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

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ABSTRACT

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

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

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

INTRODUCTION

The carrot psyllid (*Trioza apicalis* Förster, Homoptera, Psylloidea) is an economically important carrot pest in northern Europe.\(^1\) 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, like 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 non-woven 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 quality of carrots.

The aim of the present study was to investigate how carrot psyllid attack in the field affect 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.

MATERIALS AND METHODS

Field studies of carrot attack by \textit{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 length 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 randomized block design with 3 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 17 May with 1,600,000 seeds per ha. The field was fertilized as follows (ha⁻¹): 400 kg PK fertilizer (OPTI-PK™ 0-5-17), 600 kg NPK (Fullgjødsel® 11-5-18) and 300 kg N Nitrabor™ (calcium nitrate containing boron), all from Yara International, Oslo, Norway.

In Experiment B (2005), carrots of cv. ‘Merida’ were sown on 6 May with 1,500,000 seeds per ha. The field was fertilized as follows (ha⁻¹): before sowing with 450 kg NPK (Fullgjødsel® 11-5-18), after 6 weeks with 400 kg PK fertilizer (OPTI-PK™ 0-5-17) and after 8 weeks with 450 kg NPK (Fullgjødsel® 11-5-18). Thereafter, the field was top-dressed three times, every second week with 250 kg Nitrabor™.
The herbicide program was: Fenix® and Finale® (both 1 l ha\(^{-1}\), Bayer, Mannheim, Germany) prior to germination, Sencor WG® (50 g ha\(^{-1}\), Bayer) and Linuron Afalon® (250 mL ha\(^{-1}\), Agronica, Stoke, New Zealand) after germination and 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 3-4 leaf stage. Carrots were harvested after 15 and 16 weeks (8 and 5 September) for Experiment A and B respectively. No fungicides or insecticides were used in the experimental plots.

Yellow, sticky traps (20X15 cm, Rebell®, Andermatt Biocontrol AG, Grossdietwil, Switzerland) were used to monitor adult \textit{T. apicalis} attacks in the field. The traps were oriented 90 degrees against the predominant wind direction and placed 3 cm above leaves of the carrots (raised during growth of the plants). Five traps were placed in the field and registered 2 times or more per week from 18 May to 15 August 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 x 2.30 m, arranged as one bed with 3 carrot rows equally distributed on each bed.

Treatment level against \textit{T. apicalis} was regulated by using non-woven floating row covers (Agryl®, 17 g m\(^{-2}\), single layer, polypropylene fleece) 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 1 psyllid per trap per day, see Figure 1).
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 class one (damage free roots, 17-35 mm) were recorded and percentage 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 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 polymer bags at 2 °C overnight. Samples of mixed cubes for chemical analysis (100 g) were frozen in liquid nitrogen, vacuum packed and stored at -80 °C, then ground to a powder in a sub-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 with aroma and harsh, burning taste in carrots, and the sugars contribute with sweet taste and masking of bitter or harsh flavor. The polyacetylenes falcarinol and falcarindiol have attracted attention concerning health aspects and bitter taste, respectively. 6-Methoxymellein was chosen due to importance for bitter taste and increase in stress situations like ethylene exposure. The reference compounds (+)-β-pinene, R-(+)-limonene, (-)-bornyl acetate and (-)-trans-caryophyllene (purity 99%), (+)-α-pinene (purity 99.5%), R-(-)-α-phellandrene, ρ-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 were isolated from carrots by the authors as described earlier. 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, USA) 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 semi-quantitatively 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 methyl palmitate and 200 µL rose oxide (internal standards) and 30 mL 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 (Watman no 1). The extraction was repeated at room temperature with 30 mL dichloromethane and stirring for 10 min. The two extracts were placed on ice, very gently evaporated to half volume by a stream of nitrogen, 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 compounds of interest could be found in third and so forth extracts. Thus, extraction twice with dichloromethane was considered sufficient for a semi-quantitative method.

The extracts were analyzed on a GC (Agilent HP 6890, Agilent, Palo Alto, CA, USA) equipped with an HP-5MS column (25m * 0.25mm i.d., 0.25 µm film) coupled to a flame ionization detector(FID). 1 µL sample was injected with an auto sampler (Agilent 6890, Palo Alto, CA, USA) at 280 °C. The oven temperature program started at 60 °C for 10 min, increased by 3 °C min⁻¹ to 230 °C, then 10 °C min⁻¹ to 270 °C, and 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 like falcarindiol. Peaks were integrated with HP GC ChemStation software (rev. A.05.02) and identified by use of external standards and verified by analysis on a GC-MS (Agilent 6890 GC/ Agilent 5973 MS, Palo Alto, CA,
USA) at similar chromatographic conditions with further identification of the compounds with the NIST 90 Mass Spectral Library, John Wiley & sons, Hoboken, New Jersey, USA (match > 95%). The sample contents of the individual components were calculated based on rose oxide or 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 injection 1 – value injection 2) / (value injection 1 + value injection 2), where the values are the ratio: peak area of compound/peak area of internal standard. Chromatogram of a representative carrot extracts 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 ice-cold methanol for 2 min at 23 000 rpm (Polytron, PT 3000, Kinematica AG, Littau, Luzern, Switzerland), kept 10 min on ice, centrifuged for 10 min at 35,000 ×g max and 4 °C, and decanted. The pellet was re-extracted in 10 mL methanol. The combined supernatants were filtered. Part of the methanol extract was diluted to four concentrations and analyzed by the ORAC assay as applied by Aaby et al. 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 (Waldbromn, Germany, 1100 Series HPLC system) with a NUKLEOGEL® Sugar 810 Ca column, 300 mm x 7.8 mm, a guard column 30 x 4 mm (Machery-Nagel,
Düren, Germany), and a refraction index detector (Model 132, Gilford, Villiers-le-Bel, France).

Injection volume was 20 µl and the elution was at 85 °C with 0.1 mM Na$_2$Ca-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 a sensory panel 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 were served at room temperature to each panelist. All 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, USA) at significance level 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, USA). Exposure degree to the pest was considered to be a fixed effect, block and panelists were regarded as random effects. The error terms for the F-tests were based on the Satterthwaite approximation. For significant attributes (p<0.05) Tukey's pairwise comparisons 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 attacking period, while the A2 treatment was only exposed to the second attacking period and A3 nearly unexposed like the A4 carrots (Figure 1). The relatively short attack period the second year was mainly affecting B1 carrots, to minor extent B2 (end of period), but not the B3 carrots. The year differences in attack reflects the weather related differences expressed by temperature-dependent development of adults, eggs and larva as described earlier.22

The yield was clearly affected by different degrees of exposure, as seen in Table 1. For Experiment A, treatment A2 and A3 gave 30 to 70 fold increase in yield, respectively, compared to A1 carrots. For Experiment B the increases were 5 to 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, as well as 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.\textsuperscript{1-3,9,23} The significant reduction in root weight for carrots exposed from germination compared to those exposed late in the season confirm results from controlled studies by Nissinen \textit{et al.} \textsuperscript{9} showing plants to be most sensitive to psyllid attack at the 1-2 leaf stage.

**Effect of psyllid attack on root sensory quality**

Carrots from the A1 treatment had 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 results on effects of leaf stress by hail damage in field trials where a hail exposed location had enhanced sensory score for bitter taste and reduced score for sweet taste compared with an unexposed location.\textsuperscript{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), showing a 2-3 point decrease in sweet taste and 3 to 3.5 point increase in bitter taste on a 1 to 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 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) as regards
sensory or chemical characteristics (Table 2-4). Only crispiness was higher in the unexposed carrots (A4).

The most heavily exposed carrots in Experiment B (B1) showed results similar to 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).

As regards 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. 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, like wounding or bacterial attack. 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.\textsuperscript{9} and for most of the tested genotypes after mechanical stress.\textsuperscript{10,27} Other studies show falcarinol and other polyacetylenes to be affected in different directions by exposure to drought-stress in the field.\textsuperscript{28,29} This indicates a complex pattern most likely depending on degree and type of stress carrots are exposed to.

The A1 carrots were also associated with the highest level of 10 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.,\textsuperscript{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\textsuperscript{-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 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 show no clear increase by increasing psyllid exposure as content of A1 were lower than A2, but not different form 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 and other kinds of stress exposure like hail damage, mechanical stress at harvest and ethylene exposure. The decrease in sucrose, fructose and glucose found in our experiment were also found in the study by Nissinen et al. A 30% sugar reduction 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 untreated control. The decrease in total sugar content were also found for most of the tested genotypes when comparing carrots from the hail exposed location with the unexposed ones.

**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 and hail stress. An increase in phenolic antioxidants were also verified in studies of carrots exposed to wounding. The responding antioxidants in these studies were caffeoylquinic acid, 3,5-dicafeoylquinic acid and chlorogenic acid (5-caffeoylquinic acid).
The stress reaction formed in connection with wounding has been explained by two types of responses. The first one is oxidation of the existing phenolic compounds as a result of 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 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, possibly influenced by the plant pathogenic bacterium *Candidatus Liberibacter solanacearum*. 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 (R=0.98) content.
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.\textsuperscript{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.

Further, the positive correlation between sweet taste and total sugar content was in agreement or in contrast with other studies.\textsuperscript{14,27} A poor prediction for sugars to sweet taste was seen in a study by Kreutzmann \textit{et al.}\textsuperscript{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.\textsuperscript{27,32}

\textbf{PCA analysis}

The principal component analysis (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 PC2, which explains 87.2\% and 5.7\% respectively of the total variation (\textit{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 content of terpenes, falcarindiol, 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 two other groups and was
intermediate in quality characteristics as shown in the loading plot (Figure 3). In addition these samples were associated with sucrose content by the PC2 which explain 5.7% of total variation.

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 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 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 cause changes in sensory quality and content of chemical constituents of carrots.

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Figure captions

Figure 1. Number of carrot psyllids (*Trioza 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 5 traps. Dotted lines indicate 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 10 Aug.

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

Figure 3. Principal component analysis of Experiment A results. Loading plot and score plot for principal components one and two (PC1 and PC2) of the 22 sensory attributes and 18 chemical variables (all with coefficient of variation above 1). The degree of psyllid attack for treatments A1 to A4 is shown in Figure 1. Numbers above symbols refer to replicates.
Table 1. Effect of level of carrot psyllid attack on leaf damage and yield of class one and discarded carrots. Values are means of 3 field replicates. Values within each experiment and variable labeled with the same letter are not significantly different by Tukey’s multiple comparisons test at significance level 0.05.

<table>
<thead>
<tr>
<th>Exposure period to natural pest attack *</th>
<th>Yield roots (kg m⁻²)</th>
<th>Grade 1** roots (kg m⁻²)</th>
<th>Portion of discarded roots (%)</th>
<th>Portion of plants with leaf curling or discoloration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1 from germination</td>
<td>0.10 c</td>
<td>0.00 b</td>
<td>100 a</td>
<td>100 a</td>
</tr>
<tr>
<td>A2 from 5 July</td>
<td>3.11 b</td>
<td>0.18 b</td>
<td>94 a</td>
<td>80 b</td>
</tr>
<tr>
<td>A3 from 28 July</td>
<td>7.45 a</td>
<td>6.26 a</td>
<td>16 b</td>
<td>2.0 c</td>
</tr>
<tr>
<td>p ANOVA</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Experiment B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1 from germination</td>
<td>0.87 b</td>
<td>0.20 b</td>
<td>79 a</td>
<td>98 a</td>
</tr>
<tr>
<td>B2 from 4 July</td>
<td>4.87 a</td>
<td>4.43 a</td>
<td>9 b</td>
<td>1.5 b</td>
</tr>
<tr>
<td>B3 from 19 July</td>
<td>5.46 a</td>
<td>4.40 a</td>
<td>19 b</td>
<td>0 b</td>
</tr>
<tr>
<td>p ANOVA</td>
<td>0.007</td>
<td>0.001</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*) Actual attack by carrot psyllids in the exposed periods are shown in Figure 1. **) Damage free roots with diameter 17-35 mm. ^) P value from the Analysis of variance.
Table 2. Intensity of sensory attributes for carrots with different degrees of carrot psyllid attack (scores 1-9 from lowest to highest intensity).

Values are means of 3 field replicates. Values within each experiment for each variable labeled with the same letter are not significantly different by Tukey’s multiple comparisons test at significance level 0.05.

<table>
<thead>
<tr>
<th>Treatment code</th>
<th>Period of exposure to natural pest attack*</th>
<th>Taste and Flavor</th>
<th>Odor</th>
<th>Color</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Taste intensity</td>
<td>Odor intensity</td>
<td>Soil odor</td>
<td>Plastic odor</td>
</tr>
<tr>
<td>A1</td>
<td>from germination</td>
<td>8.02 a</td>
<td>7.91 a</td>
<td>6.39 a</td>
<td>4.92 a</td>
</tr>
<tr>
<td>A2</td>
<td>from 5 July</td>
<td>6.95 b</td>
<td>6.77 b</td>
<td>4.57 a</td>
<td>2.54 ab</td>
</tr>
<tr>
<td>A3</td>
<td>from 28 July</td>
<td>6.49 b</td>
<td>3.07 b</td>
<td>1.47 ab</td>
<td>2.54 ab</td>
</tr>
<tr>
<td>A4</td>
<td>unexposed</td>
<td>6.47 b</td>
<td>2.98 b</td>
<td>2.54 ab</td>
<td>1.18 a</td>
</tr>
<tr>
<td>B1</td>
<td>from germination</td>
<td>7.12 a</td>
<td>6.72 a</td>
<td>4.72 a</td>
<td>2.54 ab</td>
</tr>
<tr>
<td>B2</td>
<td>from 4 July</td>
<td>6.44 a</td>
<td>5.72 a</td>
<td>4.72 a</td>
<td>2.54 ab</td>
</tr>
<tr>
<td>B3</td>
<td>from 19 July**</td>
<td>6.37 a</td>
<td>5.72 b</td>
<td>4.72 a</td>
<td>2.54 ab</td>
</tr>
</tbody>
</table>

p ANOVA² <0.001 <0.001 <0.001 <0.001 <0.139 <0.016 <0.002 <0.031 0.463 <0.001 0.0002 <0.001 <0.0001 <0.001 <0.002 0.492

*) Levels of attack by carrot psyllid in the periods of exposure are shown in Figure 1. **) Very low attack level, below 0.1 psyllid found per trap per day. ^) P value from Analysis of variance.
Table 3. Effect of attack level by carrot psyllid on antioxidant capacity (ORAC, Trolox equivalents g\(^{-1}\) FW) and hydrophobic compounds (ng g\(^{-1}\) FW) in carrots from Experiment 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 comparisons test at significance level 0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Period of exposure to natural pest attack*</th>
<th>ORAC (ng g(^{-1}) FW)</th>
<th>falcarn-diol (ng g(^{-1}) FW)</th>
<th>6-methoxy-mellein (ng g(^{-1}) FW)</th>
<th>α-pinene (ng g(^{-1}) FW)</th>
<th>β-pinene (ng g(^{-1}) FW)</th>
<th>myrcene (ng g(^{-1}) FW)</th>
<th>α-phellandrene (ng g(^{-1}) FW)</th>
<th>β-cymene (ng g(^{-1}) FW)</th>
<th>R-(+) limonene (ng g(^{-1}) FW)</th>
<th>terpinolene (ng g(^{-1}) FW)</th>
<th>camphene (ng g(^{-1}) FW)</th>
<th>bornyl acetate (ng g(^{-1}) FW)</th>
<th>total terpenes (ng g(^{-1}) FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 from germination</td>
<td></td>
<td>5.39 a</td>
<td>25 460 a</td>
<td>11 544 a</td>
<td>4 525 a</td>
<td>1 100 a</td>
<td>711 a</td>
<td>246 a</td>
<td>356 a</td>
<td>685 a</td>
<td>9 195 a</td>
<td>92 a</td>
<td>302 a</td>
<td>24 044 a</td>
</tr>
<tr>
<td>A2 from 5 July</td>
<td></td>
<td>2.39 b</td>
<td>10 803 b</td>
<td>636 b</td>
<td>1 395 b</td>
<td>301 b</td>
<td>233 b</td>
<td>145 b</td>
<td>180 b</td>
<td>393 b</td>
<td>6 747 a</td>
<td>71 ab</td>
<td>141 b</td>
<td>15 678 b</td>
</tr>
<tr>
<td>A3 from 28 July</td>
<td></td>
<td>1.48 b</td>
<td>8 024 b</td>
<td>278 b</td>
<td>1 004 b</td>
<td>188 b</td>
<td>198 b</td>
<td>69 c</td>
<td>147 b</td>
<td>141 c</td>
<td>1 835 b</td>
<td>60 b</td>
<td>122 b</td>
<td>13 115 b</td>
</tr>
<tr>
<td>A4 unexposed</td>
<td></td>
<td>1.96 b</td>
<td>6 368 b</td>
<td>321 b</td>
<td>1 041 b</td>
<td>177 b</td>
<td>195 b</td>
<td>83 c</td>
<td>130 b</td>
<td>171 c</td>
<td>2 454 b</td>
<td>59 b</td>
<td>136 b</td>
<td>12 648 b</td>
</tr>
<tr>
<td>p ANOVA*</td>
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<td>0.002</td>
<td>0.001</td>
<td>0.006</td>
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<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.006</td>
<td>0.010</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*) Levels of attack by carrot psyllid in the periods of exposure are shown in Figure 1. *) 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. 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 comparisons test at significance level 0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Period of exposure to natural pest attack*</th>
<th>Total sugar</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>from germination</td>
<td>45.84 b</td>
<td>23.95 b</td>
<td>9.44 b</td>
<td>12.46 b</td>
</tr>
<tr>
<td>A2</td>
<td>from 5 July</td>
<td>61.54 a</td>
<td>33.88 a</td>
<td>12.66 b</td>
<td>15.01 ab</td>
</tr>
<tr>
<td>A3</td>
<td>from 28 July</td>
<td>60.80 a</td>
<td>25.33 ab</td>
<td>18.70 a</td>
<td>16.77 a</td>
</tr>
<tr>
<td>A4</td>
<td>unexposed</td>
<td>62.06 a</td>
<td>26.16 ab</td>
<td>18.72 a</td>
<td>17.18 a</td>
</tr>
</tbody>
</table>

\(\n\) p ANOVA\(^9\) = 0.010 0.033 0.004 0.018

\(^*)\) Levels of attack by carrot psyllid in the periods of exposure are shown in Figure 1. \(^\#\) P value from the Analysis of variance
Daily trap catches of *Trioza apicalis* at different exposure treatments

**Experiment A**

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of psyllids</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.5</td>
<td>30</td>
</tr>
<tr>
<td>25.5</td>
<td>25</td>
</tr>
<tr>
<td>1.6</td>
<td>10</td>
</tr>
<tr>
<td>8.6</td>
<td>5</td>
</tr>
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<td>15.6</td>
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<tr>
<td>22.6</td>
<td>20</td>
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<td>29.6</td>
<td>10</td>
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<tr>
<td>6.7</td>
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<td>13.7</td>
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<tr>
<td>27.7</td>
<td>2</td>
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<tr>
<td>3.8</td>
<td>4</td>
</tr>
<tr>
<td>10.8</td>
<td>5</td>
</tr>
<tr>
<td>17.8</td>
<td>2</td>
</tr>
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<td>24.8</td>
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<tr>
<td>31.8</td>
<td>3</td>
</tr>
<tr>
<td>7.9</td>
<td>1</td>
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</table>

**Experiment B**

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of psyllids</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.5</td>
<td>150</td>
</tr>
<tr>
<td>25.5</td>
<td>100</td>
</tr>
<tr>
<td>1.6</td>
<td>50</td>
</tr>
<tr>
<td>8.6</td>
<td>5</td>
</tr>
<tr>
<td>15.6</td>
<td>10</td>
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<tr>
<td>22.6</td>
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<tr>
<td>29.6</td>
<td>5</td>
</tr>
<tr>
<td>6.7</td>
<td>3</td>
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<tr>
<td>13.7</td>
<td>1</td>
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<tr>
<td>20.7</td>
<td>3</td>
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<tr>
<td>27.7</td>
<td>5</td>
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<tr>
<td>3.8</td>
<td>2</td>
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<tr>
<td>10.8</td>
<td>1</td>
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<tr>
<td>17.8</td>
<td>5</td>
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<tr>
<td>24.8</td>
<td>2</td>
</tr>
<tr>
<td>31.8</td>
<td>1</td>
</tr>
<tr>
<td>7.9</td>
<td>3</td>
</tr>
</tbody>
</table>

Date 2004

**Experiment A**

- A1
- A2
- A3
- A4

Date 2005

**Experiment B**

- B1
- B2
- B3
Figure 3

Loading Plot of chemical and sensory variables

Score Plot of chemical and sensory variables

Psyllid exposure
- A1 - from germination
- A2 - from 5. July
- A3 - from 28. July
- A4 - unexposed

Part of total variation explained
PC1: 87.2%
PC2: 5.7%