Polyacetylenes

-In Organic and Biodynamic Carrots

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Cover: The main character in this thesis
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Abstract

Falcarinol type polyacetylenes (FaTP) in carrots have been assigned both positive health effects and negative effects on taste, in connection with human consumption.

The aim of this thesis was to contribute to the description of factors influencing the concentrations of FaTP in carrot. Some 465 different samples, from 77 different plots, harvested at 17 different occasions during the period 2005 – 2008 were used in the investigations. The samples were analyzed concerning their size, root morphology, concentrations of soluble sugars and concentrations of polyacetylenes. Only the three major polyacetylenes in carrots; falcarinol (FaOH), falcarindiol (FaDOH) and falcarindiol-3-acetate (FaDOAc) were considered in this thesis.

No single factor was found to explain all of the variation in the levels of FaTP. Factors such as harvesting year, location of growing site, length of the growing season, storage and soil conditions were shown to have a stronger impact on the FaTP concentrations than for example manuring strategy. Increased concentrations of FaDOH, and sometimes also of FaOH, appeared to be a reaction on stress, *i.e.* a way of resisting stress.

The concentration of FaTP increased until 110-115 days after sowing and then decreased during the latter part of the season. Initially the levels of FaTP increased as the roots grew larger. However, roots heavier than 70 g exhibited lower levels of FaDOH and roots heavier than 107 g were lower also in their total concentration of FaTP. It was not possible to assign a specific root shape as an indicator of the composition of FaTP in the carrot samples.

The levels of FaOH were mainly correlated to regular changes occurring during root development, but they could occasionally increase if the carrots were grown under stressful conditions. The FaDOH concentration exhibited a pattern of variation more dependent on external factors, such as annular variation and soil conditions. The concentration of FaDOAc was more dependent on factors such as the levels of hexoses and on root length or root shape.

The variation in the concentration of FaOH indicated the existence of a critical level below which the synthesis of this compound was triggered. The enzymes transforming FaOH into FaDOH appeared to be active under similar conditions as the enzymes converting sucrose to hexoses.

Keywords: Falcarinol, falcarindiol, falcarindiol-3-acetate, polyacetylene, carrot, *Daucus carota* (L), organic agriculture, biodynamic agriculture,

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Dedication

To *Marina, Samuel, Ivan, Karin and Tomas*, with lots of love

To my supervisors, *Marie and Eva*, in great admiration for their patience

To *Karl-Erik*, with cordial thanks, for helping me with the HPLC-equipment and a lot of other things
To *Artur*, keeping me on the track
To *Mary*, probably breaking a new world record in accurately correcting a doctoral thesis in almost no time at all
To all my pupils at school, constantly trying to pull me back to reality

To everyone enjoying a tasteful carrot

"The day is coming when a single carrot, freshly observed, will set off a revolution!"
Paul Cezanne (1839-1906)

Contents

Preface

This thesis is part of a project that started some twenty years ago. At first it was more as a notion; organic carrots does not taste as much as they used to do! This led me to wonder what might had caused organic carrots to taste like most conventional carrots This rather naïve thought was the starting point of the overall project “The chase for the perfect carrot”

Initially, the outcomes from nearly 1 000 organic carrot samples were analyzed and compared. The samples were harvested all over Scandinavia from the late 1960ies and onwards. The results were compared with older research within the organic movement (Klein, 1968; Klett, 1968; Wistinghausen, 1979; Wistinghausen, 1984; Northolt *et al.*, 2004) and also with the results from more general research on carrots in Scandinavia (Hård *et al.*, 1977; Martens *et al.*, 1985; Hogstad *et al.*, 1997; Seljåsen, 2000; Suojala, 2000a; Rosenfeld, 2003).

Despite of all these studies, it seemed as there was a disagreement concerning one issue: What causes the bitter taste that sometimes spoils the eating quality of both organic and conventional carrots? Some results were published in 2003 that pointed out that falcarinol-type polyacetylenes (FaTP) might play a key role when it came to causing bitter taste in carrots (Czepa & Hofmann, 2003). At that time little was reported on how different factors influenced the levels of FaTP in carrots on a practical level.

Therefore I decided to make e a contribution…..

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Kjellenberg L, Johansson E, Gustavsson K-E and Olsson M. E: (2010). Effects of Harvesting Date and Storage on the Amounts of Polyacetylenes in Carrots, *Daucus carota*. Journal of Agricultural and Food Chemistry 58:11703-11708.

II Kjellenberg L, Johansson E, Gustavsson K-E and Olsson M. E. (2011). Polyacetylenes in fresh and stored carrots, *Daucus carota*; correlations to root morphology and sugar content. Journal of the Science of Food and Agriculture 92 (8) 1748-1754

III Kjellenberg L, Johansson E, Gustavsson K-E, Granstedt, A. and Olsson M. E. (2016). Influence of organic manures on carrot (*Daucus carota* L.) crops grown in a long-term field experiment in Sweden. Renewable Agriculture and Food Systems Renewable Agriculture and Food Systems 31 (03) 258-268

IV Kjellenberg L, Johansson E, Gustavsson K-E, Granstedt, A. and Olsson M. E. (2016). Correlations between polyacetylene concentrations in carrots, (*Daucus carota* L) and various soil parameters. FOODS 5 (3) 60, published online 2016-08-31

Papers I-IV are reproduced with the kind permission of the publishers.

The contribution of Lars Kjellenberg to the papers included in this thesis was as follows:

I Planning and executing the experiment, chemical analysis of samples, statistical analysis of data and writing the manuscript jointly with the co-authors.

II Planning and executing the experiment, chemical analysis of samples, statistical analysis of data and writing the manuscript jointly with the co-authors.

III Planning and executing the experiment, chemical analysis of samples, statistical analysis of data and writing the manuscript jointly with the co-authors.

IV Planning and executing the experiment, chemical analysis of samples, statistical analysis of data and writing the manuscript jointly with the co-authors.

Abbreviations

BA	Biodynamic agriculture
CA	Conventional agriculture
DAS	Days after sowing
FaDOAc	Falcarinol-3-acetate
FaDOH	Falcarindiol
FaOH	Falcarinol
FaTP	Falcarinol -type polyacetylene
OA	Organic agriculture
PCA	Principal Components Analysis

1 Objectives

The core objective of this doctoral thesis was to study how the concentrations of falcarinol type polyacetylenes (FaTP) vary depending on the development of the carrot, external factors or cultivation measures and how the FaTP are related to other substances, mainly soluble sugars.The following specific questions were addressed:

- How do the concentrations of FaTP vary during the season and after storage?
- Are the concentrations of FaTP related to certain stages in carrot root development and maturity?
- Are there connections between FaTP, soluble sugar and root morphology?
- How do soil conditions, annual variations and location of carrot cultivation influence the levels of FaTP?
- Does the farming system influence the concentrations of FaTP?
- Do different types of organic manures or cultivars influence the carrot crop in general and the concentrations of FaTP in carrots in particular?
- Is it possible to optimize carrot cultivation with particular regards to the content of the FaTP?

2 Introduction

Sometimes carrots develop a bitter taste making them impossible to consume. Falcarinol type polyacetylenes (FaTP) have been suggested to be correlated to this off-taste (Lund & White, 1990; Czepa & Hofmann, 2003).

Falcarinol type polyacetylenes are also reported to have health promoting traits (Brandt & Christensen, 2000; Zidorn *et al.*, 2005; Christensen & Brandt, 2006; Ohnuma *et.al.* ,2011, Zaini *et al.*, 2012, El-Houri *et.el.* 2015).

Is it possible to find ways to increase the beneficial effects of FaTP without risking the eating quality of the carrots?

2.1 What are falcarinol type polyacetylenes?

Polyacetylenes are low molecular weight compounds produced by plants as a respond to microbial attack, disease state, or abiotic stress (Minto & Blacklock, 2008). According to Bohlmann et. al. (1973) the FaTP are named after the plant *Falcaria vulgaris* Bern. from which they were originally extracted. The FaTP are formed from crepenynic acid and dehydrocrepenynic acid, which are transformed to C17-acetylenes by β-oxidation (Minto & Blacklock, 2008). Further oxidation and dehydrogenation leads to the different FaTP having two conjugated triple bonds and two non-conjugated carbon-carbon double bonds (Bohlmann *et al.*, 1973; Hansen & Boll, 1986; Minto & Blacklock, 2008; Dawid *et al.*, 2015).

Falcarinol type polyacetylenes have been isolated from several plant species, mostly belonging to the Araliaceae and Apiaceae families. Twelve different FaTP have been identified in carrots (Dawid *et al.*, 2015). The three most common FaTP in carrots are falcarinol (FaOH), falcarindiol (FaDOH) and falcarindiol-3-acetate (FaDOAc) (Figure 1). Falcarinol is described as the precursor to the other two FaTP (Minto & Blacklock, 2008).

All three major FaTP in carrot are reported to be allergenic, toxic, antifungal and to exhibit anti-inflammatory activity in macrophages and endothelial cells

(Metzger *et al.*, 2008; Dawid *et al.*, 2015). Extracts of carrot which contain FaOH, FaDOH and FaDOAc have been shown to exhibit significant inhibitory effects on both normal and cancer cell proliferation (Purup *et al.*, 2009). FaOH is reported to be most bioactive of the three major FaTP, followed by FaDOH and then FaDOAc (Kobaek-Larsen *et al.*, 2005).

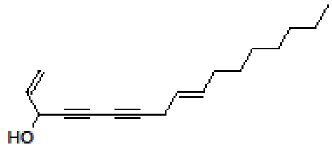
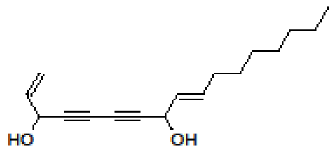
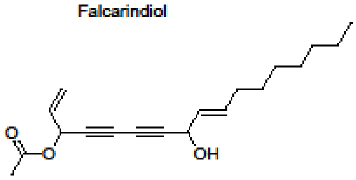
Chemical structure	Common name (abbreviation) full name
 Falcarinol	Falcarinol, (FaOH) (Z)-heptadeca-1,9-diene-4,6-diin-3-ol
 Falcarindiol	Falcarindiol, (FaDOH) (Z)-heptadeca-1,9-diene-4,6-diin-3,8-diol
 Falcarindiol-3-acetate	Falcarindiol- 3-acetate, (FaDOAc) (Z)-3-acetoxyheptadeca-1,9-diene-4,6-diin-8-ol

Figure 1. Structure of the three most common falcarinol-type polyacetylenes in carrot: falcarinol (FaOH), falcarindiol (FaDOH) and falcarindiol-3-acetate (FaDOAc).

Falcarinol, synonymous with "panaxynol" when derived from ginseng (Bernart *et al.*, 1996), is considered a toxicant (Arscott & Tanumihardjo, 2010) It is also reported to be able to elicit pro-allergic skin effect (Murdoch & Dempster, 2000; Machado *et al.*, 2002), although others suggest that systemic allergic dermatitis caused by Apiaceae root vegetables is not caused by FaOH (Paulsen *et al.*, 2014). In lower concentration FaOH has been suggested to act as a health promoting compound against *e.g.* different types of cancer (Brandt *et al.*, 2004). It has been reported that FaOH have a cytotoxic effect on different cell lines (Zidorn *et al.*, 2005), as well as a biphasic stimulatory effect on mammary epithelial cells (Hansen *et al.*, 2003). For example, in a study in which rats were fed on 10% freeze-dried carrot powder, providing 35 µg falcarinol/g feed, or falcarindiol extract, the rats exhibited reduced numbers of prenoplastic lesions in the rat colon (Kobaek-Larsen *et al.*, 2005).

Significant cytotoxic activity of FaOH and FaDOH has been reported with an LD_{50} value of 0.67 and 3.32 µg/mL respectively (Cunsolo & Ruberto, 1993). FaDOH is also recognized as being allergenic, toxic, and antifungal (Garrod *et al.*, 1978; Garrod *et al.*, 1979; Garrod & Lewis, 1982; Hansen & Boll, 1986).

There are multiple reviews on more recent findings concerning the health effects of FaTP *e.g.:* Brandt & Christensen (2000), Brandt *et.al* (2004), Zidorn *et al.* (2005), Christensen (2011), Dawid *et al.*(2015).

2.2 What is a carrot?

A more comprehensive description of the carrot and its traits is found in Kjellenberg (2007). The edible carrot, *Daucus carota var. sativus Hoffm*, is part of the Apiaceae family. The genus *Daucus* consists of just over 20 different species. *Daucus carota* includes the wild carrot and a number of varieties of the cultivated subspecies (Rubatzsky *et al.*, 1999).

Cultivated carrots can be divided into two types: Eastern (Asiatic), carrots have reddish, purple or yellow roots, pubescent leaves and a tendency for early flowering. Western carrots have orange, yellow, red or white roots, less pubescent, green leaves and show less tendency to bolt (Rubatzsky *et al.*, 1999).

Anatomy and morphology

The carrot storage root, from now on referred to only as carrot, consists mainly of two parts; the hypocotyl and the taproot (Esau, 1940). The hypocotyl is, at first, thicker and bears no lateral roots. The upper part of the hypocotyl is terminated at the cotolydonary node. The shape of the carrot is determined by the relation between the hypocotyl and the taproot, as well as by the relationship between the enlargement in length and in thickness.

During the first stages of growth the carrot is paler and increases more strongly in length than in thickness. A little later in the season the carrot becomes more colored and, starting at the hypocotyl, thickness increases (Rubatzsky *et al.*, 1999). The increase in thickness often causes the outer cell layers of the carrot to rupture. After approximately one-third of the growth period, root weight begins to increase more rapidly. This continues until harvest. At the end of the growing season the root weight increases faster than the size of the root diameter, measured at the shoulder of the carrot (Rubatzsky *et al.*, 1999).

The shape of the carrot tends to be more slender and cylindrical at first, then turning more conical, before changing to a more cylindrical shape again towards the end of the growing season. A more cylindrical shape of the carrot has been suggested as an indicator of when to harvest (Bleasdale & Thompson, 1963).

The changes in root thickness also have consequences concerning the shape of the root tip. A pointed tip is more common at the early stages of development. At harvest time the root tip is often more rounded and blunt (Rubatzsky et al., 1999). However the root tip can sometimes grow more pointed again during the late part of the season (Rosenfeld *et al.*, 2002).

Of special importance concerning the FaTP is the oil ducts situated mainly in the intercellular spaces of the carrot root pericycle (Baranska & Schulz, 2005). Esau (1940) noted that phloem parenchyma contained oil ducts arranged transversely and longitudinally. These ducts contain oil droplets high in FaTP (Garrod & Lewis, 1982). More information about the relation between root anatomy and FaTP is found below.

Chemical traits

Carrots can be analyzed into a large number of different substances, but only the two groups of compounds most relevant for this thesis are presented here. More comprehensive description can be found elsewhere (Rubatzsky *et al.*, 1999; Seljåsen, 2000; Suojala, 2000a; Rosenfeld, 2003; Kjellenberg, 2007)

Water soluble sugars

The sugar concentration varies between 30 and 70% of the dry matter. At harvest the sugar content predominantly consist of the disaccharide sucrose and the two monosaccharides, glucose and fructose (Nilsson, 1987a). The sucrose concentration varies between 20 and 45% of carrot dry matter and the concentration of the two hexoses is about 10% each (Ricardo & Sovia, 1974). Glucose is present both as α- and β-glucose (Nilsson, 1987a).

The composition of soluble sugars in carrots changes during the season. At first no soluble sugar is stored, then only hexoses are incorporated and in the third phase mainly sucrose is stored (Steingröver, 1983)

The accumulation of sucrose in the taproot seems to be more influenced by environmental factors than the storage of hexoses (Rosenfeld, 2003). Sucrose is the main sugar used in transportation from leaf to root in the plant (Nilsson, 1987b). Sucrose is then broken down into hexoses in the root during the early part of the carrot development.

The soluble sugars are mainly stored in vacuoles in the parenchymatic tissues (Nilsson, 1987a). The total sugar content does not differ much between different parts of the carrot, (Rosenfeld, 2003). The concentration of sucrose is higher in the upper part and in the phloem. Hexoses, especially fructose, are more common in the centre and lower (tip) part of the carrot and in the xylem, (Habegger *et al.*, 1996; Rosenfeld, 2003).

The ratio between sucrose and hexoses normally increases during the growing season. This ratio has been discussed, and questioned, as a useful indicator on when to harvest, mainly with respect of the storage abilities of the carrots (Goris, 1969a; Goris, 1969b; Phan *et al.*, 1973; Nilsson, 1987a; Nilsson, 1987b; Suojala, 2000a; Suojala, 2000b).

Falcarinol type polyacetylenes

The distribution and concentrations of FaTP have been reported to vary among cultivars and between cultivated and wild types of carrots (Schulz-Witte, 2011). The FaTP are most abundant in oil-filled channels, in the outer parts of the parenchymatic tissue running along the length of the carrot (Garrod & Lewis, 1979; Baranska & Schulz, 2005).

FaOH is more evenly distributed in the carrot root whereas FaDOH is more concentrated in the upper and outer part (Czepa & Hofmann, 2004; Baranska & Schulz, 2005). The concentration of FaDOH has been described being 5- to 10-fold higher in the peel than in the inner part of the carrot (Garrod & Lewis, 1979) and to increase during wound healing of carrot tissues (Garrod *et al.*, 1979; Lewis *et al.*, 1981; Lewis *et al.*, 1983).

All three FaTP have been reported to be part of the defense against different kind of pathogenic fungi, such as *Mycocentrospora acerina* (Garrod & Lewis, 1982).

2.3 A carrot in its environment

External factors, such as climate, inter-annular variation and soils have been reported to influence most carrot traits, *e.g.* root morphology (Rosenfeld, 2003) and concentrations of soluble sugars (Nilsson, 1987b). The composition of FaTP has also been reported to be influenced by external factors (Lund & White, 1990; Kidmose *et al.*, 2004; Kramer *et al.*, 2012). In general the variation depending on external factors is complex, and the influence of one single factor is difficult to analyze outside climate chambers (Rosenfeld, 2003). Annular variation and site-specific factors are reported to contribute more to the traits of the carrots than the fertilization system (Hansen, 1981).

More comprehensive reports on the importance of external factors on carrot traits are found elsewhere (*e.g.* Suojala, 2000a; Rosenfeld, 2003).

Growing site and climate

Carrots, grown in the Scandinavian climate, are said to taste sweeter, but contain less terpenoids and carotenoids, when grown in more northerly locations than samples grown in more southern parts of Scandinavia (Hård *et al.*, 1977; Rosenfeld *et al.*, 1997; Skrede *et al.*, 1997; Rosenfeld *et al.*, 1998).

Both temperature and light regime has been suggested to contribute to the differences between carrots grown at different latitudes (Rosenfeld (2003).

Samples harvested at different locations often differed in their composition of FaTP (Lund & White, 1990; Kidmose *et al.*, 2004; Kreutzmann *et al.*, 2008; Metzger & Barnes, 2009; Schulz-Witte *et al.*, 2010 ; Söltoft *et al.*, 2010; Kramer *et al.*, 2012; Tiwari *et al.*, 2013) but the reasons to these differences have not been thoroughly analyzed.

Soil

Most previous studies dealing with the influences of soil composition on carrot traits have compared samples grown in peat with samples grown in mineral soils. Newly harvested carrots have been reported to taste sweeter when grown in a peat soil than when grown in mineral soil, but after storage the carrots from the peat soil tasted more bitter (McCall & Möller, 1999). Growing carrots in peat has also been reported to be correlated more strongly with sucrose concentration and bitter taste, while when growing carrots in a mineral soil they were correlated more strongly to carotene, earthy taste and firmness, (Rosenfeld & Samuelsen, 2000). Carrots grown in peat soil have been reported to have the highest score for sweetness and the lowest scores for negatively associated characteristics such as bitterness, earthy flavour, terpene flavour and firmness (Seljåsen *et al.*, 2012). According to that study nitrate content and the amounts of forked roots were lowest on sandy soil (Seljåsen *et al.*, 2012).

Soil factors have been reported to influence the concentrations of FaTP in carrots but only in a moderate way (Czepa & Hoffmann, 2004; Kramer *et al.*, 2012). The moisture of the soil, both too much and too little water, has also been reported to influence the composition of FaTP in carrots (Lund & White, 1990; Kramer *et al.*, 2012)

2.4 Taking care of a carrot

Cultivating carrots involves choices between a number of treatments and measures having an impact on the final quality of the carrot root (Suojala, 2000a; Rosenfeld, 2003; Seljåsen *et al.*, 2013a).

Choice of farming system

The farming system comprises the unique combination of all cultivation measures and treatments implemented in order to produce agricultural products. Two groups of farming systems often discussed are conventional agriculture (CA) and organic agriculture (OA).

Organic and biodynamic carrots

The simplest description of OA is as a cultivation avoiding the use of easy soluble synthetic fertilizers and chemical pesticides allowed in conventional agriculture (CA). However this description of OA focuses on what is avoided, but gives no clue to how the OA system is implemented.

Different efforts implementing organic farming principles have led to variety of organic farming systems. One of the oldest organic farming systems is biodynamic agriculture (BA). Two main characteristics of BA are; strong emphasis on building self-supporting, sustainable, farming systems and the use of special biodynamic preparations, both in connection with manure treatments and directly on field.

Comparisons of soils and biodiversity over longer time periods have revealed differences between farming systems (Mäder *et al.*, 2002). In comparative studies, the quality of products from OA and products from CA have shown only minor or non-consistent differences (Woese *et al.*, 1997; Worthington, 1998; Dangour *et al.*, 2009; Rembialkowska & Srednicka, 2009; Paoletti *et al.*, 2012). The choice of farming system has been reported not to influence the concentrations of FaTP in carrots (Söltoft *et al.*, 2010; Kramer *et al.*, 2012; Bach *et al.*, 2015).

Choice of cultivar

The genetic factor is one of the most important single factors related to the quality and taste of carrot, (Simon & Peterson, 1979). Differences between cultivars in their contents of FaTP have been reported by many authors *e.g.*; Czepa & Hofmann (2004), Kidmose *et al.* (2004), Schulz-Witte *et al.* (2010), Kramer *et al.* (2012), Seljåsen *et al.*(2013a).

Storage

The optimal storage conditions for carrots are reported to be a temperature of approximately 0-0.5°C and a relative humidity of 98% (Apeland & Hoftun, 1974; Fritz *et al.*, 1979; Odebode & Unachukwu, 1997).

The impact of storage on the concentrations of FaTP has been discussed in several papers *e.g.*; Garrod & Lewis (1982), Mercier *et al.*(1993), Olsson & Svensson (1996), Hansen *et al.* (2003), Kidmose *et al.* (2004), Seljåsen *et al.* (2013a), Aguiló-Aguayo *et al.* (2014).

Processing

Processing has been reported to influence most carrot traits, also the levels of FaTP, but this matter is not included in this thesis. Some papers dealing with the influence of processing on the concentrations of FaTP are given below.

Stress

Drought, water logging, frost, parasites, heavily shaking during harvest and transportation or storage under unfavourable conditions, can elicit stress reactions in carrots. One reaction on stress is an increase in off-tastes (Seljåsen, 2000). In carrots harsh taste is more common to arise already in field (Simon, 1985), while bitter taste is more common after storage (Sondheimer *et al.*, 1955; Dodson *et al.*, 1956; Herrmann, 1978). Higher scores for bitterness during storage are correlated to lower scores for sweetness (Mempel, 1998; Mempel & Geyer, 1999; Seljåsen *et al.*, 2001; Seljåsen *et al.*, 2013b).

A number of factors causing stress in carrots have also been reported to influence the levels of FaTP. Some of these factors include:

Fungi: Garrod *et al.* (1979), Louarn *et.al.* (2012)
Surface wounds: Lewis *et al.*(1983)
Insects: Seljåsen *et al.*(2013b)
Water supply: Lund & White (1990), Kramer *et al.*(2012)
Storage conditions: Olsson & Svensson (1996), Hansen *et al.* (2003), Kidmose *et al.* (2004), Ahmad *et al.* (2011); Kramer *et al.* (2012), Rawson *et al.*(2012).
Processing: Hansen *et al.*(2003), Koidis *et al.* (2012), Aguiló-Aguayo *et.al.* (2014)
(The references given above are only a selection of papers within each gruoup)

The concept of stress in relation to the levels of FaTP is discussed further in paragraph 4.11.

3 Material and methods

A more detailed description on material and methods used is found in the different papers I-IV.

3.1 Sampling

Papers I and II

During 2005, 2006, and 2007 carrot samples were harvested on the same day, 3-6 times per season on three biodynamic and one organic farm in southern and central Sweden.

Plant material. The carrot cultivar ‘Kämpe’ was grown at all sites in all years. At one site the cultivar ‘Bolero’ was also grown, making it possible to evaluate cultivar differences.

Sampling and postharvest treatment. The carrot samples were taken from 6 - 7 locations spread over the field. A total of 40 - 60 carrots were harvested from each field. Within 24 h after harvest the carrots were transported in cold store boxes to the laboratory, where they were divided into two equal groups. One was directly placed into storage and the other was cut up immediately into cubes. Approximately 60 g of these carrot cubes were frozen and kept at -80 °C until further analysis. Before chemical analysis the carrot samples were freeze dried during 5 days and then milled to a powder.

Storage. Each of the stored samples was kept in small, perforated, plastic bags at approximately +1 °C and 97% relative humidity. The storage period was terminated on the 9 March in 2005 and 2006, and on the 9 June in 2007, regardless of harvesting date.

Papers III and IV

Carrots were grown, 2006, 2007 and 2008, within a long term field experiment, situated at the Biodynamic Research Station, 45 km south of Stockholm, Sweden. The effects of applications of non-composted and composted manure,

with and without biodynamic preparation treatments, were studied at three levels of application (0, 25 and 50 tons per ha). Some plots were fertilized with pelleted chicken manure and others had been left unmanured since 1992. This resulted in a total of 12 treatments with 3 (2008) or 4 (2006, 2007) replicates. A five-year crop rotation was used. Carrots were sown in plots with winter wheat in 2006 and 2008 and in first-year ley in 2007.
Plant material: An open pollinated carrot of the cultivar 'Kämpe' was used.
Sampling: Samples were harvested on the same day from all plots, three times per season, during 2006, 2007 and 2008. At all harvests 40-60 carrots were collected and brought to the laboratory in cold storage boxes within 24 hours. The sampling then followed the same routines as have been described above. Soil samples were collected in all 48 field plots in conjunction with the second harvest in 2006.

3.2 Analysis

Carrot samples were analyzed in term of their size, shape, content of polyacetylenes and content of soluble sugars. The rate of extract decomposition was measured in connection with some of the harvests. Soils and manures were analyzed according to common standards described in Paper III.

Root morphology

Root weight, length and maximum diameter were determined for every carrot harvested. The thickness of the root, in mm, was determined by measuring the diameter at points at $2/10^{th}$, (Diam. 1), $4/10^{th}$, (Diam. 2), $6/10^{th}$, (Diam. 3), $8/10^{th}$, (Diam. 4) and $9/10^{th}$ (Diam. 5) of the root length, counted from the root top. The different measurements undertaken are illustrated in Figure 2.
The cylindricity of the root was determined using a method described by Bleasdale and Thompson (1963) according to the formula;

Cylindricity= Weight (g)/ (Length (cm)* (Maximum radius (cm))^2 * π)

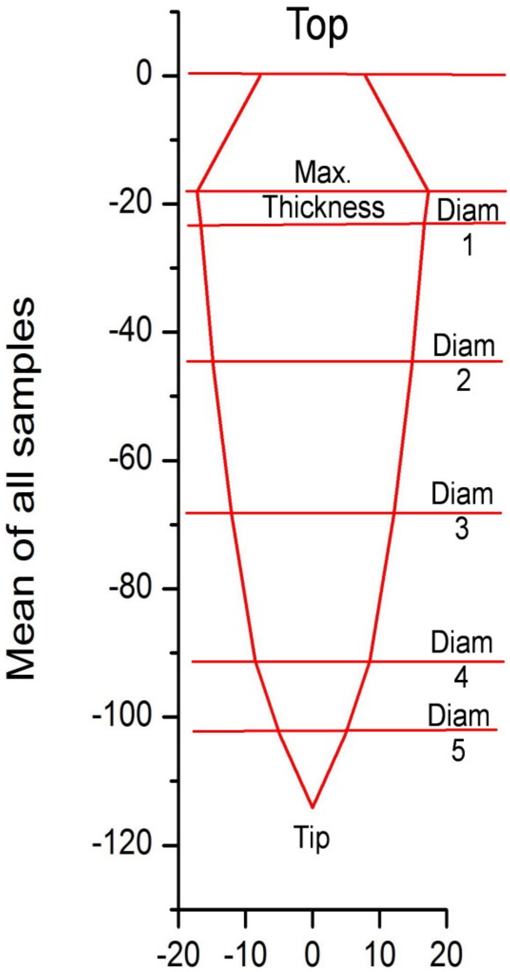


Figure 2. Mean carrot root size, mm, including all samples 2005-2008. Some of the measurements made during morphological determination are also included

giving carrots with a more cylindrical shape a value closer to 1 and roots with a more conical shape a value closer to 0.

The roundness or bluntness of the root tip was calculated by dividing the diam. 5 with diam. 4, giving carrots with a more rounded tip values closer to 1 and roots with a more pointed tip values closer to 0.

Root compactness was determined by dividing root weight, g, by root length, cm.

Carrot conical shape was determined by dividing the maximum diameter by diam. 4 with higher values corresponding to a more conical shape.

Proportion of peel was calculated by dividing the surface area or the volume of the outer 0.5 mm of the carrot by the total root volume.

The proportion of the hypocotyl was calculated by dividing the volume of the upper two-tenths of the root by its total volume. This measure gives a rough value of the proportion of the hypocotyl in relation to the total root.

Falcarinol type polyacetylenes

All carrot samples were analyzed by high-performance liquid chromatography (HPLC) for their FaTP content according to methods from Zidorn et al (2005) and Christensen and Kreutzmann (2007) with modifications. Each sample was extracted in triplicate and each extraction was analyzed separately. Data were evaluated by using Chemstation A09.03 software (Agilent Technology, USA). The different falcarinol type polyacetylenes, at a detection wavelength of 205.5 nm, were identified using retention time and data from the UV-spectra at 200-320 nm with 2 nm bandwidth. The spectra obtained were compared with those given in the literature (Christensen and Kreutzmann, 2007). The concentrations of FaTP were initially expressed as equivalents of the internal standard, 0.5% 4-chlorobenzophenone (Alfa Aesar Gmbh & Co., Germany). A standard of falcarindiol (Atomax Chemicals Co., Shenzhen, China) became available during the summer of 2009. A value of 1.235 was obtained on dividing the linear constant for 4-chlorobenzophenone at 205 nm by the corresponding constant for FaDOH at 205 nm. The factor 1.235 was therefore used to express the concentrations of FaTP as µg FaDOH equivalents /g DW. In the remainder of this thesis the concentrations of the FaTP are mostly expressed in the form µg/g DW and not in the more strictly accurate form µg FaDOH equivalents/ g DW.

Soluble sugars

Analysis of fructose, glucose and sucrose was performed by extracting each sample with 70% ethyl alcohol. The extracts were kept for 14 days at -20°C. After centrifugation at 10500*x g for two minutes, the extract was poured into glass vials and then analyzed by HPLC. Data were evaluated by using software by HPLC Technology Ltd, UK, Prime for Windows, PW- 500. The integrated area from the samples was compared with that from external standards for fructose (Merck & CO., USA), glucose (Merck & CO., USA) and sucrose (Merck & CO., USA) and determined as mg/ g DW. Triplicate analyses were performed on all samples.

Extract decomposition

Measurements of the changes in the electrical conductance (EC) were performed at 20°C with a water: carrot extract ratio of 10:1. The extract was kept at this temperature and measured on a daily basis in the same way, until the conductance no longer changed. The EC value from the fresh extract, R_0, was then subtracted from the final EC value, R_f. The rate of extract combustion was calculated as $(R_f - R_0)*100/R_0$. Triplicate analyses were performed on all samples.

Soil

Total carbon (C) and nitrogen (N) content were measured with a LECO CHN 600 element analyzer (SS-ISO 11464). Available phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were analyzed after extraction in ammonium lactate (AL) solution (SS 028310) and pH was determined according to SS-ISO 10390.

Manures

The composition of manures was analyzed using the following methods: pH: SS-ISO 10390; Total P, K, Mg, Ca and Sulphur (S): SS 028311; Total C: SS-ISO 10694; Total N: SS-ISO 13878; ammonium-nitrogen (NH4-N): ISO 11732.

3.3 Treatment of data

The computer programs Excel 2010 (Microsoft Corp. USA), Origin 8 (Origin Lab Corporation, USA), Minitab 16 (Minitab Inc. USA), SIMCA 14.0 (UMETRICS AB, Sweden) and SPSS 16.0 (SPSS Inc. USA) were used for calculations, graphics and statistical evaluations. One-way ANOVA analysis together with the Duncan post hoc test at a significance level of $P \leq 0.05$ was used to determine differences between subjects. Bivariate correlations tests were used to calculate Pearson correlation coefficients. Multivariate analysis was performed through Principal Components Analysis (PCA).

3.4 Weather conditions

Mean monthly precipitation and temperature at all cultivation sites is given, year by year, in Figure 3.

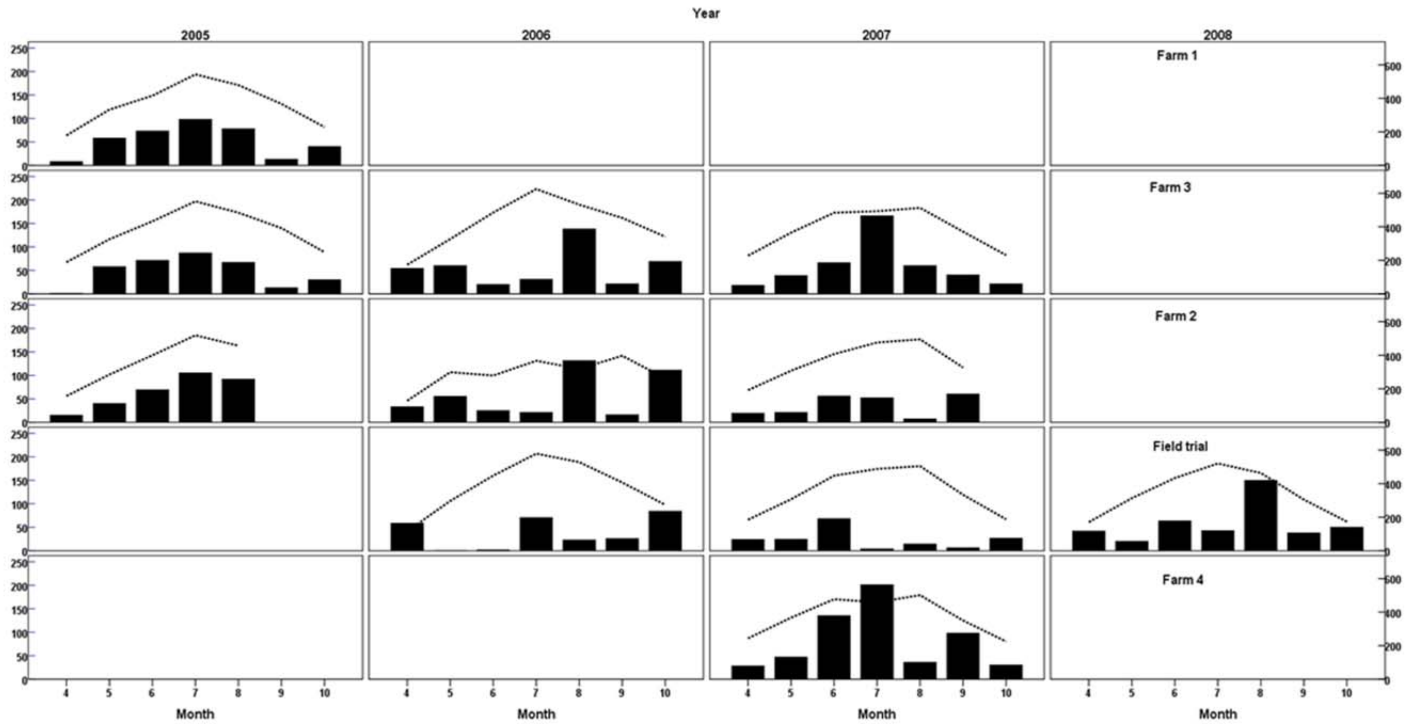


Figure 3. Temperature (right vertical axis, degree-day/month, dotted line) and precipitation (left vertical axis, mm/month, bars) at the five sampling locations included in this thesis, 2005-2008.

4 Results and discussion

The results obtained in the appendix of the printed version of this thesis, Papers I-IV, are referred to with roman numerals (I-IV), sometimes specified by the number of a certain table or figure. A compiled dataset comprising 393 different samples, from 77 different plots, harvested at 17 different occasions during the period 2005 to 2008 is also used when discussing the results.

The focus in this thesis is entirely on the three major FaTP; FaDOH, FaOH and FaDOAc. Several others FaTP have earlier been determined to be present in carrot roots (Bohlmann *et al.*, 1973; Lund, 1992) but were not considered here. Possible mixtures of FaDOAc and its allylic isomer, 1-acetoxyneptadeca-2,9-diene-4,6-diyn-8-ol (Lund, 1992) were jointly registered as FaDOAc.

4.1 Correlations between the three types of FaTP

The mean general relative composition of the FaTP in all the 393 samples was FaDOH 78.2%, FaOH 14.6% and FaDOAc 7.2%, but with a considerable variation between years, growing sites, harvesting dates and root size.

Regardless of data set, harvest occasion or growing location all three FaTP were strongly and positively correlated with each other (Table 1, Paper II: table 3, Paper IV: table 4). The concentrations of FaDOH and FaDOAc were more strongly correlated with each other than to the concentrations of FaOH (Table 1). The FaOH/ FaTP ratio was positively correlated with the concentrations of all three FaTP (Table 1). The FaDOH/ FaTP ratio was negatively correlated with the total concentrations of FaTP and also to the FaDOAc/ FaTP ratio and with the FaOH/ FaTP ratio (Table 1).

FaOH is the precursor to the other two FaTP (Minto & Blacklock, 2008; Dawid *et al.*, 2015). If influenced by factors stimulating the conversion of FaOH to FaDOH, and further to FaDOAc, theoretically the concentrations of FaOH can alter in three ways; i) the concentration of FaOH instantly decrease as the concentration FaDOH increases, ii) the concentrations of FaOH and FaDOH both increases, iii) the synthesis of FaOH occurs in parallel with the oxidation to FaDOH, increasing the levels of FaDOH, but maintaining the levels of FaOH. The results in this thesis mainly supported option (ii).

Table 1. Correlation matrix between different carrot traits, Pearson correlation coefficients, all 393 samples 2005-2008.

Variable	FaDOH	FaDOAc	FaOH	Total FaTP	FaOH/ FaTP	FaDOH/ FaTP	FaDOAc/ FaTP
FaDOH	1						
FaDOAc	0.634**	1					
FaOH	0.567**	0.531**	1				
Total FaTP	0.967**	0.717**	0.750**	1			
FaOH/FaTP	0.122*	0.196**	0.793**	0.327**	1		
FaDOH/FaTP	-0.089	-0.382**	-0.752**	-0.309**	-0.947**	1	
FaDOAc/FaTP	-0.093	0.591**	-0.069	-0.032	-0.094	-0.232**	1
Weight	-0.237**	-0.277**	0.190**	-0.153**	0.377**	-0.284**	-0.260**
Length	-0.285**	-0.029	0.131**	-0.181**	0.321**	-0.343**	0.089
Max. thickness	-0.196**	-0.310**	0.145**	-0.138**	0.328**	-0.219**	-0.314**
Mean thickness	-0.160**	-0.340**	0.129*	-0.118*	0.286**	-0.162**	-0.363**
Compactness	-0.122*	-0.323**	0.175**	-0.077	0.306**	-0.173**	-0.391**
Conical	0.113*	0.271**	0.040	0.100*	-0.095	0.017	0.234**
Bluntness	0.197**	-0.142**	0.180**	0.183**	0.064	0.056	-0.368**
Cylindricity	0.004	-0.269**	0.122*	0.011	0.109*	0.004	-0.342**
Peel %	0.205**	0.308**	-0.130**	0.148**	-0.309**	0.212**	0.278**
Hypocotyl %	0.125*	0.296**	0.077	0.143**	-0.004	-0.071	0.232**
Fructose	0.209**	0.306**	0.156**	0.229**	0.028	-0.073	0.142**
Glucose	0.260**	0.343**	0.054	0.243**	-0.105*	0.041	0.190**
Sucrose	-0.121*	-0.041	0.200**	-0.040	0.298**	-0.283**	-0.024
Soluble sugar	0.109*	0.237**	0.218**	0.164**	0.168**	-0.203**	0.121*

** Negative correlation; P<0.01 (two-tailed) and correlation coefficient > 0.5

** Negative correlation; P<0.01 (two-tailed) and correlation coefficient < 0.5

* Negative correlation; P<0.05 (two-tailed)

** Positive correlation; P<0.01 (two-tailed) and correlation coefficient > 0.5

** Positive correlation; P<0.01 (two-tailed) and correlation coefficient < 0.5

* Positive correlation; P<0.05 (two-tailed)

4.2 Factors and interactions influencing the levels of FaTP

Eleven factors have been considered in this thesis regarding their influence on the levels of FaTP in carrots:

Root size	Sites of cultivation
Root shape	Choice of cultivars
Soluble sugars	Storage
Decomposition of carrot extracts	Manuring strategies
Harvesting years	Soil conditions
Duration of growth periods	

No single factor was found to explain all of the variation in the levels of FaTP (Papers I, II, III; IV). Using principal components analysis (PCA) the interaction between different factors became more visible. Among samples from the field trial 2006 and 2007, the component scores were arranged in clusters where both harvesting year and days of carrot growth were clearly distinguishable (Paper III: figure 1). Bringing all samples from the compiled data set into a PCA also revealed clusters and patterns based on harvest years and days of carrot growth (Figure 4).

In those two PCA plots the increase in root size contributed significantly to the first principal components. The component scores of the different FaTP were constituted mainly by their relation to the second or higher principal component.

The different plots of the field trial were also arranged in distinct clusters when using PCA (Paper IV: figure 2). Here the size related variables did not contribute so much to the explained variance of the two first components. Instead it was the variance among soil traits that exhibited similarities to the variance of FaTP.

A general linear model (Table 2) revealed significant differences between years or number of cultivation days considering the concentrations of FaDOH or FaOH ($P<0.001$) and FaDOAc ($P<0.002$). The interaction between harvest year and number of cultivation days also indicated significant differences in the concentrations of FaDOH and FaOH ($P<0.001$) (Table 2). Moreover, the GLM also revealed a significant difference in the concentrations of FaDOH and FaDOAc depending on root weight (Table2).

In the following paragraphs the influence of different factors on the levels of FaTP are discussed. The factors are organized more or less according to the assumed strength of their influence.

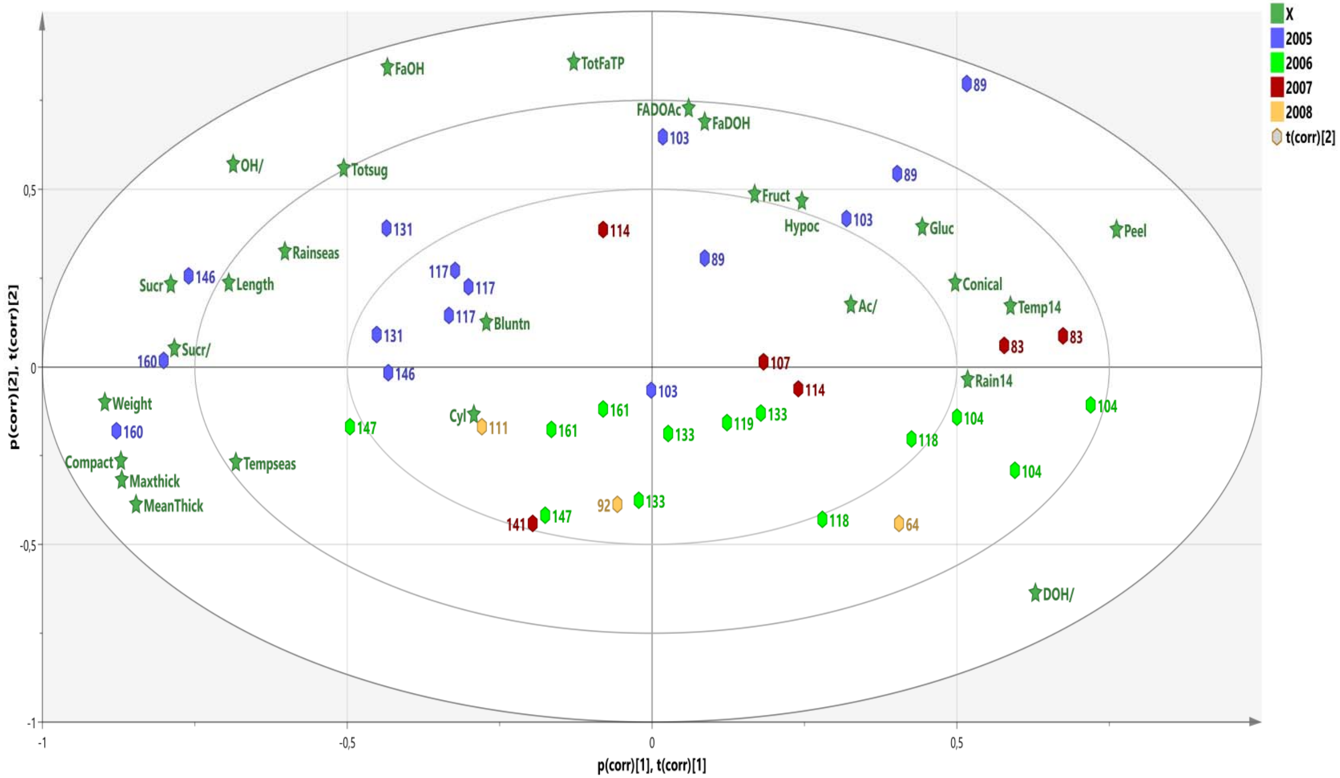


Figure 4. Biplot of PCA-principal component analysis (PCA) using all variables, 393 samples 2005-2008. Mean values from the 37 different harvests. Coloured according to harvesting year, numerals show days of cultivation. The proportion of explained variance; PC1 35%, PC2 19%, PC3 15%.

Table 2. *Univariate General Linear Model (GLM) on interaction between FaTP, harvest year, quartiles of root weight and quartiles of number of cultivation days. Tests of between-subjects effect. Type III sum of square. All carrot samples from field trials, 2006 to -2008*

	Dependent Variable: FaDOH					Dependent Variable: FaDOAc					Dependent Variable: FaOH				
Source	Sum of Squares	df	Mean Square	F	Sig.	Sum of Squares	df	Mean Square	F	Sig.	Sum of Squares	df	Mean Square	F	Sig.
Corrected model	1.26E+07[a]	26	484997	18.8	0.000	102666[a]	26	3948	8.5	0.000	725582[a]	26	27907	13.7	0.000
Intercept	2.18E+07	1	2.18E+07	846.9	0.000	146382	1	146382	313.9	0.000	725731	1	725731	357.0	0.000
Year	662480	2	331240	12.9	0.000	5877	2	2938.	6.3	0.002	27683	2	13841	6.8	0.001
Weight	362266	3	120755	4.7	0.003	4576	3	1525	3.3	0.021	10843	3	3614	1.8	0.151
Days	2041882	3	680627	26.4	0.000	6777	3	2259	4.8	0.003	221552	3	73850	36.3	0.000
Year*Weight	163658	6	27276.	1.1	0.387	2559	6	426	0.9	0.484	10866	6	1811	0.9	0.502
Year*Days	1001807	3	333935	13.0	0.000	1969	3	656	1.4	0.240	41416	3	13805	6.8	0.000
Weight*Days	296532	8	37066	1.4	0.179	3401	8	425	0.9	0.507	18722	8	2340	1.2	0.328
Year*Weight*Days	2642	1	2642	0.1	0.749	47	1	47	0.1	0.750	212	1	212	0.1	0.746
Error	8706176	338	25757			157605	338	466			687149	338	2032		
Total	1.05E+08	365				919446	365				4288700	365			
Corrected total	2.13E+07	364				260271	364				1412731	364			

[a]Adjusted R^2= 0,560   [a]Adjusted R^2= 0,348   [a]Adjusted R^2= =0 ,476

4.3 Harvesting time and cultivation site

Harvesting year had an impact on the concentrations of FaDOH and to lesser degree also the concentrations of FaOH (Figure 5, Table 3, Paper I: table 5)

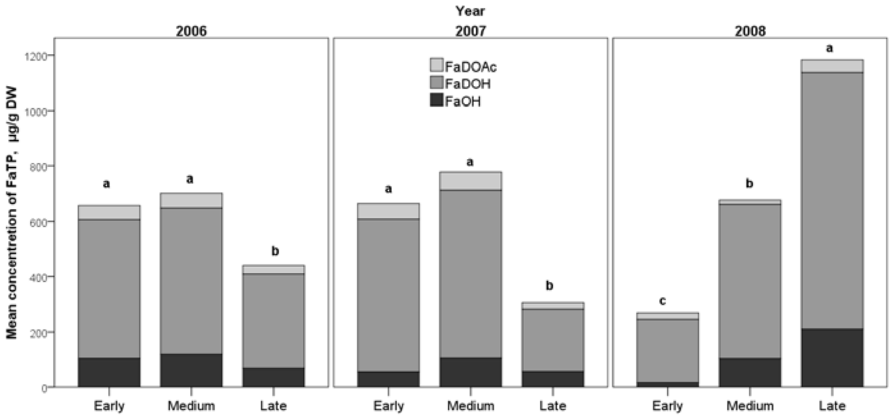


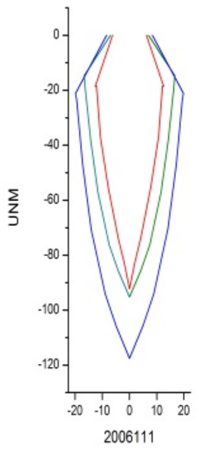
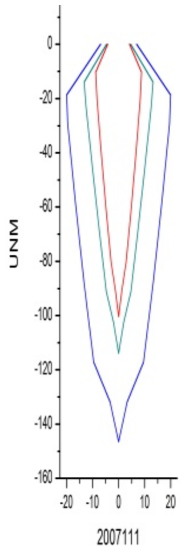
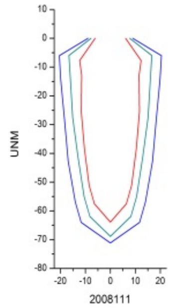
Figure 5. Levels of FaTP among samples from the field trial 2006-2008, arranged according to harvesting time. Different letters above bars indicate significant differences in total FaTP between harvests within the same year (P< 0.05).

The season 2005 differed from the three other seasons studied by high levels of FaTP, especially FaOH (Figure 4, Paper I: table 5). Samples harvested during the seasons 2006 and 2007 were similar in their concentrations of FaTP, whereas samples from 2008 were at first lower and then higher in the levels of FaTP, in comparison with the seasons of 2006 and 2007 (Table 3).

All carrot traits investigated changed significantly during the growing season (Table 4). In 2006 and 2007 the levels of FaTP among the samples fell between the second and third harvest, but during the season of 2008 there was a constant increase from harvest to harvest (Figure 5, Table 4).

The general pattern concerning the changes in FaTP during the harvest season was expressed by a high, sometimes increasing concentration of FaTP until approximately 110-115 days of growth, followed by a decrease during the latter part (Table 4). If the first harvest was performed when the carrots were very small there was also an initial decrease in the concentration of FaDOH followed by an increase before a final decrease (Paper I: table 2). The changes during the season indicate a correlation between the concentration of FaTP and the carrot root development.

Table 3. Changes in carrot shape and levels of FaTP among unmanured samples at early (inner shape line), medium term (middle shape line and late (outer shape line) harvest in the field trial 2006-2008. Means within the same row followed by different letters are significantly different according to one-way ANOVA with Duncan's post hoc test (P < <0.05)

	2006	2007	2008
Un-manured samples			

Harvest time	Variable	Year 2006	2007	2008
Early (red line)	FaDOH, µg/g DW	547.2 a	560.3 a	211.4 b
	FaDOAc, µg/g DW	53.6 a	54.3 a	20.0 b
	FaOH, µg/g DW	96.8 a	63.3 a	14.8 b
	Total FaTP, µg/g DW	697.7 a	677.9 a	246.3 b
	FaOH/FaTP, %	13.9 a	8.8 b	4.7 b
Medium term (green line)	FaDOH, µg/g DW	554.2 a	653.6 a	516.4 a
	FaDOAc, µg/g DW	62.5 a	61.8 a	19.2 b
	FaOH, µg/g DW	127.5 a	133.3 a	93.5 a
	Total FaTP, µg/g DW	744.2 a	848.8 a	629.1 a
	FaOH/FaTP %	18.2 a	16.2 a	14.5 a
Late (blue line)	FaDOH, µg/g DW	366.2 b	238.9 b	961.8 a
	FaDOAc, µg/g DW	32.4 a	25.9 a	35.1 a
	FaOH, µg/g DW	55.3 b	52.6 b	222.2 a
	Total FaTP, µg/g DW	454.0 b	317.3 b	1219.2 a
	FaOH/FaTP, %	10.3 a	13.2 a	18.0 a

Table 4. Changes during season in means of different carrot traits. Samples from 2005-2008. Means within the same row followed by different letters are significantly different according to one-way ANOVA with Duncan's post hoc test (P < 0.05).

Harvesting time	Early	Medium	Late
n=	131	132	130
Days after sowing, mean	88	111	133
FaDOH, µg/g DW	478 b	556 a	402 b
FaDOAc, µg/g DW	50 a	51 a	33 b
FaOH, µg/g DW	79 c	117 a	103 a
Total FaTP, µg/g DW	607 b	724 a	539 b
OH/FaTP, %	11.2 b	16.1 a	16.6 a
DOH/FaTP, %	80.7 a	77.0 b	76.8 b
Ac/FaTP, %	8.1 a	6.8 b	6.7 b
Weight, g	26 c	60 b	114 a
Length, mm	94 c	104 b	126 a
Maximum thickness, mm	24 c	34 b	43 a
Mean thickness, mm	18 c	26 b	33 a
Compactness, g/cm	2.8 c	5.8 b	9.0 a
Bluntness	0.53 ab	0.56 a	0.51 b
Cylindricity	0.54 b	0.58 a	0.60 a
Conical	2.60 a	2.21 b	2.06 c
Peel %	12.5 a	8.8 b	6.6 c
Hypocotyl %	28.5 a	26.0 b	25.1 b
Fructose, mg/100 gDW	154 a	142 b	131 c
Glucose, mg/100 gDW	202 a	175 b	156 b
Sucrose, mg/100 gDW	85 c	155 b	190 a
Soluble sugar, mg/100 gDW	441 b	471 a	477 a

Samples grown at different sites exhibited significant differences in their content of FaTP (Paper I: table 3) confirming the earlier findings by Kidmose et al. (2004). The differences between locations decreased slightly, but also changed expression, after storage (Paper I: table 3). Earlier studies have reported that carrots grown at more northerly latitudes in Scandinavia tasted sweeter and contained lower concentrations of terpenes, in comparison with samples grown at more southern latitudes (Hård *et al.*, 1977; Rosenfeld *et al.*, 1997; Rosenfeld *et al.*, 1998). In terms of the levels of FaTP in carrots, the two more northerly sites exhibited higher concentrations of all FaTP than a site situated some 400 to 450 km more to the south (Paper I).However, studies at more sites are needed to verify this.

4.4 Storage

The levels of FaTP have been reported to either decrease (Hansen *et al.*, 2003; Ahmad *et al.*, 2011) or to increase (Kidmose *et al.*, 2004) during storage.

The mean of all stored samples exhibited lower concentrations of FaDOH and FaOH compared to the mean of the corresponding fresh samples (Paper I:table 2). When harvested during the medium term period, stored samples exhibited lower values of FaDOH, FaDOAc and FaOH but in late-harvested samples there were higher amounts FaDOAc in the stored carrots, compared to fresh (Paper II).

Most samples low in FaOH at harvest exhibited higher concentrations by the end of storage, whereas samples high in FaOH at harvest exhibited lower concentrations by the end of storage (Paper II: figure 1). The changes in FaOH concentrations in stored samples indicate that a certain level of FaOH is crucial in the carrot root, probably as a part of the protection against external stress factors. These results also indicate the existence of an equilibrium, or critical level, concerning the FaOH concentration regulating the concentrations of FaTP in carrots.

4.5 Cultivar

Earlier reports have shown significant differences between cultivars concerning their content of FaTP (Czepa & Hofmann, 2004; Kidmose *et al.*, 2004; Baranska *et al.*, 2005; Schulz-Witte, 2011). The differences between the two cultivars used here; 'Kämpe' and 'Bolero', were not so distinct. Differences occurred mainly early during the harvest season and during storage (Paper I: table 4). This indicates that although the carrots were grown and stored side by side, there was also a genetic factor involved in the metabolism of FaTP.

4.6 Soil conditions

The field trial provided a gradient in the soil conditions running across the different blocks (Paper IV). This gradient influenced the levels of FaTP, but not root size (Paper IV). Carrots grown in soils generally low in available phosphorus, but high in total phosphorous, exhibited higher levels of FaDOH (Paper IV: figure 2, Paper IV: table 5). Of the three polyacetylenes, FaDOH concentrations were influenced most by changes in soil chemical composition (Paper IV).

The combination of high levels of total phosphorus and low levels of available phosphorus in soil are an indication that the carrots could not solubilize sufficient phosphorus. This might lead to stress reactions in the carrot root. Water stress has been reported to influence the levels of FaTP in carrots

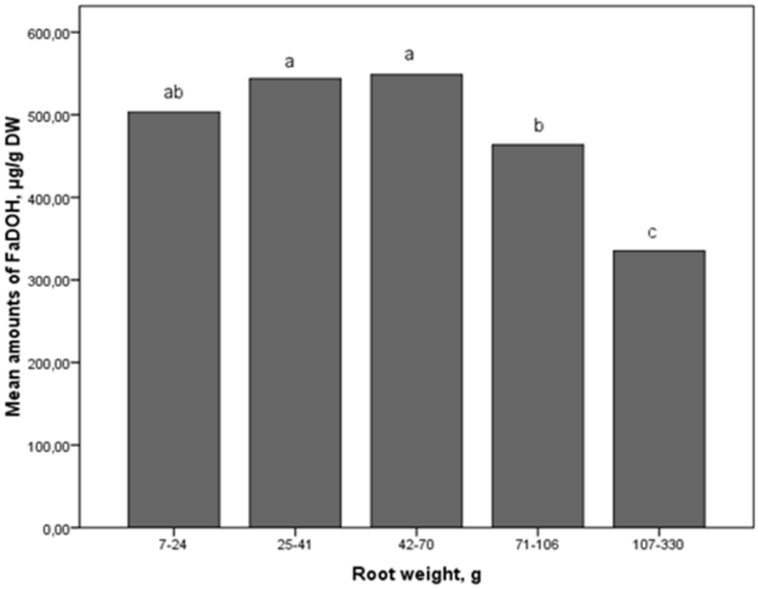
(Lund & White, 1990a; Kramer *et al.*, 2012). The higher levels of FaTP in carrots from some parts of the field trial are perhaps also a reaction on stress. There were lower sucrose/ total sugar ratio values among samples grown in these soils. This is strengthening the impression of a nutrient stress delaying the carrots in their development. The differences in the levels of FaTP can in that case be caused by differences in the pace of the root development.

In organic farming where limited amounts of easily soluble phosphorus are applied, plants must rely more on soil bacteria and arbuscular mycorrhizal fungi for phosphorus solubilization (Harrier & Watson, 2004; Gosling *et al.*, 2006; Ngosong *et al.*, 2010). The concentrations of FaTP in carrots are reported to increase in the presence of fungi (Garrod *et al.*, 1978; Garrod & Lewis, 1979; Garrod & Lewis, 1982; Lewis *et al.*, 1983). The positive correlation observed in Paper IV between the concentrations of FaDOH or FaDOAc in carrots and the levels of total phosphorus in soil might possibly be explained by higher amounts of mycorrhiza surrounding the roots.

4.7 Root size and shape

All variables connected to root size increased during the season (Table 4). The concentrations of FaDOH were strongly and negatively correlated to all parameters connected to carrot root size (Table 1). Also the concentrations of FaDOAc were negatively correlated to root weight and root thickness, but not to root length (Table 1). The concentrations of FaOH and, even more so, the proportion of FaOH, were positively correlated to carrot size (Table 1).

Roots heavier than 70 g, or thicker than 37 mm, exhibited lower concentrations of FaDOH (Table 5, Figure 6). The concentrations of FaDOAc were lower in carrots having a weight higher than 106 g or a maximum thickness larger than 37 mm (Table 5). The concentration of FaOH were higher in roots weighing more than 24 g, and the FaOH/ FaTP ratio increased in parallel with increasing root size (Table 5, Figure 6).



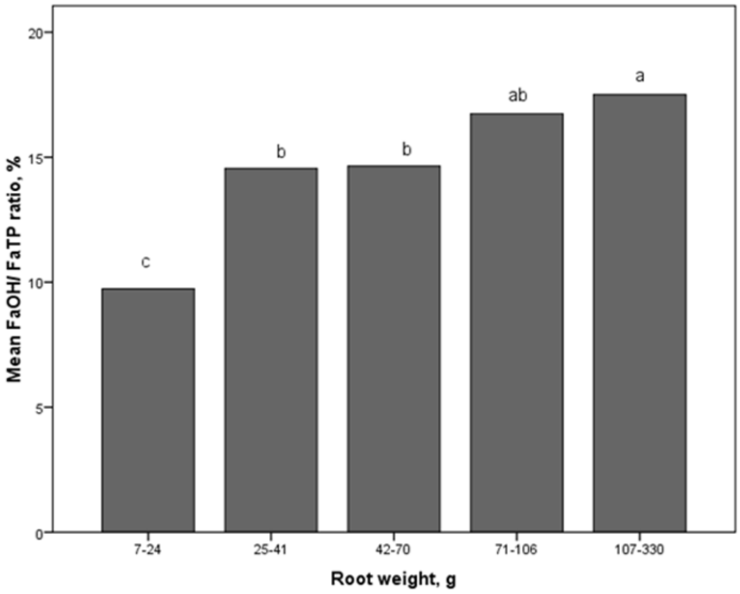


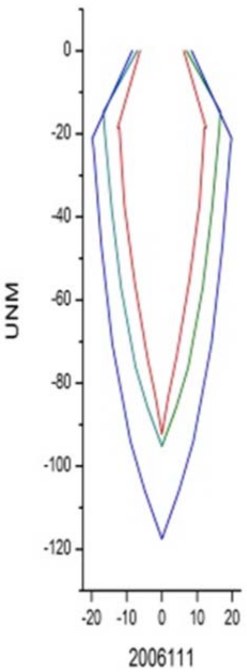
Figure 6. Connections between carrot root weight and concentrations of of FaDOH (upper diagram) and between carrot root weight and the FaOH/ FaTP ratio (lower diagram). Different letter above bars indicate significant differences , according to one-way ANOVA with Duncan's post hoc test (P < 0.05).

Table 5. *Influence of root size on the concentrations of polyacetylenes in carrots. Means within the same section of a column followed by different letters are significantly different according to one-way ANOVA with Duncan's post hoc test (P < 0.05).*

Root trait	Pentiles	N	FaDOH, µg/g DW	FaDOAc, µg/g DW	FaOH, µg/g DW	FaOH/ FaTP %
Weight,	7-24	78	503±214 ab	52±30 a	69±69 b	9,7±5 c
g	25-41	80	544±197 a	52±34 a	110±76 a	14,5±6 b
All harvests	42-70	78	549±216 a	50±30 a	105±60 a	14,6±7 b
	71-106	79	464±281 b	44±32 a	117±103 a	16,7±9 ab
	107-330	78	335±236 c	26±22 b	98±113 a	17,5±10 a
Length,	46-85	79	548±293 ab	38±35 b	102±80 a	12,9±6 bc
mm	86-102	79	564±335 a	54±27 a	104±57 a	14,0±6 bc
All harvests	103-113	79	499±185 ab	48±26 a	82±54 a	12,5±6 c
	114-129	81	480±221 b	49±31 a	106±87 a	15,4±8 b
	129-198	76	300±184 c	34±32 b	106±137 a	18,5±12 a
Maximum	14-25	78	516±232 a	54±32 a	73±85 b	9,7±6 c
thickness	26-30	79	527±154 a	55±27 a	104±71 a	14,3±6 b
mm	31-37	80	545±239 a	47±37 a	111±73 a	15,2±7 ab
All harvests	38-43	79	370±236 b	35±24 b	95±89 ab	16,4±9 ab
	44-62	77	436±292 b	32±26 b	116±112 a	17,6±10 a
Compactness	0.7-2.7	78	559±199 a	58±30 a	85±84 c	10.8±6 b
g/ cm	2.8-4.2	80	494±167 ab	51±23 a	89±55 bc	13.1±6 b
All harvests	4.3-6.6	78	515±254 ab	49±41 a	116±88 ab	16.0±8 a
	6.7-8.7	79	369±221 c	32±24 b	86±82 c	15.7±8 a
	8.8-20.9	78	459±311 b	31±25 b	124±115 a	17.7±10 a

The general shape of the carrots differed more between years than between harvests (Table 3 and Table 4). However, it was difficult to draw general conlusions based on the mean shape of all carrot samples, due to the large variation between harvesting date and growing sites. Decreasing the number of samples to only one harvesting date, one manuring strategy and one growing site made the analysis more feasible. Table 6 shows the connection between the shape of the unmanured carrots in the field trial in 2006 and the corresponding values of various carrot traits. Most variables related to root shape exhibited significant differences in their values between the first and the following two harvests (Table 6). The same pattern was displayed by the concetrations of sucrose (Table 6). Variables connected to root size increased more or less linearly during the season. The total concentrations of FaTP, FaOH and FaDOAc were all significantly different in samples harvested late, in comparison with samples harvested at the medium term period (Table 6). In short: the changes during the season exhibited different patterns. No other type of carrot trait investigated in this thesis showed a pattern completely similar to the pattern among the FaTP (Table 6).

Table 6. Changes in root shape and various traits in unmanured carrots harvested in 2006. Inner shape line represents early, middle shape line medium term and outer shape line late harvest. Table shows the corresponding values of different carrot traits, n=8. Means within the same row followed by different letters are significantly different according to one-way ANOVA with Duncan's post hoc test (P < 0.05).



Carrot shape	Carrot Trait	Early harvest	Medium term	Late harvest
	Weight, g	24 c	50 b	86 a
	Length, mm	92 b	95 b	118 a
	Maximum thickness, mm	25 c	33 b	40 a
	Compactness, g/cm	2.6 c	5,1 b	7.2 a
	Cylindricity	0.51 b	0.57 a	0.58 a
	Conical	3.23 a	2.26 b	2.26 b
	Bluntness	0.48 b	0.56 a	0.56 a
	Peel %	11.6 a	8.5 b	7.2 c
	Hypocotyl %	29.7 a	26.1 b	25.9 b
	Glucose, mg/ 100 g DW	188 a	208 a	172 a
	Fructose, mg/ 100 g DW	147 a	164 a	144 a
	Sucrose, mg/ 100 g DW	46 b	149 a	144 a
	Total sugar mg/ 100 g DW	381 b	520 a	461 ab
	Sucrose/Total sugar %	11.8 b	28.6 a	31.3 a
	FaDOH , µg/g DW	547 a	554 a	366 a
	FaDOAc, µg/g DW	54 ab	63 a	32 b
	FaOH, µg/g DW	97 a	128 a	55 b
	FaTP, µg/g DW	698 a	744 a	454 b
	FaOH/FaTP %	13.9 ab	18.2 a	10.3 b

In general the concentration of FaDOAc decreased with increasing cylindricity values (Table 7). The concentrations of FaDOH and FaDOAc were higher, but the levels of FaOH and the FaOH/FaTP ratio were lower, in carrots with a high proportion of peel (Table 7). Regarding both these connections the root weight also differed significantly between the groups, making it difficult to distinguish whether it was root size or root shape that caused the differences.

Table 7. *Influence of root shape on the concentration of polyacetylenes in carrots. Means within the same section of a column followed by different letters are significantly different according to one-way ANOVA with Duncan's post hoc test (P < 0.05)*

Root trait	Pentiles/ Quartiles	N	FaDOH, µg/g DW	FaDOAc, µg/g DW	FaOH, µg/g DW	FaOH/ FaTP %
Cylindricity,	0.36-0.50	78	545±168 a	57±28 a	83±70 b	11.1±6 c
	0.51-0.55	77	451±196 b	46±25 b	83±50 b	13.8±6 b
All harvests	0.56-0.59	78	420±233 b	44±28 b	114±107 a	18.3±10 a
	0.60-0.65	78	439±238 b	47±41 b	107±95 ab	15.9±7 ab
	0.66-0.81	81	540±321 a	30±25 c	113±100 a	14.0±8 b
Conical shape	1.2-1.7	80	550±185 a	58±27 a	91±71 b	12,.2±6 c
	1.7-2.0	77	497±189 a	50±26 ab	88±60 b	13,.3±7 bc
All harvests	2.0-2.3	84	399±206 b	40±25 c	92±85 b	15,.6±8 ab
	2.3-2.8	87	419±262 b	41±34 bc	110±108 ab	17,.2±10 a
	2.8-4.9	65	555±320 a	33±37 c	123±105 a	14,.5±10 abc
Bluntness	0.21-0.39	78	351±204 b	37±23 b	64±54 b	13.5±7 a
of root tip	0.40-0.45	82	505±199 a	52±27 a	103±91 a	14.3±9 a
	0.46-0.54	79	526±195 a	57±40 a	107±77 a	14.8±6 a
All harvests	0.55-0.74	80	474±230 a	48±30 a	113±108 a	15.9±10 a
	0.75-0.92	74	541±325 a	28±20 b	113±93 a	14.6±10 a
Proportion of	4.1-6.5	78	454±300 b	32±26 c	120±117 a	17.2±10 a
peel	6.6-7.5	79	374±236 c	33±24 c	95±85 ab	16.7±9 a
% of total	7.6-9.5	79	513±247 ab	48±40 b	110±83 ab	15.5±7 ab
volume	9.6-11.6	79	492±176 ab	52±24 ab	91±60 ab	13.1±7 b
All harvests	11.7-23.8	78	564±198 a	58±30 a	84±84 b	10.6±6 c
Proportion of	18.0-23.8	79	514±311 a	35±36 b	101±83 a	13.4±8 bc
hypocotyl	23.9-25.3	79	408±228 b	35±23 b	92±68 a	15.9±7 ab
% of total	25.4-27.1	78	414±231 b	43±31 b	100±85 a	16.6±9 a
volume	27.2-29.9	79	524±215 a	55±30 a	106±86 a	14.3±7 abc
All harvests	30.0-42.9	78	537±181 a	55±28 a	102±114 a	13.0±10 c

The connection between FaTP and root size or root shape is complex. Earlier assumptions that the concentrations of FaDOH decreases as root size increases (Kidmose *et al.*, 2004) has been confirmed, at least among carrots heavier than

70 g. However, up to 70 g the carrot roots increased in weight without any decrease in the FaDOH concentration. The total concentrations of FaTP did not decrease until the roots have reached a weight of 107 g.

The concentration of FaDOH has been reported to be higher in the peel than in the cortex of the carrot root (Czepa & Hofmann, 2004). The decrease in FaDOH concentration has therefore been described as a result attributed to a dilution, since the proportion of peel decreases as the root grow bigger (Kidmose *et al.*, 2004; Kramer *et al.*, 2012). This claim was also confirmed here. However, during the first half of the harvest seasons the proportion of peel decreased without any decrease in the concentrations of FaDOH (Table 6). Further; during the season of 2008 the proportion of peel decreased from 10.5% at the early harvest to 5.6% at the late harvest. During the same period the concentrations of FaDOH increased from 211to 962 µg/g DW (Table 3).

According to Kramer et. al. (2012) differences in the growth rate might influence the concentrations of FaDOH in the root. Paper II also indicated a positive correlation between a relative growth rate in terms of root thickness and FaDOH or FaDOAc concentrations (Paper II: table 4). However, the results from the field trial indicated a negative correlation between the absolute growth rate and the concentrations of FaDOH or FaDOAc (Table 8). This effect was mainly evident in samples from the early part of the growing season. This indicates that the changes in the concentrations of FaTP occurring during the harvest season are too complex to be explained only by differences in growth rate.

Table 8 Pearson correlation coefficients for the relationship between FaTP and growth rate i.e. increase in weight or thickness/ day. Mean of samples from field trials 2006-2008, n=264.

Differences in size between	Growth rate concerning	FaDOH	FaDOAc	FaOH	FaTP	FaOH/ FaTP	FaDOH/ FaTP	FaDOAc/ FaTP
Early and medium term harvests	Weight	-0.22**	-0.23**	0.03	-0.15*	0.23**	-0.16*	-0.18**
	Thickness	-0.26**	-0.26**	0.07	-0.19**	0.30**	-0.22**	-0.20**
Medium term and late harvests	Weight	0.08	-0.02	-0.01	-0.03	-0.07	0.10	-0.13
	Thickness	-0.12	-0.13	0.02	-0.03	0.10	-0.09	-0.04

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Growth rate calculated as mean increase in carrot weight g/day, or in root thickness mm/day, between two harvests

When carrot size increase, especially in thickness, the outer epidermis ruptures (Rubatzsky *et al.*, 1999). These wounds on the root surface might be part of the explanation to why the concentrations of FaDOH initially increase as the carrots grow bigger.

Table 9. *Influence of soluble sugars on the concentrations of FaTP in carrots. Means within the same section of a column followed by different letters are significantly different according to one-way ANOVA with Duncan's post hoc test (P < <0.05).*

Root trait	Pentiles/ Quartiles	N	FaDOH, µg/g DW	FaDOAc, µg/g DW	FaOH, µg/g DW	FaOH/ FaTP %
Glucose,	17-144	78	388±270 d	28±20 d	79±75 b	14.4±9 ab
	145-163	79	434±238 cd	39±25 c	97±77 ab	15.7±7 a
All harvests	164-181	79	489±235 bc	44±25 bc	112±100 a	15.8±10 a
	182-212	79	518±205 ab	52±36 ab	108±82 ab	15.0±7 ab
	213-324	78	567±225 a	61±36 a	103±102 ab	12.4±8 b
Fructose	67-118	78	398±289 c	32±23 c	79±72 b	14.1±9 a
	119-134	79	455±229 bc	40±25 bc	88±81 ab	14.3±7 a
All harvests	135-145	79	494±227 b	45±36 b	106±79 ab	15.0±7 a
	146-169	79	479±213 b	46±26 b	113±99 a	16.0±9 a
	170-245	78	570±221 a	61±37 a	115±116 a	13.8±9 a
Sucrose	23-79	78	480±204 ab	46±24 a	84±65 b	12.5±6 b
	80-121	79	514±232 a	44±31 a	83±52 b	12.3±6 b
	122-159	79	520±270 a	47±29 a	99±71 b	14.0±6 b
All harvests	160-190	79	460±264 ab	39±26 a	96±76 b	14.8±8 b
	191-385	78	422±230 b	46±42 a	139±139 a	19.5±12 a
Sucrose/	5-17.5	78	522±189 a	51±24 a	88±62 ab	12.6±5 cd
Total sugar	17.6-27.9	79	490±234 a	46±29 a	78±54 b	12.0±6 d
%	28.0-34.1	79	524±219 a	49±32 a	114±91 a	14.9±7 bc
	34.2-40.5	79	490±274 a	41±32 ab	110±93 a	15.7±9 ab
	40.6-61.8	78	368±258 b	35±35 b	110±120 a	17.9±11 a

4.8 Soluble sugars

The concentration of hexoses decreased and that of sucrose increased during the harvest season (Table 4). The concentrations of FaDOH and, even more, the concentrations of FaDOAc were positively correlated to the concentrations of hexoses, whereas FaOH was positively correlated to the concentrations of sucrose (Table 2). The PCA plot also revealed a close relation between FaOH and the total concentration of sugars, on the one hand, and between the concentrations of FaDOH or FaDOAc and the concentrations of hexoses, on the other hand (Figure 2).

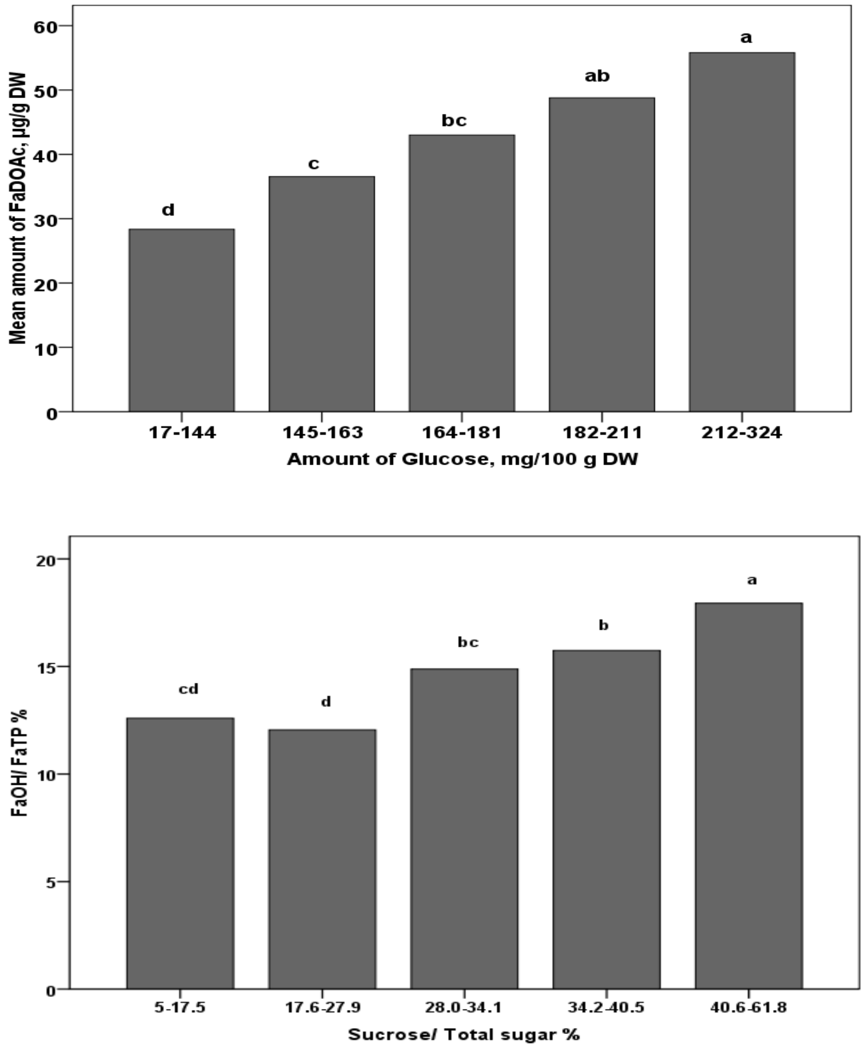


Figure 7. Relationship between concentration of glucose in carrot samples and concentration of FaDOAc (upper diagram) and between sucrose/total sugar ratio and FaOH/ FaTP ratio (lower diagram) Different letters above bars indicate significant differences according to one-way ANOVA with Duncan's post hoc test (P < <0.05)

The concentrations of both FaDOH and FaDOAc were high in samples high in hexoses (Table 9), especially glucose (Figure 7). The highest concentrations of FaOH and also the highest FaOH/ FaTP ratio were found in samples with sucrose content higher than 191 mg/ 100 g DW (Table 9). In carrots with a sucrose/total sugar ratio above 40 % the levels of FaDOH and FaDOAc were low but the levels of FaOH and the FaOH/ FaTP ratio were high (Table 9, Figure 7). Among stored samples the concentrations of all FaTP, especially that of FaDOAc, were negatively correlated with the concentrations of all types of soluble sugar (Paper II: table 4) and there was a linear, inverse relationship between the total concentration of FaTP and the total concentration of sugars (Paper II: figure 2).

There are at least two different ways to explain the connection between FaTP and soluble sugars: i) The chemical changes of FaOH to FaDOH and then further to FaDOAc needs energy. High levels of glucose provide this. ii) The chemical changes of FaOH to FaDOH and then further to FaDOAc occur in synchronicity with the breakdown of sucrose to hexoses. This will be discussed further in paragraph 4.11.

4.9 Extract decomposition

A carrot extract with a low decomposition rate exhibited higher concentrations of FaTP, in comparison with extracts from samples with a high rate (Figure 8).

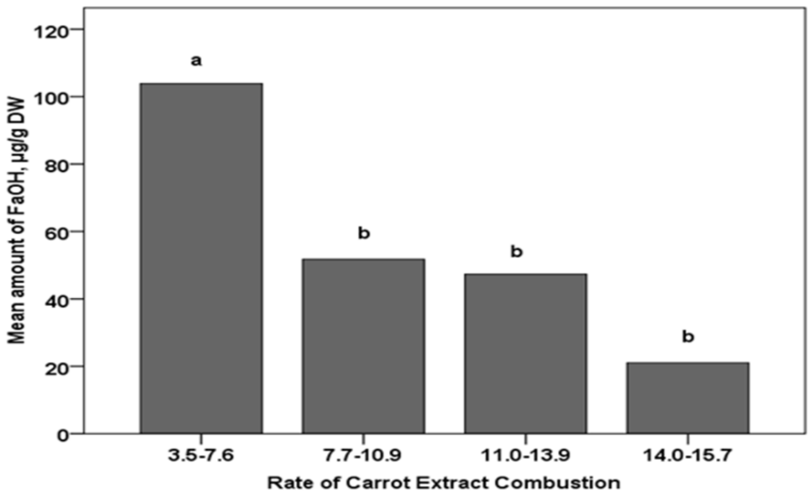


Figure 8. Connections between the rates of extract decomposition and the concentrations of FaOH. Different letters above each bar indicate significant differences according to one-way ANOVA with Duncan's post hoc test (P < <0.05)

High levels of FaTP in an extract may according to this be assumed to slow down the decomposition. The decomposition of the extract is enzymatic at first and then becomes microbial. Regarding the reported antimicrobial traits of the FaTP (Garrod *et al.*, 1979; Olsson & Svensson, 1996; Zidorn *et al.*, 2005; Dawid *et al.*, 2015) it is possible that these compounds can contribute to the prevention against decomposition. According to concepts sometimes used within the organic agriculture, low scores concerning extract decomposition rate can be interpreted as a sign of a high level of structure (Kusche *et al.*, 2010) or of "integrity" (Kahl *et.al.*, 2012), or as a high resistance to some stressing factors. If so, high levels of FaTP can be assumed to be a reaction on stress, *i.e.* a sign of the carrot ability to resist external stressing factors. However, this need to be tested also using other, more precise methods.

4.10 Farming system and manures

Earlier reports have not found any differences in the concentrations of FaTP between carrot samples from organic or conventional farming systems (Söltoft *et al.*, 2010; Kramer *et al.*, 2012; Bach *et al.*, 2015). On analyzing the results obtained in Paper III, the differences between different organic manuring systems were found to be smaller than the variation between harvest years and harvest occasions. One reason to this was probably the grass-clover ley included in the crop rotation system (Paper III). The presence of ley in the crop rotation has been reported crucial when comparing farming systems. As an example the difference in quality of potato samples from conventional and biodynamic farming systems is reported to be significantly smaller, both between systems and within the same system, when ley is included in the crop rotation than when it was not (Kjellenberg & Granstedt, 2015).

Seasonal fluctuations in FaTP were more pronounced in unmanured carrots and in samples manured with fresh or composted manure than in carrots fertilized with pelleted chicken manure (Paper III: table 3, Figure 9).

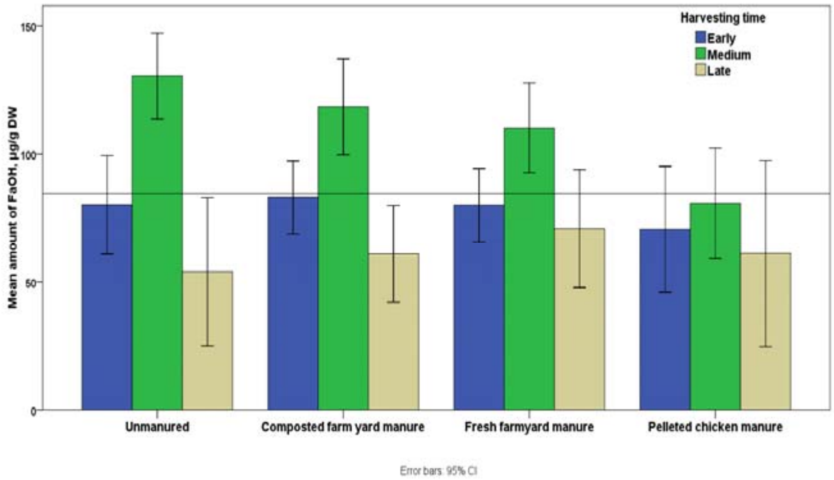


Figure 9. Concentrations of FaOH in carrots depending on type of manure and harvesting time. Samples from the field trial 2006 and 2007.Reference line indicates overall mean, error bar show 95 % confidence interval.

The results in Paper III suggest that manuring organic carrots with compost may be the most beneficial strategy regarding most carrot traits, at least in systems where fertilizer is applied only once per crop rotation, whether directly to the carrot crop or in the preceding crop.

The results in Paper III also suggests, that although there are no differences between treatments when comparing them at the final harvest, there might be significant differences earlier during the season.

4.11 Different approaches to explain the variation in FaTP in carrots

The interactions between FaTP and various factors are complex and also revealed differences between the three FaTP. Some of the variation could be explained by the fact that FaOH is the precursor to both FaDOH and FaDOAc. Some other differences were correlated instead to the differences between the FaTP concerning their spatial distribution in the carrot root. Other possible approaches to explain the variations in FaTP are root size, root development, enzymatic processes or reactions on stress. An overview on some of these approaches may shed some light upon the variation in the levels of FaTP and perhaps raise some new questions for the future.

FaTP and root development

According to the general linear models the number of days after sowing (DAS) was the single most important factor when trying to explain the variation of the FaTP in carrots (Table 2). Regardless of set of samples, *i.e.* originating from different manuring regimes, or from different years or locations, the concentrations of FaTP peaked approximately 110 to 115 DAS. One factor contributing to this similarity in rhythm was the fact that the same cultivar was used in all sets of samples. The peak 110-115 DAS was expressed in the carrots regardless of sowing or harvesting date. Even the exceptional seasonal changes during 2008 can be interpreted as an expression of this rhythm. Due to bad weather conditions that year the carrots could not be sown until the middle of June. Thus, though the levels of FaTP in the carrots increased steadily during the whole season the late harvest was conducted at only 111 DAS.

Among the FaTP it was the concentrations of FaOH that exhibited the most consistent seasonal rhythm while the concentration of FaDOH was more influenced also by the variation between harvesting years and cultivation site. This indicates that it is the concentrations of FaOH that has the strongest connection to the genetic traits of the carrot root.

There appears to be a critical change in carrot development around 110-115 DAS. The rhythm expressed by the concentrations of FaTP differed from most seasonal variations observed for other substances in carrot (Paper I). Changes in the respiration rate have been ascribed a similar, but inversed, seasonal change (Weichmann & Käppel, 1977). To confirm a correlation between FaTP concentrations and the respiration rate more detailed studies on the annual variation are needed.

For the practical farmer it would be convenient if the concentrations of FaTP corresponded to certain traits of root shape. However, none of the parameters of carrot shape tested here showed a similar pattern of change to the FaTP during the growing season. Thus the results presented in this thesis do not provide the farmer any further clue on how to determine the status of the FaTP in the carrots just by looking at root size or shape.

FaTP and enzymatic processes

The enzymatic processes associated with the formation of the different FaTP are not completely defined (Dawid *et al.*, 2015). Fungal responsive fatty acid acetylenases have been suggested important in inducing the metabolism of oleic acid to crepenynic and dehydrocrepenynic acids (Cahoon *et al.*, 2003). However, carrots are not known to accumulate relevant concentrations of crepenynic and dehydrocrepenynic acids (Dawid *et al.*, 2015). This indicates that FaOH is rapidly synthesized from its precursors. A description of the enzymatic and genetic background to the metabolism of FaTP is necessary to explain the variation of these compounds in carrot roots. While waiting for such a description parallels to other substances may help clarifying the picture. According to the results presented in Paper II there was a strong correlation in carrots between the composition of polyacetylenes and the composition of sugars among both fresh and stored samples. The connection found between sucrose and falcarinol accumulation indicates that the enzymes transforming falcarinol into falcarindiol may be active under similar conditions as the enzymes converting sucrose to hexoses (Paper II).

FaTP and stress

Stress is the most common explanation cited when discussing the variation in the concentrations of FaTP. The ability of a crop to resist stress conditions can be seen as the first reactive step in a chain of changes occurring under stress. According to the results presented here high levels of FaTP, especially FaOH and FaDOH, can be seen as indicator of the first resistance reaction to stress.

The results provided by Lund and White (1990) and Kramer *et. al* (2012) indicate that the total concentrations of the three major FaTP decreases when the carrots are subjected to prevailing water stress. Further analyze of the results from Lund and White (1990) indicates that the FaDOH/ FaTP ratio does not change, but the FaOH/ FaTP ratio decreases, and the FaDOAc/ FaTP ratio increases, as the carrots are grown in excess soil water conditions.

The results from Lund and White (1986) also show that in carrot stressed by drought over a long period neither FaDOH nor FaOH can be found, but both are present in the unstressed controls. At the same time the concentrations of FaDOAc and the concentrations of five other FaTP increased in carrots stressed over a long period (Lund & White, 1986). Similar results have not been found elsewhere, but recent papers have mostly focused on the three major FaTP.

Based on the results presented in this thesis a speculative description on how the FaTP are influenced by stress might be as follows: The biosynthesis of FaTP begins with the formation of FaOH. This synthesis appears mainly to be determined by genetic factors. When stimulated by some stressing factor the oxidation of FaOH to FaDOH then follows. As FaOH is converted to FaDOH the biosynthesis of FaOH continues at first. Depending on cultivar, root size and the strength of stress the increase in the concentrations of FaOH and FaDOH proceeds at different rates and also reaches different levels. It would seem likely that the total concentrations of the three major FaTP increases initially, as a first protective reaction to stress. If the stressing conditions persist, FaDOH is broken down, but the biosynthesis of FaOH cannot keep the levels of FaTP unchanged. At first the levels of FaOH decreases, followed by the levels of FaDOH. If the concentrations of FaOH and FaDOH gradually decreases during heavy stress this might explain why some of the samples, *e.g.* carrots from the early harvest in 2008, exhibited very low concentrations of one or both these substances.

FaTP and weather conditions

Only one previous study has examined weather conditions in connection with the FaTP. It showed that higher global radiation and a higher temperature sum display a positive correlation with increased levels of all FaTP (Bach *et al.*, 2015).

The relation between weather factors and levels of FaTP has not been analyzed in detail in any of the papers published in connection to this thesis. However, the samples from the compiled dataset exhibited some correlations to weather variables (Table 10).

Table10. Pearson correlation coefficients for the relationship between FaTP and various weather variables, n=37

Weather variable	FaDOH	FaDOAc	FaOH	Total FaTP	OH/ FaTP	DOH/ FaTP	Ac/ FaTP
Sum of precipitation during season	0.13	0.21	0.60**	0.36*	0.54**	-0.54**	-0.08
Sum of temperature during season	-0.14	-0.10	0.04	-0.08	0.28	-0.27	-0.11
Sum of precipitation during the 14 days before harvest	-0.01	0.09	-0.17	-0.07	-0.50**	0.44**	0.30
Sum of temperature during the 14 days before harvest	0.01	0.05	-0.10	-0.03	-0.37*	0.30	0.32

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

It was mainly the sum of precipitation that had a correlation to the levels of FaTP. Interestingly, higher precipitation during the whole season exhibited the opposite correlation to the proportion of FaOH and FaDOH, compared with the amount of precipitation within a fortnight before harvest. Heavy rain shortly before harvest seemed to increase the proportion of FaDOH and decrease the proportion of FAOH without any change in the levels of FaTP. This can be seen as a sign of stress. More rain during the whole season was correlated to the opposite change in the proportion of FaDOH and FaOH, but at the same time positively correlated to an increase in the FaOH concentrations. This may be interpreted as a sign of less stressing conditions among the carrot samples also indicating that the amount of precipitation during the project period was below the optimal. However, the connections to the weather were not consistent and the results are given here more as indications.

FaTP critical levels and source sink relations

The changes in the concentrations of all three FaTP during storage seemed to be dependent on the concentrations of FaOH at harvest (Paper II). This indicated the existence of a critical level of in the FaOH concentrations below which the synthesis of FaOH is triggered. Being the precursor to both FaDOH and FaDOAc the biosynthesis of FaOH sets the limits to the abundance of the two other FaTP, while external factors ultimately modify the expression of FaTP in the carrot roots.

Source-sink relations have been used to describe the distribution of assimilates between different parts of the carrot plant (Wareing & Patrick, 1975; Benjamin & Wren, 1978; Hole *et al.*, 1984; Ehness *et al.*, 1997; Roitsch, 1999; Roitsch *et al.*, 2000). Hypothetically, a source sink relation could be used to describe also the relation between the FaTP. FaOH would first act as a sink in relation to its precursors and then as a source for FaDOH or FaDOAc. The relative strength of these relations would then determine the composition of the FaTP. Under certain external conditions the sink strength of FaDOH would increase causing at first a decrease in the FaOH concentrations. This in turn might lead to an increase in the sink strength of FaOH causing the concentrations of FaOH to increase again.

4.12 Reflections on carrot off-taste and the optimal composition of FaTP

Is there a correlation between the FaTP and bitter off-taste in carrots also with regards to fresh samples? Is it possible to optimize carrot cultivation with regards to the content of the FaTP? These are two of the questions that have not found a clear answer in this thesis. However, the large number of samples collected here gives some hints, concerning these questions, which may be helpful for the future.

FaDOH has been reported to contribute to bitter off-tastes in carrots (Czepa and Hoffmann, 2003, Kreutzmann *et al.*, 2008). However, in this investigation, the highest values of FaDOH concentrations were found in small carrots harvested early. Such carrots are generally considered to be sweet in taste (Rosenfeld, 2003). In Paper I three different plausible explanations are presented to this apparent contradiction:

First, young carrots generally have a higher proportion of fructose (Goris, 1969), that is a sugar with a sweeter taste, which may conceal the bitter taste.

Second, some compounds, such as terpenes, have been reported to correlate to bitterness in carrots and to increase during the latter part of the harvest season (Rosenfeld, 2003). Also 6-methoxi-mellein has been reported correlated to bitter taste in carrots (Seljåsen, 2000). The interaction of several compounds may be necessary to experience bitter taste in fresh carrots.

Third, FaDOH may be in a more inactive form, as far as human perception of bitter taste is concerned, during the early-harvest period. Before the final stage of their synthesis FaTP have been found to be conjugated with glycerolipids (Minto and Blacklock, 2008). The chemical extraction method used here, might be more effective than the human gustatory system to separate FaDOH from its surroundings at the final stages of its synthesis.

No regular sensory test was performed as part of this thesis. However, during the preparation of the samples from the field trial, the taste of each of the samples was noted with regard to bitter off tastes. This resulted in two groups of samples: 59 samples categorized as giving a bitter off taste, and 306 samples without this trait. Comparing these two groups with regards to their levels of FaTP indicated a slight increase in the concentrations of mainly FaDOH among samples notified as having a bitter off-taste. This is shown in Figure 10.

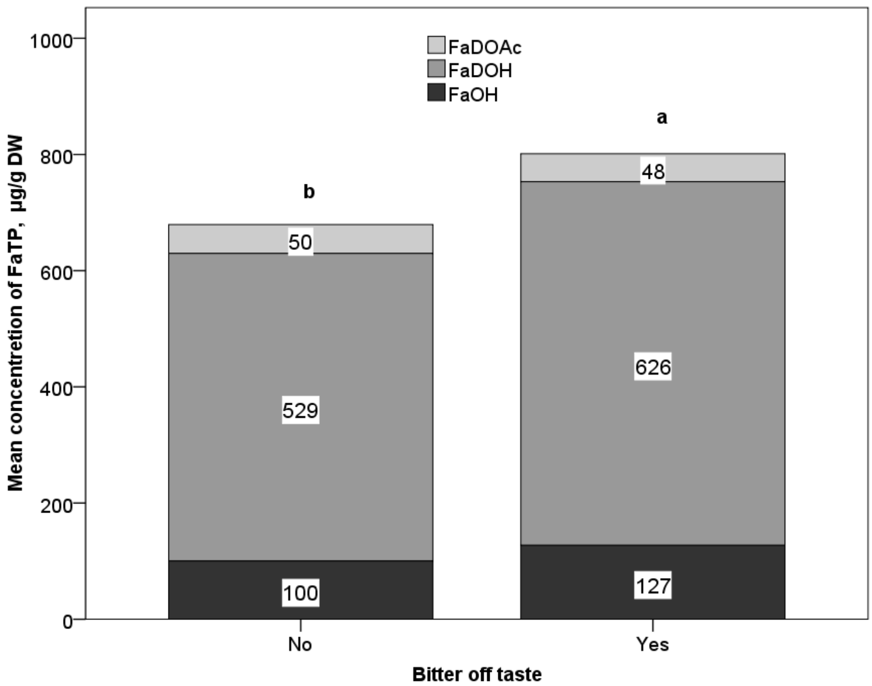


Figure 10. Levels of the three different FaTP in carrot samples from the field trials 2006-2008, classified as not bitter (No), n=306 or bitter (Yes), n=59. Different letters above bars indicate a significant difference in relation to the other bar, according to one-way ANOVA with Duncan's post hoc test (P < <0.05).

Applying current knowledge on carrots, FaDOH concentrations should be held low and the concentration and proportion of FaOH should be high. Such circumstances occurred sometimes among the samples in this thesis, *e.g.* at the harvest on the 3rd of September 2005. The profile of the carrot traits among the samples harvested at that date is illustrated in Figure 11.

Beside the concentrations of FaOH, also the values of sucrose and precipitation during the season were high on that occasion, while the percentage of peel was low. This might serve as a first, rough, indication of how to optimize the composition of the FaTP in carrots.

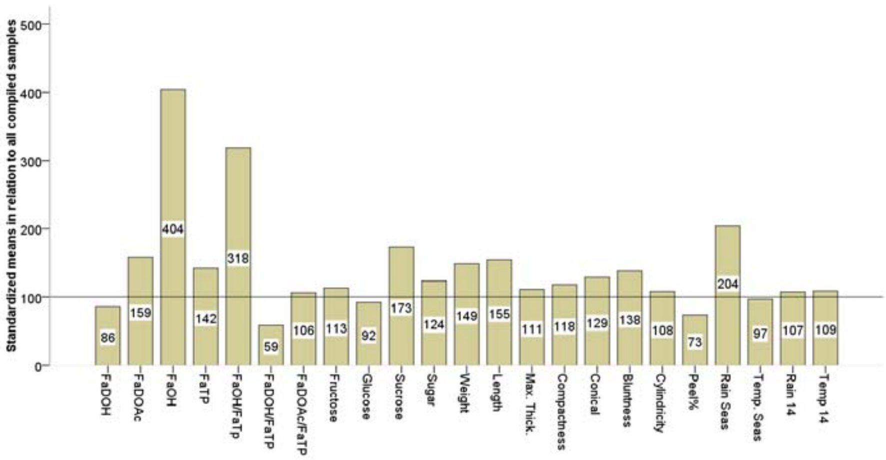


Figure 11. Profile of carrot traits harvested on the 3rd of September 2005. Values are standardized in relation to overall mean of all samples 2005-2008. Rain 14= Precipitation within a fortnight before harvest, Temp 14= Sum of degree-day within a fortnight before harvest. Reference line= overall mean

5 Conclusions

- Following analysis of 465 different carrot samples, from 77 different plots, harvested on 17 different occasions during the period 2005-2008, it was possible to draw the following conclusions:
- No single factor was found to explain all of the variation in the levels of falcarinol-type polyacetylenes (FaTP). Regular changes occurring during root development, here expressed as the number of days of cultivation, were probably the major cause to the concentration of falcarinol (FaOH). It therefore seems likely that the biosynthesis of FaOH is correlated mainly to genetic factors. However, the FaOH concentration could occasionally increase if the carrots were grown under stressful conditions.
- The concentration of falcarindiol (FaDOH) exhibited a pattern of variation more dependent on external factors, such as inter-annular variation and soil conditions.
- The concentration of falcarindiol-3acetate (FaDOAc) was more dependent on internal factors, such as the levels of hexoses and root shape or root size *i.e.* root length. Among the three falcarinol-type polyacetylenes (FaTP), the concentration of FaDOAc was most strongly, and positively, correlated with the concentration of glucose.
- Factors such as harvesting year, growing site, stress and the duration of the growing season had a stronger impact on the concentrations of FaTP than for example manuring strategy.
- There were significant differences between the harvesting seasons regarding the levels of FaTP. The causes to these differences could not be clarified. In order to investigate this further a more thorough documentation considering weather factors, soil conditions, attack of pests and cultivation measures is necessary.

- The levels of FaTP in the cultivar used exhibited a seasonal rhythm that was more or less the same from year to year. The total levels of FaTP increased until 110-115 days after sowing and then decreased during the latter part of the season. In order to achieve a better understanding of the causes of this rhythm the changes in several other substances during the season need to be recorded. Starting the harvesting season earlier and performing more harvests during the season would also give a better resolution when recording the rhythm. Using different cultivars in the comparison would also increase the understanding of the foundation for this rhythm.
- Carrots grown at different locations exhibited significant differences in the levels of FaTP. The reasons for this could not be explained within the framework of this thesis. The results indicated higher levels of FaTP in carrots grown at more northerly latitudes. Carrot samples grown under similar conditions at a larger number of locations are needed to verify this indication.
- The levels of FaTP increased initially as the roots grew bigger. However, roots heavier than 70 g exhibited lower levels of FaDOH and roots heavier than 107 g also had lower total concentrations of FaTP.
- It was not possible to assign a specific root shape as an indicator of the composition of FaTP in the carrot samples.
- The variation in the concentrations of FaOH indicated the existence of a critical level below which the synthesis of FaOH was triggered.
- There was a correlation between sucrose and falcarinol accumulation indicating that the enzymes transforming FaOH into FaDOH may be active under similar conditions as the enzymes converting sucrose to hexoses. A more detailed description of the enzymatic and genetic background to the metabolism of FaTP is necessary to comprehend the variation of these compounds in carrot roots.
- The total concentration of FaTP was positively correlated to factors stressing the carrots *i.e.* to stressingful conditions during growth and storage and to low availability of soil phosphorous. In carrot extract with a low decomposition rate the concentrations of FaTP was high. The increased concentrations of FaTP appeared to be a reaction to stressingful conditions, i.e. a way of resisting the stress caused mostly by external factors.
- The type and dose of different organic manures did only occasionally have an influence on the FaTP concentrations.

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