered (0.45 μm). Quantitation was carried out with an Agilent Technologies HPLC (Waldbronn, Germany, 1100 series HPLC system) with a NUKLEOGEL Sugar 810 Ca column, 300 mm × 7.8 mm, a guard column 30 × 4 mm (Machery-Nagel, Düren, Germany), and a refraction index detector (model 132, Gilford, Villiers-le-Bel, France). The injection volume was 20 μL, and the elution was at 85 °C with 0.1 mM Na_2Ca−EDTA at 0.5 mL min^{-1}. The individual sugars were identified by comparing their retention times with those of known standards. Quantification was based on external standard calibration curves.

Sensory Analyses. The sensory analyses were performed by means of flavor profile methods according to ISO 6564:1985-E (Sensory Analysis—Methodology—Flavor Profile Methods) using sensory panels of 8 (experiment A) and 11 (experiment B) trained panelists. The facilities for sensory analysis were designed according to ISO 8589:1989-E (General Guidance for the Design of Test Rooms). The data were recorded using "Compusense five" (Compusense Inc., Guelph, Canada) with an unstructured line scale anchored with low intensity at the left and high intensity at the right. The data were converted to a 1.0−9.0 scale.

Prior to analysis the panelists were trained according to ISO 3972:1991 (Sensory Analysis—Methodology—Method of Investigating Sensitivity of Taste) and calibrated with two of the extreme carrot samples from the experiments that were included in the sensory test (the highest and the lowest degrees of attack).

In the trial, 25 g of mixed carrot cubes from each sample was served at room temperature to each panelist. The 4 exposure levels × 3 field replicates were tested for experiment A. Due to very small roots (restrictions on available material), the B1 sample was tested as a bulked sample consisting of a combined sample of the three field replicates. For sample B3, one of the replicates was discarded due to pathogen decay and the sensory analyses performed on the two remaining replicates.

Statistics. Analysis of variance (ANOVA) was performed for each experiment separately on sensory, chemical, and morphological data.

For the chemical results and yield data the statistics were performed using Minitab 16 (Minitab Inc., State College, PA) at a significance level of 0.05. Block was regarded as a random effect and psyllid exposure degree as a fixed effect.

Sensory data were analyzed using "Proc glm" in SAS 9.1. (SAS Institute Inc., Cary, NC). Exposure degree to the pest was considered to be a fixed effect, and the block and panelists were regarded as random effects. The error terms for the F-tests were based on the Satterthwaite approximation.[21] For significant attributes ($p < 0.05$) Tukey's pairwise comparison test was used to compare differences between individual treatments (significance level 0.05).

For experiment A, correlations between the chemical variables and the sensory attributes were computed using Minitab 16. In addition, principal component analysis (PCA) was performed on 22 sensory and 18 chemical variables using Minitab 16. The coefficient variable was above 1 for all variables.

RESULTS AND DISCUSSION

Effect of Psyllid Attack on Root Yield and Leaf Damage. The level of psyllid attack measured by trap catches during the two experiments for the different degrees of physical protection of the carrots is shown in Figure 1. The carrot psyllids had a long attack period (6−7 weeks) in 2004 (experiment A) with two peaks, in contrast to a more intense, but very short attack period (2 weeks) in 2005 (experiment B). The A1 carrots were exposed to both peaks during the 6 week attack period, while the A2 treatment was only exposed to the second attack period and A3 nearly unexposed like the A4 carrots (Figure 1). The relatively short attack period the second year mainly affected B1 carrots and to a minor extent B2 (end of period), but not the B3 carrots. The year differences in attack reflect the weather-related differences expressed by temperature-dependent development of adults, eggs, and larva as described previously.[22]

The yield was clearly affected by different degrees of exposure, as seen in Table 1. For experiment A, treatments A2 and A3 gave 30−70-fold increases in yield, respectively, compared to A1 carrots. For experiment B the increases were 5−6-fold for the two similar psyllid protection treatments. In both experiments the carrots exposed to psyllids from germination had the lowest portion of grade 1 carrots and the largest fractions of discarded roots (79−100%) and roots with leaf damage (98−100%) (Table 1). The A1 and A2 treatments gave the same proportion of discarded roots (94−100%), but the total yield was lower and the proportion of plants with leaf damage was higher for carrots from treatment A1. The A3 treatment had the lowest damage (2% plants with leaf damage and 16% discarded roots).

The results from experiment B confirm the results from experiment A, showing a clear difference between the most heavily attacked carrots and the other treatments with respect to yield, portion of discarded roots, and leaf damage (Table 1).

The dramatic yield reduction and leaf curling or discoloration after high-intensity, prolonged psyllid attack in our studies are in agreement with other studies indicating this pest to be an economically important carrot pest in northern Europe.[1−3,9,23] The significant reduction in root weight for carrots exposed from germination compared to those exposed late in the season confirm the results from controlled studies by Nissinen et al. [9] showing plants to be most sensitive to psyllid attack at the one- to two-leaf stage.

Effect of Psyllid Attack on Root Sensory Quality. Carrots from the A1 treatment had the highest scores for the attributes taste intensity, bitter taste, soil flavor, terpene flavor, aftertaste, astringency, odor intensity, and toughness and at the same time the lowest scores for acidic taste, sweet taste, color hue, color strength, and crispiness (Table 2). Our results confirm the results on the effects of leaf stress by hail damage in field trials where a hail-exposed location had an enhanced sensory score for bitter taste and a reduced score for sweet taste compared with an unexposed location.[10] The impact on sensory quality was approximately at the same level by the hail exposure as by the psyllid stress in our study (Table 2),

Table 2. Intensity of Sensory Attributes for Carrots with Different Degrees of Carrot Psyllid Attack (Scores 1−9 from Lowest to Highest Intensity)[a]

treatment code	period of exposure to natural pest attack[b]	taste and flavor										odor						color			texture			
		taste intensity	acidic taste	sweet taste	bitter taste	soil flavor	plastic flavor	chemical flavor	terpene flavor	aftertaste	sickeningly sweet	astringency	odor intensity	soil odor	plastic odor	chemical odor	terpene odor	ethanol odor	whiteness	color hue	color strength	crispiness	juiciness	toughness	hardness
Experiment A																									
A1	from germination	8.02 a	1.55 c	2.28 b	7.07 a	6.21 a	2.40 a	3.80 a	5.28 a	6.52 a	2.53 a	5.38 a	7.91 a	6.39 a	2.13 a	3.56 a	4.92 a	1.91 a	4.85 a	3.34 c	3.70 c	3.90 c	3.49 b	5.30 a	6.35 a
A2	from July 5	6.95 b	3.99 b	4.40 a	4.61 b	4.39 b	1.47 ab	2.54 ab	3.93 b	3.44 b	2.16 a	3.44 b	6.77 b	4.57 a	1.84 a	2.56 a	3.47 ab	1.30 ab	4.87 a	5.10 b	5.25 b	5.05 b	4.88 ab	3.62 b	5.87 ab
A3	from July 28	6.49 b	5.07 a	4.84 a	4.03 b	2.19 c	1.16 b	1.28 b	3.07 b	4.31 bc	1.57 a	2.75 bc	5.44 c	1.93 b	1.13 b	1.38 b	2.55 bc	1.23 ab	4.43 a	6.14 a	6.48 a	5.72 b	6.17 a	2.41 b	5.45 b
A4	unexposed	6.47 b	5.69 a	4.97 a	3.58 b	2.02 c	1.09 b	1.40 b	2.98 b	4.07 c	1.4 a	2.57 c	5.29 c	2.05 b	1.20 b	1.41 b	2.49 c	1.14 b	4.38 a	6.08 a	6.33 a	6.01 a	6.16 a	2.41 b	5.41 b
	p(ANOVA)[d]	<.0001	<.0001	<.0001	<.0001	<.0001	0.01	0.001	<.0001	<.0001	0.139	<.0001	<.0001	<.0001	0.016	0.0004	0.0002	0.031	0.463	<.0001	<.0001	0.0002	<.0001	<.0001	0.002
Experiment B																									
B1	from germination	7.12 a	1.67 a	3.61 a	5.72 a	4.10 a	1.65 a	3.45 a	4.45 a	5.58 a	2.84 a	3.65 a	7.00 a	3.98 a	1.67 a	4.13 a	4.70 a	2.01 a	5.72 a	3.38 b	4.06 b	3.47 a	3.70 b	4.66 a	5.76 a
B2	from July 4	6.44 a	3.96 a	4.36 a	4.63 a	2.53 a	1.18 a	1.70 b	3.25 a	4.48 a	1.37 b	2.45 a	5.72 ab	2.44 a	1.09 b	1.58 b	2.97 b	1.49 a	3.88 b	5.32 a	5.75 a	4.93 a	5.15 a	2.92 b	5.39 a
B3	from July 19[c]	6.37 a	4.09 a	4.14 a	4.29 a	2.56 a	1.15 a	1.66 b	2.98 a	4.23 a	1.29 b	2.29 a	5.48 b	2.50 a	1.12 b	1.31 b	2.63 b	1.47 a	3.50 b	5.61 a	6.20 a	5.29 a	5.55 a	2.63 b	5.20 a
	p(ANOVA)[d]	0.293	0.153	0.508	0.364	0.239	0.306	0.027	0.194	0.155	0.008	0.106	0.045	0.055	0.032	0.000	0.024	0.460	0.001	<0.0001	0.001	0.096	0.044	0.049	0.492

[a]Values are means of three field replicates. Values within each experiment for each variable labeled with the same letter are not significantly different by Tukey's multiple-comparison test at a significance level of 0.05. [b]Levels of attack by carrot psyllid in the periods of exposure are shown in Figure 1. [c]Very low attack level, below 0.1 psyllid found per trap per day. [d]p-value from analysis of variance.

showing a 2−3 point decrease in sweet taste and 3−3.5 point increase in bitter taste on a 1−9 point evaluation scale. In the hail damage study the stressed carrots were found to be 2 points lower in preference. Carrots from the A1 and A2 treatments differed from those from the A3 and A4 treatments by having higher sensory scores for soil odor, plastic odor, chemical odor, and terpene odor (Table 2). Carrots from the shortest exposure period (A3) did not differ significantly from unexposed carrots (A4) in regard to sensory or chemical characteristics (Tables 2−4). Only crispiness was higher in the unexposed carrots (A4).

The most heavily exposed carrots in experiment B (B1) showed results similar to those from experiment A (A1), with higher sensory scores for the attributes chemical flavor, sickeningly sweet flavor, plastic odor, chemical odor, terpene odor, whiteness, and toughness and lower scores for color strength, color hue, and juiciness (Table 2).

In regard to texture parameters, the score for toughness was highest and juiciness lowest in carrots exposed from germination, compared to the other treatments in both experiments (Table 2). In experiment A the lowest level of crispiness was also found in carrots exposed from germination (A1). This indicates a negative effect of heavy psyllid attack on the texture of carrots, making them tougher and less crispy. In experiment B there were no significant differences in scores for sensory attributes between treatment B2 and B3 (Table 2).

Effect of Psyllid Attack on Hydrophobic Compounds. Numerous compounds were identified in the GC analysis of the carrot extracts from experiment A, including terpenes, 6-methoxymellein, and polyacetylenes. The heavily attacked A1 samples had the highest contents of the bitter compounds falcarindiol and 6-methoxymellein (Table 3). The increased level of 6-methoxymellein indicates biosynthesis of ethylene in the plants since ethylene is an inducer for production of 6-methoxymellein in carrots.[24] Such a stress stimulation of ethylene production is in agreement with other studies showing ethylene production to increase after exposure of plants to different kinds of stress, such as wounding or bacterial attack.[25,26] The increased content of 6-methoxymellein with increasing attack of carrot psyllid found in our study is in agreement with the controlled pot study of carrot psyllid by Nissinen et al.[9] and for most of the tested genotypes after mechanical stress.[10,27] Other studies show falcarinol and other polyacetylenes to be affected in different directions by exposure to drought stress in the field.[28,29] This indicates a complex pattern most likely depending on the degree and type of stress carrots are exposed to.

The A1 carrots were also associated with the highest level of 9 of the analyzed terpenes: α-pinene, β-pinene, myrcene, α-phellandrene, p-cymene, (*R*)-(+)-limonene, terpinolene, camphene, and bornyl acetate (Table 3). These results confirm studies by Nissinen et al.,[8] where it was found that carrot psyllid feeding induced changes in the endogenous monoterpene pool in the carrot leaves. Their findings that the terpenes β-pinene and limonene increased in leaves after carrot psyllid feeding are in accordance with our results showing these terpenes to be among the affected root terpenes after psyllid attack. No differences between the treatments were found for the following compounds (content given as mean of all treatments, ng g^{-1} FW ± SD): γ-terpinene (913 ± 244), (−)-*trans*-caryophyllene (6566 ± 1316), and falcarinol (9517 ± 1316).

Effect of Psyllid Attack on Sugar Content. Carrots exposed to psyllids from germination (A1) had lower total sugar content than carrots with different degrees of protection

Table 3. Effect of Attack Level by Carrot Psyllid on Antioxidant Capacity (ORAC, Trolox equiv g^{-1} FW) and Hydrophobic Compounds (ng g^{-1} FW) in Carrots from Experiment A[a]

treatment	period of exposure to natural pest attack[b]	ORAC	bitter compounds		terpenes								
			falcarindiol	6-methoxy-mellein	α-pinene	β-pinene	myrcene	α-phellandrene	*p*-cymene	limonene	terpinolene	camphene	bornyl acetate	total terpenes
A1	from germination	5.39 a	25 460 a	11 544 a	4 525 a	1 100 a	711 a	246 a	356 a	685 a	9 195 a	92 a	302 a	24 044 a
A2	from July 5	2.39 b	10 803 b	636 b	1 395 b	301 b	233 b	145 b	180 b	393 b	6 747 a	71 ab	141 b	15 678 b
A3	from July 29	1.48 b	8 024 b	278 b	1 004 b	188 b	198 b	69 c	147 b	141 c	1 835 b	60 b	122 b	13 115 b
A4	unexposed	1.96 b	6 368 b	321 b	1 041 b	177 b	195 b	83 c	130 b	171 c	2 454 b	59 b	136 b	12 648 b
	p(ANOVA)[c]	0.002	0.001	0.006	<0.001	<0.001	0.001	<0.001	0.001	<0.001	0.006	0.010	0.001	<0.001

[a]Values are means of three field replicates. Values within each variable labeled with the same letter are not significantly different by Tukey's multiple-comparison test at a significance level of 0.05. [b]Levels of attack by carrot psyllid in the periods of exposure are shown in Figure 1. [c]*p*-value from the analysis of variance.

Table 4. Effect of Attack Level by Carrot Psyllid on Content of Sugars (g kg^{-1} FW) in Experiment A[a]

treatment	period of exposure to natural pest attack[b]	total sugar	sucrose	glucose	fructose
A1	from germination	45.84 b	23.95 b	9.44 b	12.46 b
A2	from July 5	61.54 a	33.88 a	12.66 b	15.01 ab
A3	from July 28	60.80 a	25.33 ab	18.70 a	16.77 a
A4	unexposed	62.06 a	26.16 ab	18.72 a	17.18 a
	p(ANOVA)[c]	0.010	0.033	0.004	0.018

[a]Values are means of three field replicates (two replicates for A4). Values within each variable followed by the same letter are not significantly different by Tukey's multiple-comparison test at a significance level of 0.05. [b]Levels of attack by carrot psyllid in the periods of exposure are shown in Figure 1. [c]*p*-value from the analysis of variance.

(Table 4). The two most exposed treatments (A1 and A2) also had lower glucose content than the less exposed and unexposed carrots (A3 and A4). Fructose followed the same pattern, showing clear differences between the carrots exposed from germination and the A3 and A4 treatments. Nonetheless, sucrose showed no clear increase with increasing psyllid exposure as the content of A1 was lower than that of A2, but not different from those of A3 and A4.

The reduction in sugar content caused by psyllid attack indicates a situation with increased respiration and carbohydrate consumption due to stress and wound healing activity by the plant. This is confirmed by results from other studies of psyllid-exposed carrots[9] and other kinds of stress exposure such as hail damage,[10] mechanical stress at harvest,[27] and ethylene exposure.[14] The decreases in sucrose, fructose, and glucose found in our experiment were also found in the study by Nissinen et al.[9] A 30% sugar reduction was found in our study, when comparing carrots exposed to psyllids from germination with the unexposed ones, which is similar to the 40% sugar reduction for plants infected with one psyllid per plant at the one-leaf stage in comparison with the untreated control.[9] The decrease in total sugar content was also found for most of the tested genotypes when comparing carrots from the hail-exposed location with the unexposed ones.[10]

Effect of Psyllid Attack on Antioxidant Capacity. The most heavily attacked carrots (A1) also had the highest antioxidant capacity (ORAC value), while there were no differences between the other treatments for this variable (Table 3).

Despite the high antioxidant capacity found in these heavily attacked carrots, the contribution from the mentioned constituents, on a molar basis, could explain only part of the measured antioxidant capacity. Furthermore, most of the compounds have not been documented as (potent) antioxidants. Therefore, other compounds in carrots with antioxidant activity not analyzed in this study could have been increased due to psyllid attack, for instance, phenolic compounds, which have shown increased contents after psyllid damage[9] and hail stress.[10] An increase in phenolic antioxidants was also verified in studies of carrots exposed to wounding.[11,12] The responding antioxidants in these studies were caffeoylquinic acid,[11] 3,5-dicaffeoylquinic acid, and chlorogenic acid (5-caffeoylquinic acid).[12]

The stress reaction formed in connection with wounding has been explained by two types of responses.[30] The first one is oxidation of the existing phenolic compounds as a result of a ruptured cell membrane and the possibility for phenolics to combine with oxidative enzyme systems. The second response

is the synthesis of monomeric or polymeric phenolics to repair the wounded tissue. The damaging effect on tissue caused when psyllids insert their stylet and suck nutrients[4] can to some extent explain the high effect of this pest on antioxidant capacity and other quality-related parameters of carrots. In addition to this wounding effect, the curling of leaves and leaf discoloration indicate one or more unknown toxins to be involved and systemically transported in the plant,[5] possibly influenced by the plant pathogenic bacterium *C.* L. solanacearum.[6] These aspects were not considered in our study, and further investigations are needed to understand the mechanisms behind the effect of psyllids and possible secondary organisms.

The increase in antioxidants and antioxidant capacity occurring at high levels of psyllid attack may have little practical meaning for the consumer's health perspective since highly affected carrots will be discarded due to reduction in root size and shape.

Correlations between Sensory and Chemical Variables. Falcarindiol and 6-methoxymellein were highly correlated ($p < 0.001$) to bitter taste ($R = 0.96$ and 0.87, respectively) and aftertaste ($R = 0.95$ and 0.97, respectively). There were negative correlations between these compounds and sweet taste ($R = -0.92$ and -0.94, respectively). Antioxidant capacity was very highly correlated with falcarindiol content ($R = 0.98$).

The correlations of falcarindiol and 6-methoxymellein to bitter taste are in agreement with other studies where these compounds may have contributed to increased bitterness.[31] Correlation of these compounds to aftertaste indicates their possible involvement in the aftertaste picture, most likely together with the terpenes, which also were positively correlated to aftertaste in our study.

Furthermore, the positive correlation between sweet taste and total sugar content was in agreement or in contrast with other studies.[14,27] A poor prediction for sugars to sweet taste was seen in a study by Kreutzmann et al.[31] despite the fact that there was a large span in total sugar contents between the tested samples. The negative correlation between the bitter compounds falcarindiol and 6-methoxymellein and sweet taste indicates a possibility for bitter compounds to partially reduce the sweet taste perception. For 6-methoxymellein this correlation has been confirmed by other results.[27,32]

PCA Analysis. The PCA of the 22 sensory and 18 chemical variables for experiment A shows three groups of variables mainly grouped by principal component 1 (PC1) and to some extent by principal component 2 (PC2), which explains 87.2% and 5.7%, respectively, of the total variation (Figure 3). The samples exposed from germination (A1) were located on the right bottom side of the score plot. They were mostly associated with the contents of terpenes, falcarindiol, and 6-methoxymellein and antioxidant capacity. From the sensory point of view, these samples were associated with bitter taste, ethanol odor, chemical odor and flavor, plastic odor and flavor, and soil odor and flavor. The A3 and A4 samples formed a common group on the left bottom side of the score plot. These samples were mostly associated with the variables fructose and glucose, total sugar, acidic taste, and sweet taste, as well as with crispiness and juiciness. The A2 samples, which made a third group in the upper part of the score plot, were located between the other two groups and were intermediate in quality characteristics as shown in the loading plot (Figure 3). In addition, these samples were associated with sucrose content by PC2, which explain 5.7% of the total variation.

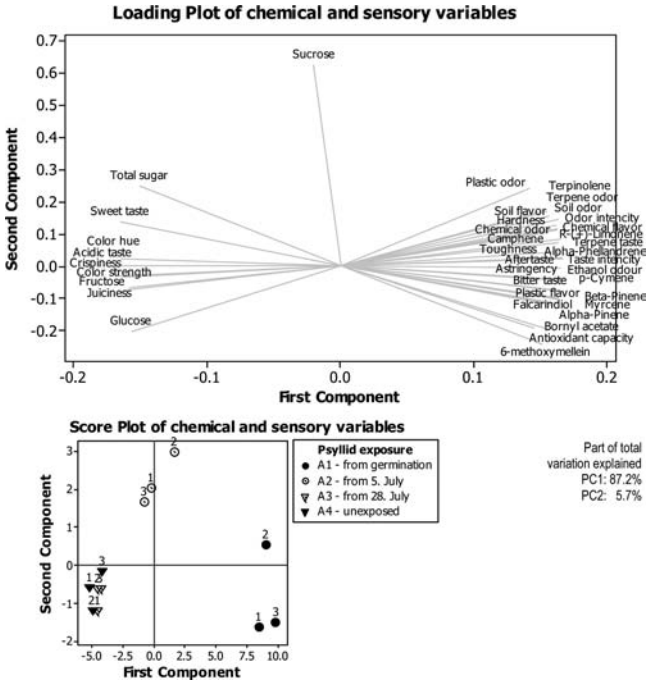

Figure 3. Principal component analysis of experiment A results. Loading plot and score plot for PC1 and PC2 of the 22 sensory attributes and 18 chemical variables (all with a coefficient of variation above 1). The degree of psyllid attack for treatments A1−A4 is shown in Figure 1. Numbers above the symbols refer to replicates.

The results from the PCA analysis were in accordance with the results from analysis of variance and Tukey's test regarding sensory and chemical quality measurements.

Psyllid attack affected the quality of carrots by increasing the bitter taste and content of bitter tasting compounds (6-methoxymellein and falcarindiol) as well as changing the terpene composition and causing an increase in terpene flavor and chemical flavor. The quality was further affected by reductions in total sugar, fructose, glucose, sweet taste, color hue, color strength, crispiness, and juiciness.

From our results it can be concluded that 8−10 week old carrot plants tolerate attack levels by psyllids corresponding to 2% plants with curling symptoms on leaves without any risks for changes in sensory quality. Since a limited number of attack levels were tested in our field study, additional controlled studies with many attack levels are needed to find the level of tolerance to psyllid attack in carrots. To avoid yield losses, plants need to be protected from germination until the attack period flattens out. However, since the end of the attack period varies between locations and years, it has to be monitored by frequent measurements of psyllids in field traps. The main result of this study is that stress by carrot psyllid attack causes changes in the sensory quality and content of chemical constituents of carrots.

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Notes

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■ ABBREVIATION

6-methoxymellein, 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin

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