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SUGAR AND B-CAROTENE ACCUMULATION IN CARROT (*DAUCUSCAROTA* L.) TAP ROOTS AS INFLUENCED BY FERTILIZATION AND BIO-STIMULANT APPLICATION UNDER GREENHOUSE CONDITIONS

Sekoli M.M.S*, Pretorius J.C and Coetzer G.M.

National University of Lesotho, Faculty of Agriculture, Department of Crop Science, P. O. Roma, 180. Lesotho.

ABSTRACT: *Greenhouse study was carried out to evaluate β-carotene and sugar accumulation in carrot tap roots, at harvest, after treatment with different fertilizer levels either separately or in combination with two commercial bio-stimulants, ComCat® and Kelpak®. Four fertilizer levels included standard recommended NPK level (100%) as well as 50%, 25% and 0% of standard. Foliar application of bio-stimulants was according to recommendations by the manufacturers. Treatments were replicated five times in complete randomized block design. Translocation of sugar was followed by labelling leaves with U-14C-glucose. Both β-carotene and sucrose content increased more or less linearly with increasing fertilization, but most significant increase was observed when carrots were treated with combination of ComCat® at 50% of standard fertilizer rate. In the case of sucrose this was in concert with a significant increase in the translocation of radio-activity from the leaves to the roots where ComCat® was applied in combination with higher fertilizer regimes. Glucose and fructose levels fluctuated rather inconsistently. Kelpak® had no effect on β-carotene content and had inhibitory effect on sucrose content.*

KEYWORDS: Carrot, β-Carotene, Sugar, Bio-Stimulants, Fertilizer Levels, U-14C-Glucose.

INTRODUCTION

In carrots glucose, fructose and sucrose are major sugars and their availability in relatively high amounts leads to the characteristic flavour of carrots (Talcott and Howard, 1999). According to the authors, carrot sweetness and overall consumer preference are enhanced by the presence of sugars and diminished volatiles. High terpenoid content, exceeding 35 to 40 ppm, masks the perception of sugars and imparts a harsh, burning turpentine-like flavour in carrots (Kleemann and Florkowski, 2003).

The total sugar content of fresh carrot ranges from 3% to 10%, with sucrose being most abundant followed by glucose and fructose, while soluble sugars make up 30% to 70% of the dry weight of storage roots (Rodriguez-Sevilla *et al.*, 1999; Cazor*et al.*, 2006). The ratio of sucrose to non-reducing sugars, glucose and fructose, increases with carrot maturity, but this varies between cultivars and is also influenced by environmental conditions (Suojala, 2000). Attempts to categorize biochemical or physiological maturity of carrot based on sugar content and the ratio of sucrose to hexoses have been done with little success. Although this has led to the observation that sucrose accumulation in carrot roots increases up to harvest, the brix reading or total sugar content does not seem to be a good indicator of optimal harvest stage or horticultural maturity (Cazor *et al*., 2006).

The production of sugar, its transport, utilization and storage are continually changing and are closely associated with the physiology of cells, environmental conditions and the stage of plant

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development (Sheen *et al*., 1999; Gupta, 2006). Carbon partitioning is largely controlled by the availability of sucrose and differences in sucrose content between the source and sink (Graham and Martin, 2000; Rolland *et al*., 2002). Leaves play a pivotal role as source of carbohydrate by ensuring an abundance of sucrose and constant carbon flow to sink organs when normal photosynthesis applies. One of the most common methods of studying carbon partitioning involves the use of sugar isotopes and then measuring the amount of radio-activity in various organs (Pritchard and Amthor, 2005).

Besides sugars, a second quality characteristic of carrots is its inherent ability to produce rather large quantities of β-carotene. Among vegetables, carrots rank as one of the highest providers of β-carotene, a precursor of vitamin A, which is associated with protective effects against human diseases (Rao and Rao, 2007). Of the six types of carotenes and related compounds in carrots, α- and β-carotene are most abundant (Alasalvar *et al*., 2001). β-carotene continues to play an important health role in the provision of vitamin A especially in the developing world (Bendich, 2004). Moreover, β-carotene confers diverse functions and actions in protection against cancers and other health benefits including antiulcer, anti-aging, increased immune response and antioxidant properties (Russell, 2004; Rao and Rao, 2007). In addition to their health benefits, carotenes function as auxiliary chromophores in photosynthesis and as photo protective agents in cell membranes. Carotenoids also function as attractants, warning and disguise compounds in the animal and plant kingdoms. In carrots carotenoids are responsible for the characteristic yellow colour.

Carrots cultivated in the greenhouse under different fertilizer regimes were used in this study. The response of carrots to the potential simulated fertilizer stress condition, either separately or in combination with two commercial bio-stimulants, ComCat® and Kelpak®, was followed. Kelpak® and ComCat® are two natural products with bio-stimulatory properties, manufactured from wild plants that have been commercialized in the past decade. Kelpak® is derived from cold water sea kelp and contains auxins, cytokinins, gibberellins, amino acids, vitamins and nutrients (Arthur *et al*., 2004; Stirk *et al*,. 2014). Foliar application of Kelpak® to agricultural crops is claimed to stimulate root development leading to improved nutrient and water uptake (Ferreira and Lourens, 2002). The action mechanisms of active compounds contained in Kelpak®, mainly natural plant hormones involved in plant growth, are well documented and widely applied in the horticultural and agricultural industries. In addition to the beneficial effects on rooting of crops, the commercial kelp extract Kelpak® is also claimed to reduce parasite infection (Robertson-Andersson *et al*., 2006).

ComCat® is derived from a combination of plant materials including brassinosteroid containing extracts from the seeds of *Lychnis viscaria* (Pretorius and van der Watt, 2008; Schnabl *et al*., 2001). ComCat® has also been reported to enhance root growth leading to efficient utilization of available nutrients and to induce resistance in crops towards abiotic and biotic stress conditions as well as to stimulate the production of sugars and inherently yield (Agraforum GMBH, 2016). The principal active compounds contained in ComCat®, brassinosteroids, belongs to a new generation of phytohormones discovered approximately 25 years ago (Roth *et al*., 2000) and is currently not widely applied in practical farming practices. ComCat® is most probably the first or one of the first brassinosteroid containing natural products to have been commercialized in recent times. The use of brassinosteroids in increasing yield and increased tolerance to biotic and abiotic stress (Bishop, 2003; Nakashita *et al*., 2003) and their ecological friendliness (Khripach *et al*., 2000) make them ideal for use in agriculture and horticulture.

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In view of the potential ascribed to the above two bio-stimulants, both were included in this study in combination with different fertilizer levels ranging from zero to 25%, 50% and 100% of the recommended NPK rate for South Africa. The response of carrots in terms of sugar translocation from the source (leaves) to the sink (roots) as well as final sugar and β-carotene content in tap roots were quantified at harvest. Sugar translocation was followed by labelling seedling leaves at the 8-leaf growth stage with U-^{14}C-glucose and measuring radio-activity in tap roots four weeks later. The main aim of this study was to determine appropriate fertilization rates for carrot cultivation in combination with the above two bio-stimulants.

MATERIALS AND METHODS

Materials

Seeds of a pre-pack carrot cultivar, Karina, were purchased from a local merchant. Two commercially available bio-stimulants ComCat® and Kelpak® were supplied by a German company, Agraforum GMBH. The following laboratory grade chemicals were from Sigma (Germany): potassium from potassium chloride (KCl), nitrogen from ammonium nitrate (NH_4NO_3) and phosphorus from phosphoric acid (H_3PO_4). U-^{14}C-glucose as well as ACS-II scintillation cocktail were obtained from Amersham, International. The sugar test kit (catalogue No.10716260035) was purchased from Boehringer Mannheim, Germany. The All-*trans*-β-carotene used as HPLC standard was from Sigma-Aldrich. All other chemicals were of the highest purity available.

Methods

Experimental design and treatments

Pot trials were conducted in the greenhouse at the University of the Free State, South Africa, in the Bloemfontein district (29°01'00"S, 26°08'50"E).A complete randomized block design with five replicates was used.

Soil collection and preparation

Top soil of the fine sandy loam Bainsvlei form (Soil Classification Working Group, 1991) was collected from the West campus experimental site of the University of the Free State. The soil was dried at room temperature, sieved through a 5 mm mesh sieve and used for growing carrots in pots under greenhouse conditions. The fertility status of the soil collected in both seasons was, in general, excellent according to local guidelines as indicated in Table 1 (FSSA, 2007).

Table 1: Physical and chemical properties of the topsoil used in pot trials

Parameter	Norms	Soil
Clay & Silt %		20
Sand %		80
Class		Sandy loam
EC (mSm^{-1})	0 – 300	48
SAR	< 5	0
pH (KCl)	5.5 – 6.5	4.63

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Ca	300-3000	566.04
Mg $_{(NH\ 4\ OAc)}$	50-300	163.19
K $_{(NH\ 4\ OAc)}$	80-250	176.53
Na	100-500	1.04
P $_{(Olsen)}$	5-10	9.26
Zn $_{(HCl)}$	2-5	0.17

Planting procedure

Carrot seed (cv. Karina) was hand-sewn thinly in three rows, 8 cm apart, in pots. A split application of nitrogen and potassium, where applicable, was done with half the fertilizer amount applied prior to sowing and the balance applied six weeks after sowing. Ammonium nitrate (NH_4NO_3) was used as nitrogen source, potassium chloride (KCl) as potassium and phosphoric acid (H_3PO_4) as phosphorus sources. Calculations for appropriate fertilizer levels (100%, 50%, 25% and 0%) were made based on a pot with a diameter of 35 cm and a depth of 35 cm. The standard fertilizer rate for carrot (100%) was calculated on carrots withdrawal rates of 95 kg N, 15 kg P and 125 kg K. In all cases the fertilizer was dissolved in distilled water and 50 ml of the fertilizer solution was applied per pot to appropriate treatments one day prior to sowing. The same application method was used with the second half of fertilizer at six weeks after sowing.

Thinning was done one week after germination to achieve an in-row spacing of 4.25 cm and an average of eight plants per row. The temperature in the glasshouse was maintained between 15-20^{0}C during the day and 9-15^{0}C at night and soil moisture was kept at field capacity. Daily irrigation was done to maintain field capacity. Recommended cultural management norms were followed for the control of pests and diseases.

Treatments

At the three to four leaf stage, corresponding to growth stage 13 (Meier, 1997), ComCat® and Kelpak® were applied as foliar sprays to designated pots at the rate of 100 g ha^{-1} and 2 L ha^{-1} respectively, according to the recommendations of the manufacturers. The volume applied, based on a spray mixture application of 400 L ha^{-1}, was approximately 9.6 ml per pot for each bio-stimulant. A second application of the bio-stimulants, at the same rate as the first application, was done at the 7 to 8 leaf stage (growth stage 18; Meier, 1997), approximately 3 weeks after the first application. Control plants only received fertilizer at the four different levels.

Sugar content measurement

Extraction of sugars from carrot roots

Extraction of sucrose, D-glucose and D-fructose from carrot root samples was performed according to a modified method outlined in the Boehringer Mannheim catalogue, No.10716260035. Two g aliquots were removed from the middle section of carrot roots, transferred to separate test tubes and covered with five ml 80% ethanol g^{-1} FW. Subsequently, the tissue was boiled in a Labconwaterbath (LabDesign, R.S.A.) set at 80^{o}C for five minutes in order to stop all chemical reactions. The ethanol that evaporated during boiling was replaced to the original volume after cooling. Subsequently, the material was homogenized for one minute at full speed in a CAT X 620 homogenizer (Germany). Two ml aliquots of the

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homogenized tissue was quantitatively transferred to separate clean Eppendorff vials and centrifuged at 12 000 rpm for 10 minutes in a Selecta Centrolit centrifuge.

After centrifugation, one ml of the supernatant was transferred to clean Eppendorf vials and transferred to an oven set at 70^0C in order to get rid of the ethanol solution that might later interfere with the enzymatic method of determining sugar content in solid tissue. The ethanol was replaced with distilled water after drying of the samples. From each replicate 50 µl aliquots were removed and sucrose, D-glucose and D-fructose levels were enzymatically determined using Boehringer Mannheim (Germany) test kits. The directions of the suppliers (Boehringer Mannheim/R-Biopharm) were followed and the sugar content calculated by means of the following equation:

$$c = \frac{V \times MW}{\epsilon \times d \times v \times 1000} X \quad \Delta A \ g \ \ell^{-1}$$

Where:c = concentration; V = final volume (ml); v = sample volume (ml); MW =molecular weight of the substance to be assayed (g mol^{-1}); d = light path (cm); ϵ =extinction coefficient of NADPH at 340 nm (= 6.3). Sucrose, D-glucose and D-fructose content was expressed as µmol g^{-1} fresh weight.

Radioactive Labelling

At the 8-leaf growth stage (stage 18-19; Meier, 1997) 15 ml of U-^{14}C-glucose per replicate, with specific activity 16.7 µCi or 0.617 MBq $mmol^{-1}$, was sprayed on leaves of carrot plants. The isotope solution was prepared by dissolving 8.0 ml of isotope (1.6 mCi = 1600 µCi) in 192 ml distilled water yielding a concentration of 8 µCi per millilitre. A hand sprayer (Merck TLC sprayer, Germany) was used to deliver a fine spray of the isotope. The application was timed to ensure uniform and equal amounts of the solution per pot. The number of carrots per pot was counted and recorded for subsequent use in calculation of the amount of isotope sprayed per plant. The remaining isotope solution was frozen for later use, as a standard, in the calculation of disintegrations per minute (DPM's) on sprayed plants.

Extraction of radio-active labelled samples

Each carrot root was divided into three cross-sections: top (close to the crown/shoulder), middle and bottom. A two g sample of the carrot root cortex (phloem) from each of the three sections was thinly diced and placed in a test tube. Ten ml of 80% ethanol (5 ml ethanol per gram fresh material) was added to the sample and brought to boiling point for 2 minutes in a Labcon waterbath (LabDesign, R. S. A.) set at 80°C. The ethanol that evaporated during boiling was replaced to the original level after cooling. The material was homogenized (CAT X 620, Germany) for one minute at full speed. Ethanol was again added up to the original level, thoroughly mixed and the material allowed to settle. A 1.5 ml aliquot of supernatant of each carrot section was separately transferred to marked Eppendorff vials and centrifuged at 12 000 rpm for 10 minutes in a Selecta Centrolit centrifuge.

After centrifugation, one ml of the supernatant was transferred to other marked Eppendorf vials and the supernatant slowly dried in an oven at 70°C. After the drying process, one ml of distilled water was added to the dry material and vigorously mixed. A 500 µl aliquot of each

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sample was transferred to marked scintillation vials and 4 ml of ACS-II scintillation cocktail was added and mixed thoroughly. The material was allowed to settle for 24 hours prior to reading DPM's. The DPM's of the original isotope solution used to label plants were read from a solution made up of 5 µl radioactive glucose and 4 ml ACS-II scintillation cocktail.

Subsequent to removing the supernatant, the carrot pellets were blotted dry to remove excess supernatant. A 0.2 g aliquot of the carrot pellet was measured, placed in a scintillation vial and 4 ml of ACS-II scintillation cocktail was added and mixed thoroughly. A Beckham LS 6500 scintillation counter was used for measurement of DPM's in the supernatant and the carrot pellet. Calibration of the scintillation counter for quenching was done previously by using a series of carbon tetrachloride standards to set up calibration curve in conjunction with the internal standard of the scintillation counter. The DPM counts for the supernatant and for the pellets of carrot roots and the original solution were read and pooled. The values were used to determine the total amount of isotope absorbed by the carrot plants as well as the amount that remained in the leaves, the amount translocated to the roots and the amount lost to the atmosphere.

Previously frozen carrot leaves were thoroughly rinsed in running water to remove any unabsorbed isotope on the surface of the leaves. The leaves were blotted dry and a two g sample of as many leaves as possible was weighed. The same procedure as for extraction of carrot root was used and similarly DPM counts were read for the leaf supernatant and pellet. Radio-activity was expressed as a percentage of the absorbed isotope.

β-carotene extraction and measurement

Carotene extraction and measurements were done from carrot samples collected at harvest. The carotene extraction method was adapted from Sadler *et al*., 1990. A two gram root sample from each treatment was weighed and dissected into small pieces. The samples were placed in separate test tubes covered with aluminium foil to protect against light. Six ml 100% ethanol was added to the carrot sample and homogenized for 5 minutes. The homogenate was transferred to glass bottles covered with foil. Six ml acetone and 12 ml hexane were added to constitute the ratio of 50 : 25 : 25 (hexane **:** acetone **:** ethanol) and the bottles were agitated vigorously for 10 minutes on a mechanical shaker. Subsequently, 15 ml distilled water was added to each bottle and shaken for an additional 5 minutes. The mixture was transferred to a foil-wrapped separating funnel and allowed to settle and separate into polar and non-polar layers.

The non-polar hexane layer containing carotene was at the top. A 500 µl aliquot was removed from the hexane layer ensuring exclusion of light and transferred to a foil covered Eppendorf vial in readiness for carotene reading. The procedure was repeated for all samples.

β-carotene levels were measured with a Shimadzu HPLC system equipped with a SPD 20AV detector and LC 20 AT pump (Shimadzu Corporation, Japan). A C30 analytical column (Acclaim; 5 µm; 4.6 x 150 mm) was used with Acetonitrile: Methanol as mobile phase. The latter was applied in a gradient (0-20 min: Acetonitrile 25-15% : Methanol 75-35%; 25 min: Acetonitrile 15% : Methanol 35%; 25-30 min: Acetonitrile 25% : Methanol 75%) at a flow rate of 1 ml min^{-1}. *Trans*-β-carotene (≥ 97%; Fluka) was used as external standard at a concentration of 240 mg ℓ^{-1}. Five µl was injected and absorbance read at 475 nm. β-carotene content was calculated from the measured peak area of the standard.

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Statistical analysis of data

Data was analysed using the NCSS 2000 statistical package for identification of differences in the treatments. The Tukey Kramer least significant difference (LSD; $P<0.05$) procedure was used for separation of treatment means (Steele and Torrie. 1980). Significant differences are highlighted within figures and tables in the results section.

RESULTS

Sugar content in tap roots

In terms of glucose content no significant differences between treatments were observed at any of the fertilizer levels at harvest (Figure 1A). The fructose content, however, was significantly decreased at the 0% and significantly enhanced at the 100% fertilizer level by the addition of Kelpak® (Figure 1B). ComCat® had no significant effect on the fructose level in tap roots at any of the fertilizer levels.

The most marked differences in tap root sugar content were observed for the disaccharide sucrose (Figure 1C) at harvest. ComCat® treatments tended to markedly, but not significantly, increase the sucrose level in two (25 and 100%) of the four fertilizer levels at harvest. Alternatively, Kelpak® treatments markedly, but not significantly, decreased the sucrose content in two (25 and 50%) of the four fertilizer regimes. At the 25% fertilizer level, the amount of sucrose in the Kelpak® treated roots was significantly lower than that of the ComCat® treatment ones.

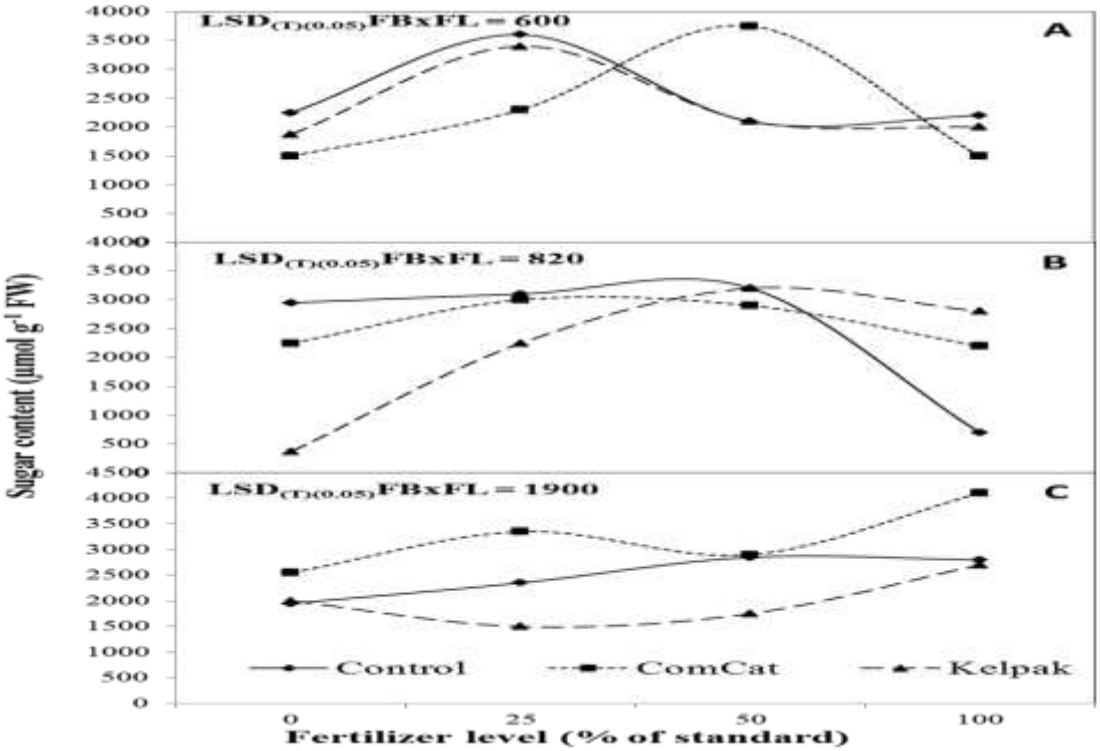

Figure 1: Effect of fertilizer, applied at different levels, either separate or in combination with two commercial bio-stimulants, ComCat® and Kelpak®, on sugar content in carrot tap roots at harvest. A) = glucose, B) = fructose and C) = sucrose.

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Translocation of radio-active label from leaves (source) to roots (sink)

Where fertilizer was applied on its own, radio-activity decreased in leaves at the same rate as it increased in the roots (sink) as the fertilizer level was increased in increments (0%, 25%, 50% and 100% of the standard recommended rate (Figure 2A). At the standard fertilizer level, the amount of radio-activity calculated in the leaves and roots was similar and settled between 40-50% of the absorbed isotope.

Radio-active ^{14}C-label partitioning between leaves and roots were different for the bio-stimulant-fertilizer combination treatments than that for the fertilizer only treatments (Figure 2B & C). The application of ComCat® had no effect at the zero fertilizer level but, as the fertilizer level was increased in increments and applied in combination with ComCat®, radio-active label translocation from leaves to roots was accelerated markedly (Figure 2B). In combination with 50% of the standard fertilizer more ^{14}C-label had already been translocated to the roots than what remained in the leaves.

Kelpak® treatment had the same enhancing effect on radio-active label partitioning between leaves and roots as the ComCat® treatment, when fertilizer was increased from zero to 25% of the standard (Figure 2C). More of the radio-active label was still found in large quantities in leaves and less in roots at the 100% fertilizer level in Kelpak® treated carrots.

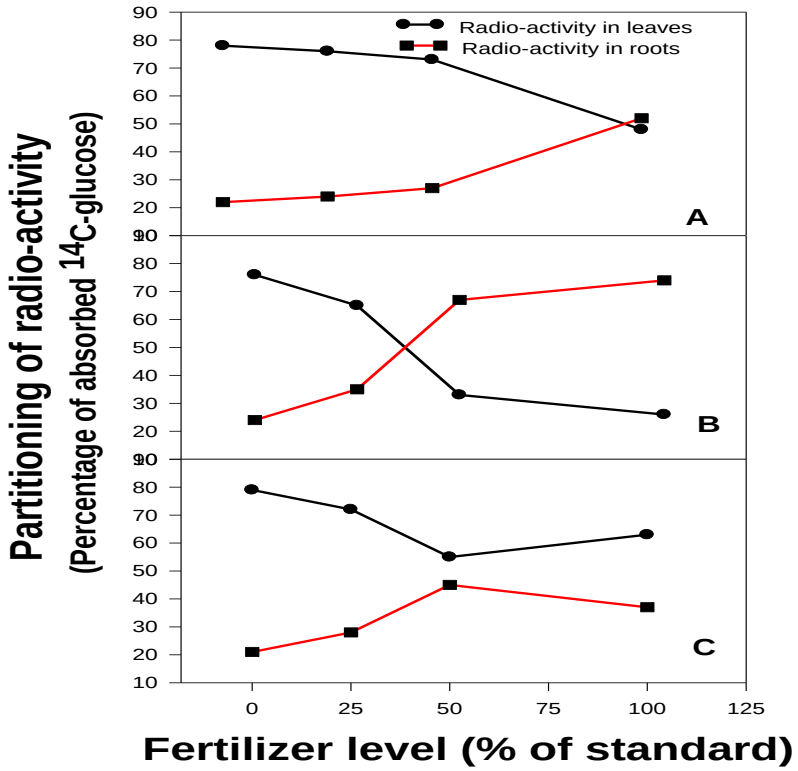

Figure 2: Effect of fertilizer applied at different levels, either separate or in combination with two commercial bio-stimulants, ComCat® and Kelpak®, on radio-activity partitioning in carrots four weeks after labelling leaves with U-^{14}C-Glucose at the 8-leaf growth stage under greenhouse conditions. A) = fertilizer only, B) = ComCat® treated and C) = Kelpak® treated.

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β-Carotene content in carrot roots

There was no effect on β-carotene accumulation in carrot tap roots as the fertilizer application was elevated in increments (Figure 3). Application of ComCat® in combination with all of the elevated fertilizer levels produced carrots with significantly (Figure 3) higher β-carotene content than the control. A combination of ComCat® with 50% of the standard fertilizer contributed to the highest β-carotene accumulation compared to all of the other treatments, including the fertilizer only controls. There was no significant difference in β-carotene accumulation between the fertilizer only controls and the Kelpak® treated carrots except in the case where Kelpak® was applied in the absence of fertilizer where a significant reduction in β-carotene content was observed.

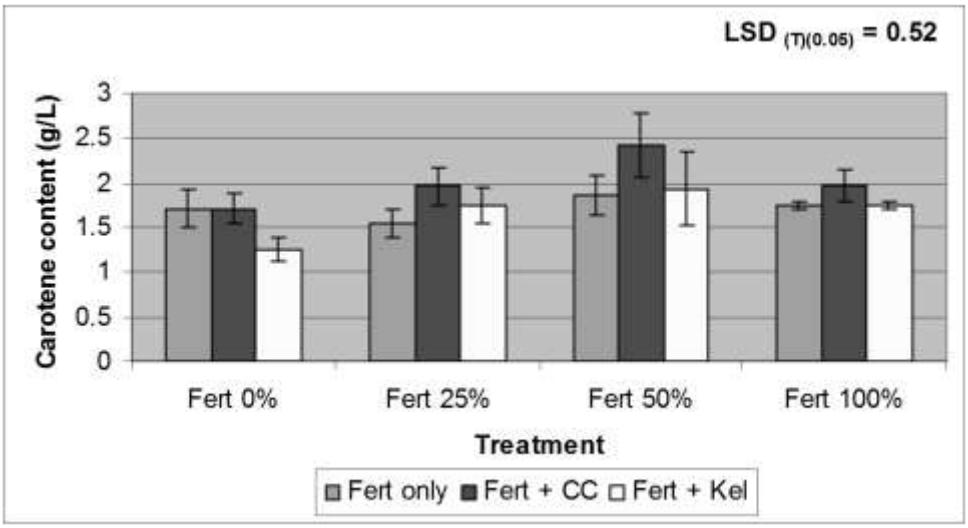

Figure 3: Effect of fertilizer and bio-stimulants applied at different levels, either separate or in combination with two commercial bio-stimulants, ComCat® and Kelpak®, on β-carotene content (g ℓ^{-1}) in carrot tap roots at harvest.

DISCUSSION

The study indicated that β-carotene (a terpene) content was significantly increased by elevated fertilizer levels and the highest content (2.42 mg g^{-1} FW) was measured where 50% of the standard fertilizer was applied in combination with ComCat®. ComCat® contributed to higher β-carotene levels in combination with all fertilizer levels while the application of Kelpak® alone had an inhibitory effect on carotene content accumulation and contributed to the lowest carotene content measured (1.25 mg g^{-1} FW). The results are in agreement with that of Ali *et al*. (2003) and Hochmuth *et al*. (1999). In the study by Hochmuth *et al*. (1999) carotene concentration increased as fertilizer level increased and the highest carotene content (55 mg kg^{-1} FW) was recorded with 160 kg ha^{-1} nitrogen fertilization. Ali *et al*. (2003) reported enhanced carotene content of 21.85 mg g^{-1} FW at 250 kg ha^{-1} potassium fertilization as opposed to 9.45 mg g^{-1} FW where no potassium fertilizer was applied.

In contrast to earlier findings that carotene content increased with increased fertilization (Hochmuth *et al.* (1999), the authors found no enhancing effect of potassium fertilization between 0 and 188 kg ha^{-1} in a follow-up study seven years later (Hochmuth *et al*., 2006). They

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concluded that there might have been sufficient soil potassium to achieve high carrot yield and quality without the additional amount. In the present study, the carotene content reached the highest level at half the recommended fertilizer level indicating that high carotene concentration could be achieved under lower fertilizer application levels. Thus, application of 50% of the recommended standard fertilizer seems to ensure acceptable carotene content, a quality parameter in carrot.

Concerning carrot tap root sugar content, sucrose is the form in which carbohydrate is translocated from the leaves to the roots where it is partially stored and partially hydrolyzed to the two monosaccharide forms, glucose and fructose, that are metabolized via standard metabolic pathways (Krook *et al*., 2000). Because of the latter, it is difficult to follow sucrose transport and how it is metabolized if only sugar content is measured in plant tissue. For this reason, carrot leaves were labelled with U-^{14}C-glucose at the 8-leaf growth stage and radio-activity measured in both the leaves (source) and the roots (sink) four weeks later at harvest. Radio-activity was expressed as a percentage of the original isotope that was absorbed by the leaves. The objective with this approach was to ascertain whether the differences in sugar content observed at different fertilizer levels, separate or in combination with two different bio-stimulants, corresponded with sugar translocation to the tap roots of carrots.

In the present study, compared to the non-fertilized control, the sucrose content tended to increase linearly with increased fertilization while the monosaccharide sugar levels showed the opposite trend at harvest. This tendency for sucrose content to increase and D-glucose and D-fructose levels to decrease as maturity approached corresponded with the findings of Suojala (2000) and Korolev *et al.* (2000a) in carrot. Especially the work of Korolev *et al.* (2000a, b) showed a predominance of glucose and fructose 30 to 50 days after seed germination while sucrose levels increased substantially from 50 days after germination to harvest. The build-up of sucrose as the carrot root matured indicates that the utilization of sucrose, the carbohydrate source, declined at the latter stages of development.

Compared to the fertilizer only and Kelpak® treatments, the application of ComCat® in combination with varying levels of fertilizer increased the sucrose level markedly at harvest but, especially at the standard fertilizer level. This indicates that either more sucrose eventually accumulated in the final sink (carrot tap roots) due to elevated translocation from the leaves under the influence of ComCat® or less sucrose was utilized during the latter stages of development. Accelerated radio-activity partitioning to the roots in ComCat® treated plants four weeks after spraying carrot leaves with U-^{14}C-glucose, especially at the higher fertilizer levels and compared to the fertilizer only control, strongly suggests that ComCat® had an enhancing effect on sucrose translocation. This is in agreement with the claim made by the manufacturers (www.agraforum-gmbh.com) that ComCat® has an energizing effect on plant membranes leading to accelerated translocation of carbohydrate from source to sink. Although Kelpak® showed the same tendency to accelerate radio-active partitioning to the roots at the higher fertilizer regimes, slightly less ended up in the roots at that stage of development, compared to the ComCat® treatment. Although there were sometimes differences in amounts of labeled^{14}C recovered in root pellet and root supernatant, at the three carrot areas investigated, they were mostly insignificant.

At any specific stage of a plant's development all three forms, i.e. sucrose, glucose and fructose, will be present in the storage tissue of carrot tap roots. Either the conversion of glucose and fructose to sucrose or the hydrolysis of sucrose to the monosaccharide forms depends on the biochemical requirements at a specific stage and this is finely regulated in plants (Krook *et al.*,

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2000). Although it is, therefore, difficult to use only sucrose content data to follow sucrose utilization in sinks, quantification of D-glucose and D-fructose levels can give an indication of a trend to convert sucrose to its monosaccharide forms at a specific stage of development.

At harvest, glucose and fructose levels showed a decreasing trend with increased fertilization and this was in agreement with the findings of Ali *et al*. (2003) who reported the highest reducing sugar level from treatments that received no nitrogen and potassium fertilization. Fertilization with the highest levels of nitrogen (200 kg ha^{-1}) and potassium (250 kg ha^{-1}) produced carrots with the lowest reducing sugar content. The results of Ali *et al*. (2003) as well as of the present study were contrary to the report by Schaller and Schnitzler (2000) who indicated that lower application rates of nitrogen fertilizer led to higher content of sucrose and essential oils whereas the content of glucose and fructose were lower. However, in the latter case, the application of high rates of nitrogen led to increased glucose and fructose and lower concentration of sucrose.

Kaack *et al*. (2001) reported that fructose, glucose and sucrose levels were not enhanced by application of mineral nitrogen at fertilizer levels ranging from 22 to 162 kg ha^{-1}. The variation of sugar content under varying fertilizer types and levels has also been discussed in other reports and, in general, this is partly explained by the inherent varietal genotype as well as soil and environmental factors prevailing during carrot growth (Rosenfeld *et al*., 2000; Suojala, 2000; Nakagawa *et al*., 2003). According to Nilsson (1987), sucrose accumulation in carrots continued up to the final harvest when carrots are regarded to be mature. The author maintained that, at maturity, it is accepted that metabolic activity has declined to the extent that less sucrose is converted to its monosaccharide forms leading to an accumulation of sucrose in the tap root.

Despite the necessity to measure sucrose, glucose and fructose levels separately, interpretation of the results can be problematic. In this regard addition of all the measured sugar values to obtain a total sugar content value might be a way to circumvent this complexity (Suojala, 2000). However, the author warned that the total sugar content in carrot roots is influenced by prevailing growth conditions and this has to be considered in any interpretation. In the study of Suojala (2000), the total sugar content in carrot roots was higher during the colder compared to warmer growing seasons. In the present study a difference in the total sugar content was also observed in different morphological parts of the carrot tap root (results not shown). The highest total sugar content was found in the section of the root closest to the crown and the lowest amount of sugars was in the lower part of the root next to the tip. This was in agreement with the radio-active labelling study confirming that sugar partitioning is also an important factor to keep in mind when sugar content data is interpreted.

CONCLUSION

The study indicated that foliar treatment of carrots with ComCat® improved carbohydrate partitioning to the harvestable parts while also being beneficial to β-carotene accumulation even at fertilizer levels lower than the recommended standard. However, the ability to accumulate carotenoids differs depending on the cultivar as well as varying planting dates (Hochmuth *et al.,* 2006) and application of commercial bio-stimulants will have to be verified using different carrot cultivars. Further research of the effect of ComCat® and Kelpak® on plant growth specially in combination with fertilization need to be carried out to better explain

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the influence of genotype and environmental conditions on carotene, sugar accumulation and photosynthate translocation to carrot roots

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