GROWTH, YIELD AND PHYSIOLOGICAL RESPONSE OF CARROT (*Daucus carota* L.) TO DIFFERENT FERTILIZER LEVELS AND BIO-STIMULANTS

BY

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DEDICATION

This dissertation is dedicated to my loving husband Bruno Tšeliso Sekoli for the patience of caring for the family in my absence and the encouragement he gave for the duration of the study. A special thanks to my daughter who was supportive throughout the period and viewed this as a source of encouragement for her to do better.

DECLARATION

I declare that the dissertation submitted by me for the degree Doctor of Philosophy at the University of the Free State, South Africa is my own independent work and has not previously been submitted by me to another University. I furthermore concede copyright of the dissertation in favour of the University of the Free State.

Signed in Bloemfontein, South Africa

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Sebina Magdalena 'Mabataung Sekoli

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CHAPTER 1

INTRODUCTION AND RATIONALE FOR THE STUDY

Carrot, *Daucus carota* L., belongs to the family Apiaceae (Umbelliferae) that includes other vegetables such as celery, celeriac, parsley and other herbs. Carrot is a cool season crop that is utilized for the edible taproot and is adapted to temperate climates (Rubatzky *et al*., 1999). The growth of carrot is slow with the taproot quality and yield being adversely affected by weed competition and poor agronomic practices. Carrots are one of the major contributors to world vegetable trade and are utilized in fresh market and processing industries. The area under carrot production in South Africa was 4 000 thousand hectares in 2003 that produced an average yield of 24.5 ton ha^{-1} (FAO, 2003). In the mean time yields have been increased substantially in South Africa but, yields obtained in neighbouring countries are still lower than the 2003 average for South Africa on account of, amongst others, low fertilizer inputs.

Healthy eating guidelines being advocated throughout the world have popularized the consumption of fresh fruit and vegetables. Amongst vegetables, carrot consumption is popular due to the pleasant flavour and health benefits accruing from the presence of carotenes, vitamins, minerals and fibre. Carotenoids are synthesized and stored in the photosynthetic apparatus of higher plants and amongst their varied functions they are also involved in the plant light harvesting system and in antioxidant defence mechanisms against photo-oxidative damage by quenching free radicals produced during photosynthesis and respiratory metabolism (Stahl and Sies 1999).

Six types of carotenes and related compounds are found in carrots. The principal carotenoids in carrots include α-carotene (3140 μg 100 g^{-1}) and β-carotene (9700 μg 100 g^{-1}). However, other carotenoids such as lutein (220 μg 100 g^{-1}) and γ-carotene are found in minute quantities. The most abundant pro-vitamin A precursor is β-carotene (Rubatzky *et al*., 1999; Marx *et al*., 2000), often exceeding 50% of the vitamin A content. The consumption of fresh and processed carrots provides a major source of vitamin A for an increasing number of people worldwide. Recommending increased consumption of fruit,

yellow and green vegetables that contain carotenoids is one of the strategies followed by governments in an attempt to decrease micronutrient deficiencies in humans, especially in economically disadvantaged communities (Takyi, 1999; Faber *et al*., 2002; van Jaarsveld *et al*., 2005). Epidemiological studies have indicated that human consumption of foods rich in carotenoids and anti-oxidants lead to diminished risk against cardiovascular diseases and some forms of cancer (Heinrich *et al*., 2003; Rissanen *et al*., 2003).

Vitamin A deficiency is one of the major public health problems especially in developing countries that can be reduced via carrot consumption. Besides vitamin A deficiency being one of the major nutritional diseases among young children and the leading cause of child blindness, deficiency increases the risk of other diseases and even death from severe infections. In pregnant women, deficiency in vitamin A leads to night blindness and increased risk of maternal mortality (Christian *et al*., 2001).

Despite the nutritional importance of carrots, production levels in small-holdings are stagnant or declining. Some of the major problems in carrot production are sporadic and delayed seedling emergence leading to low population and resultant root yield (Lada *et al*., 2004). Further, despite the fact that application of NPK fertilizer at recommended rates ensures successful production of carrots, yields are often low especially in many developing countries. The main reason is indisputably of an economic nature as subsistence farmers simply cannot afford inorganic fertilizer at the recommended rates and at the current prices. As a result the application of fertilizer varies from no application at all in certain regions to the application of sub-optimal levels in other areas. This supplied the rationale to follow the response of carrot to different fertilizer levels, ranging from 0% to 25%, 50% and 100% of the recommended rate in South Africa

However, other interventions to counteract stagnant or declining yields in developing countries, probably to circumvent fertilizer practices, include the use of crude extracts from wild plants that have either plant growth stimulatory, pesticidal or herbicidal activity (Arthur *et al*., 2003; Cespedes *et al*., 2004; Ganapaty *et al*., 2004; Chon and Kim, 2004). Indigenous knowledge on wild plants with these characteristics has been transferred from generation to generation in these countries. However, over the past two decades many

researchers from a number of developed countries embarked on the screening of wild plants for their potential antimicrobial, pesticidal, herbicidal and bio-stimulatory properties (Dwivedi and Shukla, 2000; Nteso and Pretorius, 2006). In developed countries the use of plant extracts or secondary plant metabolites as alternatives to synthetic products for improving crop production was given impetus by consumer pressure to implement farming systems that are environmentally friendly. Other advantages of plant extracts include their short life span in the environment and low toxicity to fauna.

Plant secondary metabolites are synthesized by higher plants. These compounds do not seem to have a recognized role in primary plant processes but are important in the interaction of the plant with the environment (Vardhini and Rao, 2003). Secondary metabolites have been extensively studied over the past two decades and have been ascribed many functions including their allelopathic effect on other plants (Economou *et al*., 2002; Fukuhara *et al*., 2004), their herbicidal (Chon and Kim, 2004), insecticidal (Abou-Fakhr Hammad *et al*., 2000; Cespedes *et al*., 2004; Ganapaty *et al*., 2004), antimicrobial (Dutt *et al*., 2000; Pretorius *et al*., 2003; Salvat *et al*., 2004) and bio-stimulatory properties (Roussos *et al*., 2002; Arthur *et al*., 2003; Lin *et al*., 2004).

Two natural products with bio-stimulatory properties, manufactured from wild plants, have been commercialized in the past decade namely Kelpak® and ComCat®. Kelpak® is derived from cold water sea kelp and contains auxins, cytokinins, gibberellins, amino acids, vitamins and nutrients (Arthur *et al*., 2003; Arthur *et al*., 2004). Foliar application of Kelpak® to agricultural crops is claimed to stimulate root development leading to improved nutrient and water uptake (Ferreira and Lourens, 2002). 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*. 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, 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. However, the principal active compound contained in ComCat®, brassinosteroids, belongs to a new generation of phytohormones discovered approximately 20 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.

In view of the potential ascribed to the above two bio-stimulants in terms of their ability to enhance root growth and nutrient uptake, possibly leading to increased yields, as well as the low cost compared to standard fertilizer recommendations, 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 main aim of the study was to determine appropriate fertilization rates for carrot cultivation in combination with the above two bio-stimulants. The objectives included determination of:

- the growth and yield response of carrot to different fertilizer levels, both separately and in combination with two commercially available bio-stimulants, under greenhouse conditions over two seasons (Chapter 3),
- the growth and yield response of carrot to different fertilizer levels, both separately and in combination with two commercially available bio-stimulants, under field conditions over two seasons (Chapter 4),
- β-Carotene and sugar accumulation in as well as sucrose translocation to carrot tap roots as a response to treatment with different fertilizer levels separately and in combination with commercial bio-stimulants under field conditions over one season, and

- the respiratory response of carrot tap roots, including *in vitro* activities of regulatory enzymes of the glycolytic and oxidative pentose phosphate pathways to treatment with different fertilizer levels separately and in combination with commercial bio-stimulants under field conditions.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Many people in developing countries manage to survive on cereal staples and consumption of animal products is often low, thus predisposing such communities to low intakes of vitamin A. The meals are often not diverse and sometimes lack the appropriate quantity of nutrients. This socially and economically disadvantaged stratum of society is prone to a wide range of micronutrient deficiencies as a result of poor dietary quality and inadequate intake (Ramakrishnan and Huffman, 2001). Animal products contain high quantities of readily absorbable retinol and plants, particularly fruits and vegetables, contain relatively large amounts of provitamin A carotenoids which are converted by the body to vitamin A. Improvement of vitamin A status is however higher with intake of preformed vitamin A or purified β-carotene in comparison to intake of β-carotene from fruits and vegetables.

Recent estimates indicate that over two billion people are micronutrient malnourished in relation to vitamin A, iodine and / or iron. Other micronutrient deficiencies of public health concern are zinc, folate and vitamin B. The highest prevalence of micronutrient deficiency is in south East Asia and sub-Saharan Africa (Ramakrishnan and Huffman, 2001; Ramakrishnan, 2002). Women of reproductive age are amongst the most affected as demands on nutrients during pregnancy and lactation are especially high. A global estimate of children under five years who are deficient in vitamin A ranges from 140 to 250 million (WHO, 2007). Approximately one-third of children in developing countries are affected to varying levels by vitamin A deficiency and this invariably leads to impairment of their growth, development, vision and immune system function (Hurtado *et al*., 1999, WHO, 2007). In conditions of extreme vitamin A deficiency the consequences are blindness and death. The incidence figures of sub-clinical vitamin A deficiency for children under 6 years of age for some sub Saharan African countries are as follows: Zambia 66%, Malawi and Namibia 59%, Lesotho 54%, and the lowest prevalence rate of 26% for

Mozambique. Other vitamin A prevalence figures are 28% for Zimbabwe, 30% for Botswana 33% for South Africa and 38% for Swaziland (Micronutrient Org, 2007 A & B).

The 1993 Lesotho National Micronutrient Survey reported a 13% incidence of vitamin A deficiency for children aged 2 to 6 years with only 6.5% of the children having normal serum retinol levels (MOHSW & FNCO, 1993). Prior to the 2004-2005 Lesotho Demographic Survey (LDHS, 2004) vitamin A supplements were administered to 55% of children aged 6 to 59 months and 49% of 3-year old children were consuming vitamin A rich food. Micronutrient deficiencies, especially vitamin A, invariably lead to exceptional learning disabilities in children, higher morbidity and mortality rate, considerably lower worker productivity and high health costs. The above factors have a negative impact on human potential and happiness and tend to reduce national economic development (Welch and Graham, 2004).

Interventions to combat vitamin A deficiency are the supply of β-carotene supplements, food fortification and improvement of dietary intake combined with nutrition education. The most commonly practised strategy for improvement of vitamin A status of low-income populations is increased daily consumption of carotene-rich fruits and vegetables as opposed to synthetic vitamin intake (West *et al*., 2002). Generally food based interventions aim to (a) improve the production, availability and accessibility of foods that are rich in vitamin A, (b) elevate the consumption of vitamin A rich foods, (c) increase the amount of vitamin A absorbed and utilized by the body subsequent to ingestion of vitamin A rich foods. Food fortification with multiple micronutrients is commonly practised to improve the nutrient status of original foods. Wheat flour and maize are the most common vehicles of micronutrient fortification (Semba and Bloem, 2001). However, β-carotene rich carrot in its natural form is probably underestimated as a supplement food.

Within the succulent vegetable grouping, carrots rank second in popularity in the world after potato. Apart from their nutritive value, carrots are economically important due to their: popularity and low cost for consumers, relative ease of production, ability to be harvested over a long period of time, comparative ease of shipment and long storage life under appropriate low temperatures (Yamaguchi, 1983). Countries that have high carrot production in

terms of area planted and volume include China, United States of America, Russia, Japan, France and the United Kingdom (World Carrot Museum, 2007). Carrots are amongst vegetables that contribute one of the highest levels of carotene in the human diet. Carotene, a source of provitamin A, has a wide range of protective effects in the human body including prevention of oxidative stress and damage (Handelman, 2001).

Crop micronutrients such as minerals and vitamins can be increased through plant breeding. The need to improve micro-nutrient availability for humankind, including minerals and vitamins, has led to investigation into crop micronutrient increase through plant breeding. The availability of micronutrients can also be increased by breeding for reduced anti-nutrients (Welch and Graham, 2004). Production of staple food crops with high density of micronutrients through breeding could address the global human health and nutritional problems (Welch and Graham, 2004; Lucca *et al*., 2006). Additionally, the application of bio-stimulants to manipulate the growth, yield and quality of crops has become popular over the past decade as a result of the commercialization of a number of products.

However, public concern on the impact of toxic and environmentally unfriendly conventional synthetic chemicals has led to increased focus on the use of sustainable agricultural production technologies (Laegreid *et al*., 1999). Some of the challenges encompassed in sustainable production are maintenance of soil productivity and better resource management especially of naturally occurring non renewable products. Due to the positive benefits derived from the use of natural plant products ComCat® and Kelpak® were included in this study. There is a paradigm shift on the part of researchers, environmentalists and industrialists alike to implement and support sustainable crop production strategies that utilize products that are bio-degradable and non toxic to non-target organisms. Evidence thus far indicates that natural plant products generally have a much shorter half-life in the environment than synthetic pesticides.

2.2 Biology of carrots (*Daucus carota* L.)

2.2.1 Country of origin and distribution of carrots

The carrot, *Daucus carota* L., is native to Europe, west Asia, northern Africa and northern America. It is a major cultivated Umbellifer (*Apiaceae*) in the world. Records from Europe indicate cultivation of carrots as early as the tenth century and introduction to China occurred during the thirteenth to fourteenth centuries, whereas introduction to Japan occurred later, during the seventeenth century (Yamaguchi, 1983). By the seventeenth century carrots were also been grown in America.

2.2.2 Botanical description of carrots

Daucus carota L, belongs to the family Apiaceae. This family has over 2500 species including parsley, celery, dill and cumin. Included in this family are some poisonous species such as poison hemlock and fools parsley and ornamentals such as sea holly and blue lace flower. *Daucus carota* L. has 13 subspecies of which twelve are wild taxa and one is the cultivated taxon. The cultivated carrot, which is a hybrid of the wild carrot, can be an annual in tropical regions or a biennial in temperate areas (Yamaguchi, 1983; Rubatzky *et al.*, 1999; World Carrot Museum, 2007). The plant is an erect herbaceous dicotyledon that reaches a height of 20 to 50 cm at maturity and extends to 120 to 150 cm at flowering. The fleshy taproot is usually straight, conical to cylindrical in shape. The length of the roots ranges from 5 to 50 cm and diameter at the shoulder varies from 2 to 5 cm. The colour of the carrot flesh ranges from white, yellow, orange, red purple to dark purple. The orange fleshed carrots are the most popular although other colours especially the maroon coloured carrots are slowly being brought back into cultivation in the UK.

There are basically two types of cultivated carrots namely; eastern Asiatic and western types. The eastern Asiatic types of carrots are characterised by reddish-purplish or yellow roots, greyish green foliage and tend to flower early. The leaves are slightly dissected, the roots are branched

and the plant is an annual. The western type of carrots have orange, yellow or white roots with less green leaves and a lower tendency to flower until they are exposed to continuous low temperatures. The leaves are strongly dissected and the roots are not branched (Yamaguchi, 1983; Rubatzky *et al.*, 1999). The flower stalks of the inflorescence radiate from a central point thus forming the umbrella shape. The general name “umbellifers” or “umbels” for this family is derived from this compound umbrella shaped inflorescence.

2.2.3 Cultivation of carrots

Carrot is a cool season crop with mean temperatures between 15^0 and 21^0C being most suitable for root and foliage growth and for the development of an appropriate shape and root colour (Rubatzky *et al.*, 1999). There is a reduction in growth of foliage and the development of strong flavour in roots when carrot plants are exposed to air temperatures around 28^0C. Additionally, at relatively low temperatures, 13^0C, the carrot roots tend to be long and thin with minimum foliage growth. Conversely, at higher temperatures, 24^0C, the roots become shorter and thicker (Yamaguchi, 1983; Rubatzky *et al.*, 1999).

Carrots grow well in a variety of soils but ideal soils should be deep, well drained and have a loam texture. Carrots grow well in medium texture or loam soils and a pH ranging from 5.0 to 8.0 produces best results. Heavy clay soils or compact soils tend to lead to development of forked and conically shaped roots as opposed to the desirable long tapered roots that develop in medium textured or loam soils (Yamaguchi, 1983; Rubatzky *et al*., 1999). The influence of soil type on carrot shape was demonstrated by Rosenfeld *et al.* (2000). The carrots grown on mineral soil were firm and cylindrical in shape, whereas those from organic soil were conical.

Provision of adequate moisture throughout carrot growth is important in ensuring optimum root development and high quality. The total water requirement for carrots ranges from 450 to 900 mm water, including rainfall. Provision of limited amount of water invariably leads to carrot roots with a strong pungent flavour. Conversely, excessive soil moisture and waterlogged soils lead to development of poorly coloured roots (Yamaguchi, 1983). A reduction in the standard irrigation rate led to a decline in leaf biomass and the

total percentage of marketable carrot roots. The main cause of carrot productivity decline under reduced irrigation was due to a reduction in leaf growth that in turn led to reduced leaf area (Gibberd *et al*., 2003). Crop production in arid and semi-arid regions has often led to the use of saline water. A study into the effects of irrigation with saline water was undertaken by De Pascale and Barbeiri (2000). The authors observed reduced number and yield of marketable carrots with increased salinity.

2.3 Nutritional value and health benefits of carrots

2.3.1 Nutritional value of carrots

The nutrient content of carrots varies amongst cultivars and is also influenced by soil type, fertilizer, and climatic conditions. Research on the effects of fertilization on carrot nutritive characteristics led Zdravkovic *et al*. (2007) to conclude that fertilization with manure produced significantly higher yield than inorganic fertilizers. The content of ash, dry matter, proteins, nitrates and nitrites varied widely based on fertilization and cultivar.

According to Yamaguchi (1983), the main constituent of carrots is water which accounts for 86 to 89% of the root fresh mass. The other macro nutrients are protein (0.8 – 1.0 g per 100 g carrot root), fat (0.2 g per 100 g carrot root) and carbohydrates (6.6 - 7.7 g per 100 g carrot root). Carrots contain relatively high amounts of provitamin A carotenes, β-carotene (70-80%), and α-carotene (20-30%). There is a higher concentration of carotenoids and sugar in the phloem tissues compared with the core or xylem (Rubatzky *et al*., 1999). Carrots also contain other vitamins such as vitamins B1, B2 and C as well as minerals calcium, iron, magnesium and phosphorus. Relatively low amounts of amino acids are also found, 8 - 56 mg per 100 g fresh mass (Yamaguchi, 1983). Apart from its' use as a nutrient, β-carotene has been used widely as a drug, as a colourant in industries and for inclusion in cosmetics (Diplock, 1997).

2.3.2 Health benefits of carrots

The last two decades have witnessed a lot of studies and reports on human micronutrient deficiencies. These deficiencies especially during human infancy lead to poor growth but most importantly to increased risk of morbidity and mortality from a variety of infectious diseases and to delayed psychomotor development (Savage-King and Burgess, 1993; Hurtado *et al*., 1999; Ramakrishnan and Huffman, 2001). Incidence rates of micronutrient malnutrition in Asia and sub-Saharan Africa indicate that more than two billion people worldwide are deficient in vitamin A, iodine and iron. Of this figure women and young children are at the greatest risk (Ramakrishnan, 2002; Mhenga *et al*., 2005). The devastating impact of vitamin A deficiencies especially among young children has been a major impetus behind impact and implications studies on micronutrient deficiencies.

Retinol, a pre-form of vitamin A, is the most active and readily useable form of vitamin found in animal foods. However, a large portion of vitamin A intake in developing countries is derived from carotenoids in plant based diets (van den Berg *et al*., 2000). β-Carotene and pro-vitamin A carotenoids are constituents of many fruits and vegetables and they are usually absorbed and converted to vitamin A. Although animal sources are rich in vitamin A, contribution from these sources is minimal as animal products are often beyond the purchasing power of most people due to socio-economic constraints and / or are not accessible. In developing countries, the main dietary source of vitamin A is β-carotene although in comparison to pure β-carotene its conversion to serum retinol is less efficient than was previously thought (West *et al*., *2002*; Li *et al*., 2006).

An integrated strategy for vitamin A provision was implemented by Faber *et al*. (2002) in home gardens in a rural area of KwaZulu-Natal, South Africa. For improved efficacy, the home gardens were linked to nutritional education and primary health care activities. Following increased habitual intake of vitamin A rich vegetables, the authors reported significant increases in serum retinol / vitamin A concentrations in children aged 2-5 years. The programme thus ensured that the relation between vitamin A and health as well as the importance of dark-green and yellow vegetables as vitamin A sources were

highlighted. As a result, implementation of home gardens played an important role in the improvement of intake of vitamin A rich foods in this area. In another study, a higher intake of food rich in β-carotene and α-carotene was also associated with lower incidence of coronary artery disease (Osganian *et al*., 2003).

These results corroborated those of Takyi (1999), who found that vitamin A status of pre-school children with vitamin A deficiency were enhanced by the consumption of dark-green leafy vegetables. The coupling of food based interventions with increased dietary fat intake increased the bio-availability of carotenoids (van Lieshout *et al*., 2001). Administration of pure β-carotene was the most effective in enhancing the concentrations of serum retinol. Increased agricultural production of vitamin rich vegetables and education of communities on preparation methods of food to preserve nutrients are necessary strategies towards provision of nutrients (Mhenga *et al*., 2005).

In addition to investigation into their provitamin activity, major research efforts have focused on carotenoid antioxidant activity, their protective effects against ultra violet light (Stahl *et al*., 2001), their ability to lower the risk of the development of several diseases including cancer, cardiovascular and neurodegenerative diseases (Klipstein-Grobusch *et al*., 1999; Toniolo *et al*., 2001; Heinrich *et al*., 2003; Tamimi *et al*., 2005). Studies involving carrots cooked in a conventional manner, grated carrots and carrot juice indicated increased β-carotene bio-availability with processing (Ncube *et al*., 2001; Edwards *et al*., 2002).

2.3.3 Preservation of nutrients in harvested carrots

In light of the health benefits outlined above, the preservation of carotenoids and other nutrients in post-harvested vegetables, e.g. carrots, seems imperative. However, due to the high water content in vegetables, they are highly perishable and their quality and appeal after harvest is very limited. To optimize the use of vegetables especially carrots as a source of vitamin A, appropriate methods of processing and preservation including freezing, blanching and drying have been investigated (Mayer-Meibach and Spieb, 2003; Prakash *et al*., 2004; Fan *et al*., 2005; and Wang and Xi, 2005).). These

methods were effectively used to preserve carrot carotene carrots while still maintaining quality.

Studies involving carrots cooked in a conventional manner, grated carrots and carrot juice indicated increased β-carotene bio-availability with processing (Ncube *et al*., 2001; Edwards *et al*., 2002).

2.4 Effect of fertilization on the growth and yield of crops

2.4.1 Nitrogen fertilization in crop production

Nitrogen is a constituent of most organic compounds including amino acids, nucleic acids, enzymes and energy transfer compounds such as chlorophyll, ADP (adenosine-di-phosphate and ATP (adenosine-tri-phosphate). Additionally, it is a major component of plant dry matter (Jones, 1982; Troeh and Thompson, 2005) together with carbon, oxygen and hydrogen. Formation of new cells is dependent on the availability of nitrogen. Regardless of the production and availability of photosynthate, unavailability of nitrogen prevents production of proteins, nucleic acids and enzymes (Troeh and Thompson, 2005). Carbon, oxygen and hydrogen can be obtained from the atmosphere and large amounts of nitrogen have to be obtained from the soil by non-leguminous plants. Most agricultural soils rarely contain enough nitrogen thus necessitating fertilization to attain maximum plant growth.

A majority of higher plants utilize nitrate as a major source of inorganic nitrogen (FSSA, 2007) and a large portion of these nitrate ions is translocated to the leaf where it is assimilated and metabolized into organic compounds via photosynthesis. Nitrate ions function as substrate for both assimilation and signalling molecules that partly regulate the pattern of growth and development by controlling the expression of various genes (Takei *et al*., 2002). The genes whose expression is dependent on availability of nitrate ions, so-called nitrate-specific genes, include genes involved in nitrate uptake and reduction, ammonium assimilation and supply of reducing agent, biosynthesis of co-factors, supply of carbon skeleton for nitrogen assimilation and root architecture. On the other hand the genes that are broadly-responsive to nitrogen include those involved in amino acid metabolism, protein storage,

photosynthesis and cell cycling that are controlled by nitrogen sources including, ammonium ions, nitrate and amino acids.

Field grown Chinese cabbage and carrots had higher nitrogen uptake with increasing nitrogen fertilization and higher marketable yield (Chen *et al*., 2004). The authors reported that the nitrogen supply for Chinese cabbage was 349 kg nitrogen ha^{-1} and that for carrots 227 kg nitrogen ha^{-1} for the production of 120 tons and 65 tons per hectare respectively. The application of nitrogen fertilizer above the amount required for maximum yield increased cracking and cracking severity as well as susceptibility to cracking and breakage observed subsequent to the removal of the periderm (Hartz *et al*., 2005). The general guideline for nitrogen fertilization for carrot production in South Africa is 70 to 120 kg ha (FSSA, 2007). These guidelines are often adjusted based on soil, leaf analysis and crop production practices.

The effect of nitrogen on plant growth was further demonstrated by Cruz *et al*. (2003). They reported increased shoot to root ratio as a result of elevated shoot dry mass compared to root dry mass of cassava under increased nitrogen supply. Ali *et al*. (2003) reported increased carrot root yield at the nitrogen fertilization rate of 200 kg ha^{-1}, compared to the control. This fertilization rate also led to the highest content of β-carotene and the lowest carotene content was recorded for plants that did not receive any nitrogen.

In addition to nitrogen effects on yield, increasing nitrogen supply increased the content of total soluble saccharides, non-reducing saccharides and inorganic phosphate in the leaves of cassava. On the other hand, the roots accumulated less reducing saccharides and starch. Further, the rate of photosynthesis was reduced under nitrogen deficiency (Cruz *et al*., 2003).

2.4.2 Phosphorus fertilization in crop production

Phosphorous is directly involved in most plant growth processes such as carbohydrate breakdown, cell division, transfer of inherited characteristics, stimulation of early root growth and development, hastening maturity of plants, fruiting and seed development as well as energy transformation. It is found in highest concentrations in seeds and growing points (Jones, 1982; Troeh and Thompson, 2005). The importance of phosphorous is evidenced by its role in

the breakdown of the products of photosynthesis while there is reduced formation of amino acids and protein under phosphorous deficiency. Of significant importance is the role of phosphorous in reproductive and inheritance processes within plants. Phosphorous is present in nucleotides, which are found in the nucleus where cell division occurs (Jones, 1982).

The occurrence of phosphorus in soils is relatively high. However, it's availability to plants is low due to high immobility (FSSA, 2007). The ease with which applied phosphorus can be converted to an insoluble form limits its availability to plants. Fixation of phosphorus is exacerbated by the presence of clay minerals and either low or high pH values (Wolf, 1999; Redel *et al*., 2007). A furrow slice of soil generally contains 1 kg phosphorous in solution out of a total phosphorous content of 1000 kg ha^{-1} (Troeh and Thompson, 2005) This low solubility is, however, advantageous in keeping leaching losses low. Availability of phosphorous in soils is influenced by soil pH, with slightly acid conditions (pH 6 to 6.5) being more suitable than lower or higher values. At a soil pH less than 6, phosphorus is fixed by iron and aluminium ions in soil solution, especially found in clayey soils, and at pH values greater than 6.5, phosphorus is precipitated by calcium and magnesium (Foth, 1978). The application of lime, therefore, has an effect on phosphorus availability. When basic materials including limestone are added to soils with neutral pH, the availability of calcium phosphates is reduced. Alternatively, the addition of limestone to acidic soils, which contain iron and aluminium phosphates, will increase phosphorus availability by increasing formation of more soluble calcium phosphates.

Nielsen *et al*. (2001) observed that some genotypes of common bean, *Phaseolus vulgaris,* grown under low phosphorous availability had reduced growth compared with those under high phosphorous availability. Plants under low phosphorous availability also showed higher root respiration resulting in only a small amount of carbon being left for organ development. Additionally, genotypes that were efficient in phosphorus utilization under phosphorus stress produced more adventitious roots than phosphorus-inefficient genotypes. The importance of these results was deemed to be the fact that adventitious root formation is increased under stress. Adventitious roots are particularly important in facilitating enhanced phosphorus acquisition. The metabolic

importance of adventitious root formation is that they utilize lower metabolic energy than basal roots during their formation and in addition the adventitious roots are longer compared with basal and tap roots. The plants grown under phosphorus stress had considerably less growth than plants under high phosphorus levels.

Olivera *et al*. (2004) reported increased leaf area, plant dry mass (shoot and root), nodule biomass as well as the content of phosphorus in the shoots and roots of *P. vulgaris* plants fertilized with phosphorus. The application of phosphorus however caused a decrease in total soluble sugars and amino acids in the leaves, root and nodules and reduced growth of shoots was due to reduced leaf initiation and expansion (Liao and Yan, 1999; Olivera *et al*. 2004). Soluble sugar accumulation in the roots and nodules were thought to serve as a carbon source for growth including their involvement in increasing osmotic pressure of root cells and influencing ion uptake capacity (Ciereszko and Barbachowska, 2000). Low phosphorus availability caused higher biomass allocation to the roots with the result that there was a higher root to shoot ratio which might increase phosphorus uptake (Liao and Yan, 1999; Nielsen *et al*., 2001; Olivera *et al*. 2004). Surprisingly, higher levels of phosphorus did not correlate with plant biomass, nutrient accumulation and seed yield as the percentage increase of shoot dry weight was five times larger than seed increase. This indicated that the yield potential might be controlled by factors other than phosphorus.

2.4.3 Potassium fertilization in crop production

The importance of potassium in plant growth is not easy to categorise and measure as it does not form permanent organic structures in plants, but is rather found in soluble inorganic or organic salts. Potassium is involved in plant physiological processes including photosynthesis, cell division, translocation of sugars, enzyme activity, reduction of nitrates and subsequent synthesis into proteins (Jones, 1982).

Potassium is found in relatively high concentration in clay and insoluble minerals in soil. Due to its unavailability addition of potassium in crop production has to be done (FSSA, 2007).

Potassium requirements of crops are similar to those of nitrogen and better nitrogen utilization can be achieved in the presence of potassium. Potassium is critical for enhancement of disease resistance (FSSA, 2007). The quality of carrots is affected by potassium and nitrogen availability (Ali *et al*, 2003). Carrots fertilized with a combination of 200 kg ha $^{-1}$ nitrogen and 250 kg ha $^{-1}$potassium had the highest carotene content compared with carrots without potassium fertilization. Conversely, application of potassium had adverse effect on sugar accumulation. The 250 kg ha $^{-1}$ fertilizer level produced carrots with the lowest reducing sugar content and the highest reducing sugar was from carrots without potassium fertilization.

Breeding of cultivars that are tolerant to soil mineral deficiencies is invaluable in supporting sustainable farming systems and contribute towards reduction in production costs and overdependence on mineral fertilizers (Grusak *et al*., 1999). The search for and /or breeding of cultivars that are high yielding and tolerant to low fertility would contribute towards attainment of environmentally friendly sustainable farming systems and increased profit margins for farmers. Though different breeding approaches have been used to produce crops with high nutrient content, no report of increased micronutrient content in the edible part was found (Lucca *et al*., 2006).

2.5 Environmental effects on carrot yield

Carrot splitting is a worldwide phenomenon that contributes significantly to reduced carrot yield and marketable product. Studies to determine factors governing splitting have produced widely varying results. Hole *et al.* (1999) investigated susceptibility of carrots to splitting especially the strength of carrot tissues, ease of tissue fracture (brittleness) and internal mechanical stress causing splitting. The results indicated that susceptibility to splitting differed between cultivars, developmental stage and environmental conditions (Hole *et al*., 1999). The strength of carrot tissues, ease of tissue fracture and internal mechanical stress could not be clearly explained by the size of the cell, temperature changes and tissue water status.

Photosynthesis is a major factor contributing to dry matter accumulation and yield of crops. Therefore improved photosynthesizing ability and

partitioning of assimilates could lead to increased yield (Salisbury and Ross, 1985). The leaf tissue availability of macro elements, nitrogen, phosphorus and potassium in crops ensures optimal photosynthesizing capacity. Deficiencies of these nutrients lead to reduced growth and subsequently reduced leaf area and reduced photosynthesis per unit area (Pettigrew and Gerik, 2007).

The other environmental factor influencing carrot production is the colour and intensity of light. The colour of light reflected from the soil to the developing leaves had a major influence on yield and chemical composition of carrot roots (Antonious and Kasperbauer, 2002). In cases where the colour of light reflected was in the far-red to red light the carrots produced had the highest shoot mass and lowest root to shoot mass ratio. In cases where the plots were covered with yellow or white panels, the levels of β-carotene and ascorbic acid were highest especially in the cortical tissues as opposed to the xylem tissues. Phenolic compounds were found in highest concentration in carrots covered with yellow and black plastic. The allocation of growth to shoots and roots was influenced by the colour of light reflected to developing leaves and the concentration of compounds that are responsible for carrot root flavour and nutrition could thus also be influenced by the quantity and colour of light.

The positive effect of radiation on carrot growth was examined by Kyei-Baahen *et al*. (2003). The leaf net photosynthetic rate of the four carrot cultivars examined increased as the photosynthetic active radiation increased. The leaf net photosynthetic rate increased up to 800 µmol m^{-1} s^{-1} irradiance after which the rate declined. The growth of carrot was reduced and morphology of the tap root was partly modified when the available photosynthetically active radiation per plant was reduced. Growth reduction occurred when irradiation level per area was reduced by shading and available area per plant was reduced by increasing plant density (Klug-Andersen and Nielsen, 2000).

An increase in carbon dioxide in air invariably leads to an increase in photosynthetic ability of the crop and subsequently increased growth. The accumulation of dry matter is enhanced by higher carbon dioxide concentrations especially in conjunction with high temperatures (Salisbury and Ross, 1985).

2.6 Carrot diseases and pests

Carrots are infected in the field and during storage by several fungal diseases. These diseases often attack carrot foliage leading to development of spot and blight. Several *Alternaria* species affect foliage and roots leading to the symptoms highlighted above. Once the leaves are infected they become weak and this particularly poses a problem during mechanical harvesting where carrots are pulled out by their leaves (Rubatzky *et al*., 1999). *Alternaria* black rot, caused by the seed-borne *Alternaria radicina* is prevalent in most carrot growing regions of the world and affects both roots and leaves (Farrar *et al.,* 2004).

Another economically important carrot disease is Cavity spot caused by two fungi *Pythium violacea* and *Pythium sulcatum.* The disease symptoms are sunken brown spots on the roots. The disease severity is compounded by prolonged rainy period, combined with poor drainage and low soil pH (Cooper *et al*., 2004). The value of the crop is reduced due to blemishes on the root and in severe disease attacks the crop is often ploughed under instead of incurring harvesting costs.

No chemicals are registered for specific use in the control of carrot pests in South Africa. Moderate to severe reductions in yield often occur due to infestations of root knot nematode, *Meloidogyne spp*. The roots exhibit nodular thickenings especially on the lateral roots. Severe pest attack commonly occurs in hot weather and on light textured soils. The most effective method of control is soil fumigation prior to carrot sowing.

The other carrot pests are aphids, red spider mites and worms and millipedes. Although aphids are not a major problem they reduce carrot growth due to their sucking feeding habit that reduces photosynthates. Under warm, dry conditions the number of red spider mites can quickly increase thus warranting control. Various fireworms, cutworms and millipedes pose a problem in carrot production especially if pest attack occurs late in development. The most effective control measures are crop rotation, baiting and soil turning to expose the pests.

2.7 Sugar transport, sensing and signalling in plants

Production of sugars by photosynthesis is vital for the provision of carbon source and energy for plant growth and development. Additional to their use in metabolic, growth and developmental events in plants, sugars have hormone-like functions where they act as signals (Smeekens, 2000; Gazzarani and McCourt, 2001; Finkelstein and Gibson, 2002; Rolland *et al*., 2002; Leon and Sheen, 2003; Gibson, 2005). Sugars also interact with light, stress factors and hormones to elicit plant responses which are varied and whose mechanisms of action are not fully understood to date. Finally, sugars contribute to the dry mass accumulation of harvestable parts and therefore yield.

2.7.1 Classification of sugar transporters

There are two distinct families of sugar transporters in higher plants viz. the disaccharide transporters and monosaccharide transporters that transport sugars in various plant tissues at different stages of growth and under varying environmental conditions. The disaccharide transporters often termed SUT or SUC, mainly mediate sucrose transport. Whereas, the monosaccharide transporters, STP, MST, HEX, ST, catalyse transport of a range of monosaccharides including glucose, fructose and mannose (Lalonde *et al*., 1999; Williams *et al*., 2000). Coordination of sugar transport in various tissues and organs during plant development, under a wide range of environmental conditions, is mediated by these transporters. The specificity of sugar transporters for particular carbon substrates dictate their location at membranes in source and sink tissues (Lalonde *et al*., 1999). Genes encoding sugar carriers or transporters have been studied to decipher the spatial and temporal expression of the transporters (Williams *et al*., 2000).

The transport of sucrose and its derivatives represents a major method of transporting photosynthetic assimilates throughout the plant and the efficiency of transport subsequently determines yield. Apart from it being the main metabolite transported in plants, the disaccharide sucrose is the main osmotic metabolite contributing to mass flow in the phloem. It is also a signal molecule that activates or depresses the action of specific genes in various parts of the plant (Sheen *et al*., 1999; Smeekens, 2000).

The uptake of sugars into the phloem and its retrieval from the apoplasm is accomplished by proton-coupled transporters. Based on information from transgenic yeast studies, the localization of sugar transporters in higher plants is assumed to be at the plasma membrane level (Weise *et al*., 2000). The sucrose uptake transporters are categorized into high affinity/low capacity (HALC) and low affinity-high capacity (LAHC) components. SUT1 transporters fit the high affinity-low capacity component, whereas the SUT4 transporters comply with low affinity-high capacity component. Due to the high fluxes of sucrose envisaged in phloem loading sites such as minor veins, the low affinity-high capacity components may be important for loading. Alternatively, the high affinity-low capacity transport system is necessary for maintenance of sucrose gradient along the transport route (Weise *et al*., 2000). The expression of high affinity glucose transporters occurs once low levels of glucose are sensed and under high glucose concentrations low affinity glucose transporters are expressed.

The principal sucrose transporter, SUT1, in potato, tomato (Solanaceae) and tobacco is responsible for loading sucrose across membranes from the apoplasm into the sieve elements and can thus be found in the sieve elements of leaves, petioles and stems in source and sink tissues as well as along the translocation path (Barker *et al*., 2000; Weise *et al*., 2000; Lalonde *et al*., 2004). SUT1 was also found in epidermal cells of developing cotyledons where there is high affinity sucrose uptake into the developing embryos. The necessity of SUT1 in phloem loading and long distance transport of sucrose in Solanaceae has been demonstrated by a reduction in tuber size and number of potatoes per plant, as well as, abnormal accumulation of sugars in leaves under low expression levels of SUT1.

Another group of transporters, the SUT4 transporters which fit the low affinity- high capacity component are found in minor veins and sink tissues (Weise *et al*., 2000). In tomato (Solanaceae), all three groups of transporters, SUT1, SUT2 and SUT4, were found in sieve elements (Barker *et al*., 2000). Both SUT2 and SUT1 were found to interact with SUT4 (Reinders *et al*., 2002). SUT4 transporters are characterized by being a high capacity phloem loading system and also supply the sink organs. Alternatively, the transporters SUT1

and its prototype SUC2 maintain the apoplasmic sucrose levels low at loading and in transit and are needed for phloem loading at low supply.

The third group of sucrose transporter-like proteins, SUT2, has similarities to the other sucrose transporters but also has features similar to sugar sensors. SUT2 has two subgroups with one subgroup having a central extended loop, whereas in the other subgroup the loop is absent. The potential of the extended loop in relation to sucrose transport was investigated and the loop was not functional in the transport of sucrose. However its similarity to yeast hexose transporters that act as sensors, led to the postulation that SUT2 transporters might be sucrose sensors (Barker *et al*., 2000; Meyer *et al*., 2000). SUT2 shows a lot of similarity to yeast sugar sensors RGT2 and SNF3 that have low expression and express no detectable or weak sucrose transport activity (Barth *et al*., 2003). Localization of SUT2 in tomato is in the sieve elements where it co-localizes with SUT1 and SUT4. The localization of SUT2 in the plasma membrane might be necessary for regulation of sucrose fluxes across the plasma membrane of sieve elements in plants (Barker *et al*., 2000; Lalonde *et al*., 1999).

In carrot, one transporter, DcSUT1, was predominantly found in the lamina of source leaves, suggesting linkage to loading of sucrose into the phloem. The other transporter, DcSUT2, was located in sink tissues, especially the storage parenchyma cells of the phloem and xylem (Shakya and Sturm, 1998). The expression of DcSUT1 in the lamina was highest during the day, suggesting higher activity of sucrose transport. On the other hand, expression of DcSUT2 was not detected.

2.7.2 Effects of sugars on growth processes

The movement of metabolites in the phloem, at the speed of at least 0.5 m per hour, is dependent on the functions of three types of plasma membrane proteins: (a) H^+/ sucrose symporters that accumulate sucrose to relatively high concentrations in the phloem, (b) H^+/ATPases that provide energy necessary for active transport and (c) water transporters that take up water from the xylem. The proton-coupled sucrose and monosaccharide uptake transporters remain the only clearly identified component of the photoassimilate transport

system (Lalonde *et al*., 1999; Lalonde *et al*. 2004). Potato plants that have reduced levels of sucrose transporters exhibit reduced tuber yield and sugar accumulation in leaves is elevated due to impaired sugar translocation. Antisense plants had retarded growth and exhibited dwarf phenotype due to the inability to transport sucrose and starch out of leaves i.e. reduced sucrose transport led to reduced carbon partitioning and photosynthesis. The reduced level of sucrose export from the leaves of mutants as well as reduced supply of sucrose to sinks support the theory that the presence of SUT1/SUC2 (SUC2 from *Arabidopsis*) along the translocation path might be necessary to maintain high osmotic pressure in sieve elements.

Monosaccharide transporters are required for uptake of hexoses subsequent to hydrolyzation of sucrose after unloading. The uptake of hexoses enhances cell division and storage in sink tissues and also increases sugar gradients to ensure sink supply (Sherson *et al*., 2003). The best known monosaccharide transporters are those from *Arabidopsis* and rice and their expression pattern is consistent with the main function of hexose uptake from the sinks. Monosaccharide transporters are localized in pollen grains, developing seeds, root tips and guard cells as well as in phloem fibres where they supply hexoses needed for cell wall synthesis.

The availability of sucrose during plant development was found to be crucial for the determination of shoot to root ratio and influenced developmental processes such as onset of flowering (Havelange *et al*., 2000). The coordination of photosynthesis and sucrose transport from source leaves to sink organs was observed to be necessary for ensuring controlled whole plant responses. The effects of environmental factors and endogenous signals also compounded the regulation of sucrose transport (Bush, 1999; Lalonde *et al*., 1999). Thus the inter-connectedness of the plant's regulatory system and sucrose transport regulation are valuable for ensuring control of developmental and physiological processes.

The entire lifecycle of plants including growth and development has been shown to be controlled by sugar sensing and signalling. In order to efficiently partition photoassimilates produced in leaves, plants need information on carbohydrate status of different plant components as well as the intended use of imported carbohydrate. Additional to this, sugar regulates its

own production and use. Low sugar levels within the plant enhanced photosynthesis, reserve mobilization and export to sink tissues and organs, leading to increased plant growth and storage reserve accumulation. On the other hand abundant levels of sugars lead to inhibition of photosynthetic genes. The genes responsible for triggering the onset of photosynthesis were reported to be at the highest levels towards midday, whereas those responsible for sugar transport, use and storage had the highest expression towards the end of the day and the starch mobilization genes were found in highest levels at night (Lalonde *et al*., 1999; Rook and Bevan, 2003).

In potato tubers, transportation of sucrose through the phloem was determined as necessary for growth of sprouts and low concentration of sucrose acted as a signal for sucrose demand in the sinks and also served as a regulator of storage reserve mobilization in source tubers (Hajirezaei *et al*., 2003). Low levels of sucrose thus regulated starch mobilization from potato tubers.

Leaf senescence, which coincided with reduced leaf chlorophyll and photosynthetic activity, was also found to be regulated by sugars (Dai *et al*., 1999; Quirino *et al*., 2000). The involvement of the enzyme hexokinase in sensing sugar levels in photosynthetic tissues and its' control of leaf senescence was reported by Dai *et al*. (1999). When photosynthetic tissues over expressed hexokinase, there was stunted growth and the rates of photosynthesis were lower. Fruit weight, accumulation of starch in young fruit and the amounts of total soluble solids in mature fruits decreased as the activity of hexokinase increased. Photosynthetic gene expression was reduced by endogenous sugars especially, glucose and fructose, which tended to be high during leaf senescence as opposed to decreased starch content (Dai *et al*., 1999; Xiao *et al*., 2000). Although exogenous application of sugars induced expression of senescence genes in some cases, senescence genes in *Arabidopsis* were down regulated by sugars in leaves undergoing senescence (Noh and Amasino, 1999).

2.8 Energy levels in crop development

The role of photosynthesis in plants is to harness solar energy and utilize it to synthesize carbohydrate from carbon dioxide and water. Sucrose is a fundamental product of photosynthesis and it is transported from source leaves to plant parts and organs where it is used as a substrate for further reactions or stored. Photosynthetic reactions can be divided into two distinct phases, the light and dark reactions. During light reactions of photosynthesis, light energy is utilized to produce energy rich compounds, NADPH and ATP. The dark reactions or light independent reactions, where carbon atoms are fixed, utilize the previously formed NADPH and ATP to synthesize carbohydrates, sucrose and starch, from carbon dioxide and water (Foth, 1978; Hames and Hooper, 2005).

All active plant cells respire continuously with an influx of oxygen (O_2) and carbon dioxide (CO_2) efflux. The amount of oxygen utilized and carbon dioxide released is dependent on the type of compound being oxidised (Salisbury and Ross, 1985). For substrates whose principal constituent is starch, the amount of oxygen used is approximately equal to the amount of carbon dioxide released. On the other hand, for oily compounds carbon dioxide efflux is less than oxygen influx. A major component of the energy released during respiration is heat and most of which is lost to the atmosphere and to soil and thus has minimal benefit to the plants except in few instances where the heat might stimulate metabolism under cold conditions. The most profound use of heat released during respiration is its' incorporation into ATP where it is later used for energy provision in growth processes and for ion accumulation (Salisbury and Ross, 1985).

Plant roots are continually challenged by oxygen limitation brought about by excess water from soil flooding, heavy rainfall, excess irrigation and water seepage from water tables (Wolf, 1999; van Dongen *et al*., 2003). During this period of oxygen limitation, mitochondrial respiration is limited and this consequently leads to energy deficiency and reduced metabolic activity. The transport of sugars from the shoots to roots is reduced under oxygen stress (van Dongen *et al*., 2003) and photosynthesis is inhibited.

The importance and activity of respiration during light period were debated for a long time and certain studies concluded that respiration was

completely inhibited during daytime or under light conditions. Currently, it is known that cellular respiration is active during light periods and that certain respiratory pathways such as electron transport chain are necessary for optimal photosynthetic performance (Dutilleul *et al.*, 2003). Competition for carbon substrate partitioning between respiration and biomass accumulation has been found to be slightly offset by mitochondrial and/or respiratory processes that support photosynthetic metabolism such as supply of adenosine triphosphate for sucrose synthesis (Carrari *et al*., 2003; Rasmusson and Escobar, 2007).

Wounding of plant tissue has been found to lead to an increase in respiration. Surjadinata and Cisneros-Zevallos (2003) observed respiration rates that were several times higher for grated carrots compared with whole carrots. Root respiration rates were best when measured as soon as possible after shoot removal, as the rates measured immediately after shoot removal were markedly different from the stable rates observed after considerable time had elapsed

2.9 The use of bio-stimulatory products in crop production

The use of synthetic chemicals in agriculture has been practiced for a long time and their efficacy has been proved numerous times. However, many negative effects against humans, non-target plants, animals and the environment have been reported in the literature (Brown and Morra, 1999; Khai *et al.,* 2007). These deleterious effects have pressured scientists to search for long-term strategies that preserve natural resources while increasing agricultural productivity with minimum adverse impact to the environment. The increased resistance to chemicals by pests and weeds, greater environmental pollution, health hazards including surface and ground water pollution and the presence of chemical residues in agricultural commodities are posing serious ecological questions towards the use of synthetic chemicals (Brown and Morra, 1999; Narwal, 1999; Pilgeram and Sands, 1999). Use of synthetic herbicides also tends to be uneconomical and impractical for use on rangelands and forests where weeds are well established. Additionally, most synthetic chemicals have a relatively long environmental life span compared to natural compounds. Conversely, the environmental life of botanical chemicals is short, thus making

their large scale use or replacement of chemical chemicals unrealistic (Isman, 1999).

The production of allelochemicals by plants has been researched for over six decades. These chemicals are produced in small quantities and have stimulatory and/or inhibitory effects on other plants growing in the vicinity. The allelochemicals affect growth processes including seed germination, plant growth, vigour and development of plants as well as growth and survival of micro-organisms (Mallik, 1999). There is great plant variation in the capacity to produce and in the susceptibility to these chemicals thus leading to the ability of plants to take advantage for growth and reproduction (Rice, 1984). The allelochemicals are released from plants through volatilization, rain washing and leaching, root exudates and root tissue degradation. These chemicals thus have an important influence on plant succession and the composition of plants in the ecosystem.

Knowledge of the factors that are involved in the production and release of the allelochemicals is minimal. However, some issues to be incorporated in further research to unravel these phenomena include effects of environmental factors such as quality, intensity and duration of light, temperature, water and mineral nutrition stress (Alves *et al*., 1999). This is especially important as the activity of plant extracts is dependent on the plant part used, the test organism, the time and method of collecting the plant material, the method of extraction and the solvent used. This is clearly evident in instances where the active ingredients sometimes have low stability and are lost during extraction and purification (Alves *et al*., 1999). The authors also commented that the application of allelopathy in sustainable agriculture is a novel idea considering the positive benefits especially to the environment. However, they are not meant to replace synthetic chemicals but to apply allelopathy as a component of the complex interactions in the agro-ecosystem.

Large amounts of root growth promoting compounds were found in olive knot extract as a result of infection by the bacterium *Pseudomonas savastanoi* pv. *savastanoi*. Amongst the compounds found in the olive knot extract were high levels of auxins, phenols and an unknown compound. These compounds together with auxins enhanced rooting of mung bean cuttings at concentrations ranging from 50 to 60 mg L^{-1} (Roussos *et al*., 2002). A combination of the

extract with indolebutyric acid slightly enhanced root number per cutting. The effect was ascribed to the higher level of naturally occurring auxin in the knots than in healthy olive shoots. There was higher accumulation of plant growth regulators in the vicinity of the gall that was formed subsequent to plant infection by the bacterium. Other substances found in the extract that could affect rooting response were phenolics which are involved in plant defence mechanisms as well as having a slight auxin like activity.

Similar concentration dependent effects on growth stimulation were reported by Marino *et al*. (2004). They reported increased shoot and root development of *in vitro* kiwifruit plant cultures following the application of low concentrations (0.02 ml extract per 100 ml) of amaranth extract to the culture medium. However, higher concentrations had strong inhibitory effects on shoot growth and callus development. Generally, the shoots grown on media enriched by up to 2 ml extract had higher photosynthetic activity and the leaves were greener than the control.

Effects of bio-stimulants were further reported by Zhou *et al*. (2003) who found that application of the rhizome extract of *Paris polyphylla* var. *yunnanensis* had moderate growth stimulation of root hairs of *Paris japonicus* var. *major* while shoot multiplication of *P. polyphylla* var. *yunnanensis* was increased. They concluded that shoot growth stimulation of *P. polyphylla* var. *yunnanensis* and stimulation of root hair growth of *P. japonicus* var. *major* was due to two oligosaccharides which were isolated from the extract. The importance of these findings is the possibility of the oligosaccharides acting as growth stimulants in the species of origin and on a close botanical relative.

The bio-stimulatory product Kelpak®, used in this study, contains natural plant growth regulators with high levels of auxin, low concentration of cytokinin and micronutrients (Arthur *et al*., 2003; Arthur *et al*., 2004; Linwood Supply, 2007) Kelpak® is manufactured from *Ecklonia maxima*, an edible species of brown seaweed. It is non toxic to humans, animals, birds and insects, is biodegradable and is neither explosive nor flammable. Some of the plant growth processes influenced by Kelpak® are stimulation of root formation, increased cytokinin levels as well as improved nutrient and water uptake as a result of improved growth of the root system. The application of Kelpak® at 2 L ha^{-1} increased yield of canola, *Brassica napus*, when applied at the three-leaf

growth stage compared with treatments without Kelpak® and those with higher concentrations of Kelpak. Results from another location in the same study indicated that the yield performance of canola sprayed with 3L ha^{-1} at the three or five-leaf stages was similar and did not show any significant difference from the other treatments (Ferreira and Lourens, 2002). The application of Kelpak® at the rate of 2 L ha^{-1} was more effective in increasing yield when applied at the three-leaf stage. Application of higher concentrations of Kelpak® during later stages of growth was less effective in increasing yield (Ferreira and Lourens, 2002).

In another study on the effect of Kelpak® on fruit production, Mansy et *al*., (2004) reported varying effects of seaweed foliar applications on two cultivars of strawberry. Both products, Kelpak® SL and Goemar BM 86, increased yield of one variety but did not affect the yield of the other cultivar. Additionally, both products reduced fruit firmness in both test cultivars. Further positive effects of the application of Kelpak® were reported by Arthur *et al*. (2003) in a study on pepper, *Capsicum annum*. They observed that application of Kelpak at varying stages during growth, from transplanting to fruit set, led to an overall increase in size and number of marketable pepper fruits. The best treatment was the one where the seedlings were soaked in 0.4% Kelpak® solution and later had three foliar applications of 0.4% Kelpak solution at regular intervals (Arthur *et al*., 2003).

In a review of the effects of liquid fertilizers and natural products, including those derived from seaweed (Kelpak® included), fish waste, vegetables and animal products, Edmeades (2002) concluded that these products were not effective in increasing yield at the rates applied in cited literature. The conclusion from this review was that although the extracts have a potential to increase yield based on the fact that they contain nutrients, organic matter and plant growth hormones and the application rates would have to be at several orders of magnitude over the recommended rates. Edmeades (2002) concluded that the effects on yield have a normal distribution with relatively equal responses reported as positive or negative. Additionally, the nutrients, organic matter and plant hormones in the products were perceived as not being available in sufficient concentrations to elicit the responses reported in literature based on the recommended application

dosages. Based on this conclusion, further quantification of the effects of Kelpak and similar biostimulants is indicated in order to further clarify their effectiveness on plant growth and development.

ComCat®, a new natural bio-stimulant registered in Germany (Agraforum, 2006), was also applied in this study. ComCat® is a unique family of natural products that are based upon a combination of bio-stimulants derived from plant materials. ComCat® products have demonstrated consistent plant growth enhancement and physiological efficiency in the treated plant's utilization of available nutrients. The products nurture and enhance the health of vegetables, flowers and agricultural crops. ComCat® is not a fertilizer substitute but, instead, it is a biological enhancer which stimulates the plant to more properly utilize available nutrients, it activates and induces allelopathy and disease resistance in the treated plant and stimulates greater production of sugars, which are the building blocks for cellulose and fruiting bodies. The result is a more productive, healthier plant with stronger plant stalks, better flowering and greater fruit biomass.

The most cost effective strategies to improve food security include implementation of food based interventions that incorporate improved and diversified crop production. The importance of food security and its effects on human health and the major impacts of food insecurity on cognitive and neurological development with the subsequent lowered learning capability and reduced productive ability of adults are currently better understood (Pelletier *et al*., 2001; Welch and Graham, 2004). The current human health and nutritional anomalies are compelling reasons for plant crop specialists to improve crop production through interventions including biostimulant use and for breeders to improve micronutrient content of crops especially staple crops.

In this study the effects of the interactive effects of biostimulants and fertilizers on growth of carrots will be determined. Of particular interest will be a range of physiological changes following treatment with biostimulants and this will be compared and contrasted with known developmental growth changes. Changes in processes involved in crop development, quality and yield attainment such as sugar and carotene content, sugar translocation; enzyme activity and respiration will be documented. Respiratory activity of carrots throughout growth will be traced to determine potential enhancement of

enzyme synthesis, especially ATP- dependent phosphofructokinase enzyme that is involved in carbohydrate breakdown. It is envisaged that the study will assist in further elucidation of the mode of action of both ComCat and Kelpak.

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CHAPTER 3

Growth response of carrots (*Daucus carota* L.) to different fertilizer levels and commercial bio-stimulants under greenhouse conditions

Abstract

Greenhouse studies were undertaken over two seasons during 2006 and 2007 in order to assess the effect of combined applications of NPK fertilizer and two commercially available bio-stimulants, ComCat® and Kelpak®, on the yield and growth characteristics of carrot (*Daucus carota* L.), cv Karina. The studies were conducted at the University of the Free State's experimental greenhouse and twelve treatments, replicated five times, were laid out in a randomized complete block design. The two bio-stimulants, ComCat® and Kelpak®, were applied either alone or in combination with four fertilizer levels namely the recommended standard (100%) as well as 0%, 25% and 50% of the standard. Increasing levels of fertilizer consistently contributed to increasing carrot root mass, width and length as well as leaf mass and length at the growth stages measured. Application of bio-stimulants led to inconsistent effect on growth components although these differences were not always significant. However, Kelpak®, tended to have a slightly more pronounced growth stimulatory effect than ComCat® over the two seasons in terms of root mass, length and width while the latter mainly influenced leaf length. Interestingly, treatment combinations of bio-stimulants together with the highest fertilizer level (100%; RSA Standard), led to higher measured values for growth components than the combinations with lower fertilizer levels. Intermediate fertilization levels (25% and 50%) in combination with bio-stimulants produced intermediary levels of growth, the same trend that was observed for sole fertilizer application.

Keywords: Carrot, NPK fertilizer, bio-stimulants, ComCat®, Kelpak®, growth components

3.1 Introduction

Carrots, *Daucus carota* L., are economically important worldwide and are popular due to their pleasant flavour and carotene content which confers a variety of health benefits. Carrots are preferred for incorporation in a variety of foods including shredded carrots which are gaining popularity (Alasalvar *et al*. 2001). In South Africa approximately 10 000 hectares are under carrot production annually with a total market share of 9 million South African Rands (South African Department of Agriculture, 2007). The average carrot yield in 2003 was 24.5 tons ha^{-1} based on 4 000 hectares harvested (FAO, 2003). A wide variety of carrots are available for production in South Africa but five main types that target specific markets are grown including those for processing and fresh market (bunching, cut and peel). Premium prices for carrots are received in March to April and to a lesser extend in May due to difficulty of carrot production under hot summer weather conditions (South African Department of Agriculture, 2007). Some carrots are exported to neighbouring countries to meet production constraints in those countries. Of the total 830 tons vegetables imported into Lesotho from South Africa approximately 30 tons are carrots and this is ranked sixth highest in vegetable quantities imported (MITCM, 2003).

Epidemiological evidence in support of the association between food consumption and reduction in chronic disease has triggered the elevated research interest in carrots. Further, consumer interest in the health enhancing aspect of carrots has aided increased production levels. Reports by Russell (2004) as well as Rao and Rao (2007) emphasized that the consumption of food containing β-carotene, lycopene and other carotenoids may be effective against certain types of cancer. Additionally, numerous studies recently highlighted the positive mitigation by antioxidants, including β- carotene, against damage by reactive oxygen species in man (Winklhofer-Roob *et al*., 2003; Dimitrios, 2006).

However, difficulties are encountered in the production of carrots due to erratic seed germination and seedling emergence. The poor plant density often obtained influences yield. When the plant density is low the roots tend to be large, prone to splitting and marketable yields decline. On the other hand, high plant

density tends to produce thin often twisted roots of lower quality (Reid, 2005; Kamariddin, 2007). Sporadic carrot seed germination and emergence inevitably result in variable plant density that in turn results in reduced crop stands thereby negatively influencing root growth and yield (Rajasekaran *et al*., 2002). Conversely, yields of greenhouse grown vegetables tend to be higher and of better quality than field vegetable crops and production can be done throughout the year (Kamariddin, 2007).

The continuing alteration of environmental nitrogen cycling by humans through agricultural activity has adverse effects on water sources such as lakes, rivers and estuaries (Hatano *et al*., 2002; Janzen *et al*., 2003; Ju *et al*., 2006; 2007). This has led to the necessity for better understanding of nitrogen dynamics within the watersheds and the overall impact of nitrogen sources to nitrogen loading into the environment and eventually its influence on water quality (Hatano *et al*., 2002; Janzen *et al*., 2003; Rock and Mayer, 2006; Yang *et al*., 2007). According to Gutezeit and Fink (1999), the groundwater nitrogen concentration of intensive vegetable growing regions was found to be higher than the recommended WHO and European limit of 50 mg NO_3^- per litre. They recommended that better nutrient planning; including reduced levels of nitrogen in carrot production programs should limit nitrate contamination of soils.

Carrot nitrogen requirements are low and a good yield is possible with 70 kg ha^{-1} nitrogen application. Higher levels of nitrogen, 120 kg ha^{-1}, are commonly applied to attain higher yields. The phosphorus requirement of carrots is 40 to 80 kg ha^{-1} (FSSA, 2007) and the nutrient is important for root vigour and growth. Carrot potassium requirement is 60 to 100 kg ha^{-1} and potassium has been associated with better colour development and enhancement of keeping quality (shelf life). The most practical fertilizer application method for carrots is split application where most of the fertilizer is applied at planting and the balance at approximately 4 to 8 weeks after sowing when leaves are expanding (KwaZulu Natal, 2008).

Although nitrates are naturally present in fruits and vegetables, their content is compounded by those from water and additives (Oztekin *et al*., 2002; Prasad

and Chetty, 2008). According to the authors, additional to environmental concerns following fertilizer application, is the accumulation of nitrates in carrots as the amount of nitrates in baby foods and dietary juices might be higher than the prescribed quality requirements. The nitrate content of carrot roots utilized in the production of baby foods must be as low as possible and should not exceed prescribed threshold values (Belpomme *et al*., 2007). The consumption of these chemicals by humans in meat, vegetable products and drinking water is of great concern as nitrate is broken down to nitrites and eventually carcinogenic nitrosoamines (Camargo and Alonso, 2006; Irigaray *et al*., 2007). The application of nitrogen fertilizers during production, therefore, has to be regulated to control the level of nitrate in the soil profile and crops.

In this study, two commercially available natural bio-stimulants with root enhancing properties were used to investigate their effect at four different fertilizer regimes. The two bio-stimulants are: ComCat®, which contains a seed extract of the plant *Lychnis viscaria* L., (German catch fly) as well as Kelpak®, a sea weed extract from marine alga or kelp, *Ecklonia maxima.* The main aim was to ascertain whether foliar applications of the bio-stimulants on their own, or in combination with fertilizer at lower than the recommended level, were capable of producing acceptable yields. The latter approach additionally aimed at addressing environmental concerns regarding extreme fertilizer application.

3.2 Materials and Methods

3.2.1 Materials

Pot trials were conducted in the greenhouse at the University of the Free State during the 2006 and 2007 growing seasons. A pre-pack carrot cultivar, Karina, was used during both seasons. Two commercially available bio-stimulants ComCat® and Kelpak® were used. The following laboratory grade chemicals were used for the supply of various nutrients: potassium from potassium chloride (KCl), nitrogen from ammonium nitrate (NH_4NO_3) and phosphorus from phosphoric acid (H_3PO_4).

3.2.2 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, University of the Free State, in the Bloemfontein district (29°01'00"S, 26°08'50"E). The soil was dried at room temperature, sieved through a 5 mm mesh sieve and used for growing carrots in pots in the greenhouse during both growing seasons. The fertility status of the soil collected in both seasons was, in general, excellent according to local guidelines as indicated in Table 3.1 (FSSA, 2007).

Table 3.1: Physical and chemical properties of the topsoil collected in 2006 and 2007.

	Norms	2006	2007
Clay & Silt %		20	20
Sand %		80	80
Class		Sandy loam	Sandy loam
EC (mSm^{-1})	0 – 300	89	48
SAR	< 5	0.5	0
pH $_{(KCl)}$	5.5 – 6.5	4.57	4.63
Nutrients ($mg\ kg^{-1}$)			
Ca	300-3000	571.3	566.04
Mg $_{(NH\ 4\ OAc)}$	50-300	191.35	163.19
K $_{(NH\ 4\ OAc)}$	80-250	227.45	176.53
Na	100-500	31.98	1.04
P $_{(Olsen)}$	5-10	9.53	9.26
Zn $_{(HCl)}$	2-5	0.91	0.17

Determined with standard procedures (The Non-affiliated Soil Analysis Working Committee, 1990).

3.2.3 Treatments and experimental design

Fertilizer treatments applied to the carrots was based on the nutrient withdrawal amounts (FSSA, 2003) for South African conditions. For a potential yield of 25 ton ha^{-1} (Hygrotech, 2006) carrots are calculated to withdraw 95 kg N, 15 kg P and 125 kg K ha^{-1}. Four fertilizer levels, the standard recommended level ($NPK_{100\%}$), half ($NPK_{50\%}$), a quarter ($NPK_{25\%}$) and none ($NPK_{0\%}$) of the recommended level were applied. Two commercial bio-stimulants (ComCat® and Kelpak®) were applied singly or in combination with the various fertilization regimes. The resultant treatments are shown in Table 3.2.

Table 3.2: Different fertilizer and bio-stimulant treatments

Treatment	Fertilizer (%)	N (kg ha $^{-1}$)	P (kg ha $^{-1}$)	K (kg ha $^{-1}$)	ComCat (g ha $^{-1}$)	Kelpak (L ha $^{-1}$)
1	100	95	15	125	0	0
2	100	95	15	125	100	0
3	100	95	15	125	0	2
4	50	47.5	7.5	62.5	0	0
5	50	47.5	7.5	62.5	100	0
6	50	47.5	7.5	62.5	0	2
7	25	23.75	3.75	31.25	0	0
8	25	23.75	3.75	31.25	100	0
9	25	23.75	3.75	31.25	0	2
10	0	0	0	0	0	0
11	0	0	0	0	100	0
12	0	0	0	0	0	2

A randomized complete block design was used and each treatment combination was replicated five times. 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. All the phosphorus fertilizer application was done one month prior to soil use. The phosphoric acid was dissolved in distilled water and evenly sprayed on the soil heap using a backpack sprayer. The soil was regularly turned over to ensure even distribution of the sprayed material. After application of the phosphoric acid the soil was stored for one month before it was used to fill the pots. 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 were made based on a pot with a diameter of 35 cm and a depth of 35 cm. 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.

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) approximately 3 weeks after the first application.

3.2.4 Production aspects

Carrot seed (cv. Karina) was hand-sown thinly in three rows, 8 cm apart, in pots. 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.

3.2.5 Growth measurements

Two carrots per treatment were taken from all five replicates at four growth stages coinciding with the development of vegetative plant parts (Meier, 1997). Samples were taken at the following stages of growth: when 30% of the expected leaf number was reached (growth stage 43); 60% expected leaf number (growth stage 46); 80% expected leaf number (growth stage 48) and final development when the leaves started yellowing (growth stage 49). Measurements of leaf length, root length, root width, leaf and root mass were done directly after removing the plants from the pots. All length and width measurements were done using a digital

caliper. For leaf length, measurement was taken from the base of the petiole to the tip of the leaf blade. Root length was taken from the collar to the base of the storage root and root width was taken in the region of the crown, within 2 cm from the collar.

3.2.6 Statistical analysis

The Number Cruncher Statistical Software, NCSS 2000, (Hintze, 1999) was used to perform analysis of variance (ANOVA) on the data in order to identify differences between the treatment means. Separation of treatment means was performed using the Tukey-Kramer Multiple Comparison Test and expressed as least significant difference (LSD) at the 5% ($P<0.05$) probability level (Steele and Torrie, 1980).

3.3 Results

3.3.1 Root fresh mass

During both seasons, and for all growth stages tested, the same tendency of steady root fresh mass increase as the fertilizer level was increased from 0 – 100%, was observed (Table. 3.3). However, the response of carrots to treatment with bio-stimulants in terms of root fresh mass was not always significantly different when measured at different growth stages.

At 30% development there was no significant difference in the interaction (FBxFL) between the bio-stimulant treatments (FB) and fertilizer levels (FL) in terms of root fresh mass, and this trend applied for both seasons. However, for both seasons fertilizer application at the standard (100%) and at 50% of the standard fertilizer level significantly increased root mass compared to the control (0%). Although the Kelpak® treatment showed a significant increasing effect on the average root mass in 2006, compared to the ComCat® treatment, it did not differ significantly from the control treatment and this tendency was not repeated in 2007.

Table 3. 3: Effect of fertilizer and bio-stimulants applied at different levels on the mean fresh mass of carrots (g/carrot) at different growth stages

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
30% Plant development								
100	9.42	9.96	8.74	**9.37**	19.02	20.38	20.98	**20.13**
50	7.92	5.84	10.37	**8.04**	15.48	16.96	14.46	**15.63**
25	6.93	7.11	7.61	**7.21**	10.84	12.04	14.06	**12.31**
0	7.10	5.18	8.15	**6.81**	7.54	10.24	10.24	**9.34**
Ave FB	**7.84**	**7.02**	**8.72**		**13.22**	**14.91**	**14.94**	
$LSD_{(T)(0.05)}$ FBxFL	ns				ns			
$LSD_{(T)(0.05)}$ FL	1.94				3.72			
$LSD_{(T)(0.05)}$ FB	1.52				ns			
60% Plant development								
Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	42.82	41.24	64.48	**49.51**	47.16	51.96	46.18	**48.43**
50	34.22	45.76	44.22	**41.40**	32.97	45.53	44.11	**39.87**
25	25.84	36.92	42.06	**34.94**	35.46	33.58	38.26	**35.77**
0	27.92	28.60	29.68	**28.73**	26.69	25.03	30.21	**27.31**
Ave FB	**32.70**	**38.13**	**45.11**		**35.57**	**38.27**	**39.69**	
$LSD_{(T)(0.05)}$ FBxFL	12.02				7.83			
$LSD_{(T)(0.05)}$ FL	5.31				3.46			
$LSD_{(T)(0.05)}$ FB	4.16				2.71			
80% Plant development								
Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	71.10	106.13	93.98	**90.40**	58.76	48.52	58.72	**55.33**
50	74.78	79.88	97.50	**84.05**	41.10	48.16	42.68	**43.98**
25	94.85	70.70	88.80	**84.78**	25.96	37.06	35.06	**32.69**
0	71.58	58.05	70.76	**66.80**	30.62	31.92	34.50	**32.35**
Ave FB	**78.08**	**78.69**	**87.76**		**39.11**	**41.42**	**42.74**	
$LSD_{(T)(0.05)}$ FBxFL	31.07				ns			
$LSD_{(T)(0.05)}$ FL	13.72				7.13			
$LSD_{(T)(0.05)}$ FB	ns				ns			
At harvest								
Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	108.80	89.56	106.70	**101.69**	62.82	70.12	71.02	**67.99**
50	97.18	86.50	76.16	**86.61**	59.64	53.58	50.60	**54.61**
25	82.16	101.22	77.48	**86.95**	51.24	44.18	44.64	**46.69**
0	84.68	60.06	59.22	**67.99**	44.66	33.40	34.48	**37.51**
Ave FB	**93.21**	**84.34**	**79.89**		**54.59**	**50.32**	**50.19**	
$LSD_{(T)(0.05)}$ FBxFL	34.24				10.69			
$LSD_{(T)(0.05)}$ FL	15.12				4.72			
$LSD_{(T)(0.05)}$ FB	11.86				3.70			

Except for 2007 at 80% development, significant interaction (FBxFL) between the bio-stimulant treatments (FB) and fertilizer levels (FL) in terms of root fresh mass was observed from 60% vegetative growth up to harvest (Table 3.3). At 60% development and in 2006, root fresh mass increased significantly where 25% or 100% fertilizer was applied in combination with Kelpak® compared to the corresponding fertilizer only treatments. However, in 2007 the same tendency only applied for Kelpak® at the 50% fertilizer level while the combination treatment with ComCat® showed significant differences at both the 50% and 100% fertilizer levels. At 80% development, but only in 2006 at the 100% fertilizer level, root fresh mass significantly increased in both cases where bio-stimulants were applied in combination. Although the interaction was also significant at harvest, addition of the bio-stimulants to different levels of fertilizer treatments rather had a reducing effect on root fresh mass. From the above it is clear that a rather erratic picture emerged under greenhouse conditions.

3.3.2 Root length

As was the case with root fresh mass, root length growth followed the same trend to increase as fertilizer application was elevated (Table 3.4). Although this tendency was observed in both seasons and at all development stages, it was less pronounced during 2006. However, differences were statistically significant (FL) in 2007 at all development stages but, especially between the 0% and higher fertilizer regimes. The root length growth response after application of the two bio-stimulants, ComCat® and Kelpak®, was rather erratic when measured at the different growth stages. As a result the statistical interaction (FBxFL) between bio-stimulant treatment (FB) and fertilizer level (FL) did not follow the same pattern at all development stages. For instance, FBxFL was statistically significant in both seasons only at 30% development and at harvest.

However, this significance did not apply when cross comparisons between the controls and bio-stimulant treatments on specific fertilizer levels were made (horizontally in rows) at 30% plant development in 2006 and at harvest in 2007

(Table 3.4). At harvest, in 2006, ComCat® and Kelpak® significantly decreased carrot root length where no fertilizer was applied and at the 30% and 60% development stages Kelpak®, in combination with the 100% fertilizer level, significantly increased carrot root length.

Table 3.4: Effect of fertilizer and bio-stimulants applied at different levels on the mean carrot root length (mm) at different growth stages

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
30% Plant development								
100	87.33	73.15	69.55	**76.68**	114.34	102.00	135.77	**117.32**
50	64.39	65.98	92.30	**74.22**	104.29	110.03	114.39	**109.57**
25	67.86	69.17	78.70	**71.91**	96.79	116.88	112.39	**108.69**
0	60.40	67.01	70.28	**65.89**	78.29	97.97	99.25	**91.83**
Ave FB	**70.00**	**68.83**	**77.71**		**98.43**	**106.72**	**115.45**	
$LSD_{(T)(0.05)}$ FBxFL	30.05				14.52			
$LSD_{(T)(0.05)}$ FL	ns				6.41			
$LSD_{(T)(0.05)}$ FB	ns				5.03			
60% Plant development								
Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	126.85	109.98	107.36	**114.73**	125.44	128.69	151.97	**135.37**
50	110.79	135.25	104.65	**116.89**	147.08	117.70	132.22	**132.33**
25	110.20	110.00	104.30	**108.16**	125.54	124.29	134.08	**127.97**
0	99.22	124.38	92.09	**105.23**	87.83	96.91	114.62	**99.79**
Ave FB	**111.76**	**119.90**	**102.10**		**121.47**	**116.90**	**133.22**	
$LSD_{(T)(0.05)}$ FBxFL	ns				22.79			
$LSD_{(T)(0.05)}$ FL	ns				10.07			
$LSD_{(T)(0.05)}$ FB	14.18				7.89			
80% Plant development								
Fertilizer level %	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	133.73	117.92	138.86	**130.17**	140.22	134.14	143.88	**139.41**
50	142.17	132.28	129.32	**134.59**	133.86	133.51	119.17	**128.84**
25	158.54	125.95	147.18	**143.89**	103.04	116.39	112.64	**110.69**
0	126.04	123.38	120.24	**123.22**	109.20	109.66	114.20	**111.02**
Ave FB	**140.12**	**124.88**	**133.90**		**121.58**	**123.42**	**122.47**	
$LSD_{(T)(0.05)}$ FBxFL	ns				ns			
$LSD_{(T)(0.05)}$ FL	ns				14.97			
$LSD_{(T)(0.05)}$ FB	ns				ns			
At harvest								
Fertilizer level %	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	131.87	130.98	142.87	**135.24**	145.95	123.20	132.72	**133.96**
50	120.68	126.81	120.82	**122.77**	109.97	101.37	118.88	**110.07**
25	124.82	136.55	126.57	**129.31**	88.07	101.23	96.94	**95.41**
0	133.01	108.87	107.05	**116.31**	82.77	101.71	111.52	**98.66**
Ave FB	**127.59**	**125.80**	**124.33**		**106.69**	**106.88**	**115.01**	
$LSD_{(T)(0.05)}$ FBxFL	22.35				30.30			
$LSD_{(T)(0.05)}$ FL	17.10				13.38			
$LSD_{(T)(0.05)}$ FB	ns				ns			

Root width

Although the statistical interaction (FBxFL) between fertilizer level and treatment with bio-stimulants was non-significant across all development stages during both the 2006 and 2007 growing seasons, except at harvest in 2007, consistent increases in root width subsequent to elevated fertilizer application (FL) were observed (Table 3.5). The latter was significant in both seasons at the 60% development stage and at harvest as well as at the 30% and 80% plant development stages in 2007.

Once again treatment with the two bio-stimulants had no significant effect on root width except at harvest (Table 3.5) during 2007. Treatment with ComCat® and Kelpak® in combination with the 100% fertilizer level had a significant increasing effect on root width at harvest in 2007. The application of 50% and 100% fertilizer levels consistently increased root width during both seasons.

Table 3.5: Effect of fertilizer and bio-stimulants applied at different levels on the mean root width (mm) of carrots at different growth stages

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
30% Plant development								
100	12.11	12.18	11.92	**12.07**	13.24	13.23	14.01	**13.49**
50	10.88	11.18	12.58	**11.55**	12.33	12.62	12.08	**12.34**
25	11.33	10.36	11.86	**11.18**	10.75	11.08	12.30	**11.38**
0	11.14	10.69	10.97	**10.93**	9.57	10.35	10.76	**10.22**
Ave FB	**11.37**	**11.10**	**11.83**		**11.47**	**11.82**	**12.29**	
$LSD_{(T)(0.05)}$ FBxFL	ns				ns			
$LSD_{(T)(0.05)}$ FL	ns				1.30			
$LSD_{(T)(0.05)}$ FB	ns				ns			
60% Plant development								
Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	19.97	20.55	22.05	**20.85**	20.42	19.98	21.54	**20.65**
50	20.41	19.57	21.86	**20.61**	21.25	20.86	21.03	**21.05**
25	18.71	19.63	19.07	**19.13**	18.37	19.25	18.44	**18.68**
0	17.47	17.92	18.13	**17.83**	17.82	18.61	18.85	**18.42**
Ave FB	**19.13**	**19.41**	**20.27**		**19.46**	**19.67**	**19.97**	
$LSD_{(T)(0.05)}$ FBxFL	ns				ns			
$LSD_{(T)(0.05)}$ FL	2.16				1.77			
$LSD_{(T)(0.05)}$ FB	ns				ns			
80% Plant development								
Fertilizer level %	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	26.22	25.98	24.87	**25.69**	22.56	21.93	22.75	**22.41**
50	26.02	24.69	26.08	**25.60**	20.70	21.41	20.39	**20.83**
25	27.30	25.59	27.03	**26.64**	18.75	19.43	20.46	**19.55**
0	24.14	22.72	22.42	**23.09**	19.93	19.16	19.39	**19.49**
Ave FB	**25.92**	**24.74**	**25.10**		**20.49**	**20.48**	**20.75**	
$LSD_{(T)(0.05)}$ FBxFL	ns				ns			
$LSD_{(T)(0.05)}$ FL	ns				1.81			
$LSD_{(T)(0.05)}$FB	ns				ns			
At harvest								
Fertilizer level %	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	31.62	28.58	29.42	**29.87**	25.03	28.09	28.94	**27.35**
50	27.05	29.31	26.84	**27.73**	23.19	24.37	23.64	**23.73**
25	27.56	27.42	25.37	**26.78**	22.30	21.98	20.57	**21.62**
0	26.16	25.53	25.75	**25.81**	19.24	19.83	18.78	**19.29**
Ave FB	**28.10**	**27.71**	**26.84**		**22.44**	**23.57**	**22.98**	
$LSD_{(T)(0.05)}$ FBxFL	ns				2.57			
$LSD_{(T)(0.05)}$ FL	2.74				1.13			
$LSD_{(T)(0.05)}$ FB	ns				0.89			

3.3.4 Leaf fresh mass

More or less the same, rather inconsistent, pattern in terms of the effect of different treatments on leaf fresh mass emerged as was the case for root data (Table 3.6). An interesting feature in leaf growth was the fact that leaf mass was, generally, substantially lower (up to five times) during the 2007 season compared to 2006 while the root mass (Table 3.3), albeit not so pronounced, followed a similar tendency. It was therefore not surprising that the FBxFL interaction was non-significant at all growth stages in the one season (2006) but significant in the other (2007).

Despite the non-significant interaction between fertilizer level and bio-stimulant treatment, elevated fertilizer levels contributed to a significant increase in leaf fresh mass during both seasons and at virtually all growth stages in a more or less linear fashion (Table 3.6). This was more pronounced at the 50% and 100% fertilizer levels that in most instances also differed significantly from each other, while highest fertilizer level (standard) constantly contributed to the highest leaf fresh mass.

Application of bio-stimulants once again gave precarious results in terms of leaf fresh mass. During 2007, treatment with both ComCat® and Kelpak® contributed to significant leaf mass increases at the early development stages (30% to 80% development; Table 3.6). In contrast, the opposite prevailed at harvest where treatment with the bio-stimulants had an inhibiting effect during the same season. Leaf mass was not enhanced by bio-stimulant application in 2006 across all development stages.

Table 3.6: Effect of fertilizer and bio-stimulants applied at different levels on the mean leaf fresh mass of carrots (g/carrot) at different growth stages

30% Plant development

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	16.25	17.03	15.32	**16.20**	8.45	11.73	10.90	**10.36**
50	13.93	12.31	16.63	**14.29**	8.35	9.20	8.50	**8.68**
25	11.69	12.18	11.69	**11.85**	6.10	7.02	7.17	**6.76**
0	10.52	9.79	11.86	**10.72**	5.18	7.07	6.55	**6.26**
Ave FB	**13.10**	**12.83**	**13.87**		**7.02**	**8.76**	**8.28**	
$LSD_{(T)(0.05)}$FBxFL	ns				1.97			
$LSD_{(T)(0.05)}$ FL	3.85				0.87			
$LSD_{(T)(0.05)}$ FB	ns				0.68			

60% Plant development

Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	34.68	48.64	44.36	**42.56**	9.07	11.43	12.41	**10.97**
50	36.30	26.92	42.52	**35.25**	9.44	9.10	10.72	**9.75**
25	27.12	26.86	23.90	**25.96**	6.89	7.64	7.62	**7.38**
0	17.08	21.02	21.54	**19.88**	6.97	4.95	6.06	**5.99**
Ave FB	**28.80**	**30.86**	**33.08**		**8.09**	**8.28**	**9.20**	
$LSD_{(T)(0.05)}$FBxFL	ns				2.33			
$LSD_{(T)(0.05)}$ FL	9.43				1.03			
$LSD_{(T)(0.05)}$ FB	ns				0.81			

80% Plant development

Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	36.54	38.40	37.06	**37.33**	10.12	12.36	14.08	**12.19**
50	40.74	29.62	36.66	**35.67**	6.88	9.68	8.06	**8.21**
25	41.40	31.46	31.10	**34.65**	5.66	5.50	5.78	**5.65**
0	20.90	22.98	32.46	**25.44**	5.20	5.82	5.92	**5.65**
Ave FB	**34.90**	**30.62**	**34.32**		**6.97**	**8.34**	**8.46**	
$LSD_{(T)(0.05)}$FBxFL	ns				3.38			
$LSD_{(T)(0.05)}$ FL	ns				1.49			
$LSD_{(T)(0.05)}$ FB	ns				1.17			

At harvest

Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	34.20	29.56	27.26	**30.34**	11.16	9.10	6.00	**8.75**
50	26.10	29.96	22.44	**26.17**	5.62	6.22	7.10	**6.31**
25	21.54	25.18	18.84	**21.85**	3.72	3.98	3.42	**3.71**
0	19.56	18.44	23.88	**20.63**	4.22	4.52	3.98	**4.24**
Ave FB	**25.35**	**25.79**	**23.11**		**6.18**	**5.96**	**5.13**	
$LSD_{(T)(0.05)}$FBxFL	ns				3.75			
$LSD_{(T)(0.05)}$ FL	9.92				1.66			
$LSD_{(T)(0.05)}$ FB	ns				ns			

3.3.5 Leaf length

Interestingly, where the measured leaf fresh mass was generally lower during the 2007 season compared to 2006, this was not the case with leaf length (Table 3.7). However, interaction between fertilizer level and bio-stimulant treatment (FBxFL) was insignificant in most cases except at the 30% and 60% development stages in 2007.

In 2007 and at the 30% development stage, Kelpak® in combination with the 50% fertilizer level significantly decreased leaf length compared to the control and ComCat® treatments. The opposite was observed at the 60% development stage where Kelpak® in combination with 100% fertilizer significantly increased leaf length compared to the control and ComCat® treatments.

At all growth stages and during both seasons elevation of fertilizer levels linearly enhanced carrot leaf length although not always significantly. At harvest in 2006 both bio-stimulants tended to decrease leaf length although there was significance only where Kelpak® was applied. However, in 2007 ComCat® significantly increased leaf length whereas Kelpak® significantly decreased leaf length compared to the control.

Table 3.7: Effect of fertilizer and bio-stimulants applied at different levels on the mean leaf length of carrots (mm) at different growth stages

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
30% Plant development								
100	281.70	278.52	280.28	**280.17**	284.30	302.21	299.41	**295.31**
50	269.56	250.52	260.71	**260.27**	285.86	280.72	233.12	**266.57**
25	244.21	270.47	251.37	**255.35**	264.54	281.98	277.60	**274.71**
0	247.79	243.21	249.07	**246.69**	271.14	289.13	258.68	**272.98**
Ave FB	**260.82**	**260.68**	**260.36**		**276.46**	**288.51**	**267.20**	
$LSD_{(T)(0.05)}$ FBxFL	ns				41.38			
$LSD_{(T)(0.05)}$ FL	28.02				18.28			
$LSD_{(T)(0.05)}$ FB	ns				ns			
60% Plant development								
Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	331.99	362.69	347.13	**347.27**	281.58	284.67	320.70	**295.65**
50	329.72	322.66	358.75	**337.04**	293.18	292.14	272.22	**285.85**
25	323.86	329.55	291.21	**314.87**	274.12	284.00	292.76	**283.63**
0	287.62	303.08	281.47	**290.72**	290.17	289.76	287.59	**289.18**
Ave FB	**318.30**	**329.49**	**319.64**		**284.76**	**287.64**	**293.32**	
$LSD_{(T)(0.05)}$ FBxFL	ns				39.42			
$LSD_{(T)(0.05)}$ FL	35.87				ns			
$LSD_{(T)(0.05)}$ FB	ns				ns			
80% Plant development								
Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	368.61	380.89	357.14	**368.88**	310.00	283.53	290.44	**294.66**
50	343.49	336.93	376.16	**352.19**	292.21	313.65	271.66	**292.51**
25	348.48	336.04	314.26	**332.93**	248.13	275.12	266.17	**263.14**
0	291.44	301.33	292.97	**295.25**	289.05	285.83	273.28	**282.72**
Ave FB	**338.00**	**338.80**	**335.13**		**284.85**	**289.53**	**275.39**	
$LSD_{(T)(0.05)}$ FBxFL	ns				ns			
$LSD_{(T)(0.05)}$ FL	40.18				23.67			
$LSD_{(T)(0.05)}$ FB	ns				ns			
At harvest								
Fertilizer level %	Fertilizer + Bio-stimulant							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	364.81	337.16	329.09	**343.69**	284.38	328.14	276.34	**296.29**
50	341.52	306.06	333.64	**327.07**	298.12	296.56	254.14	**282.94**
25	329.23	319.91	304.69	**317.94**	257.45	268.21	245.40	**257.02**
0	281.20	289.45	272.67	**281.11**	248.80	273.30	245.34	**255.81**
Ave FB	**329.19**	**313.14**	**310.02**		**272.19**	**291.56**	**255.31**	
$LSD_{(T)(0.05)}$ FBxFL	ns				ns			
$LSD_{(T)(0.05)}$ FL	21.88				21.44			
$LSD_{(T)(0.05)}$ FB	17.15				16.81			

3.4 Discussion

The fertilizer treatments applied in this greenhouse study included levels equal to and lower than the recommended standard for carrot production in South Africa. The four fertilizer treatments enabled the study of carrot growth under a range of fertilizer regimes including sub-optimal levels representing trends in many areas of Lesotho. These included the standard (100%) as well as 50%, 25% and 0% of the standard.

During both the 2006 and 2007 growing seasons, and as could be expected, the general trend observed was that the increase in levels of NPK fertilizer from 0% to 100% consistently had a positive influence on the measured growth parameters namely carrot root width, length and mass as well as carrot leaf mass and length in a more or less linear fashion. Although at times erratic, this trend was repeatedly observed at all four growth stages (30%, 60% and 80% vegetative growth as well as at harvest) and during both growing seasons. However, although increasing fertilization accelerated vegetative growth measured by means of different parameters, the relationship between the fertilizer level and the outcome was not always significant. Nevertheless, the general increase in vegetative growth over time and at different fertilizer regimes was typical for carrots (Rubatzky *et al*., 1999) and also conformed to expected higher uptake of nutrients by plant roots in fertilized crops (Krishna and Rosen, 2002).

The stimulating effect of increasing fertilizer levels on carrot growth was consistent with the growth enhancing effect of fertilizer on crop growth in general (Ryan, 2002). This positive influence of fertilizers was recently reported by Hailu *et al*. (2008) who noted that pre-harvest application of organic phosphorus and inorganic nitrogen fertilizer increased the yield and yield components of carrots. Similar results to those of this study were reported by Ali *et al*. (2003) who observed increasing root yield when increasing levels of nitrogen and potassium were applied to carrots. Overall the highest level of fertilization had the highest effect on growth. Significant increases in yield were also reported by Gutezeit (2001) with application of 150 kg ha^{-1} nitrogen fertilizer compared to zero fertilization on both sandy and loam soils.

Contrary to the expectation of increased growth and yield with increasing fertilizer application, Gutezeit (1999) and Pettipas *et al.* (2006) did not observe substantial increase in growth and yield subsequent to fertilizer application. Nitrogen applied up to 200 kg ha^{-1} did not significantly increase growth, yield or quality of carrots (Gutezeit, 1999; Pettipas *et al.*, 2006). The root fresh mass, leaf fresh mass and leaf length were also not significantly different regardless of the level of nitrogen fertilizer applied by Pettipas *et al.* (2006). Instead, the carrots that received zero nitrogen fertilization produced a significantly higher quantity of fancy grade carrots compared with nitrogen fertilization at the rate of 200 kg ha^{-1}. The lack of response to fertilizer application can be partially explained by the ability of the deep rooted carrot plants to access residual nutrients in the soil (Thorup-Kristensen and van den Boogaard, 1999), particularly the direct uptake of nitrogen adsorbed to soil particles (Pettipas *et al*., 2004). Carrots developed well up to harvest and utilized a limited amount of available soil nitrogen despite the deep roots (Thorup-Kristensen and van den Boogaard, 1999). Excessive nitrogen application thus diminishes the ability of carrots to deplete available soil nitrogen.

No deficiencies of phosphorus were detected in the present study thus indicating possible adequacy and availability of this nutrient throughout both seasons at all the levels of fertilizer used. Determination of phosphorus utilization and efficiency in cabbage, carrot and potato was undertaken by Dechassa *et al.* (2003). The results indicated that carrot reached only 4% of its maximum yield at no phosphorus application. The uptake of phosphorus by root hairs was found to be 3% of the total phosphorus absorbed for carrot compared to 50% for cabbage and potato. In light of the growth and full development of carrots in 2006 and 2007 under glasshouse conditions it can be concluded that phosphorus was sufficient to attain full growth of carrot under the implemented fertilization regimes.

One of the main objectives of this study was to ascertain whether the application of new generation bio-stimulants, such as ComCat® and Kelpak®, could contribute to elevated growth and yield in carrots at lower fertilizer regimes. The constituent auxin-like substances in Kelpak® are thought to induce crop growth by stimulating formation of adventitious roots and enhancing water and

nutrient uptake from the soil (Linwood Supply, 2005). In the case of ComCat® it is brassinosteroids (Roth *et al*., 2000) that are responsible for its root growth enhancing effect. However, a rather precarious correlation between treatment and growth was detected in this study where different fertilizer levels were combined with foliar applications of the two products. Two foliar applications of Kelpak®, at the three to four leaf growth stage and again at the 7 to 8 leaf stage (three weeks after the first application), increased carrot growth characteristics in an inconsistent manner. During both growing seasons root fresh mass was positively (i.e. in terms of number of growth stages found with positive growth) influenced by Kelpak® at early stages of growth (30% to 80% development), although not always significantly. Root length and width were also mainly positively influenced by Kelpak® during early growth stages while its effect on leaf mass and length was minimal compared to root length, mass and width. This apparent beneficial influence of Kelpak® on carrot growth was, however, not evident at harvest where growth was actually inhibited in some instances.

Research conducted previously on the yield effects of Kelpak® on canola (*Brassica napus*) indicated an improvement in yield when the bio-stimulant was applied at certain critical stages of growth (Ferreira and Lourens, 2002). Likewise the study done by Arthur *et al.* (2003) indicated that application of Kelpak® to pepper (*Capsicum annuum*) positively influenced fruit number and quality of pepper. Contrary to expectations and to what has been documented by among others Ferreira and Lourens (2002) and Arthur *et al*. (2003) the application of Kelpak® in this study did not always lead to the expected increase in growth characteristics of carrots. The likely explanation might be related to the exact timing of application. Crops seem to respond to treatment with Kelpak® when it coincides with regular and repeated application throughout growth and development.

In the literature varied controlled studies on the effects of seaweed extracts are found and with mixed results. Seaweed extract, of which Kelpak® is an example, have been shown to improve yield in one crop but not another grown under the similar conditions. Further, regardless of the numerous studies

conducted the mode of action of the seaweed extracts is not clear. Although Kelpak® contains plant growth regulators such as cytokinins and auxins it has not been conclusively proven that they alone are responsible for the improved growth and yield reported. This emphasizes how important it has become to elucidate the mechanism of action of these types of products in order to unravel the current limited understanding of their physiological control in crop development.

A strongly positive response of carrot leaf length growth to treatment with the other bio-stimulant, ComCat®, compared to the sole fertilizer treatments, was evident during both growing seasons. Although not always significant, application of ComCat® tended to increase carrot root fresh mass, but mostly at the 100% fertilizer level. This was not always significantly different from the Kelpak® effect, especially at harvest, indicating that under the greenhouse conditions in this study neither of the two products was superior. Further, the same trend was not observed for root length and width in carrots treated with ComCat®. Thus, in instances where combinations of different fertilizer levels and bio-stimulants were applied, the general trend was for slightly higher growth attainment where high levels of fertilizer were used compared to instances where fertilizer was absent or applied at a low level (e.g. 25% fertilization).

The literature pertaining to the use of ComCat® in agriculture is scarce and does not necessarily relate to carrots or root crops, thus making comparison on efficacy difficult. However, Roth *et al*. (2000) reported on the enhanced resistance to viral and pathogen attack in tobacco, cucumber and tomato treated with aqueous extracts from *Lychnis viscaria* L. seeds, a constituent of ComCat®. The presence of brassinosteroids, 24-Epi-secasterone and 24-epi-castasterone from *Lychnis viscaria* L. seeds was confirmed by Friebe *et al*. (1999). Hayat *et al*. (2000) concluded that application of the brassinosteroid, 28-homobrassinolide, increased vegetative growth and dry matter production of mustard as a result of enhanced photosynthetic capacity of the treated plants. Brassinosteroids are the main active compounds of ComCat®.

In this study, application of elevated nitrogen, phosphorus and potassium fertilizer levels generally showed a consistent positive effect on the growth of

carrots. The results indicated that at least the 50% and standard (100%) fertilizer regimes were sufficient to satisfy the growth requirements of carrots up to maturity. Although soil nitrogen was not determined in this study, the satisfactory performance of carrots under varying fertilizer levels, including some below the recommended rate might indicate the ability of carrots to access nutrients available from the soil. This aspect per se is of great interest in the case of subsistence farmers that cannot afford fertilizers as well as in light of the recent fertilizer price hikes, from a commercial perspective. Westerveld *et al.* (2006) observed high nitrogen use efficiency in carrots under minimal or no nitrogen fertilization. However, crop production under very low or deficit nutrient levels pose the danger of soil mining (D'Haene *et al*., 2007). This ability of carrots to produce marketable yield at low fertilizer levels might offer an opportunity for growers to reduce fertilizer application without reducing yields and while still being assured of recovery of good quality grades of carrots.

In conclusion, while combination treatments of bio-stimulants and fertilizer were not always significantly better than the fertilizer controls in this pilot study under greenhouse conditions, the possibility that the bio-stimulants could still be viable options under field conditions prevailed. This was particularly the case for medium fertilizer levels which often gave results similar to higher fertilization levels or combination treatments with the bio-stimulants. A field study is reported in Chapter 4.

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CHAPTER 4

Growth and yield response of carrots (*Daucus carota* L.) to different fertilizer levels and commercial bio-stimulants under field conditions

Abstract

Two field experiments were conducted at the experimental farm of the University of the Free State, South Africa in 2006 and 2007 with the objective to evaluate the effects of different inorganic NPK fertilizer levels on their own and in combination with two commercial bio-stimulants, ComCat® and Kelpak®, on vegetative growth and yield of carrot, *Daucus carota* L. cv. Karina. The two bio-stimulants were applied either alone or in combination with four fertilizer levels namely the recommended standard (100%) as well as 0%, 25% and 50% of the standard. Application of various fertilizer levels and bio-stimulants had inconsistent and mostly insignificant effects on growth characteristics, fresh root weight, root width and root length as well as leaf length and fresh weight in 2006. However, significant differences were observed in 2007. During the second season application of fertilizer at the standard rate and at 50% of the standard rate produced significantly higher yields than the 25% and zero fertilizer regimes. The yield obtained at the standard (100%) fertilizer level was 15.5 ton ha^{-1} higher than that of the non-fertilized control. Further, application of ComCat® during this season led to an additional and significant increase in root yield at all four fertilizer regimes, compared to the non bio-stimulant treated controls, while Kelpak® showed the same tendency at the lower but not the standard (100%) fertilizer level. In general, bio-stimulant treatments in combination with 50% of the standard fertilizer produced similar yields to the standard fertilizer level indicating that the bio-stimulant application might have some beneficiary influence on input costs for carrot production.

Keywords: Bio-stimulants, carrot, ComCat®, growth, Kelpak®, NPK fertilizer, yield

4.1 Introduction

The increase in global population growth which is projected to be 7.5 billion by 2020 at the present growth rate of 1.3% puts more pressure on crop production systems. With the projected population growth the pressure to meet global food demand often leads to poor land utilization and soil management with resultant soil degradation (Lai, 2001). Additionally, misuse of land resources invariably leads to negative changes in greenhouse gases. Adoption of sound agricultural practices and the use of natural plant growth enhancing products are integral components of sustainable management practices in the drive to attain global food security (Lai, 2001).

High yield and quality are important factors for consideration by growers of high value crops such as carrots, *Daucus carota* L. Among a variety of vegetables, carrots have a potential for export as either fresh or processed products. In 2002 approximately 21 035 metric tonnes of carrots were produced worldwide on 984 000 hectares of land. Globally, carrots might seem like a minor crop but it is one of the major field vegetables. Carrots are also economically important in South Africa and approximately 4 000 hectares were under cultivation in 2002 with production of 111 000 tonnes and yield in excess of 24 000 kg ha^{-1} (FAO, 2003). The latest figures indicate annual cultivated hectarage of approximately 10 000 hectares with a market value of 9 million South African Rands (South African Department of Agriculture, 2007).

A major quality parameter for carrots is root size particularly when carrots are grown for export purposes where specific size standards have to be adhered to (Anonymous, 2008). Although there is wide variation in the preferred specifications for the size and variety of carrots for different countries, the general quality characteristics and criteria include carrots that are firm, straight from shoulder to tip, smooth with little residual hairiness, sweet with no bitter or harsh taste and with no cracks or sprout development. Some countries prefer the blunt ended Nantes type, whereas others prefer Kuroda types with a wide top and tapering towards the end of the carrot. On the production side, therefore, factors

that influence attainment of premium size and quality attributes have to be stringently adhered to.

Soil fertility is one of the main factors determining agricultural productivity and it is generally supplemented by addition of inorganic fertilizer. Commercial scale production of high value crops such as carrot pose a problem of nitrate leaching due to the often excessively high fertilization levels and irrigation regimes adopted (Allaire-Leung *et al*., 2001; Kraft and Stites, 2003). In addition to deposition of nitrate-nitrogen and phosphorus in soil, soil Cadmium concentration is increased through the application of fertilizers and manures. Deposition of Cadmium also occurs from the atmosphere (McLaughlin *et al*., 1999). Accumulation of phosphorus due to long-term application of fertilizer levels above the optimum crop demand has also led to deterioration of water quality and eutrophication of nearby water bodies (Elliot and O'Connor, 2007). Environmental concerns relating to pollution of ground water, the escalating costs of inorganic fertilizers and high costs of fertilizer transport have led to consideration of alternative methods of fertilization. The application of bio-stimulants with yield increasing potential is one of these methods.

As a result, research into the efficacy of plant extracts in increasing crop growth and stress tolerance (Butler *et al*., 2007; Butler and Hunter, 2007), production and yield of various crops (Ferreira and Lourens, 2002; Arthur *et al.* 2003; Linwood Supply, 2005) has been undertaken for decades with varying results. A range of combinations of plant extracts with fertilizers or on their own have been investigated. Of the natural products, seaweed extracts are probably the best known and also the most widely researched. Seaweed products contain natural growth hormones that have been researched on various crops to determine their efficacy in increasing yield and quality of crop plants.

This field study investigated the effect of two commercially available natural bio-stimulants on vegetative growth and yield of carrots at four different fertilizer levels. The two bio-stimulants used were: ComCat®, which contains a seed extract of the plant *Lychnis viscaria* L., (German catch fly) as well as Kelpak®, a sea weed extract from marine alga or kelp, *Ecklonia maxima.* The main aim was

to ascertain whether foliar applications of the bio-stimulants on their own, or in combination with fertilizer at lower than the recommended level, were capable of producing acceptable yields under field conditions. The latter approach additionally aimed at addressing environmental concerns regarding extreme fertilizer application.

4.2 MATERIALS AND METHODS

4.2.1 Materials

F_1 hybrid carrot (*Daucus carota* L. cv Karina) seed (Peto seed, California, USA) was purchased from a local seed merchant. The fertilizers used in this study included potassium chloride (KCl 50%), limestone ammonium nitrate (LAN 28%) and super phosphate (10.5%). The commercial bio-stimulant, Kelpak®, was purchased from the local Cooperative while ComCat® was generously supplied by Agraforum AG (Germany). Carrot nutrient withdrawal guidelines for South Africa (FSSA, 2003) for production of 25 tonnes ha^{-1} (Hygrotech, 2006) were followed.

4.2.2 Methods

4.2.2.1 Field soil preparation

The study was carried out over two seasons, 2006 and 2007, at the experimental farm of the University of the Free State in Bloemfontein, South Africa (29°01'00"S, 26°08'50"E), on a sandy loam soil with 20% clay content. During both seasons the experimental area was ploughed and harrowed to a fine tilth prior to sowing. The characteristics of the top 30 cm of soil were acquired through chemical analysis for both seasons (Table 1, Chapter 3; 3.2.2).

Fertilization was done based on the soil analysis and fertilizer withdrawal rates for carrots. Basal phosphorous and potassium were broadcast and worked into the soil prior to sowing at the rate of 15 kg and 125 kg ha^{-1} respectively to meet the expected fertilizer withdrawal levels for carrots. A split application of nitrogen was used with half being broadcast at sowing and the other half was applied six weeks after planting to make up total levels of the selected treatment values.

4.2.2.2 Trial layout and experimental design

A randomized complete block design with five replicates was used during both seasons of the study. Four fertilizer levels, the standard recommended level (100%), half (50%) of the recommended level, a quarter (25%) of the recommended level and no fertilization (0%), were applied alone and in combination with two commercial bio-stimulants (ComCat® and Kelpak®). A total of twelve (12) treatment combinations were used for the two seasons of the study. The resultant treatments were as detailed in Table 3.2, Chapter 3. The total experimental area was 603.5 m^2, the plot size was 2.5 m x 3 m with eight rows $plot^{-1}$ and an inter-row spacing of 30 cm.

The bio-stimulants, ComCat® and Kelpak®, were applied at the 3 to 4 leaf stage (code 13 of stage 1– Meier, 1997) and repeated at the 7 to 8 (code 18 of stage 1) leaf stage. For ComCat® the application rate was 1.5 g ComCat® mixed in 15 ℓ of water (100 g ha^{-1} recommended rate) and 30 ml Kelpak® was mixed in 15 ℓ of water (2 litres ha^{-1} recommended rate) translating to 0.75 ℓ of bio- stimulant spray solution per plot. For both bio-stimulants the delivery rate was 1000 ℓ water ha^{-1}. Regular irrigation was undertaken as needed to maintain adequate soil moisture and a standard recommended disease and pest management schedule was followed throughout the development period.

4.2.2.3 Seeding

Carrot (cv. Karina) seed was weighed out for each plot and was hand sown on April 7, 2006 and April 11, 2007. At the 3 to 4 leaf stage the plants were thinned to a spacing of 5 cm between plants to achieve a plant population of 1 500 000 plants ha^{-1}.

4.2.2.4 Growth measurements

A sample of ten carrots was taken from each plot at four stages of development, namely, 30%, 60%, 80% and 100% leaf development. A hand held caliper was used to measure the lengths of the leaves and root, as well as root

width of the samples. Leaf and root mass were also measured and recorded after removing the plants from the field. Leaf length was measured from the base of the petiole to the tip of the leaf blade. Root length was measured from the collar to the base of the storage root and root width was taken in the region of the crown, within 2 cm from the collar. The roots and leaves from all the sampling dates were oven dried at 70^0C for two weeks. Subsequently, dry mass of leaves and roots were separately recorded.

At maturity, two linear meters of the central two rows in each plot were harvested. A record of carrot number for this harvest area, fresh mass of carrot roots and aerial parts was made. The yield per plot was calculated from the harvest area and extrapolated to yield per hectare. Additionally, the width and length of 10 carrots per harvest area were measured as detailed above and recorded for all treatments.

4.2.2.5 Calculations and statistical methods

Data was analyzed using the NCSS 2000, (Hintze, 1999) statistical package for identification of differences in the treatments. The Tukey Kramer LSD ($P<0.05$) was used for separation of treatment means (Steele and Torrie, 1980).

4.3 RESULTS

4.3.1 Root mass

The interaction between the fertilizer levels (FL) and bio-stimulant treatments (FB) significantly influenced the root fresh mass, in both seasons except at the 30% fertilizer level in 2007 (Table 4.1). However, this significance in interaction was rather inconsistent and was seldom applicable to differences within a specific fertilizer level when data was compared horizontally from left to right in Table 4.1. In 2006 and at 30% development Kelpak® and ComCat® in combination with the 100% fertilizer level and Kelpak® in combination with the 50% fertilizer level increased root mass significantly compared to the control treatments. Additionaly, Kelpak® in combination with the 50% fertilizer level significantly increased carrot root mass compared to both control and ComCat®

treatments at 80% development. At the same plant development stage where ComCat® was applied without any fertilizer there was a significant decrease in carrot root mass compared to the control or Kelpak® treatment. At harvest, both ComCat® and Kelpak® in combination with the 25% fertilizer level significantly increased carrot root mass in comparison with the control. However, Kelpak® applied in the absence of fertilizer significantly decreased carrot root fresh mass compared to the control.

In 2007, at the 60% plant development stage both bio-stimulants in combination with 100% fertilizer level significantly reduced root mass in comparison with the control. A significant increase in root mass compared to the control occurred where ComCat® and Kelpak® were applied in combination with 25% fertilizer level. At harvest, root mass decreased significantly only where Kelpak® was applied in combination with 50% fertilizer level. Further, although the linear increase in root fresh mass as fertilizer levels was systematically increased from 0 to 100%, as was seen in the preliminary greenhouse trial (Chapter 3), was not as marked during the 2006 season as during 2007, it was nevertheless statistically significant in almost all cases except for 2006 at 80% plant development.

In 2006, a rather unexpected trend was observed in the mean root mass during the early growth stages (30% and 60% plant development) where the root fresh mass decreased as the fertilizer level (FL) was increased from 0% to 50% (Table 4.1) and this was statistically significant especially at these lower fertilizer levels. At 80% development and at least for the standard fertilizer application the tendency was reversed albeit statistically non-significant. However, at harvest the root fresh mass followed the expected trend of increasing with an increase in fertilizer level, but this was statistically significant only at the 50% fertilizer level when compared to 0% fertilizer. In 2007 this steady and expected tendency to increase root mass as fertilizer levels were increased in increments from 0% to 100%, was observed at all growth stages except at the 80% development stage.

Table 4.1: Effect of fertilizer levels and bio-stimulants on the mean carrot root mass (g/carrot) at different growth stages under field conditions

30% Plant development								
Fertilizer level % (FL)	**Fertilizer + Bio-stimulant (FB)**							
	2006				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	124.10	234.22	268.85	**209.06**	44.03	54.20	45.35	**47.86**
50	131.40	170.27	224.63	**175.43**	43.75	35.43	35.10	**38.09**
25	199.90	216.63	124.9	**180.48**	23.57	27.08	18.7	**23.12**
0	231.80	273.93	207.77	**237.83**	7.2	6.77	6.88	**6.95**
Ave FB	**171.80**	**223.76**	**206.54**		**29.64**	**30.87**	**26.51**	
$LSD_{(T)(0.05)}$FLxFB	**39.97**				**ns**			
$LSD_{(T)(0.05)}$ FL	**17.66**				**6.20**			
$LSD_{(T)(0.05)}$ FB	**13.84**				**ns**			
60% Plant development								
Fertilizer level % (FL	**2006**				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	326.73	276.33	321.27	**308.11**	153.57	70.47	120.08	**114.71**
50	249.70	273.60	270.37	**264.56**	99.50	98.95	100.20	**99.55**
25	239.40	241.20	261.50	**247.37**	47.50	98.42	59.80	**68.57**
0	329.52	278.20	315.90	**307.87**	37.34	29.37	37.32	**34.65**
Ave FB	**286.34**	**267.33**	**292.26**		**84.48**	**74.30**	**79.33**	
$LSD_{(T)(0.05)}$FLxFB	**55.48**				**12.21**			
$LSD_{(T)(0.05)}$ FL	**24.51**				**5.39**			
$LSD_{(T)(0.05)}$ FB	**19.21**				**4.23**			
80% Plant development								
Fertilizer level %(FL	**2006**				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	377.47	361.06	371.97	**370.17**	276.90	252.33	230.35	**253.19**
50	292.83	318.67	456.45	**355.98**	143.27	126.20	176.53	**148.67**
25	377.80	323.13	306.23	**335.72**	123.17	90.51	179.73	**131.14**
0	385.10	268.88	367.38	**340.45**	206.70	118.43	144.22	**156.45**
Ave FB	**358.30**	**317.93**	**375.51**		**187.51**	**146.87**	**182.71**	
$LSD_{(T)(0.05)}$FLxFB	**82.95**				**38.14**			
$LSD_{(T)(0.05)}$ FL	**ns**				**16.85**			
$LSD_{(T)(0.05)}$ FB	**28.73**				**13.21**			
At harvest								
Fertilizer level % (FL)	**2006**				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	560.00	549.67	521.67	**543.78**	652.01	660.15	641.84	**651.34**
50	576.00	626.00	634.25	**612.08**	570.33	516.64	396.23	**494.40**
25	439.33	577.75	652.00	**556.36**	360.74	447.39	347.02	**385.05**
0	507.00	581.00	426.33	**504.78**	359.63	318.53	443.65	**373.94**
Ave FB	**520.58**	**583.60**	**558.56**		**485.68**	**485.68**	**457.18**	
$LSD_{(T)(0.05)}$FLxFB	**134.33**				**172.05**			
$LSD_{(T)(0.05)}$ FL	**59.33**				**76.00**			
$LSD_{(T)(0.05)}$ FB	**46.52**				**ns**			

Application of the two bio-stimulants (FB) at different fertilizer levels once again resulted in rather erratic fluctuations in carrot root mass (Table 4.1). In 2006 the ComCat® treatment tended to increase the root mass early in the growing season (30% plant development) and again at harvest and in both cases the differences were statistically significant. However, this tendency was reversed in 2007 where ComCat® either had no effect (30% development and at harvest) or decreased root mass significantly at harvest. Kelpak® tended to decrease root mass in 2007, albeit it not at all fertilizer levels, while the opposite was true for the 2006 growing season.

4.3.2 Root length

A rather unexpected feature during the 2006 season was that the root length almost reached its maximum early during the season at the 30 – 60% development stage (Table 4.2). Again this points towards an exceptional 2006 season. Further, plots that received fertilizer did not significantly differ from the zero fertilizer plots in terms of root length during the 2006 season. Not surprisingly, the fertilizer-bio-stimulant combination treatments followed the same indiscriminate pattern during this season, in terms of root length, at all of the development stages.

In contrast, during the 2007 season, the root length increased linearly as fertilizer levels were increased and this trend was observed at all development stages except at 30% plant development (Table 4.2). Further, root length also increased linearly over time reaching its maximum at maturity. Foliar treatment with neither ComCat® nor Kelpak® contributed to root length growth during the 2007 season for 30% and 80% development stages. At 60% plant development, both of the Kelpak® and ComCat® combination treatments with 100% fertilizer significantly reduced root length while ComCat® in combination with 25% of the standard fertilizer significantly increased root length. At harvest, ComCat® also significantly reduced root length.

Table 4.2: Effect of fertilizer levels and bio-stimulants on the mean carrot root length (mm) at different growth stages under field conditions

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
30% Plant development								
100	113.95	110.41	121.24	**115.20**	86.46	98.08	90.39	**91.65**
50	114.71	123.40	126.59	**121.57**	94.97	86.68	93.46	**91.70**
25	113.00	117.10	98.19	**109.43**	77.26	75.74	73.59	**75.53**
0	119.21	131.31	111.72	**120.74**	56.03	59.26	61.20	**58.83**
Ave FB	**115.22**	**120.56**	**114.44**		**78.68**	**79.94**	**79.66**	
$LSD_{(T)(0.05)}$FLxFB	ns				ns			
$LSD_{(T)(0.05)}$ FL	12.28				8.45			
$LSD_{(T)(0.05)}$ FB	Ns				ns			
60% Plant development								
Fertilizer level % (FL	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	143.10	138.70	173.48	**151.76**	127.61	92.79	102.20	**107.53**
50	160.04	122.06	139.88	**140.66**	105.68	104.98	109.54	**106.73**
25	127.54	121.42	133.08	**127.35**	93.22	116.14	92.90	**100.75**
0	129.86	131.11	130.85	**130.61**	84.97	86.77	90.79	**87.51**
Ave FB	**140.14**	**128.32**	**144.32**		**102.87**	**100.17**	**98.86**	
$LSD_{(T)(0.05)}$FLxFB	20.86				8.60			
$LSD_{(T)(0.05)}$ FL	9.21				3.80			
$LSD_{(T)(0.05)}$ FB	7.22				2.97			
80% Plant development								
Fertilizer level %	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	116.79	118.30	142.47	**125.85**	135.99	137.75	132.53	**135.42**
50	113.05	128.27	140.99	**127.44**	131.05	122.83	130.45	**128.11**
25	134.55	111.90	116.98	**121.14**	109.75	121.41	122.35	**117.84**
0	117.88	129.12	136.36	**127.79**	119.54	111.51	122.32	**117.93**
Ave FB	**120.57**	**121.90**	**134.20**		**124.08**	**123.38**	**126.91**	
$LSD_{(T)(0.05)}$FLxFB	26.27				ns			
$LSD_{(T)(0.05)}$ FL	ns				8.44			
$LSD_{(T)(0.05)}$ FB	9.10				ns			
At harvest								
Fertilizer level %	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	146.35	154.71	154.43	**151.83**	164.37	158.03	158.52	**160.30**
50	146.69	140.29	141.49	**142.92**	148.23	136.15	151.09	**145.16**
25	121.7	160.13	149.65	**143.83**	150.98	137.88	139.47	**142.78**
0	149.99	161.17	145.08	**152.08**	137.90	132.01	139.94	**136.62**
Ave FB	**141.18**	**154.08**	**147.66**		**150.37**	**141.02**	**147.26**	
$LSD_{(T)(0.05)}$FLxFB	22.63				ns			
$LSD_{(T)(0.05)}$ FL	10.00				11.31			
$LSD_{(T)(0.05)}$ FB	7.84				8.87			

4.3.3 Root width

Although root width increased over time (Table 4.3) during the 2006 season, no significant differences between plots fertilized differently could be observed. Likewise, treatment with ComCat® and Kelpak® across fertilizer levels for all growth stages was not significant compared to the controls and this was confirmed by the non-significant interaction (FL x FB) between the bio-stimulant treatments (FB) and fertilizer levels (FL) in terms of the mean root width measured during 2006, except at the 80% plant growth stage. At this stage Kelpak® in combination with 50% of the standard fertilizer significantly increased root width. However, both Kelpak® and ComCat® increased root width at harvest. The similarities in trends between root length (Table 4.2) and root width (Table 4.3) confirmed the unreliability of the 2006 data. However, as was the case with root length, root width increased linearly and significantly as fertilizer increased as well as over time during 2007 (Table 4.3), but the combination treatments with the two bio-stimulants did not contribute significantly to root width.

Table 4.3: Effect of fertilizer levels and bio-stimulants on the mean carrot root width (mm) at different growth stages under field conditions

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
30% Plant development								
100	18.33	19.36	17.47	**18.39**	11.20	11.10	10.69	**10.99**
50	17.70	18.72	18.74	**18.39**	10.65	9.16	10.83	**10.21**
25	17.37	18.68	15.31	**17.12**	8.64	7.89	6.82	**7.78**
0	18.60	20.24	17.83	**18.89**	4.56	5.01	4.53	**4.70**
Ave FB	**18.00**	**19.25**	**17.33**		**8.76**	**8.29**	**8.22**	
$LSD_{(T)(0.05)}$FLxFB	ns				ns			
$LSD_{(T)(0.05)}$ FL	1.21				1.03			
$LSD_{(T)(0.05)}$ FB	0.95				ns			
60% Plant development								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	20.89	20.08	23.38	**21.45**	16.09	12.88	14.54	**14.50**
50	19.44	21.08	21.83	**20.78**	14.59	13.31	14.90	**14.27**
25	20.86	20.59	20.40	**20.61**	12.79	13.05	12.32	**12.72**
0	22.33	21.74	21.81	**21.96**	9.67	10.40	10.60	**10.22**
Ave FB	**20.88**	**20.87**	**21.85**		**13.28**	**12.41**	**13.09**	
$LSD_{(T)(0.05)}$FLxFB	ns				ns			
$LSD_{(T)(0.05)}$ FL	ns				1.47			
$LSD_{(T)(0.05)}$ FB	ns				ns			
80% Plant development								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	25.46	24.83	26.75	**25.68**	20.94	19.85	23.12	**21.30**
50	23.44	22.95	27.96	**24.78**	18.78	17.23	19.73	**18.58**
25	25.19	23.74	23.70	**24.21**	16.20	14.61	17.08	**15.96**
0	24.34	22.90	22.89	**23.38**	17.80	15.89	16.71	**16.80**
Ave FB	**24.61**	**23.60**	**25.33**		**18.43**	**16.90**	**19.16**	
$LSD_{(T)(0.05)}$FLxFB	3.90				ns			
$LSD_{(T)(0.05)}$ FL	1.72				2.05			
$LSD_{(T)(0.05)}$FB	1.35				1.61			
At harvest								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	26.55	29.11	27.37	**27.68**	32.03	32.19	30.43	**31.55**
50	28.3	30.08	30.44	**29.61**	27.41	26.84	30.18	**28.14**
25	25.26	28.32	29.53	**27.71**	27.20	25.91	23.78	**25.63**
0	25.97	27.80	26.98	**26.98**	24.52	23.25	25.21	**24.33**
Ave FB	**26.52**	**28.88**	**28.58**		**27.79**	**27.05**	**27.40**	
$LSD_{(T)(0.05)}$FLxFB	ns				ns			
$LSD_{(T)(0.05)}$ FL	2.03				2.60			
$LSD_{(T)(0.05)}$ FB	1.60				ns			

4.3.4 Leaf length

Despite the non-responsiveness of below soil vegetative growth during the 2006 season, leaf length growth followed the unexpected pattern of increasing linearly with increased fertilizer application as well as over time and this applied to all of the growth stages (Table 4.4). Fertilization at the 100% level was significantly different from the non-fertilized control for all the growth stages. The interaction between fertilizer levels and bio-stimulants was significant at 30% and 60% plant development as well as at harvest. During the early growth stages (30% and 60% plant development) bio-stimulants in combination with fertilizer did not significantly influence leaf growth at any of the fertilizer levels. However, at harvest Kelpak® in the absence of fertilizer significantly increased leaf growth while ComCat® in combination with the standard fertilizer significantly decreased leaf growth in 2006.

Results obtained during the 2007 season were less erratic and followed a logical trend. Leaf length was linear with increased fertilizer levels as well as over time (Table 4.4). At all growth stages the interaction between fertilizer levels and bio-stimulants significantly influenced leaf growth. However, at the 30% plant development stage the application of bio-stimulants alone or in combination with different fertilizer levels did not significantly influence carrot leaf length. Kelpak®, in combination with 100% fertilizer, significantly increased leaf growth compared to ComCat® in combination with the same fertilizer level at 60% plant development. At 80% plant development, ComCat® in combination with the standard fertilizer significantly increased leaf growth compared to the control and Kelpak® in combination with fertilizer treatments. Both Kelpak® and ComCat® in combination with 50% of the standard fertilizer had a significant decreasing effect on leaf growth at harvest. The increase in leaf length at the other fertilization levels was not consistent across the growth stages during this season.

Table 4.4: Effect of fertilizer levels and bio-stimulants on the mean carrot leaf length (mm) at different growth stages under field conditions

30% Plant development

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	152.16	161.61	164.47	**159.40**	80.60	85.85	80.36	**82.27**
50	153.18	141.38	159.38	**151.31**	78.58	64.98	78.43	**74.00**
25	143.19	156.58	132.43	**144.07**	72.40	65.54	61.39	**66.44**
0	133.18	144.71	150.08	**142.66**	57.65	55.80	57.64	**56.97**
Ave FB	**145.42**	**151.07**	**151.60**		**72.31**	**68.04**	**69.41**	
$LSD_{(T)(0.05)}$FLxFB	**24.57**				**15.35**			
$LSD_{(T)(0.05)}$ FL	**10.85**				**6.78**			
$LSD_{(T)(0.05)}$ FB	**ns**				**ns**			

60% Plant development

Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	190.11	189.57	212.47	**197.38**	103.33	98.59	110.62	**104.18**
50	191.26	182.18	173.02	**182.15**	104.13	94.22	97.61	**98.65**
25	167.76	183.77	154.42	**168.65**	98.66	99.54	96.08	**98.09**
0	176.17	176.66	171.20	**174.68**	83.88	76.35	88.85	**83.03**
Ave FB	**181.32**	**183.05**	**177.78**		**97.50**	**92.18**	**98.29**	
$LSD_{(T)(0.05)}$FLxFB	**27.90**				**9.70**			
$LSD_{(T)(0.05)}$ FL	**12.33**				**4.28**			
$LSD_{(T)(0.05)}$ FB	**ns**				**3.36**			

80% Plant development

Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	246.23	258.24	261.92	**255.46**	157.75	193.88	165.40	**172.34**
50	256.07	248.69	242.25	**249.00**	159.59	154.03	157.15	**156.92**
25	262.78	231.89	238.94	**244.54**	138.28	155.53	155.19	**149.66**
0	229.26	232.92	229.49	**230.56**	124.71	138.94	132.16	**131.93**
Ave FB	**248.59**	**242.94**	**243.15**		**145.08**	**160.59**	**152.47**	
$LSD_{(T)(0.05)}$FLxFB	**ns**				**22.01**			
$LSD_{(T)(0.05)}$ FL	**15.27**				**9.72**			
$LSD_{(T)(0.05)}$ FB	**ns**				**7.62**			

At harvest

Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	357.87	309.62	344.98	**337.49**	297.07	319.51	286.45	**301.01**
50	342.93	305.63	305.35	**317.97**	326.86	257.11	268.48	**284.14**
25	325.33	296.62	327.28	**316.41**	251.23	257.54	245.08	**251.29**
0	266.01	310.34	317.43	**297.93**	250.82	250.20	240.49	**247.17**
Ave FB	**323.04**	**305.55**	**323.76**		**281.49**	**271.09**	**260.13**	
$LSD_{(T)(0.05)}$FLxFB	**48.80**				**34.80**			
$LSD_{(T)(0.05)}$ FL	**21.56**				**15.37**			
$LSD_{(T)(0.05)}$ FB	**16.90**				**12.05**			

4.3.5 Leaf fresh mass

There was a significant interaction between fertilizer level and bio-stimulants during both seasons across all plant development stages except at the 30% plant development stage in 2006. ComCat® foliar treatment increased the leaf fresh mass significantly at the 30% growth stage. In 2006 and at 80% plant development ComCat® in combination with 25% of the standard fertilizer significantly decreased leaf mass while Kelpak® significantly increased it at the 50% fertilizer level. However, leaf fresh mass was significantly increased where the two bio-stimulants were applied on their own in the absence of additional fertilizer while ComCat® had the same effect at harvest in combination with the standard fertilizer. From this it seems that despite the high precipitation during 2006, the response of leaf growth to different fertilizer regimes as well as treatment with the bio-stimulants followed a different pattern than did the below soil parts.

In 2007 the linear accumulation of leaf fresh mass, as fertilizer level was increased, prevailed at all vegetative growth stages (Table 4.5). In 2007, Kelpak® in combination with the standard fertilizer had a significant increasing effect on leaf fresh mass at the 30% and 80% plant development stages. ComCat® in combination with both the standard and 50% of the standard fertilizer significantly decreased leaf fresh mass at 60% plant development. ComCat® and Kelpak® in combination with 50% fertilizer significantly enhanced leaf fresh mass at the 80% plant development stage. At harvest, ComCat® combined with the standard fertilizer level significantly increased carrot leaf fresh mass compared to both the control and Kelpak® in combination with the standard fertilizer level. However, ComCat® significantly reduced carrot leaf mass compared to both the control and Kelpak® in combination with the 50% fertilizer level. The opposite was observed with Kelpak® in combination with the 25% fertilizer level having a decreasing effect on carrot leaf mass.

Table 4.5: Effect of fertilizer levels and bio-stimulants on the mean carrot leaf mass (g) at different growth stages under field conditions

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
30% Plant development								
100	47.86	58.68	38.28	**48.27**	20.03	24.58	25.70	**23.43**
50	49.42	63.48	53.96	**55.62**	18.50	18.37	18.37	**18.40**
25	47.20	55.00	31.82	**44.67**	13.78	12.75	9.70	**12.08**
0	46.60	61.08	40.58	**49.42**	6.30	6.25	5.80	**6.12**
Ave FB	**47.77**	**59.56**	**41.16**		**14.65**	**15.49**	**14.88**	
$LSD_{(T)(0.05)}$FLxFB	ns				5.30			
$LSD_{(T)(0.05)}$ FL	6.67				2.34			
$LSD_{(T)(0.05)}$ FB	5.23				ns			
60% Plant development								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	83.80	72.68	87.98	**81.49**	38.15	21.48	34.73	**31.45**
50	72.56	63.98	65.20	**67.25**	29.3	23.68	29.87	**27.61**
25	74.50	76.22	85.52	**78.75**	23.23	25.80	19.65	**22.89**
0	86.68	72.52	72.92	**77.37**	13.90	13.23	13.13	**13.42**
Ave FB	**79.38**	**71.35**	**77.91**		**26.15**	**21.04**	**24.34**	
$LSD_{(T)(0.05)}$FLxFB	15.27				4.86			
$LSD_{(T)(0.05)}$ FL	6.74				2.15			
$LSD_{(T)(0.05)}$ FB	5.29				1.68			
80% Plant development								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	113.80	128.08	121.92	**121.27**	34.33	67.73	85.03	**62.37**
50	93.43	109.00	121.62	**108.02**	37.95	68.35	55.30	**53.87**
25	125.10	88.40	107.73	**107.08**	31.73	29.27	35.67	**32.22**
0	92.85	97.16	104.62	**98.21**	31.43	29.68	36.10	**32.40**
Ave FB	**106.30**	**105.66**	**113.97**		**33.86**	**48.76**	**53.03**	
$LSD_{(T)(0.05)}$FLxFB	18.46				12.42			
$LSD_{(T)(0.05)}$ FL	8.15				5.49			
$LSD_{(T)(0.05)}$ FB	6.39				4.30			
At harvest								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	172.20	208.68	198.50	**193.13**	161.53	179.55	158.93	**166.67**
50	170.00	173.20	164.00	**169.07**	151.83	101.68	133.35	**128.78**
25	123.80	120.00	143.80	**129.20**	109.18	95.65	70.62	**91.82**
0	98.50	153.00	144.00	**131.83**	77.05	67.75	74.90	**73.23**
Ave FB	**141.13**	**163.72**	**162.58**		**124.90**	**111.16**	**109.45**	
$LSD_{(T)(0.05)}$FLxFB	33.61				17.09			
$LSD_{(T)(0.05)}$ FL	14.85				7.55			
$LSD_{(T)(0.05)}$ FB	11.64				5.92			

4.3.6 Yield

The application of neither different fertilizer levels nor bio-stimulants led to significant differences in the final yield of carrots (Figure 4.1), expressed as ton ha^{-1} based on area harvested, during the 2006 season that was characterized by exceptionally high precipitation. As could be expected, the interaction between variables was also insignificant (Table 4.6). Nevertheless, foliar application of ComCat® tended to have a slight enhancing effect on yield under these circumstances albeit at the low fertilizer regimes only (Figure 4.1). A slight yield response was observed at 100% fertilizer level where Kelpak® was applied in combination. At the elevated fertilizer levels (50% and 100%) the bio-stimulants rather seemed to have a repressive effect on yield, during 2006, with the range of decline lying between 6.43% and 19.95% across treatments.

A totally different pattern in terms of final yield, compared to 2006, emerged during the 2007 season (Figure 4.1) for both the fertilizer and bio-stimulant treatments. The yield increased linearly as fertilizer levels were elevated in increments and the difference between the zero and standard fertilizer was a significant 17 ton ha^{-1}. The foliar application of ComCat® and Kelpak® contributed to substantial yield increases compared to plants that received only fertilizer at different levels (Table 4.6). However, the interaction between fertilizer level and bio-stimulant treatment was not statistically significant. The standard deviations were rather high probably contributing to the fact that fertilizer x bio-stimulant treatment in terms of final yield was not regarded significant at the 5% ($P < 0.05$) level.

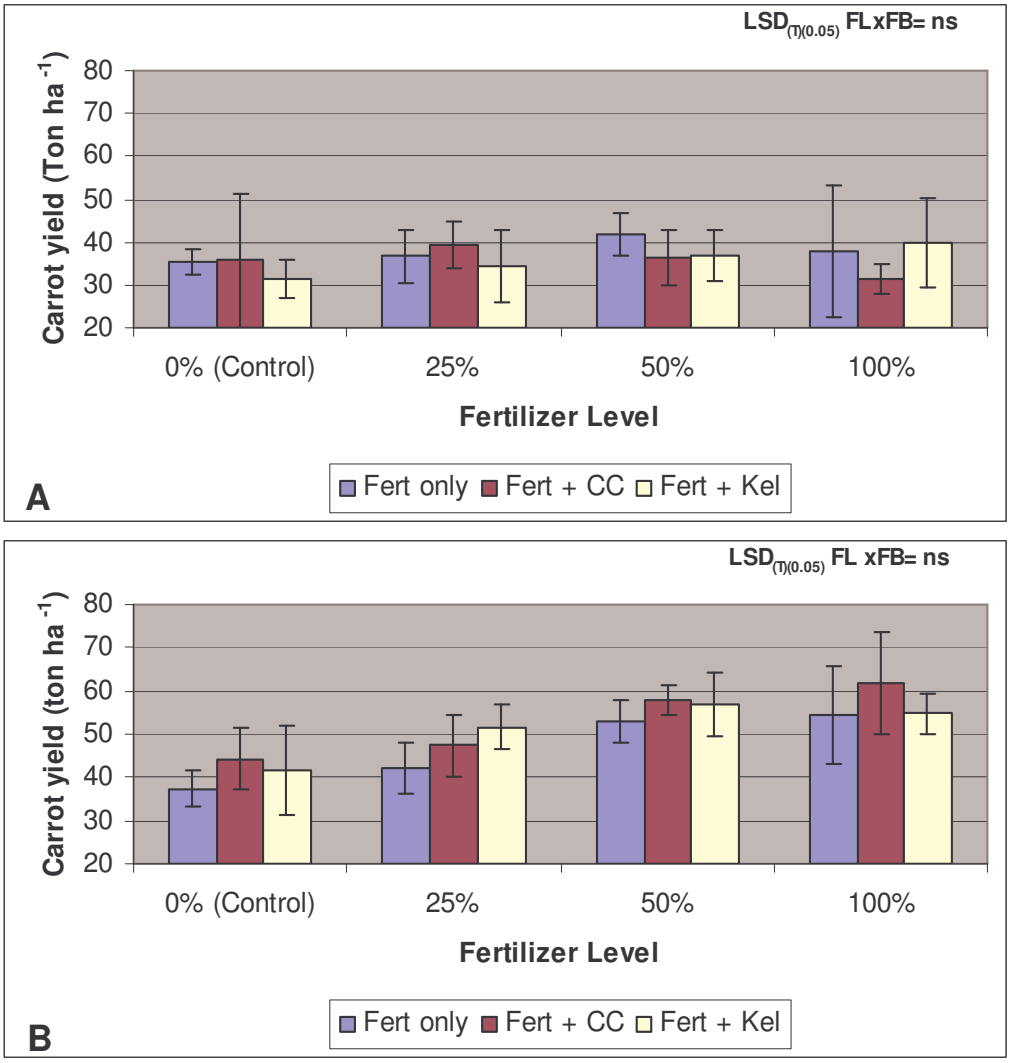

Figure 4.1: Effect of fertilizer and bio-stimulants on carrot yield (ton ha^{-1}) under field conditions, based on area harvested for 2006 (A) & 2007 (B).

Table 4.6: Fertilizer and bio-stimulant effects on the mean carrot yield (ton ha^{-1}) under field conditions								
At harvest								
Fertilizer level % (FL)	**Fertilizer + Bio-stimulant (FB)**							
	2006				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	37.72	31.46	39.94	**36.37**	54.45	61.78	54.78	**57.00**
50	41.82	36.45	36.95	**38.41**	52.88	57.82	56.77	**55.82**
25	36.73	39.47	34.51	**36.90**	42.02	47.32	51.72	**47.02**
0	35.28	35.76	31.31	**34.12**	37.40	44.27	41.72	**41.13**
Ave FB	**37.89**	**35.79**	**35.68**		**46.69**	**52.80**	**51.25**	
$LSD_{(T)(0.05)}$FLxFB **$LSD_{(T)(0.05)}$ FL** **$LSD_{(T)(0.05)}$ FB**	**ns** **ns** **ns**				**ns** **7.46** **5.85**			

4.3.7 Dry root and leaf mass

4.3.7.1 Dry root mass

Dry mass data was measured in an attempt to ascertain whether the rather inconsistent fresh mass data obtained during the 2006 season showed a different pattern when expressed as dry mass. However, application of various fertilizer levels led to a similar, rather erratic, root dry mass accumulation pattern, for all of the vegetative developmental stages in 2006 (Table 4.7). Foliar application of both bio-stimulants had an inconsistent effect on dry root mass accumulation with marginally higher mass accumulation at higher fertilizer levels, especially the later two stages of development. During both seasons and at all development stages there was significant interaction between fertilizer levels and bio-stimulants. In 2006 and at 30% development ComCat® combined with 50% fertilizer level significantly increased carrot dry root mass in comparison with the control. On the other hand, Kelpak® combined with 25% fertilizer level significantly decreased dry root mass compared with a combination of ComCat® at the same fertilizer level. In 2006 and at the 60% plant development stage, Kelpak® in combination with the 100% fertilizer level significantly increased dry root mass compared to the

combination of 100% fertilizer level with ComCat®. At harvest, both bio-stimulants in combination with 25% fertilizer level increased dry root mass accumulation compared to the control, whereas Kelpak® applied alone significantly enhanced accumulation of dry root mass compared to the control.

In 2007, ComCat® in combination with 100% fertilizer significantly decreased carrot root dry mass at the 60% and 80% development stages and increased it significantly at 30% development and at harvest. However, at 60% development where ComCat® was applied in combination with the 25% fertilizer level carrot root dry mass increased significantly. At harvest, ComCat® in combination with 50% fertilizer, significantly decreased carrot root dry mass. At 30% development, a combination of Kelpak® and 50% fertilizer significantly increased carrot root dry mass. Additionally, at 80% development, Kelpak in combination with 100% fertilizer significantly decreased carrot dry root mass in comparison with the control. However, a combination of Kelpak® with 50% and 25% fertilizer levels significantly enhanced carrot root dry mass accumulation. At harvest, Kelpak® in combination with 25% fertilizer level significantly decreased carrot dry root mass in comparison with the control whereas when Kelpak® was applied alone carrot root dry mass increased significantly.

As was the case with the fresh mass data, the pattern of root dry mass accumulation was different during the 2007 season as opposed to 2006 for fertilizer application (Table 4.7). Dry mass accumulation showed a linear increase with increased increments of fertilizer for all stages of development.

Table 4.7: Effect of fertilizer levels and bio-stimulants on the mean carrot root dry mass (g) at different growth stages under field conditions

30% Plant development								
Fertilizer level % (FL)	**Fertilizer + Bio-stimulant (FB)**							
	2006				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	25.30	27.07	23.67	**25.34**	5.51	6.68	5.62	**5.93**
50	15.63	27.53	25.90	**23.02**	3.58	4.41	5.74	**4.58**
25	23.90	31.27	15.10	**23.42**	3.03	3.22	2.42	**2.89**
0	26.20	30.68	22.60	**26.49**	0.88	0.93	0.98	**0.93**
Ave FB	**22.76**	**29.13**	**21.82**		**3.25**	**3.81**	**3.69**	
$LSD_{(T)(0.05)}$FLxFB	**9.49**				**0.89**			
$LSD_{(T)(0.05)}$ FL	**ns**				**0.39**			
$LSD_{(T)(0.05)}$ FB	**3.29**				**0.31**			
60% Plant development								
Fertilizer level % (FL)	**2006**				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	34.73	27.98	43.60	**35.44**	16.5	7.75	13.93	**12.73**
50	23.23	29.73	28.97	**27.31**	10.95	9.95	11.68	**10.86**
25	25.80	32.83	29.90	**29.51**	6.60	11.3	7.57	**8.49**
0	37.13	30.33	33.97	**33.81**	4.46	3.98	4.04	**4.16**
Ave FB	**30.22**	**30.22**	**34.11**		**9.63**	**8.24**	**9.30**	
$LSD_{(T)(0.05)}$FLxFB	**9.99**				**2.87**			
$LSD_{(T)(0.05)}$ FL	**4.41**				**1.27**			
$LSD_{(T)(0.05)}$ FB	**3.46**				**0.99**			
80% Plant development								
Fertilizer level % (FL)	**2006**				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	40.05	45.30	45.67	**43.67**	33.27	27.40	22.12	**27.60**
50	36.08	44.95	49.90	**43.64**	16.27	14.00	23.31	**17.86**
25	44.63	34.47	39.23	**39.44**	15.00	13.13	20.68	**16.27**
0	36.47	32.28	45.00	**38.25**	11.17	13.53	16.53	**13.74**
Ave FB	**39.31**	**39.50**	**44.95**		**18.93**	**17.02**	**20.66**	
$LSD_{(T)(0.05)}$FLxFB	**12.23**				**5.35**			
$LSD_{(T)(0.05)}$ FL	**5.40**				**2.36**			
$LSD_{(T)(0.05)}$ FB	**4.24**				**1.85**			
At harvest								
Fertilizer level % (FL)	**2006**				**2007**			
	Control	**ComCat**	**Kelpak**	**Ave FL**	**Control**	**ComCat**	**Kelpak**	**Ave FL**
100	55.60	54.97	52.17	**54.25**	61.27	72.87	63.40	**65.85**
50	57.60	62.60	63.43	**61.21**	53.13	35.90	48.67	**45.90**
25	43.93	57.78	65.20	**55.64**	38.37	42.40	28.00	**36.26**
0	50.70	60.85	64.07	**58.54**	27.03	33.58	45.97	**35.53**
Ave FB	**51.96**	**59.05**	**61.22**		**44.95**	**46.19**	**46.51**	
$LSD_{(T)(0.05)}$FLxFB	**10.97**				**9.46**			
$LSD_{(T)(0.05)}$ FL	**4.85**				**4.18**			
$LSD_{(T)(0.05)}$ FB	**3.80**				**ns**			

4.3.7.2 Leaf dry mass

Dry leaf mass accumulation was enhanced by application of increased levels of fertilizer regimes for all stages of development in 2006, except at 60% plant development (Table 4.8), and this followed the same tendency as was observed with the fresh mass data. Again, foliar application of both bio-stimulants in combination with fertilizer had no significant effect on leaf dry mass accumulation.

Interestingly, compared to 2006, the leaf dry mass was much lower at all plant development stages and for all treatments during 2007 except at harvest where dry mass accumulation markedly surpassed that measured in the previous season (Table 4.8). During all plant development stages in 2007 and at 30% development in 2006, the interaction between fertilizer levels and bio-stimulants was significant. At 30% development and where Kelpak® was applied without fertilizer carrot leaf dry mass decreased significantly compared to where ComCat® was applied. In 2007, Kelpak® in combination with 25% fertilizer also significantly decreased carrot leaf dry mass compared to the control. At 60% plant development, carrot leaf dry mass accumulation was significantly decreased by application of ComCat® in combination with either 100% or 50% fertilizer levels in comparison with the control and Kelpak® treatments. On the other hand carrot leaf dry mass was significantly enhanced by application of a combination of ComCat® and 25% fertilizer compared to the control. At 80% plant development carrot leaf dry mass was significantly enhanced by application of combinations of Kelpak® with either the 100% or 50% fertilizer level but where Kelpak® was applied without fertilizer leaf dry mass was significantly lower. Application of ComCat® in combination with 100% fertilizer also significantly increased carrot leaf dry mass but where ComCat® was applied in combination with the 25% fertilizer level leaf dry mass was significantly decreased. At harvest, ComCat® in combination with the standard fertilizer level significantly increased leaf dry mass whereas at the 50% fertilizer level ComCat® reduced leaf dry mass significantly.

Table 4.8: Effect of fertilizer levels and bio-stimulants on the mean carrot leaf dry mass (g) at different growth stages under field conditions

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
30%Plant development								
100	9.33	12.13	9.38	**10.28**	5.52	5.42	5.73	**5.56**
50	9.05	12.30	9.83	**10.39**	4.15	3.86	4.12	**4.04**
25	9.68	9.85	10.73	**10.09**	3.58	2.80	2.11	**2.83**
0	9.00	11.20	7.73	**9.31**	1.20	1.31	1.20	**1.23**
Ave FB	**9.26**	**11.37**	**9.42**		**3.61**	**3.35**	**3.29**	
$LSD_{(T)(0.05)}$FLxFB	**3.27**				**1.17**			
$LSD_{(T)(0.05)}$ FL	**1.13**				**0.52**			
$LSD_{(T)(0.05)}$ FB	**ns**				**ns**			
60% Plant development								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	16.83	15.53	19.17	**17.17**	7.60	4.05	6.77	**6.14**
50	13.62	11.47	14.36	**13.15**	5.73	4.70	5.63	**5.35**
25	12.30	15.95	15.23	**14.49**	4.17	5.10	4.30	**4.52**
0	16.07	14.27	15.23	**15.19**	2.68	2.20	2.67	**2.52**
Ave FB	**14.70**	**14.03**	**16.00**		**5.04**	**4.01**	**4.84**	
$LSD_{(T)(0.05)}$FLxFB	**ns**				**0.90**			
$LSD_{(T)(0.05)}$ FL	**2.33**				**0.40**			
$LSD_{(T)(0.05)}$ FB	**ns**				**0.31**			
80% Plant development								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	25.23	27.28	27.83	**26.78**	6.23	11.04	15.46	**10.91**
50	20.88	21.23	24.13	**22.08**	6.89	5.21	9.59	**7.23**
25	21.37	20.78	22.67	**21.60**	6.66	4.68	7.38	**6.24**
0	23.15	21.24	25.27	**23.22**	8.90	5.29	5.48	**6.56**
Ave FB	**22.66**	**22.63**	**24.97**		**7.17**	**6.56**	**9.48**	
$LSD_{(T)(0.05)}$FLxFB	**ns**				**2.27**			
$LSD_{(T)(0.05)}$ FL	**2.60**				**1.00**			
$LSD_{(T)(0.05)}$ FB	**2.04**				**0.79**			
At harvest								
Fertilizer level % (FL)	2006				2007			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	17.23	20.87	19.85	**19.32**	27.33	32.88	28.00	**29.40**
50	17.00	17.33	17.50	**17.28**	25.4	18.53	22.67	**22.20**
25	12.37	13.05	14.37	**13.26**	19.55	17.6	12.00	**16.38**
0	11.23	15.30	14.40	**13.64**	15.16	12.35	13.37	**13.63**
Ave FB	**14.46**	**16.64**	**16.53**		**21.86**	**20.34**	**19.01**	
$LSD_{(T)(0.05)}$FLxFB	**ns**				**4.18**			
$LSD_{(T)(0.05)}$ FL	**1.86**				**1.85**			
$LSD_{(T)(0.05)}$ FB	**1.46**				**1.45**			

4.4 Discussion

In general, carrot seedling establishment and development during early stages of growth more or less progressed as expected for all four fertilizer level treatments during the 2007 growing season. A distinct feature was that the effect of various fertilizer levels was visually noticeable at the early stages of growth while the high fertilizer rate clearly contributed to carrots with distinctly larger and lush foliage. Interestingly, it seemed that vegetative growth was not hindered significantly by nutrient deficiency during the early growth stages but, analyses of the growth parameter data showed an increase in size, weight or length of the applicable morphological features as the fertilizer levels were increased and as the growing season progressed.

However, as will be discussed, the data obtained during 2006 followed a different pattern than that obtained during the 2007 season. Especially the expected growth differences between plants fertilized at different levels were not as pronounced during 2006 as they were in 2007 and were also rather erratic in terms of the reaction of plants to treatment with different bio-stimulants. The most apparent reason is that 2006 was characterized by exceptionally high rainfall that caused flooding and probable sideways leaching of fertilizer within plots.

As a result, during the 2006 season, inconsistent root growth responses to different fertilization were observed with a tendency for better growth at the low (0 and 25%) fertilizer levels. Under normal circumstances this would be difficult to explain but, due to the high rainfall pattern during this season, chances are that lateral movement of fertilizer could have been responsible for contamination of some or all of the zero or low fertilized plots. Although an increase in root fresh mass was measured at different growth stages the increase was not linear with systematical elevation of the fertilizer level at specific growth stages. Nevertheless, application of both bio-stimulants contributed to significant increases in root mass. Under the same circumstances root length and width growth followed a similar erratic pattern except that, during the 2006 season, initial root length growth seemed to be rather fast during the early growth stages while no

significant differences in root length growth between differently fertilized plots were observed.

However, compared to the growth pattern of below soil parts, leaf length growth showed the opposite and rather unexpected tendency to increase linearly as the fertilizer level was elevated with increments between 0 and 100% during 2006 and this applied for all growth stages. Moreover, foliar application of both bio-stimulants contributed to further increased leaf length growth especially at the higher fertilizer levels. Interestingly, the leaf fresh mass did not follow exactly the same pattern under these circumstances and bio-stimulant treatment had a much more marked effect on fresh mass increase than it did on leaf length growth. Despite the 2006 circumstances, vegetative growth data collected during this season coincided with non-significant differences in root fresh mass, leaf fresh mass, leaf length and number of roots per meter of field carrots cultivated under different fertilizer levels as reported by Pettipas *et al*. (2006). However, the coincidence with the data of Pettipas *et al*. (2006) in the same season is questionable due to the high rainfall experienced in the case of data reported in this manuscript. Finally, no significant differences in carrot yield were observed during 2006 that probably confirms that this was an exceptional year. For this reason the 2006 results can probably not be accepted as a good representation of the outcome.

During 2007 the rainfall was more evenly spaced and at no time did water logged conditions apply. For this reason the data obtained during this season is regarded as a better indicator of how carrots reacted to different fertilizer levels and foliar application of the two commercial bio-stimulants under normal rain fed conditions. Root fresh mass, length and width showed a linear increase as fertilizer levels increased confirming a positive correlation and this was true for all of the development stages where data was collected except at 80% development. The increase in size or mass of the roots, as fertilizer levels were elevated in increments, is consistent with the known role of fertilizers, especially nitrogen, in promoting plant growth and yield (Gastal and Lemaire, 2002; Lawlor, 2002). However, Rubatzky *et al*. (1999) indicated that medium levels of fertilization

tended to produce median vegetative growth in carrots but the final yield was not affected significantly. From this the authors deduced that prevailing environmental conditions and not soil fertility only, determine cessation of carrot growth.

Interestingly, treatment with both bio-stimulants had no significant effect on root fresh mass at the early growth stages and at the lower fertilizer regimes but, treatment with ComCat® contributed to a non-significant (+>7 ton ha^{-1}) increase at final harvest where 100% fertilizer was applied. At all of the lower fertilizer levels and at harvest the tendency of ComCat® to increase root fresh mass was observed, although not significant in all cases. Kelpak® tended to increase root fresh mass at the lower fertilizer levels but, not at the standard level, and this was only significant where 25% of the standard fertilizer was applied.

In terms of final yield the plant stand for 2006 was 841 389 plants ha^{-1} whereas the number for the 2007 season was 866 806 plants ha^{-1} (3% difference). The final yield for the two seasons ranged between 31 – 42 tons ha^{-1} in 2006 and 37 - 62 tons ha^{-1} for the 2007 season and was generally within the expected range (25 to 50 ton ha^{-1}) for the recommended standard (100%) fertilizer level used (Hygrotech, 2006).

A short discussion on the effect of bio-stimulant application together with different fertilizer levels on the final root yield in 2007 seems appropriate at this stage. Although not significant, a combination of ComCat® and Kelpak® together with 100% fertilizer increased the yield by 7.33 ton ha^{-1} (13.5%) and 0.33 tons ha^{-1} (0.6%) respectively. Where only 50% of the standard fertilizer was applied, the yield of control plots (53 ton ha^{-1}) was almost the same as where standard fertilizer (54 ton ha^{-1}) was applied while ComCat® increased the yield by 4.94 ton ha^{-1} (9.34%) and Kelpak® by 3.89 ton ha^{-1} (8.9%). Again this was not statistically significant. Where only 25% of the standard fertilizer was applied at planting, the yield dropped substantially by 13 ton ha^{-1}, compared to the standard fertilizer regime. However, although not statistically significant, both ComCat® and Kelpak® contributed to a yield increase of 7 and 10 ton ha^{-1} respectively at this fertilizer regime and this almost brought the yield on par with the standard fertilizer application. The latter emphasized the potential of bio-stimulant products to play a

role in stabilizing carrot yield in the case where subsistence farmers traditionally supply sub standard fertilizer dosages especially under drier conditions.

The increase in yield after fertilizer application is consistent with research by Ali *et al*. (2003) who reported a significant increase in carrot root yield subsequent to the application of increasing levels of nitrogen and potassium. The authors observed increases of 136% over the control subsequent to an increase of 200 kg ha^{-1} nitrogen. Root yield increased significantly and progressively with increasing application of potassium up to the highest rate of 250 kg ha^{-1}. On the contrary, trials in which different rates of phosphorus were applied to sandy and clay soils, van Wijk *et al*. (2002) found a higher requirement of carrot for available phosphorus on sandy soil than on soil with higher clay content. Generally, the application of phosphorus had minimal influence on yield and the only significant differences were between 0 kg ha^{-1} and 300 kg ha^{-1}. The greatest influence in yield, according to the authors, was due to the level of soil phosphorus rather than the phosphorus application rate but, no growth or physiological explanation was endeavoured by them. Neither van Wijk *et al*. (2002) nor Ali *et al*. (2003) determined dry mass accumulation in either leaves or roots.

In an attempt to explain the differences in final root yield observed in this study between the differently fertilized plots and the fact that fresh mass can be deceiving due to the role water plays, dry mass accumulation was followed in both the leaves and roots. As carbohydrate photosynthate is primarily supplied by the leaves, root and leaf dry weight were both determined in order to ascertain a possible link between dry matter accumulation and final yield for all treatments. Root dry mass increased from the first sampling date, already at 30% development up to harvest during both seasons of the study confirming that dry matter accumulation increased with progression of growth. This was also the case with incremental fertilizer elevation but more so in 2007. As the 2007 season was more of a normal season in terms of rainfall, further discussion will focus on this season's data only.

The standard (100%) fertilizer level consistently produced carrots with higher dry matter accumulation than the other fertilizer levels, while non-fertilized

carrots showed the lowest dry mass. This is consistent with the study by Westerveld *et al*. (2006) who observed linear increase of carrot root dry matter and nitrogen accumulation from approximately 50 to 60 days after seeding up to harvest, when fertilizer levels were increased in increments. Ramesh *et al*. (2002) also observed that increasing nitrogen application, from 0% to 100% of the recommended dose of nitrogen, led to increased dry matter accumulation and productivity in wheat. However, elevated nitrogen application produced unexpected results in a study conducted by (Pettipas *et al*., 2006) where a nitrogen increase of up to 200 kg ha^{-1} did not lead to significant dry mass accumulation in neither carrot roots nor leaves.

In this study, the application of ComCat® contributed to a significant increase in dry mass accumulation, compared to the fertilizer only control, but, only at harvest and where the standard fertilizer was applied. This corresponded with the increase in yield at this stage of development and at this fertilizer regime. Dry matter accumulation where Kelpak® was applied, on the other hand, was much lower than the untreated controls during the early growth stages and at almost all fertilizer regimes but, only slightly higher than the controls at harvest and at the highest (100%) fertilizer regime. Again this corresponded with the slight, but non-significant, final root yield increase following Kelpak® treatment.

Leaf dry mass accumulation followed exactly the same linear pattern as did root dry mass accumulation in all cases, whether fertilizer was applied on its own or whether bio-stimulants were applied additionally. However, again at only the standard (100%) fertilizer regime and at harvest the ComCat® treatment contributed to significant dry mass accumulation in leaves, compared to the fertilizer only treatment, whereas Kelpak® did not. From this it seems that a positive correlation existed between dry matter accumulation in the leaves and roots, an aspect that was followed up with isotope and metabolite content as well as other related physiological studies (Chapter 5).

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CHAPTER 5

β-Carotene and sugar accumulation as well as sucrose translocation in carrots (*Daucus carota* L.).

Abstract

The response of carrots to treatment with different fertilizer levels either separately or in combination with two commercial bio-stimulants, ComCat® and Kelpak®, were investigated in terms of sugar accumulation in, sucrose translocation to and β-carotene content in the tap roots over one growing season. Sucrose, glucose and fructose levels were measured in field carrot roots at different growth stages. The translocation of sucrose was followed under greenhouse conditions by labelling leaves with U-^{14}C-glucose at early carrot development and determining the partitioning of radio-active label to the roots at harvest three weeks later. The β-carotene content in carrot roots was measured at harvest. Generally, sucrose levels increased more or less linearly with increasing fertilization, reaching the maximum level already where only 50% of the standard fertilizer was applied, and this tendency was observed at all growth development stages where sucrose was measured. Foliar application of ComCat® contributed to a significant increase in sucrose content at the 30% growth stage compared to both the fertilizer only control and the Kelpak® treatment, but only in combination with the standard fertilizer. This tendency prevailed at harvest. The latter 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 the higher (standard and 50% of the standard) fertilizer regimes. Glucose and fructose levels, on the other hand, fluctuated rather inconsistently at the lower fertilizer regimes (0% and 25% of the standard), but tended to stabilize where the standard and 50% of the standard fertilizer was applied. In the case of both glucose and fructose ComCat® and Kelpak® combination treatments tended to decrease their content in roots, especially where the standard and 50% of the standard fertilizer was applied. The β-carotene content generally increased with increasing fertilization but the most

significant increase was observed when carrots were treated with a combination of ComCat® and half of the recommended standard fertilizer. The Kelpak® treatment had no effect on the β-carotene content.

Key words: sugar, accumulation, translocation, β-carotene, fertilizer levels, bio-stimulants

5.1 Introduction

In photosynthetic tissue of plants triose phosphates, mainly 3-P-glyceraldehyde and dihydroxyacetone phosphate, are transported out of chloroplasts and into the cytosol via an antiport carrier system after three turns of the Calvin cycle (Salisbury and Ross, 1992). This leads to a net appearance of triose phosphates in the cytosol where it is utilized as substrate to form sugars, cell wall polysaccharides and hundreds of other compounds of which the plant is made. The synthesis of sucrose in the cytosol is of special importance as it is, together with starch, the principal leaf storage product accumulating in daylight and is also the form in which most plants translocate carbohydrate from the source to sinks via the phloem (Salisbury and Ross, 1992).

Photosynthetic assimilates partitioned to various plant tissues and organs are utilized for growth during the vegetative growth stage. Sucrose metabolizing enzymes such as invertase and sucrose synthase play a major controlling role on the rate of sucrose utilization by sinks thus creating a high demand for sucrose (Pollock and Farrar, 1996; Graham and Martin, 2000). However, a large amount of carbohydrate is stored in storage tissue that is eventually translocated to harvestable parts in the form of sucrose where it determines the final yield.

Carbon partitioning is, therefore, 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). In this regard 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).

In carrots, glucose, fructose and sucrose are major sugars and their availability in relatively high amounts, inter alia, 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 authors maintained that the volatile content is probably associated with the genetic heritage of carrots but that warm, humid climatic conditions also contribute to an unwanted flavour whereas cool, dry conditions and sandy soil contribute to less harsh-flavoured carrots.

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).

Moreover, 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 development (Sheen *et al*., 1999). For example, variation of light intensity as well as other abiotic stress conditions, especially drought and cold stress, substantially reduce the photosynthesis rate and, in turn, sugar translocation to sink tissues (Gupta, 2006). Limited or depleted

sugar levels eventually lead to cessation of growth that correlates positively with a reduced respiration rate via a decline in glycolytic pathway activity (Yu, 1999).

Further, the ability of plants to adapt to low temperatures has been attributed to soluble sugar (sucrose, glucose, fructose) accumulation that stabilizes membranes during freezing desiccation thus preventing phase separation and membrane fusion (Hoekstra and Buitink, 2001). Under stress conditions the mono- and disaccharide levels increase and are part of the protective osmotic effects (Gupta, 2006).

Besides sugars, a second quality characteristic of carrots is its inherent ability to produce rather large quantities of β-carotene. In fact, 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, they function as auxiliary chromophores in photosynthesis and as photoprotective 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.

However, carotenoids are sensitive to heat, oxygen and light exposure and, therefore, highly susceptible to oxidation due to the ease of destruction of the conjugated double bonds in their highly unsaturated structure (Gross, 1991). This instability necessitates extreme care in carrot handling procedures in order to minimize carotene loss. The large industry of catering and food services which has the capability of producing massive amount of meals per year thus needs to invest in specialized facilities and professionals to ensure retention of nutrients.

Carrots cultivated in the field during the 2007 season under different fertilizer regimes were used in this study for β-carotene and sugar content measurements. The response of carrots to the potential simulated fertilizer stress condition, either separately or in combination with two commercial bio-stimulants, was followed in terms of sugar and β-carotene production while the translocation of sucrose from the source (leaves) to the sink (roots) was measured at the 60% vegetative development stage, approximately four weeks after labelling seedlings at the 8-leaf growth stage with D-U^{14}C-glucose.

5.2 MATERIALS AND METHODS

5.2.1 Materials

5.2.1.1 Plant material

Seeds of a pre-pack carrot cultivar, Karina, were hand-sown thinly in the same manner as for the greenhouse trial (Chapter 3; 3.2.1) 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.

5.2.1.2 Other materials

A test combination kit (UV method Cat. Nr. 10 716 260 035), purchased from Boehringer Mannheim (Germany), was used to determine sucrose, D-glucose and D-fructose content in fresh carrot root material. In labelling experiments, D-U-^{14}C-glucose as well as ACS-II scintillation cocktail was obtained from Amersham, International. All other chemicals were of the purest quality obtainable.

5.2.2 Methods

5.2.2.1 Experimental design and treatments

A greenhouse study for β-carotene and sugar translocation by means of radio-active labelling was done. A randomized complete block design with five replicates was used for the study. Soil from the University of the Free State

experimental farm, that was previously analyzed, was used for growing carrots in pots in the greenhouse. Exactly the same procedures and treatments that were followed in the previous greenhouse trial applied (Chapter 3; 3.2.3 and 3.2.4). A field trial was used for measurement of β-carotene and the same procedures in Chapter 4. 4.2 were followed.

5.2.2.2 Radioactive labelling

An isotope solution was made up by dissolving 8.0 ml of D-U^{14}C-glucose (specific activity 0.617 MBq mmol^{-1}) or 1600 µCi in 192 ml distilled water to reach a final concentration of 8 µCi ml^{-1}. At the 8-leaf stage (stage 18-19) 15 ml (120 µCi) of the isotope solution was sprayed on plants in each of 12 pots (representing the 12 treatments; Chapter 3; 3.2.3; Table 2). Plants in each pot was covered with 32 µCi isotope and taken to represent a replicate. A hand sprayer (Merck TLC sprayer, Germany) was used to deliver a fine spray and the application was timed to ensure uniform and equal amounts of solution per pot. The remaining isotope solution was frozen for later use, as a standard, in order to calculate the amount of radio-activity absorbed by and translocated in the plant.

5.2.2.3 Extraction of labelled samples three weeks after labelling plants

A modified method of Hendrix and Peeley (1987) was used. Three weeks (approximately the 30% growth stage), each carrot root was first weighed and, subsequently, divided into three cross-sections: top (close to the crown/shoulder), middle and bottom. Two g aliquots of the three sections was thinly diced and placed separately in test tubes. Ten ml 80% ethanol (5 ml ethanol g^{-1} FW) was added to the sample and boiled for five minutes in a Labcon waterbath (LabDesign, R.S.A.) set at 80^{0}C in order to stop all chemical reactions. The ethanol that evaporated during boiling was replaced to the original volume after cooling and the material was homogenized for one minute at full speed (CAT Homogenizer X620, Germany). Ethanol was again added up to the original level,

thoroughly mixed and the material allowed to settle. A 1.5 ml supernatant aliquot of each homogenized 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 clean Eppendorf vials and transferred to an oven set at 70^0 C in order to rid the solution of ethanol that might later interfere with radio-activity readings through quenching. Some of the supernatant was kept in the freezer for later use and the rest of the supernatant was decanted. After the drying process, one ml of distilled water was added to the dry material and vigorously mixed. A 500 µl aliquot of each 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 determining radio-activity by reading disintegrations per minute (DPM's) with a Beckham LS 6500 scintillation counter. Prior to reading of DPM's the scintillation counter was calibrated for quenching by using a series of carbon tetrachloride standards to set up calibration curve in conjunction with the internal standard of the scintillation counter.

Subsequent to removing the supernatant from centrifuged samples, the carrot pellets were blotted dry to remove excess supernatant. A 0.2 g aliquot of the pellet from each carrot section was transferred to a scintillation vial, 4 ml ACS-II scintillation cocktail added and mixed thoroughly. Disintegrations per minute were counted in pellets and the sum of counts for the supernatant and pellets taken as representatitive of the amount of radio-activity g^{-1} FW. The sum of DPM-counts for the supernatant and pellets, and for replicas separately, was taken as the total radio-activity in the tissue at harvest. The original weight of the carrot was considered in calculations to extrapolate the data obtained with aliquots to the whole root.

Radio-activity remaining in the leaves was also measured in order to establish the amount of radio-activity that was not translocated to the roots. Previously frozen carrot leaves were thoroughly rinsed in running water to remove any unabsorbed isotope on the surface, blotted dry with tissue paper and 2 g

aliquots treated in exactly the same way as the root sections. Again the sum of radio-activity in the supernatant and pellets of each replica, for all treatments, were taken as the total radio-activity in the leaf tissue at harvest. Calculations were made in the same way as for roots and the total radio-activity in the whole leaf system determined through extrapolation.

Further, the total radio-activity of the original isotope solution was also determined from a solution made up of 5 µl radioactive D-U-^{14}C-glucose (original isotope solution used to spray plants) and 4 ml ACS-II scintillation cocktail. The latter was essential to calculate the amount of radio-activity absorbed by the plant. Partitioning of radio-active label was expressed as a percentage of the absorbed isotope.

5.2.2.4 Sugar content measurement

5.2.2.4.1 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 Labcon waterbath (LabDesign, R.S.A.) set at 80^{0}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 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 70^0 C in order to 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 of all treatments 50 µl aliquots were

removed for the determination of sucrose, D-glucose and D-fructose content by following the directions accompanying the Boehringer Mannheim test kits.

5.2.2.4.2 Principle of the Boehringer Mannheim enzymatic technique for determining sucrose, D-glucose and D-fructose content in solid tissue

The D-glucose concentration is determined before and after the enzymatic hydrolysis of sucrose. D-fructose is determined subsequently to the determination of D-glucose.

Determination of D-glucose before inversion:

At pH 7.6 the enzyme hexokinase (*HK*) catalyses the phosphorylation of D-glucose by adenosine-5'-triphosphate (ATP) with the simultaneous formation of adenosine-5'-diphosphate (ADP) [1].

$$\text{D-Glucose + ATP} \xrightarrow{HK} \text{Glucose-6-phosphate + ADP} \quad \text{.....[1]}$$

In the presence of *glucose-6-phosphate dehydrogenase* (*G-6-PDH*), the D-glucose-6-phosphate (G-6-P) formed is specifically oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH $+H^+$) [2].

$$\text{Glucose-6-phosphate + } NADP^+ \xrightarrow{G\text{-}6\text{-}PDH} \text{gluconate-6-phosphate +NADPH + } H^+ \quad \text{.....[2]}$$

The NADPH formed in this reaction is stoichiometric to the amount of D-glucose and is measured by means of its absorbance at 340 nm.

Determination of D-fructose:

Hexokinase (*HK*) also catalyzes the phosphorylation of D-fructose to fructose-6-phosphate (F-6-P) in the presence of ATP [3].

$$\text{D-Fructose + ATP} \xrightarrow{HK} \text{Fructose-6-phosphate + ADP} \quad \text{.....[3]}$$

On completion of the reaction (3) **F-6-P** is converted by *phosphoglucose isomerase (PGI)* to Glucose-6-phosphate [4].

$$\text{Fructose-6-phosphate} \xrightarrow{PGI} \text{Glucose-6-phosphate} \quad \text{..... [4]}$$

G-6-P reacts again with NADP to form gluconate-6-phosphate and NADPH [2]. The amount of NADPH formed is now stoichiometric with the amount of D-fructose.

Enzymatic inversion:

At pH 4.6, sucrose is hydrolyzed by the enzyme *β–fructosidase (invertase)* to D-glucose and D-fructose [5].

$$\text{Sucrose + } H_2O \xrightarrow{\beta\text{–fructosidase}} \text{D-glucose + D-fructose} \quad \text{.....[5]}$$

The determination of D-glucose after inversion (total D-glucose) was carried out according to the principle outlined above. The sucrose content was calculated from the difference of the D-glucose concentrations before and after enzymatic inversion.

Procedure:

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:

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

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)

It follows for sucrose:

$$c = \frac{3.02 \times 342.3}{\varepsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{sucrose} = \frac{10.34}{\varepsilon} \times \Delta A_{sucrose} \text{ g } \ell^{-1}$$

for D-glucose:

$$c = \frac{3.02 \times 180.16}{\varepsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{D\text{-}glucose} = \frac{5.441}{\varepsilon} \times \Delta A_{D\text{-}glucose} \text{ g } \ell^{-1}$$

for D-fructose

$$c = \frac{3.04 \times 180.16}{\varepsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{fructose} = \frac{5.477}{\varepsilon} \times \Delta A_{fructose} \text{ g } \ell^{-1}$$

Sucrose, D-glucose and D-fructose content was expressed as µmol g^{-1} fresh weight.

5.2.3 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 2 g 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 exclude 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, 6 ml acetone and 12 ml hexane were added to constitute the ratio of 50 hexane**:** 25 acetone**:** 25 ethanol and the bottles were

agitated for 10 minutes. Fifteen ml distilled water was added to the bottles 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. Carotene levels were measured using a Shimadzu HPLC system with SPD 20AV detector and LC 20 AT pump (Shimadzu Corporation, Japan). Carotene amount was calculated from the measured area of the trans-carotene graph and the supplied 240 mg L^{-1} carotene standard.

5.2.4 Calculations and statistical methods

Data was analyzed using the NCSS 2000 statistical package (Hintze, 1999) for identification of differences in the treatments. The Tukey Kramer LSD ($P<0.05$) was used for separation of treatment means (Steele and Torrie, 1980). Significant differences are highlighted within figures in the results section.

5.3 RESULTS

5.3.1 Carrot root sugar content

5.3.1.1 Glucose content

Where no fertilizer was applied the **root glucose content** (µmol g^{-1} FW) was significantly enhanced by the application of both bio-stimulants at the 30% and 80% development stages (Figure 5.1A & C), judged on the LSD value representing the interaction (FLxFB) between fertilizer level and bio-stimulant effects. This was neither the case at 60% development (Figure 5.1B) nor at harvest (Figure 5.1D) where no significant differences were observed.

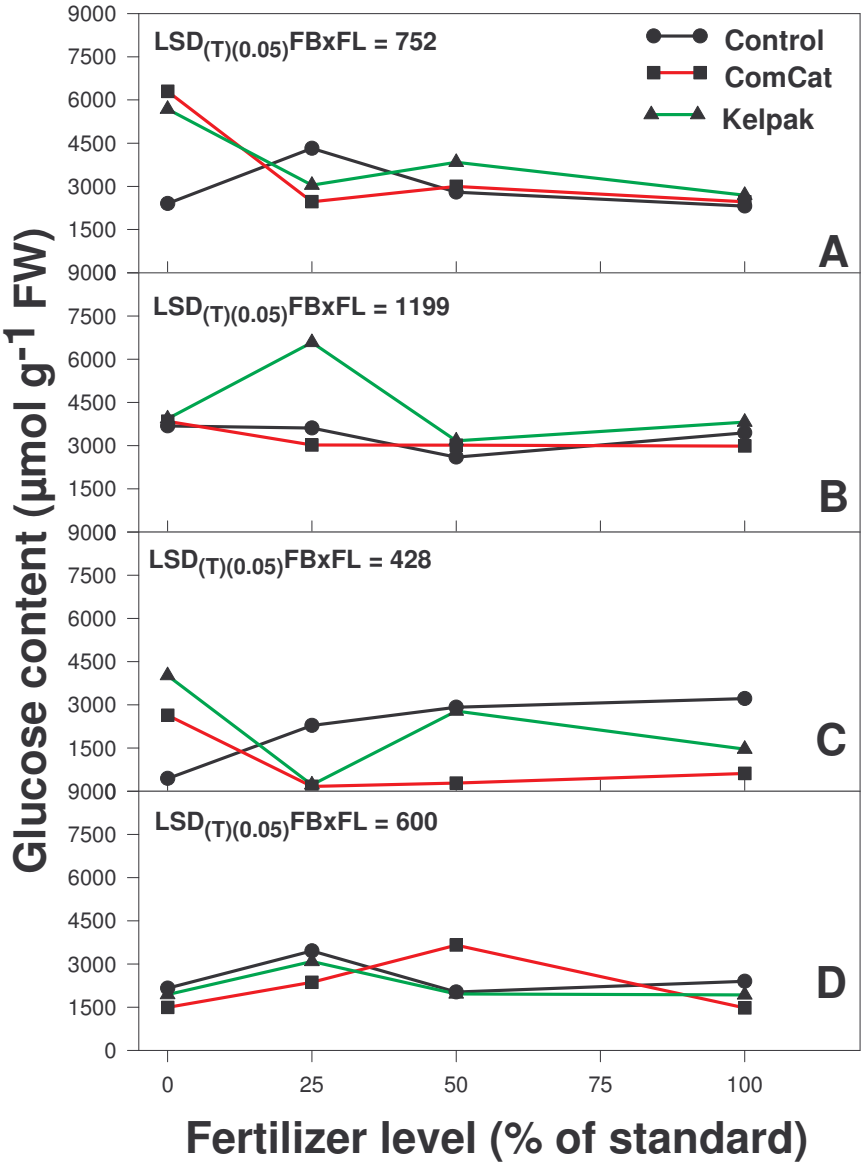

Figure 5.1: Effect of fertilizer, applied at different levels, either separate or in combination with two commercial bio-stimulants (ComCat® and Kelpak®) on **glucose content** of carrot roots (µmol g^{-1} FW) at different growth stages in 2007. A = 30% development, B = 60% development, C = 80% development and D = at harvest.

When the fertilizer level was increased to 25% of the standard, treatment with both bio-stimulants tended to decrease the glucose content in roots at all stages of development except at 60% development where Kelpak® (Figure 5.1B) contributed

to a significant increase. The decrease in glucose content where bio-stimulants were applied was statistically significant only at 80% development (Figure 5.1C) and only for ComCat® at harvest (Figure 5.1D).

No significant differences in glucose content between the different treatments, i.e. where 50% of the standard fertilizer was applied separate or in combination with the two bio-stimulants, was observed during the early growth stages but, only ComCat® contributed to a significant decrease of glucose at 80% development (Figure 5.1C) and a significant increase at harvest (Figure 5.1D).

Exactly the same tendency as was observed at the 50% fertilizer level prevailed where standard fertilizer (100%) was applied during the early growth stages (Figure 5.1A & B). However, at 80% vegetative development as well as at harvest both bio-stimulants contributed to a decrease in root glucose content (Figure 5.1C & D) where100% fertilizer was applied, although the decrease was only significant at the 80% growth stage.

5.3.1.2 Fructose content

Where no fertilizer was applied, the fluctuation of **fructose content in carrot roots** followed almost the same pattern as did the glucose content (Figure 5.1) in as much as the tendency to be increased at 80% development (Figure 5.2C) and decreased at harvest (Figure 5.2D) was similar. However, at the early growth stages (Figure 5.2A & B) no significant differences in fructose content between the fertilizer only and bio-stimulant combination treatments were observed.

Elevation of the fertilizer level to 25% of the standard had no significant effect on the fructose content, at any growth stage, whether it was applied separately or in combination with the bio-stimulants, except in the case of the Kelpak® treatment at 60% growth development. Interestingly, Kelpak® contributed to a significant decrease in fructose content at the same fertilizer regime and at the same growth stage (Figure 5.2B) where it significantly increased the glucose level (Figure 5.1B).

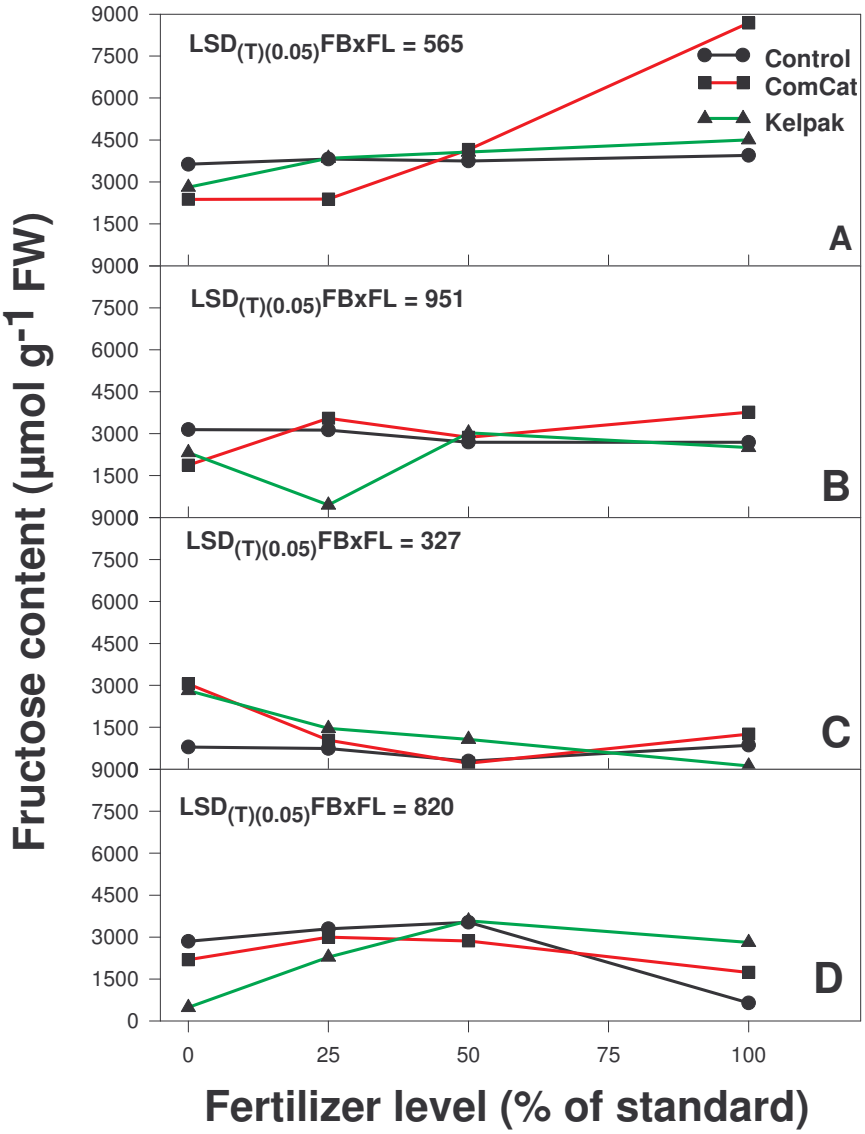

Figure 5.2: Effect of fertilizer, applied at different levels, either separate or in combination with two commercial bio-stimulants (ComCat® and Kelpak®) on **fructose content** of carrot roots (µmol g^{-1} FW) at different growth stages in 2007. A = 30% development, B = 60% development, C = 80% development and D = at harvest.

Except for a rather sharp and significant increase in the fructose content at the 30% development stage (Figure 5.2A) where ComCat® was applied together with standard fertilizer, no significant differences were observed at the other growth

stages either at the 50% or 100% fertilizer regimes. The only other feature worth mentioning is that the fructose level was slightly higher at harvest where both bio-stimulants were applied in combination with the standard fertilizer (Figure 5.2D).

5.3.1.3 Sucrose content

The **root content of the disaccharide sucrose** was lower than that of the two monosaccharide sugars at all stages of development and at all fertilizer regimes (Figure 5.3). Compared to the two monosaccharide sugars (Figures 5.1 and 5.2) the sucrose content also showed greater variation at either different fertilizer regimes or different growth stages or both. Where no fertilizer was applied, and especially from the 60% development stage onwards (Figure 5.3A, B & C) it was only the ComCat® treatment that tended to increase the sucrose level in roots. This was significant at both 60% (Figure 5.3B) and 80% (Figure 5.3C) vegetative development.

No clear pattern emerged where 25% of the standard fertilizer was applied except that the sucrose content was slightly higher at times compared to the zero fertilizer treatment. This difference was, however, only significant at the 80% vegetative growth stage (Figure 5.3C). The sucrose content where Kelpak® was applied in combination with 25% fertilizer, did not differ significantly from the fertilizer only control except at the later growth stages (Figure 5.3C & D) where this treatment tended to decrease the sucrose content in roots. The combination treatment with ComCat®, on the other hand, tended to increase the root sucrose content initially (Figure 5.3B) as well as at harvest (Figure 5.3D).

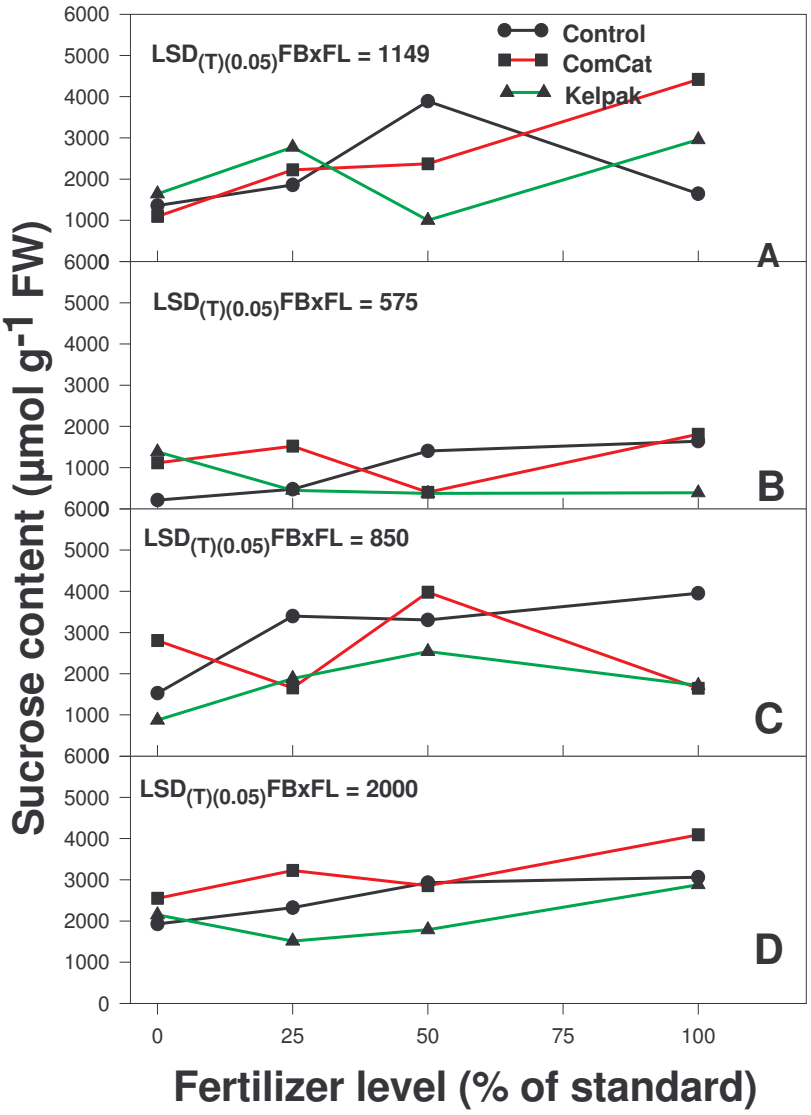

Figure 5.3: Effect of fertilizer, applied at different levels, either separate or in combination with two commercial bio-stimulants (ComCat® and Kelpak®) on **sucrose content** of carrot roots (µmol g^{-1} FW) at different growth stages in 2007. A = 30% development, B = 60% development, C = 80% development and D = at harvest.

Interestingly, when the fertilizer level was increased to 50% of the standard a sharp increase in root sucrose content occurred compared to the lower fertilizer regimes at 30% vegetative development (Figure 5.3A). This was statistically significant when compared to the sucrose level at both the 0% and 25% level as

well as with both the bio-stimulant combination treatments. Importantly, at this early growth stage both bio-stimulants had a significant decreasing effect in the measurable sucrose content in roots. However, although no significant differences in root sucrose content between the 50% fertilizer control and both bio-stimulant combination treatments were observed during later growth stages, Kelpak® constantly tended to have a decreasing effect on measurable sucrose at these growth stages (Figure 5.3B, C & D).

At 30% growth and where standard fertilizer (100%) was applied, the direct opposite was observed compared to the 50% fertilizer treatment in the sense that both bio-stimulants significantly enhanced the sucrose content (Figure 5.3A). However, this tendency was not repeated at the later growth stages where, in fact, the same tendency to decrease the sucrose content as was observed for the lower fertilizer regimes prevailed (Figure 5.3B, C & D). Although not significant, the standard fertilizer/ComCat® combination treatment tended to enhance the sucrose content in carrot roots at harvest (Figure 5.3D).

5.3.1.4 Total sugar content

When the total sugar content (mmol g^{-1} FW) was calculated as the sum of sucrose, glucose and fructose for each fertilizer level at different growth stages, an interesting repetitive pattern emerged (Table 5.1). In all cases the total sugar content decreased as vegetative growth progressed. The total sugar content was considerably less than at the early development stage and remained at low and comparable levels between 60% development and harvest. There was a tendency for increased total sugar content as fertilizer was incrementally increased. The application tended to have a slight enhancing effect on sugar content but this tendency was erratic across fertilizer levels and growth stages often with an inhibitory effect on sugar accumulation.

Table 5.1: Total carrot sugar concentration (mmol g^{-1} FW) at different growth stages as influenced by different fertilizer levels, both separately and in combination with bio-stimulants.

Fertilizer level % (FL)	Fertilizer + Bio-stimulant (FB)							
	30% Plant development				80% Plant development			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	7.44	12.43	8.97	**9.61**	2.68	1.18	1.10	**1.65**
50	10.44	8.67	8.90	**9.34**	2.17	1.49	2.13	**1.93**
25	9.99	6.48	9.67	**8.71**	2.14	1.29	1.53	**1.65**
0	7.10	9.36	10.13	**8.86**	0.92	2.83	2.57	**2.11**
Ave FB	**8.74**	**9.24**	**9.42**		**1.98**	**1.70**	**1.83**	
$LSD_{(T)(0.05)}$FLxFB	**3.85**				**0.41**			
$LSD_{(T)(0.05)}$ FL	**ns**				**0.18**			
$LSD_{(T)(0.05)}$ FB	**ns**				**0.14**			
Fertilizer level % (FL)	**60% Plant development**				**At harvest**			
	Control	ComCat	Kelpak	Ave FL	Control	ComCat	Kelpak	Ave FL
100	2.59	2.86	2.24	**2.56**	2.70	2.44	2.54	**2.56**
50	2.20	2.10	2.19	**2.16**	2.83	3.13	2.44	**2.80**
25	2.41	2.70	2.49	**2.53**	3.03	2.86	2.30	**2.73**
0	2.35	2.28	2.55	**2.39**	2.32	1.89	2.01	**2.07**
Ave FB	**2.39**	**2.49**	**2.37**		**2.72**	**2.58**	**2.32**	
$LSD_{(T)(0.05)}$FLxFB	**0.49**				**0.85**			
$LSD_{(T)(0.05)}$ FL	**0.22**				**0.37**			
$LSD_{(T)(0.05)}$ FB	**ns**				**0.29**			

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

Sucrose is the form in which carbohydrate is translocated from the leaves (source) to the roots (sink) 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 the 60% growth development stage, in order to establish sucrose translocation trend midway

through the vegetative growth phase. Radio-activity was expressed as a percentage of the original isotope that was absorbed by the leaves.

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 5.4A). 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 compared to that for the fertilizer only treatments (Figure 5.4B & 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 5.4B). 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 and this was similar to radio-activity partitioning where the standard fertilizer was applied.

Although the 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, this was not as marked in the case of the 50% and 100% fertilizer regimes (Figure 5.4C).

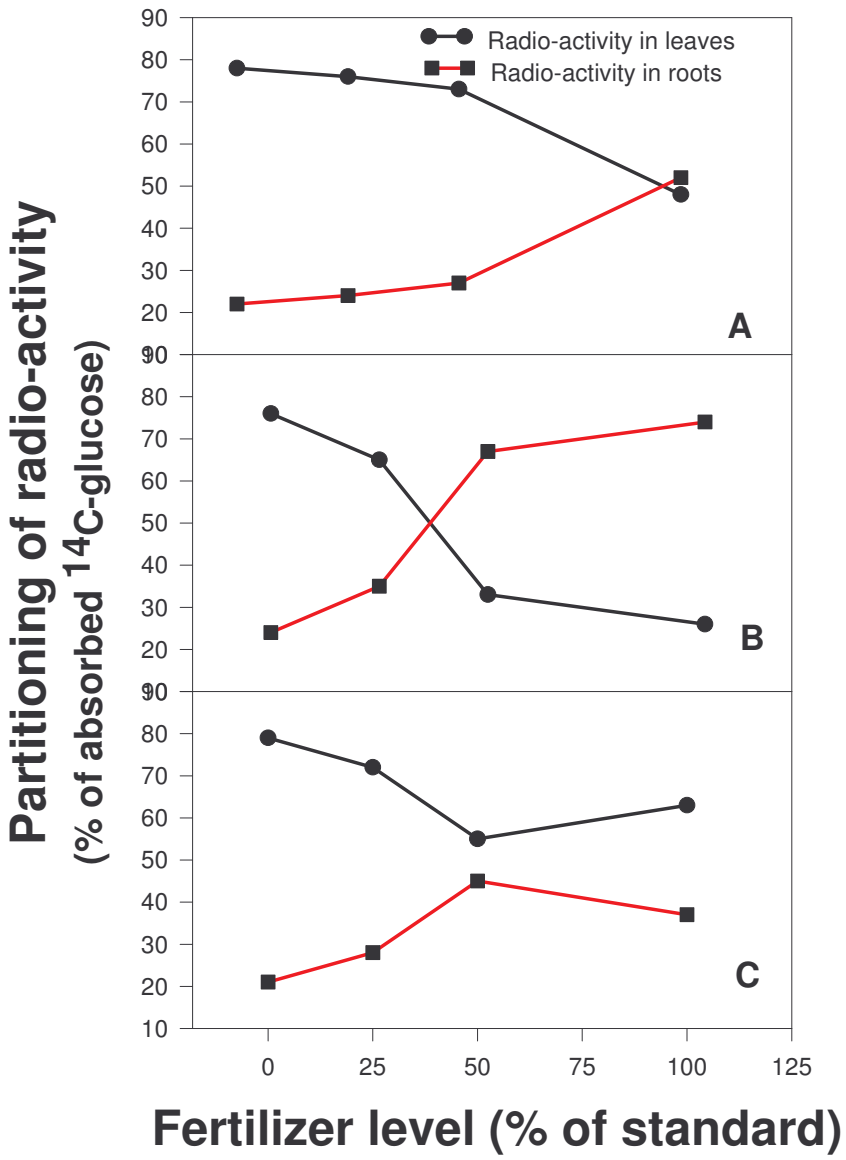

Figure 5.4: Effect of fertilizer, applied at different levels, either separate or in combination with two commercial bio-stimulants (ComCat® and Kelpak®) on **radio-activity partitioning** in carrots four weeks after spraying with U-^{14}C-Glucose at the 8-leaf growth stage under greenhouse conditions. A = fertilizer only, B = ComCat® treated and C = Kelpak® treated.

5.3.3 β-Carotene content in carrot roots

There was a slight and non-significant tendency for enhanced β-carotene accumulation in carrot roots as the fertilizer application was elevated in increments (Figure 5.5). Application of ComCat® in combination with all of the elevated fertilizer regimes produced carrots with significantly (Table 5.2) higher β-carotene content than the control. Interestingly, ComCat® in combination 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.

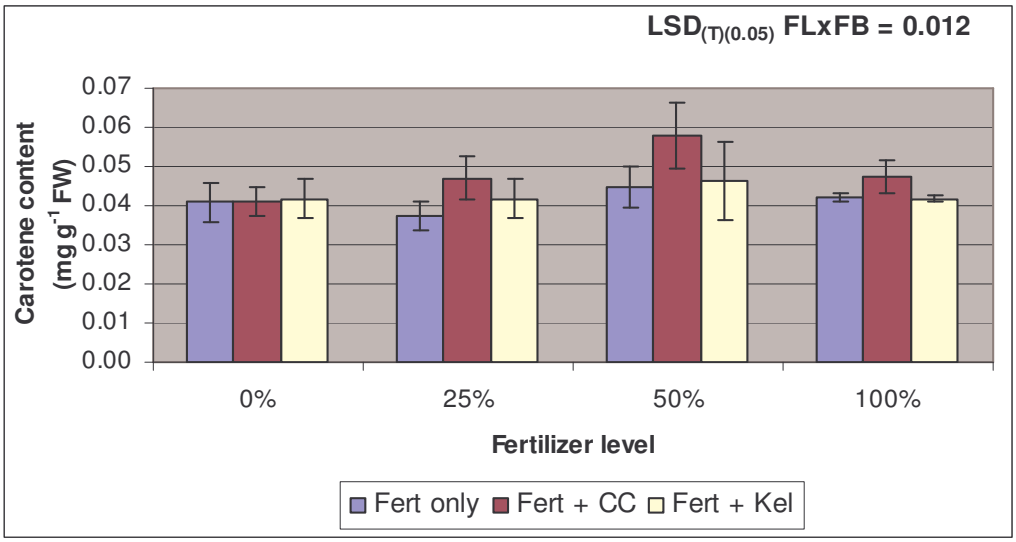

Figure 5.5: Effect of different fertilizer levels, both separately and in combination with bio-stimulants, on β-carotene content of carrots at harvest in 2007.

Table 5.2: Statistical analysis of the fertilizer level and bio-stimulant effect on carrot root β-carotene content (mg g^{-1} FW) at harvest in 2007				
Fertilizer level % (FL)	**Fertilizer + Bio-stimulant (FB)**			
	Control	**ComCat**	**Kelpak**	**Ave FL**
100	0.0420	0.0473	0.0418	**0.0437**
50	0.0447	0.0580	0.0463	**0.0496**
25	0.0372	0.0471	0.0417	**0.0420**
0	0.0410	0.0411	0.0417	**0.0413**
Ave FB	**0.0412**	**0.0484**	**0.04293**	
$LSD_{(T)(0.05)}$FLxFB	**0.0123**			
$LSD_{(T)(0.05)}$FL	**0.0054**			
$LSD_{(T)(0.05)}$FB	**0.0042**			

5.4 Discussion

In this chapter the response of carrot (*Daucus carota* L.) to varied fertilizer levels both separate and in combination with two commercial bio-stimulants was followed under field conditions in terms of sucrose, D-glucose and D-fructose as well as β-carotene content at different growth stages. Additionally the translocation of radio-active sucrose from the leaves to the roots, four weeks after spraying plants with U-^{14}C-glucose at the 8-leaf growth stage, was measured under glasshouse conditions. The objective with this approach was to ascertain whether the differences in vegetative growth and yield obtained with different fertilizer levels and bio-stimulants (chapter 4) corresponded with selected physiological activities within the taproot. In order to put this approach in perspective, it is necessary to supply a short summary of the known aspects concerning carbohydrate production, transolocation and utilization in plants under normal conditions.

In most plants the monosaccharide sugar D-glucose is the end product of photosynthesis which, in carrot, proceeds only in the above soil parts. However, in higher plants, and in the case of carrot, carbohydrate is translocated from the source (leaves) to the sink (storage roots) in the form of the disaccharide sucrose

(Dale, 1984). Even though Lemoine *et al*. (1989) stated 20 years ago that only a sucrose carrier has been identified in plant membranes at that time, it still holds true today. The implication is that monosaccharide photosynthate must be converted to sucrose before it can be translocated to storage tissue. In this regard it is accepted that the partitioning of radio-activity followed in this study was due to the partial conversion of U-^{14}C-glucose to radio-active D-fructose and eventually to radio-active sucrose before translocation could commence.

The synthesis of sucrose in higher plants takes place in the cytosol of cells by an interesting sequence of group-transfer reactions (Salisbury and Ross, 1992). Subsequently, phloem loading of sucrose proceeds in the leaves via the apoplast or symplast or both (Lucas and Madore, 1988) and is then translocated downwards into the carrot tap root. Following phloem unloading of sucrose in storage tissue, e.g. cortex parenchyma of carrot tap roots, a portion of the sucrose is hydrolyzed to hexose sugars, glucose and fructose, by sucrose phosphate synthase and invertase. The activities of these enzymes and the compartmentation of sucrose and hexoses determine the net accumulation or breakdown of sucrose as was found in *Acer pseudoplatanus* (Huber and Akazawa, 1986) and *Daucus carota* (Lee and Sturm, 1996). While a portion of the sucrose translocated from the leaves (source) to the tap root (sink) of carrot is stored, the products of the hydrolyzed portion are utilized as energy source by standard biochemical pathways including the oxidative pentose phosphate (OPP) pathway and glycolysis (Krook *et al*.,1998; Krook *et al*., 2000). From this it becomes clear that sucrose is subject to cycling by a continuous process of synthesis and degradation (Wendler *et al*., 1990).

In the present study the sucrose content in roots was much lower than that of the two monosaccharide sugars at all stages of development and at all fertilizer regimes. This is understandable as sucrose is partially hydrolyzed and metabolized in sinks. However, compared to the non-fertilized control, the sucrose content tended to increase linearly with increased fertilization and also increased as growth proceeded to reach the highest level at later stages of development while the monosaccharide sugar levels showed the opposite trend. 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) 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.

Interestingly, compared to the fertilizer only and Kelpak® treatments, the application of ComCat® in combination with varying levels of fertilizer increased the sucrose level significantly at 30% growth development and again at harvest but, only at the standard fertilizer regime. This indicates that either more sucrose eventually accumulated in the final sink of 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 regimes and compared to the fertilizer only control, strongly suggest that ComCat® had an enhancing effect on sucrose translocation. This is in concert with the findings of the company, Agraforum AG, that ComCat® has an energizing effect on plant membranes leading to accelerated translocation of carbohydrate from source to sink (Hüster, personal communication, Agraforum AG. March, 2008). 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.

It must be expected that 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*., 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.

Glucose levels decreased with increased fertilization especially during the early carrot development stage whereas fructose levels tended to increase with increasing fertilization. The tendency for a reduction in glucose content as fertilizer level increased is similar to results by 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 the present study were contrary to the report by Schaller and Schnitzler (2000) that 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. On the other hand, the application of high rates of nitrogen led to increased glucose and fructose and lower concentration of sucrose.

To further complicate the relationship between fertilization and sugar content in carrot, 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. 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 (results not shown) confirming that sugar partitioning is also an important factor to keep in mind when sugar content data is interpreted.

Korolev *et al*. (2000b) studied sucrose partitioning even more exactly and found the highest concentration of sucrose in the xylem and phloem parenchyma tissues and minimal amounts in the pith and periderm. The deposition of hexose monosaccharide sugars was found in carrot root to be divided between different tissues. Glucose was mainly found in the phloem and fructose in the xylem. The pith mainly stored water and ions whereas the periderm, which acts as a protective layer, had minimal nutrient content. Further, the distribution of sugars as well as insoluble carbon was high around the cambium confirming the strong sink capacity of the cambium which also corresponded to active growth of the region (Korolev *et al*., 2000b). The authors also showed that transport of photoassimilate in carrot occurred both radially and longitudinally with the latter transport mode being faster than radial transport.

Apart from being utilized as storage compounds, sugar content is particularly important from a consumer point of view as it contributes to the perception of sweet or bitter taste. The content of the non-volatile constituents, sugars and amino acids, and volatile compounds gives specific carrot taste sensation (Rosenfeld *et al*., 2004). The author reported that rather high terpene content results in poor carrot taste whereas carrots with a lower sugar and terpene content are preferred. Further, carrots contribute one of the highest levels of carotene in the human diet. Carotene, a source of provitamin A, has a wide range

of protective effects in the human body including prevention of oxidative stress and damage (Handelman, 2001). Carotene also has a variety of non antioxidant properties that affect cellular signalling pathways, modify the expression of some genes and can act as inhibitors of regulatory enzymes (Stahl *et al*., 2001).

In the present study, the β-Carotene (a terpene) content was not affected by elevated fertilizer application. However, where 50% of the standard fertilizer was applied in combination with ComCat® the highest β-Carotene content (0.058 mg g^{-1} FW) was measured. In fact, ComCat® contributed to higher β-Carotene levels in combination with all fertilizer regimes while the application of Kelpak® alone had an inhibitory effect on carotene content. The results are in contradiction with those of Hochmuth *et al*. (1999) and Ali *et al*. (2003). Hochmuth *et al*. (1999) reported an increase in carotene content as the fertilizer level was 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), there was 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). The authors 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 content 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. The application of bio-stimulants, in particular ComCat®, was beneficial to carotene accumulation even at below standard fertilizer levels. 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.

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CHAPTER 6

Respiratory response of carrots (*Daucus carota* L.) to treatment with different fertilizer levels separately and in combination with commercial bio-stimulants under field conditions

Abstract

Carrot plants (cv. Karina) were cultivated at different fertilizer levels (0%, 25%, 50% and 100% of the recommended rate). Two bio-stimulants, ComCat® and Kelpak®, were foliar applied either separately or in combination with the different fertilizer regimes, at the 4-leaf and again at the 30% root development growth stage. Selected respiratory metabolic events were followed including measurement of root respiration rates (both O_2 utilization and CO_2 release) at different growth stages and *in vitro* activities of regulatory enzymes of both the glycolytic (PFK and PFP) and oxidative pentose phosphate (OPP) pathway (G-6-PDH) at the 30% growth stage. The latter corresponded with the second bio-stimulant application time. As far as possible the relationships between these metabolic events were followed. Respiration rate in terms of O_2 consumption declined as fertilizer was incrementally increased from zero to the recommended standard for carrots. A similar tendency prevailed as growth progressed from early development to harvest. Application of both bio-stimulants, ComCat® and Kelpak® led to a significant increase in O_2 consumption but only where no fertilizer was applied and during early development. Application of fertilizer alone led to a linear increase in PFK activity as fertilizer was incrementally increased. However, although PFP activity did not follow the same trend, it was significantly higher than that of PFK at zero and 25% fertilization. Thereafter, application of fertilizer alone at the half and standard levels led to lower PFP activity. Application of fertilizer in combination with both ComCat® and Kelpak® led to higher PFP activity than that of PFK at the corresponding levels. The high PFK activity induced by bio-stimulants

in the absence of fertilizer coincided with a high oxygen consumption rate. Activity of G-6-PDH was highest where carrots were cultivated under zero or very low fertilizer conditions. The activity of this regulatory enzyme of the OPP-pathway settled at a twofold lower level where the standard and 50% of the standard fertilizer was applied. Foliar application of the two bio-stimulants tended to reduce the activity of G-6-PDH at the zero fertilizer level. However, ComCat® in combination with medium fertilizer regimes tended to increase the activity of this enzyme whereas Kelpak® had the opposite effect. Except at the zero fertilizer level, no correlation between G-6-PDH and CO_2 release rates was observed. The relatively high activity of G-6-PDH and PFP in carrot tap roots at the low fertilizer regimes may be indicative of the plant's response to low nutrient stress. The induction of PFP and G-6-PDH activity by ComCat®, especially at the higher (50% and 100%) fertilizer levels, correlated positively with previous findings in terms of the enhancing effect of the bio-stimulant towards yield and sucrose content at harvest.

Key words: Respiration rate, enzyme activity, fertilizer levels, bio-stimulants

6.1 Introduction

In the previous chapter (Chapter 5) sucrose, glucose and fructose levels were measured at different developmental stages of *Daucus carota* L. Mention was made of the complexity to interpret the data due to the fact that a portion of carbohydrate, produced on a daily basis through photosynthesis, is metabolized in the root after being translocated from the leaves in the form of sucrose (Dale, 1984). According to Sheen *et al*. (1999), it is the partitioning of respiratory substrate in terms of storage, conversion and metabolism that contributes to fluctuations in their levels and that complicates the interpretation of obtained data. In this chapter the breakdown of carbohydrate in carrot via respiratory metabolism, as a response to treatment at different fertilizer levels both separately and in

combination with commercial bio-stimulants, was followed in order to obtain a broader picture of metabolic events.

Respiratory rate data can be a handy tool when compared to measured respiratory substrate levels in order to interpret possible fluctuations in the latter. Emphasis was placed on the respiration rate, in terms of oxygen consumption by the roots, as well as the activity of selected regulatory enzymes of glycolysis (phosphofructokinase; PFK; EC 2.7.1.11 and fructose 6-phosphate-1-phosphotransferase; PFP; EC 2.7.1.90) and the oxidative pentose phosphate pathway (glucose-6-phosphate dehydrogenase; G-6-PDH; EC1.1.1.49) in roots.

The most acceptable aerobic respiratory rate parameter is oxygen consumption by plant tissue and not CO_2 release data as not only the respiratory pathways (glycolysis and the Krebs cycle) release CO_2 but also the oxidative pentose phosphate pathway (Krook *et al*., 2000). In the presence of sufficient oxygen, carbohydrates are completely broken down to carbon dioxide, water and energy during aerobic respiration that includes both the glycolysis pathway and the Krebs cycle (Salisbury and Ross, 1992). Glycolysis is independent of oxygen but the Krebs cycle cannot proceed in the absence of oxygen (Salisbury and Ross, 1992). When oxygen consumption is, therefore, used as parameter to measure respiration rate it is essentially the mitochondrial breakdown of respiratory substrate via the Krebs cycle that is measured (Ap Rees, 1980). According to the author the oxidizable substrate of mitochondrial respiration is, in most cases, provided by the degradation of carbohydrate. However, the rate of mitochondrial oxygen consumption represents the total aerobic respiration rate but, indirectly includes that of glycolysis in the cytosol as the latter provides pyruvic acid from glucose at the same rate as pyruvic acid is broken down via the Krebs cycle in the mitochondrion (Ap Rees, 1980).

Importantly, respiratory metabolism in plants is finely regulated depending on the energy status of the plant (Nadas *et al*., 2008). The role of regulatory enzymes involved in carbohydrate synthesis and breakdown, namely the key enzymes of glycolysis and gluconeogenesis, are very important in the regulation of sugar metabolism. The PFK/PFP enzyme system plays a crucial role in the

regulation of glycolysis and gluconeogenesis in plants (Lea *et al.*, 2002; Widodo *et al.*, 2003). ATP-dependant PFK catalyzes the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate in the glycolysis direction while the PPi dependent PFP can catalyze the mentioned reaction in both the glycolysis and gluconeogenesis direction (Stitt, 1990). According to the author, PFP is often more active than PFK. Diverse roles have been proposed for PFP including a role in glycolysis, gluconeogenesis and general adaptability to stress (Paul *et al.*, 1995).

On the other hand, the oxidative pentose phosphate pathway (OPPP) is an alternative catabolic route for hexoses. Glucose-6-phosphate dehydrogenase (G-6-PDH) is the first enzyme of the OPPP and catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconolactone, concomitant with reduction of NADP to NADPH. This first enzyme, G-6-PDH, is the only regulatory enzyme and controls carbon flux through the OPPP (Hauschild and Schaewen, 2003). However, glycolysis and the OPP pathways have common substrates namely glucose 6-phosphate, fructose 6-phosphate and glyceraldehyde 3-phosphate (Tobin and Bowsher, 2005).

Where the main function of glycolysis, and for that matter the Krebs cycle, is to continually supply living tissue with energy (ATP), the main functions of the OPPP includes the production of reduction power in the form of NADPH, the conversion of hexoses to pentoses such as ribulose 5-phosphate that is used in synthesizing nucleic acids (Hames and Hooper, 2005) as well as supplying erytrose-4-phosphate that is utilized for the production of shikimic acid, a precursor of aromatic rings (Herrman and Weaver, 1999). The NADPH produced is, inter alia, utilized for inorganic nitrogen assimilation and fatty acid biosynthesis and contributes towards protection of cells against oxidative stress (Debman *et al.*, 2004).

In this chapter the total respiration rate of carrot tap roots was correlated with the activities of key regulatory enzymes of glycolysis and the OPPP under different fertilizer regimes, both separately and in combination with commercial bio-stimulants. This was an attempt to obtain an overview of respiratory

metabolism that could assist in the interpretation of sugar level data presented in the previous chapter (see chapter 5) under the mentioned experimental conditions.

6.2 Materials and methods

6.2.1 Materials

Coupling enzymes, triose phosphate isomerase, glycerol-3-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and fructose-1,6-bisphosphate aldolase, as well as fructose-2,6-bisphosphate and commercial fructose 6-phosphate-1-phosphotransferase (PFP) were purchased from Sigma, St. Louis, USA. The chemicals ADP, ATP, NAD^+ and NADH were obtained from Boehringer-Mannheim, Germany. All other chemicals were of the highest purity available.

6.2.2 Methods

6.2.2.1 Respiration rate measurement

Field carrot samples were collected at growth stages coinciding with 30%, 60% and 80% vegetative development as well as at harvest. Immediately following sample collection the roots were separated from the leaves and individually weighed. A whole carrot root was separately placed in a 1000 cm^3 Schott bottle for simultaneous O_2 consumption and CO_2 release measurements at 25^0C using a Pasco Meter **(PASCO™, USA)**, equipped with both oxygen and carbon dioxide sensors. Data was electronically captured for 15 minutes per sample using Data Studio Software. The amount of oxygen consumed and carbon dioxide released by the roots was expressed as ppm min^{-1} g^{-1} root fresh weight. O_2 consumption rates were compared to the activity of the two glycolysis enzymes, PFK and PFP, as the rate at which glycolysis proceeds is equal to the Krebs cycle rate where oxygen is consumed (Ap Rees, 1980). The CO_2 release rates were compared to the activity of the regulatory enzyme of the OPP-pathway, G-6-PDH.

6.2.2.2 Extraction of enzymes

For extraction of PFK and PFP the method of Botha and Small (1987) was used while the extraction method of Pretorius and Small (1992) was followed for extracting G-6-PDH. Two tap roots per replicate from all treatments of field grown carrots were sampled only at the 30% growth stage, 24 h after spraying with bio-stimulant solutions, and weighed. From each root sample a two g aliquot was collected and aliquots from the two roots pooled. This was done in triplicate. Each two g aliquot was homogenized for 1 minute at full speed (Homogenizer CAT X 620, Germany), and enzymes extracted with 3 ml g^{-1} of extraction buffer. For PFK and PFP the extraction buffer consisted of 100mM Tris HCl (pH 8), 2 mM $MgCl_2$, 1 mM EDTA, 14 mM mercapto-ethanol, 10% glycerol and 2 mM phenylmethylsulfonyl fluoride (PMSF). The extraction buffer used for G-6-PDH consisted of 100 mM Tris HCl (pH 7.5), 2 mM EDTA, 10 mM mercapto-ethanol and 10% glycerol at a pH of 7.5.

The finely homogenized material was centrifuged for 2 minutes at 12 000 rpm using a Selecta Centrolit centrifuge. The supernatant was transferred to clean Eppendorff vials, kept on ice until all samples were extracted and enzyme activity determined.

Estimation of PFK, PFP and G-6-PDH activities in crude carrot tap root extracts

PFK and PFP

The *in vitro* activities of PFK and PFP were determined spectrophotometrically in triplicate at 25°C with a temperature controlled Shimadzu UV-2450 spectrophotometer (Shimadzu Corporation, Japan) by following the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate in the glycolysis direction at 340 nm.

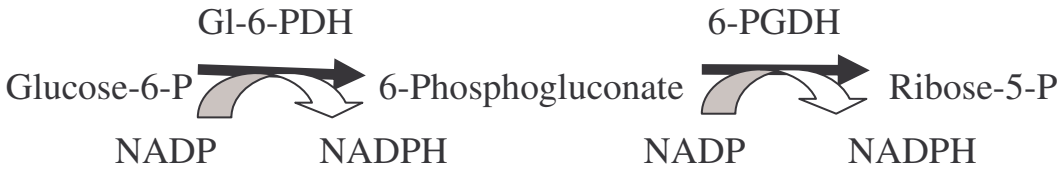
In the case of PFK the assay mixture contained 100 mM HEPES buffer (pH 7.5), 10 mM Fru-6-P, 5 mM $MgCl_2$, 1 mM ATP, 0.1 mM NADH and a coupling

enzyme mixture (1 U Fru-1,6-bisphosphate aldolase, 10 U triosephosphate isomerase and 1 U α-glycerin-3-phosphate dehydrogenase). The A_{340} was determined for ten minutes and the reaction started by addition of the crude enzyme extract.

In the case of PFP the assay mixture contained 100 mM HEPES buffer (pH 8.0), 10 mM Fru-6-P, 1 mM $MgCl_2$, 0.1 mM NADH, 1 mM PPi, 10 µM Fru-2,6-bisphosphate and a coupling enzyme mixture (1 U Fru-1,6-bisphosphate aldolase, 10 U triosephosphate isomerase and 1 U glyceraldehyde-3-phosphate dehydrogenase). The A_{340} was determined for ten minutes and the reaction started by addition of the crude enzyme extract. Enzyme activities were expressed as pmol NADPH min^{-1} g^{-1} fresh weight.

G-6-PDH

The determination of G-6-PDH activity is complicated by the fact that the initial conversion of glucose-6-phosphate to 6-phosphogluconate by the enzyme as well as the reaction that follows, i.e. the conversion of 6-phosphogluconate to Ribose-5-phosphate under the control of the enzyme 6-phosphogluconate dehydrogenase both produce NADPH, which is measured during the assay.



In order to determine the G-6-PDH activity only, the procedure of Gossling and Ross (1979) was adopted. Dehydrogenase activity was determined in the presence of an excess of commercially obtained 6-phosphogluconate dehydrogenase (0.044 U cm^{-3}) reaction mixture which, according to the authors, is five times higher than the highest activity measured in crude extracts. Under these conditions, 50% of the total activity is due to G-6-PDH activity. This is calculated by dividing the final calculated dehydrogenase activity by 2.

Assay conditions were optimized for both enzymes in the crude extracts. The final reaction mixture for G-6-PDH contained 50 mM Tris-HCl buffer (pH 7.5), 0.1 mM $NADP^+$, 10 mM $MgCl_2.6H_2O$, 0.044 U of the coupling enzyme 6-PGDH and 2.5 mM glucose-6-phosphate. The reaction was started by addition of the substrate, glucose-6-phosphate, and determined in triplicate.

6.2.2.3 Statistical analysis

Analysis of variance (ANOVA), using the Number Cruncher Statistical Software, NCSS 2000, (Hintze, 1999) was performed on the data in order to identify differences between the treatment means. Separation of treatment means was performed using the Tukey-Kramer Multiple Comparison Test and expressed as least significant difference (LSD) at the 5% ($P<0.05$) probability level (Steele and Torrie, 1980).

6.3 Results

6.3.1 Respiration rate

Generally, after an initial increase, oxygen consumption by whole carrot tap roots decreased linearly with increasing fertilization at the early stages of development. At 30% development the root respiration rate was significant higher where 25% and 50% of the standard fertilizer were applied, compared to plants that received no fertilizer (Figure 6.1A). Although not significant, the respiration rate of roots cultivated at the standard (100%) fertilizer level was lower than at all three of the other fertilizer-only treatments. Foliar applications of ComCat® and Kelpak® at the 30% development stage, where no fertilizer was additionally applied, increased the respiration rate fourfold (Figure 6.1A). However, where the two bio-stimulants were applied in combination with 25% of the standard fertilizer this tendency was reversed as both bio-stimulants contributed to significantly lower respiration rates than the fertilizer-only control. This trend prevailed where 50% of the standard fertilizer was applied. No significant differences in respiration rate were observed

between treatments where the standard fertilizer was applied on its own or in combination with the bio-stimulants.

At 60% development the respiration rate was the highest, almost threefold, where no fertilizer was applied, but decreased gradually as the fertilizer level was increased in increments (Figure 6.1B). Where both ComCat® and Kelpak® was applied in combination with all four fertilizer levels no significant difference in respiration rate was observed except that it was significantly lower than where either no fertilizer was applied or 25% of the standard. The bio-stimulant combination treatments with the standard and 50% of the standard fertilizer regimes showed no significant difference in terms of the oxygen consumption rate of tap roots. The latter trend prevailed at both the 80% (Figure 6.1C) development stage and at harvest (Figure 6.1D), albeit at a substantially lower rate at harvest.

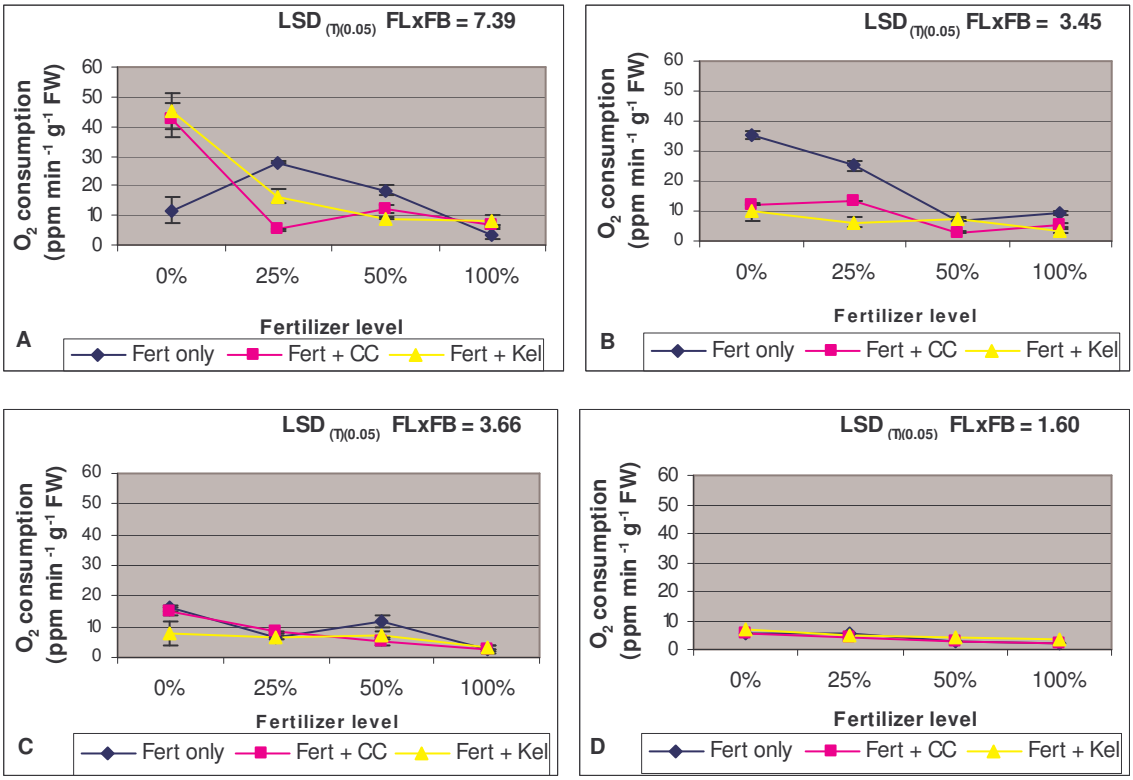


Figure 6.1: Effect of different fertilizer levels, both separately and in combination with commercial bio-stimulants, on the respiration rate of carrot tap roots expressed in terms of oxygen consumption at different growth stages under field conditions in 2007. A = 30% development; B =60% development; C =80% development; D = at harvest

6.3.2 Enzyme activity in carrot tap roots at 30% development, 24 hours after treatment with bio-stimulants

The activities of two regulatory glycolysis enzymes, PFK and PFP, as well as the only regulatory enzyme of the OPP-pathway, G-6-PDH, was measured at 30% root development and after foliar application of the two bio-stimulants, ComCat® and Kelpak®. This growth stage was chosen as it coincided with the second bio-stimulant application as suggested by the manufacturers, and its possible influence on these chosen metabolic enzymes was measured 24 h after foliar application of the bio-stimulants.

6.3.2.1 Phosphofructokinase (PFK) activity

Where no fertilizer was applied the PFK activity was extremely low (Figure 6.2A) in carrot tap roots and this correlated positively with the low respiration rate (compare with Figure 6.2B) measured at this growth stage and under these circumstances. However, PFK activity increased linearly as the fertilizer level was increased in increments (Figure 6.2A). The latter correlated positively with the respiration rate only where 0% and 25% (compare with Figure 6.2B) of the standard fertilizer was applied, after which the relationship between fertilizer level and respiration rate was reversed where the standard and 50% of the standard fertilizer was applied.

Foliar application of ComCat® and Kelpak® on its own, where no fertilizer was applied, contributed to significant increases in both PFK activity (Figure 6.2A) and respiration rate (Figure 6.2B). Although to a lesser extent, this increase in PFK activity (Figure 6.2A) by both bio-stimulants was still significant where the bio-stimulants were applied in combination with 25% of the standard fertilizer. However, the corresponding respiration rate (Figure 6.2B) decreased to a significantly lower level and remained at this level at all fertilizer regimes. In general, Kelpak®-fertilizer combination treatments tended to contribute to higher

PFK activity than the fertilizer-only controls, as well as the ComCat® combination treatments with all fertilizer regimes, except at the standard (100%) fertilizer level.

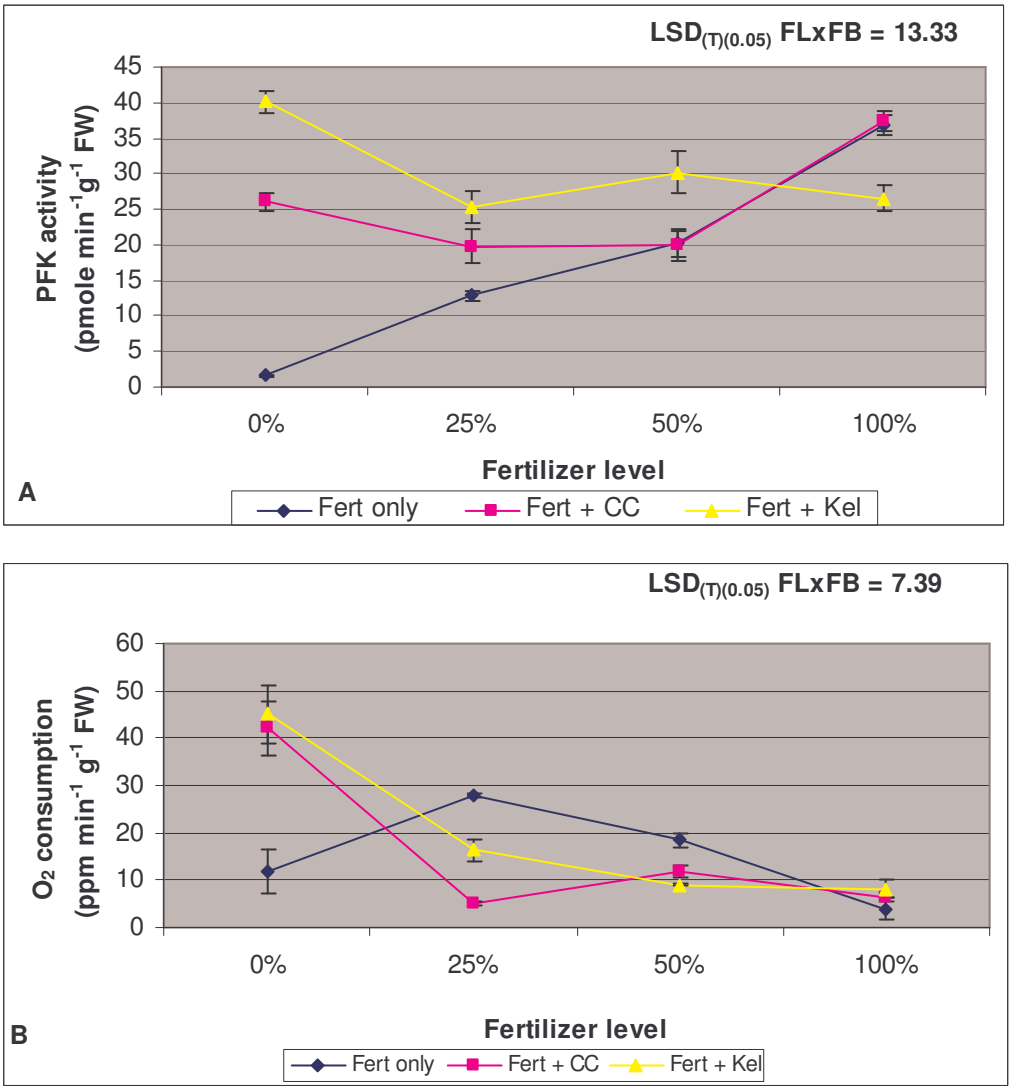


Figure 6.2: Effect of different fertilizer levels, both separately and in combination with commercial bio-stimulants on A) phosphofructokinase (PFK) activity and B) the respiration rate of carrot tap roots, expressed in terms of oxygen consumption, 24 h after spraying plants with bio-stimulants at the 30% growth stage under field conditions in 2007.

6.3.2.2 Fructose 6-phosphate-1-phosphotransferase (PFP) activity

When the fertilizer level was increased to 25% of the standard, PFP activity increased significantly but, interestingly, the opposite prevailed at the two highest fertilizer regimes (Figure 6.3A). Overall, foliar application of ComCat® in combination with fertilizer enhanced PFP activity linearly as the fertilizer level was elevated in increments (Figure 6.3A) but, compared to the fertilizer-only treatments, this differed significantly at all fertilizer regimes. Compared to the ComCat® treatment, PFP activity tended to decrease significantly where Kelpak® was applied either on its own (0% fertilizer) or in combination with standard fertilizer (100%).

Where the fertilizer level was elevated from 0% to 25% of the standard, PFP activity showed a positive relationship with respiration rate as the latter increased following the application of fertilizer to the soil (Figure 6.3B). However, the opposite prevailed where standard fertilizer and 50% of the standard was applied. Interestingly, PFP activity correlated negatively with respiration rate (compare with Figure 6.3B) in all cases where ComCat® was applied in combination with different fertilizer levels. In the latter case the respiration rate decreased as PFP activity increased whether no fertilizer was added to the soil or at the other three fertilizer regimes. Treatment with Kelpak® followed the same pattern except in combination with the standard fertilizer where PFP activity decreased as the respiration rate decreased.

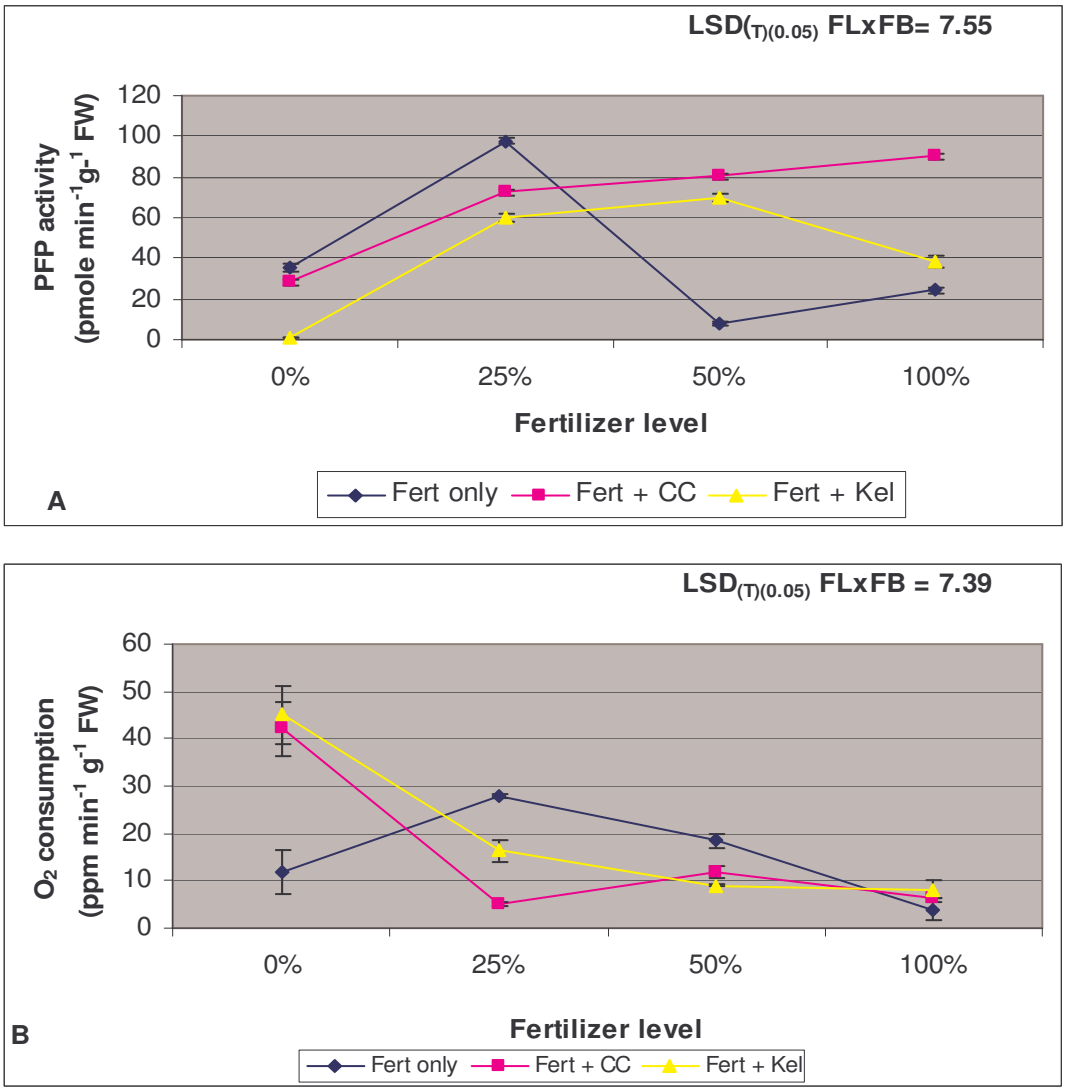


Figure 6.3: Effect of different fertilizer levels, both separately and in combination with commercial bio-stimulants on A) fructose-6-phosphate-1-phosphotransferase (PFP) activity and B) the respiration rate of carrot tap roots, expressed in terms of oxygen consumption, 24 h after spraying plants with bio-stimulants at the 30% growth stage under field conditions in 2007.

6.3.2.3 Comparison of PFK and PFP activities

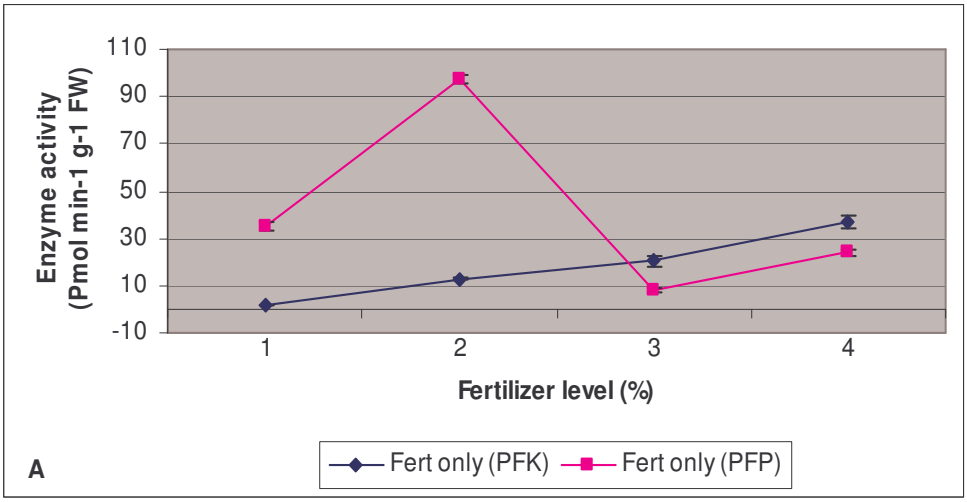
Phosphofructokinase (PFK) converts fructose-6-phosphate to fructose-1,6-bisphosphate only in the glycolysis direction. However, PFP not only catalyzes the same reaction, but also the reverse in the gluconeogenesis direction. For this

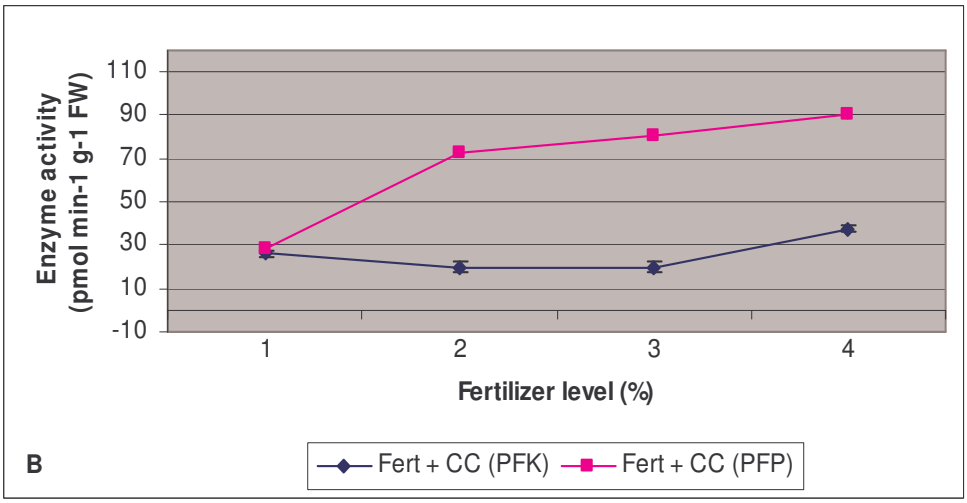
reason it was necessary to compare the *in vitro* activities of these two enzymes (Figure 6.4) in an attempt to evaluate the levels at which the enzymes operated in carrot tap roots 24 h after foliar application of the two bio-stimulants.

In most cases, but not all, PFP activity was much higher than that of PFK (Figure 6.4). Where fertilizer was applied on its own (Figure 6.4A) PFP activity did not follow a fixed pattern. In the absence of fertilizer (0%) the activities of both enzymes were lowest, but PFP activity was fourfold higher than that of PFK. Addition of 25% of the standard fertilizer to the soil at planting increased the activities of both enzymes when measured at the 30% growth stage. However, the increase in PFP activity was sevenfold higher than that of PFK. Interestingly, where the standard and 50% of the standard fertilizer was applied at planting, PFK and PFP activities were much lower and at the same level, compared to the activities measured at the 25% fertilizer regime.

Foliar application of ComCat® (Figure 6.4B) on its own, where no fertilizer was added, contributed to a higher PFK but slightly lower PFP activity compared to the zero-fertilizer treatment (Figure 6.4A). However, where ComCat® was applied in combination with fertilizer, PFP activity increased linearly as the fertilizer level was elevated in increments and this was approximately three-fold higher in all instances compared to PFK activity.

The Kelpak® treatment (Figure 6.4C) had more or less the same effect on PFP activity as did ComCat® when combined with 25% and 50% of the standard fertilizer. But, the opposite prevailed where either no fertilizer or the standard level was applied at planting.





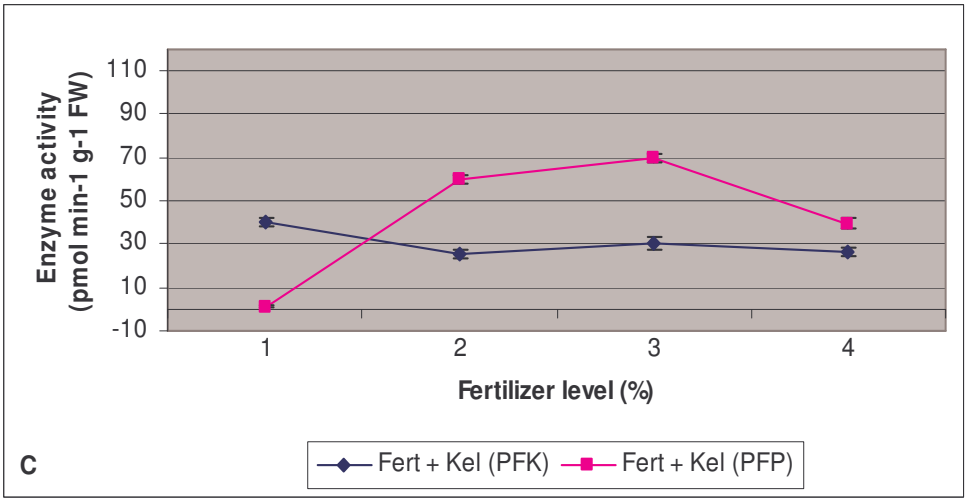


Figure 6.4: Effect of different fertilizer levels A) separately as well as in combination with B) ComCat® and C) Kelpak® on PFK and PFP activities in carrot tap roots at the 30% growth stage and 24 h after foliar treatment with the bio-stimulants.

6.3.2.4 Glucose-6-phosphate dehydrogenase (G-6-PDH) activity

Where no fertilizer was applied G-6-PDH activity was two-fold higher than in the case of both bio-stimulant only treatments that were at the same level (Figure 6.5A). The *in vitro* activity of the enzyme was further and significantly increased when 25% of the standard fertilizer was added to the soil at planting. However, at the 50% and 100% fertilizer levels G-6-PDH activity was markedly lower than where no fertilizer was applied.

Although foliar treatment with ComCat® significantly increased G-6-PDH activity when combined with 25% and 50% of the standard fertilizer, the same increase was not observed in combination with the standard fertilizer application (Figure 6.5A). In terms of G-6-PDH activity the Kelpak®-fertilizer combination treatments followed more or less the same pattern as in the case of ComCat® when combined with 25% and 50% of the standard fertilizer, but at a significantly lower activity level. Further, in combination with the standard fertilizer, Kelpak® contributed to slightly higher G-6-PDH activity compared to the fertilizer-only and ComCat® combination treatments. The differences at this fertilizer application were, however, non-significant.

The carbon dioxide release rate by carrot tap roots (Figure 6.5B) was measured at the same growth stage where G-6-PDH activity (Figure 6.5A) was determined. This was done in order to compare the activity of the only regulatory enzyme of the OPP-pathway with respiration rate, expressed in terms of CO_2-release and not O_2-consumption, as CO_2 is released via both the Krebs cycle and the OPP-pathway. More or less positive correlations existed between G-6-PDH activity and the CO_2-release rate where no fertilizer was applied as well as where the two bio-stimulants were applied on their own (compare Figures 6.5A and B at the 0% fertilizer level). However, at the 25% fertilizer level the pattern was reversed as G-6-PDH activity increased while the CO_2-release rate decreased whether fertilizer was applied on its own or in combination with the two bio-stimulants. At the higher fertilizer regimes (50% and 100%) no clear correlation between G-6-PDH activity and the CO_2-release rate existed.

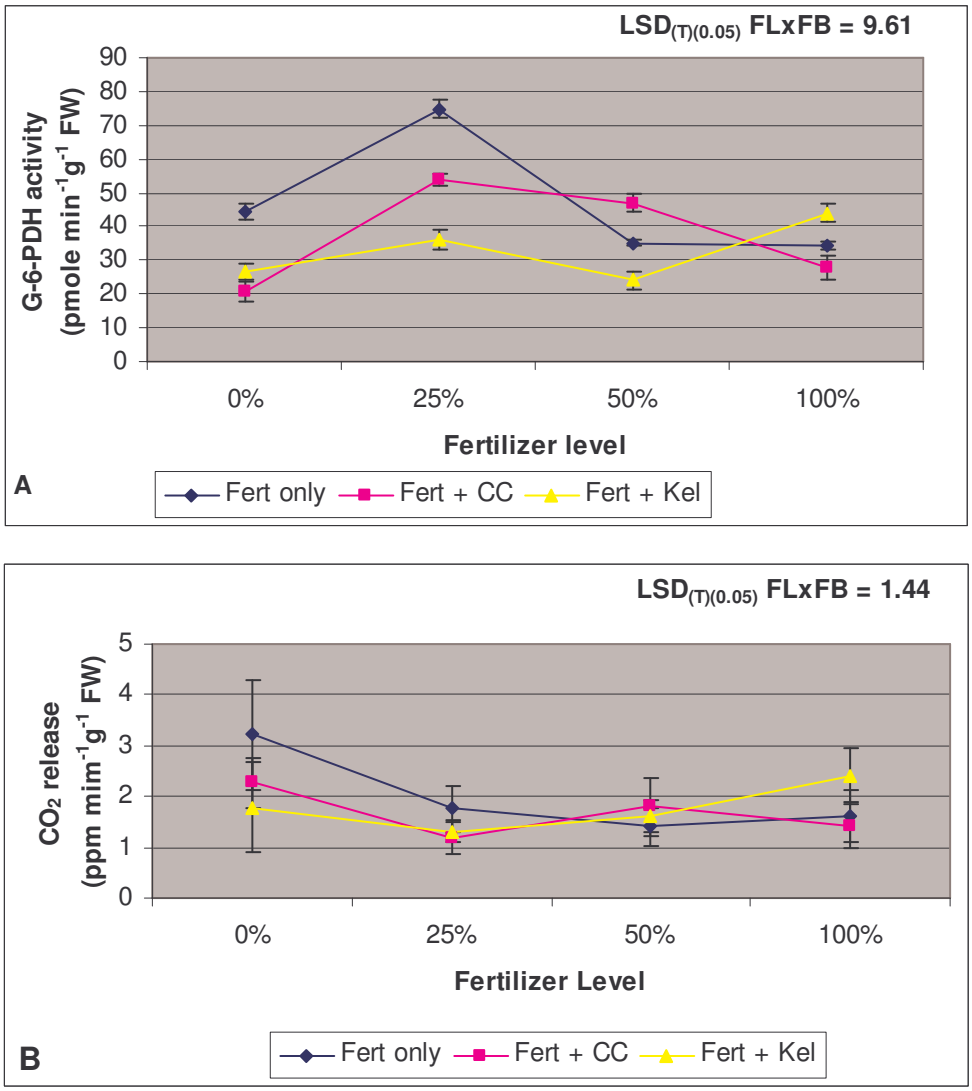


Figure 6.5: Effect of different fertilizer levels, both separately and in combination with commercial bio-stimulants on A) glucose-6-phosphate dehydrogenase (G-6-PDH) activity and B) the respiration rate of carrot tap roots, expressed in terms of carbon dioxide release, 24 h after spraying plants with bio-stimulants at the 30% growth stage under field conditions in 2007.

6.4 Discussion

Glycolysis represents a set of reactions that convert glucose and other monosaccharides to generate ATP and to provide intermediates that are precursors for other biosynthetic pathways (Hames and Hooper, 2005). The process of glycolysis is highly flexible and has various entry and exit points of metabolites. Two glycolytic pathways have been identified in plants namely in the plastids and the cytosol. The sequence of glycolytic enzymes in the plastids is nearly complete and is spatially different and distinct from those in the cytosol (Givan, 1999). The enzymes in the plastid seem to be more tightly controlled than those in the cytosol. However, there is interchange of intermediates from one compartment to the other (Flugge, 1999). Thus, it seems that glycolysis can occur in the cytosol or plastids dependent on the type of glycolytic intermediates necessary and metabolic demand (Tobin and Bowsher, 2005).

Glycolysis is, inter alia, regulated by the irreversible reaction catalyzed by phosphofructokinase (PFK) and, according to Atkinson (1968; as cited by Salisbury and Ross, 1992) this control is dependant on the energy status of cells or ATP availability. Under ATP abundance phosphofructokinase activity is inhibited and the activity is promoted when ATP is limited. Phosphofructokinase is also inhibited by citrate, a Krebs cycle intermediate. Thus, when the levels of citrate are high during an active Krebs cycle, the breakdown of glucose through glycolysis is inhibited via allosteric feedback inhibition and under high fructose-6-phosphate levels PFK activity, and hence glycolysis, is enhanced (Hames and Hooper, 2005). Under ATP limitation pyrophosphate fructose 6-phosphate 1-phosphotransferase (PFP) can substitute PFK by utilizing pyrophosphate (PPi) as a phosphoryl donor in order to maintain glycolytic flux (Hames and Hooper, 2005).

In plants fructose-2,6-bisphosphate (Fru-2,6-P_2) is a signal molecule that contributes to the coordination of reactions in sucrose synthesis and partitioning of photosynthate between sucrose and starch (Nielsen *et al*. 2004). A major effect of Fru-2,6-P_2 is in the modulation of the glycolytic pathway and respiratory process via PFP activity. A near equilibrium state of PFP activity is maintained under normal physiological conditions. Under sub optimal conditions Fru-2,6-P_2 provides

adaptive capability to plants (Nielsen *et al*. 2004). The ability of Fru-2,6-P_2 in adjusting to salt, drought, cold and osmotic stress is mainly due to its ability to regulate starch turnover on a diurnal basis (Reddy, 2000; Banzai *et al*., 2003). Surjadinata and Cisneros-Zevallos (2003) reported that environmental stress and wounding of plant tissue increased the respiration rate and attributed this to increased synthesis of respiratory pathway enzymes such as PFK.

In this study, incremental increase in the application of fertilizer led to a linear decrease in the respiration rate of carrot tap roots, in terms of the O_2-consumption rate. The highest oxygen consumption rate was generally associated with zero fertilization, especially during early growth stages and where no bio-stimulants were applied. Foliar treatment with both bio-stimulants on its own, in the absence of additional fertilizer increased the respiration rate significantly compared to the fertilizer only control, but only at the 30% growth stage. As the growth of carrots progressed through the season this enhancing effect on the respiration rate by bio-stimulants was not observed. In fact, bio-stimulant application tended to reduce the oxygen consumption rate compared to the fertilizer-only controls at similar fertilizer levels. A similar reduction in oxygen consumption in plants supplied with adequate nutrition was reported by Lambers (2005). The authors showed that root respiration of herbs cultivated in adequate nitrate was low compared to slower growing herbs cultivated in sub-optimal fertilizer levels. They concluded that the higher rate of carbon utilization via respiration in slow growing herbs was due to high respiratory costs which were associated with nitrate uptake (Lambers, 2005).

In this study the significant burst in oxygen consumption observed during early growth stages and especially where bio-stimulants were applied in combination with the low fertilizer regimes, can possibly be associated with the ability of the products in aiding the carrot plants to acquire nutrients during this early development stage, as claimed by the manufacturers. However, as the season progressed, especially during the latter stages, as well as at the higher fertilizer regimes, this pattern was not repeated. A possibility is that the more mature roots obtained the ability to acquire sufficient nutrients and that the

respiration rate declined as a result of a diminished demand for nutrients closer to harvest, as proposed by Lambers (2005).

On the other hand, the rate of oxygen consumption has also been associated with the level of stress experienced by plants. Under conditions of stress, including abiotic, wounding, salt and mechanical stress, oxygen consumption increased considerably from the normal steady state levels as reported by a number of authors (Seljasen *et al*., 2001; Surjadinata and Cisneros-Zevallos, 2003; Suzuki *et al*., 2005). In almost all of these studies a typical additional response to stress, namely an increase in the *in vitro* activities of respiratory enzymes such as PFK, was reported. In the present study carrot plants placed under nutrient deficiency stress reacted in a similar fashion by increasing their respiration rates in terms of oxygen consumption. Higher fertilization levels, on the other hand, possibly reduced or eliminated nutrient acquisition stress to the level that carrot respiration, as reflected by the oxygen consumption rate, was reduced.

In order to get a clearer picture of the carbohydrate utilization in carrot physiology the rate of oxygen consumption was compared with the activities of the two glycolysis enzymes, PFK and PFP. In contrast with the findings of Suzuki *et al*. (2005), PFK activity in carrot tap roots increased linearly as the fertilizer level was elevated. However, although PFP activity did not follow the same pattern, its *in vitro* activity was significantly higher than that of PFK especially in roots cultivated in the absence of fertilizer or at the low (25%) level. In this regard it seems that the nutrient stressed carrot roots reacted to the condition by increasing the PFP activity rather than the PFK activity. Interestingly, O_2 consumption by the root tissue was also higher under these circumstances possibly indicating that PFP was more active in the glycolysis direction.

Assuming that nutrient stressed carrots experienced an energy shortage, it seems that the latter is in agreement with the findings of Hames and Hooper (2005) as well as Graham *et al*. (2007). In essence it indicates that the PPi-dependant PFP was more active in these nutrient stressed plants due to the fact that PFP operating in the glycolysis direction is an energy saving step compared to

PFK that utilizes an ATP. Especially due to the fact that PFP activity was almost threefold higher under nutrient stress conditions than under normal fertilizer conditions, there is a strong indication that PFP provided adaptive capability to the nutrient stressed carrot plants as suggested by Nielsen *et al*. (2004). The fact that foliar application of ComCat® maintained energy saving PFP activity in the glycolysis direction even in combination with the higher (standard and half of the standard) fertilizer levels, indicates that the product can induce a crop's natural defence mechanisms against stress conditions as claimed by the manufacturers.

Somewhat contradictory, Kovacs *et al*. (2006) reported enhanced PFP activity as well as 3-phosphoglycerate and hexose phosphate levels, due to high levels of Fru-2,6-P_2 in non-stressed carrot plants. However, the authors also showed that PFP was active in the glycolytic direction and its activity did not decrease in carrot roots exposed to cold and drought stress conditions. This is a possible indication that glycolytic flux is maintained in carrots through the substitution of ATP dependant PFK by PPi-dependant PFP when the plant is in a low energy status due to stress. Although PFP has been studied widely and its regulation by Fru-2,6-P_2 well established, its *in vivo* involvement in stress related adaptation in plants is still not clearly understood (Theodorou and Kruger, 2001; Nadas *et al*., 2008).

The oxidative pentose phosphate (OPP) pathway is widely accepted as an alternative respiratory route. An active OPP-pathway in plant tissue is also associated with the supply of reductive power in the form of NADPH (Neuhaus and Emes, 2000; Kruger and von Schaewen, 2003) as well as carbon skeletons necessary for nucleotide, aromatic amino acid and phenylpropanoid synthesis (Herrmann and Weaver, 1999). In this study the measured G-6-PDH activity at least confirmed an operative OPP-pathway. The G-6-PDH activity increased significantly when the fertilizer level was increased from zero to 25% of the standard. However, at the higher fertilizer levels (standard and 50% of the standard) the activity of the enzyme was two-fold lower and remained at the same level in both cases. Although combination treatments of fertilizer with bio-stimulants had an increasing effect on G-6-PDH activity, the level of activity

remained below that observed in the fertilizer only treatments. The question to be answered is whether this can, in any way, be related to a survival mechanism in carrot roots under nutrient stress.

In this regard, increased levels of NADPH in cold-stressed plants have been associated with increased activity of the OPP-pathway (Maciejewska and Bogatek, 2002) and increased resistance towards cold stress. According to the authors, G-6-PDH activity was more pronounced compared to that of glycolytic enzymes in cold stressed plants leading to higher OPP-pathway than glycolysis activity. In the present study the level of G-6-PDH activity was similar (between 20 and 80 pmol min^{-1} g^{-1} fresh weight) to the levels of glycolytic enzymes, PFK and PFP. This might indicate that the nutrient stress treatments did not necessarily lead to a situation where up scaling of the OPP-pathway was required in order to address stress conditions.

The OPP-pathway is operative in both the cytosol and plastids (Krook *et al*., 1998) of cells and removes the C-1 carbon from hexose phosphates and releases it as CO_2 (Wagner *et al*., 1985). In this study the CO_2 release rate from carrot roots was only positively correlated where plants were cultivated under severe nutrient stress (zero fertilizer) conditions. This corresponded with a high O_2 utilization rate possibly indicating that both aerobic respiration and the OPP-pathway were highly active under these circumstances if kept in mind that CO_2 is also released during aerobic respiration. In roots from plants cultivated at the standard as well as 25% and 50% of the standard fertilizer level both the O_2 utilization and CO_2 release rates were much lower and not out of the ordinary. The latter confirms an inadequate rationale for excluding either normal respiratory metabolism or the OPP-pathway as the principle activity operative in carrots under nutrient stress. However, what was interesting from this study is that foliar application of both bio-stimulants, but especially ComCat®, either induced or maintained PFP and G-6-PDH activity where fertilizer was applied at three different levels and this correlated positively with marked yield increases at both the standard and 50% of the standard fertilizer level (Chapter 4; 4.3.6).

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The efficacy of inorganic fertilizer application in increasing crop growth and yield has long been acknowledged. Generally, optimal amounts as well as time of application of macro- and micro-elements have been determined and published for different crops. Despite the availability of this data, many subsistence farmers in Lesotho and other African countries simply cannot afford inorganic fertilizer and have to rely on either the soil itself or additional organic fertilization, e.g. cattle and/or chicken manure. On the other hand increasing levels of fertilization and irrigation under field conditions have raised concerns on considerable environmental pollution from agricultural effluent (Zhu *et al*. 2005; Thompson *et al*. 2007).

Crop producers find themselves in the middle of these arguments and have to reconcile agronomic, economic, product quality and environmental constraints during production. The aims of this study, therefore, were to evaluate the morphological, physiological and yield responses of carrot (*Daucus carota* L. cv. Karina), to varying levels of NPK fertilizer, especially levels lower than the standard recommended rate, both separately and in combination with two commercial bio-stimulants, ComCat® and Kelpak® under greenhouse and field conditions. The four fertilizer levels included in this study enabled the monitoring of carrot growth under a range of fertilizer levels, including sub-optimal levels representing trends in many areas of Lesotho. These included the standard (100%) as well as 50%, 25% and 0% of the standard.

Both Kelpak® and ComCat® are natural products. The former is an extract from sea weed while the latter is an extract from, among others, *Lychnis viscaria* seed. Kelpak® is reputed to contain cytokinins, auxins, gibberellins and vitamins that are produced in plants via the secondary metabolic pathways, namely the mevalonic acid and shikimic acid pathways, as well as amino acids and micro-nutrients that collectively enhance crop growth and production (Arthur *et al*., 2004).

As far as could be ascertained, the use of this product on carrots has not been published previously. Similarly, ComCat® contains natural compounds such as brassinosteroids, a new generation of plant hormones, flavonoids and other naturally occurring plant hormones (Agraforum, 2002). The manufacturers of ComCat® claim that it enhances seed germination, promotes seedling growth and induces resistance towards abiotic and biotic stress conditions as well as flower bud formation that can collectively lead to increased crop yields. Likewise, no publications on the response of carrot to ComCat® could be found in the literature.

The use of naturally occurring plant secondary metabolites in agricultural crop production has gained popularity over the past 50 years as they are involved in complex interactions between plants, plants and micro-organisms as well as plants and animals (Seigler, 1998). Evidence has accumulated indicating that secondary metabolites have a primary ecological role and that they are necessary in plant interactions with competitors, pathogens and herbivores while protecting plants from abiotic stresses, such as drought, cold and mineral deficiency, as well as biotic stressors, such as bacteria and fungi (Bourgaud *et al*., 2001). The latter characteristics make plant extracts contenders for potential natural products to be used in agriculture. Kelpak® and ComCat®, both plant extracts with naturally occurring secondary metabolites as active compounds, have been included in this study as representatives of natural products currently in use for manipulating crops exogenously.

On the other hand, the use of inorganic fertilizers has been practiced in agriculture for many decades and its efficacy proven by numerous studies (Cooke, 1982; Isman, 1999). Despite its efficacy, the abundant use of inorganic fertilizers has come under criticism from an ecological perspective. Crop production management, therefore, has to include strategies that minimize nutrient loss to the environment as well as utilization of cultivars with high nutrient use efficiency (Loneragan, 1997). In general and in light of the above, studies dealing with efforts to reduce fertilizer application in agriculture are welcomed (Narwal, 1999; Pilgeram and Sands, 1999); an aspect that was addressed in this study in terms of

carrot cultivation. Greenhouse and field trials were conducted simultaneously during 2006 and 2007.

Under semi-controlled greenhouse conditions, and during both growing seasons, the incremental elevation of NPK fertilizer levels consistently contributed to improved vegetative growth of carrot in terms of all growth parameters measured. Although not at all times statistically significant, this trend applied for all growth stages. This was in concert with a report by Rubatzky *et al*., (1999) for carrots and conformed to expected higher uptake of nutrients by plant roots under these conditions (Krishna and Rosen, 2002). At harvest root fresh mass, representing the only yield parameter under greenhouse conditions, followed the same trend as above soil part growth with elevated fertilizer levels contributing to increased root fresh mass. The results conformed to a recent report of Hailu *et al*. (2008) that indicated increased yield of carrots under a combination treatment of organic fertilizer and urea. However, in terms of the need to decrease fertilizer application with subsistence farmers in mind, yield results indicated that a rate of 50% of the recommended standard was sufficient to satisfy the growth requirements of carrots up to maturity under greenhouse conditions. The latter was supported by the fact that growth and yield of carrot at the standard and half-standard fertilizer level were not significantly different; an aspect that was verified under field conditions during both growth seasons.

Although application of the two bio-stimulants in combination with different fertilizer levels under glasshouse conditions resulted in a rather erratic growth response from carrots, as measured at different growth stages, both contributed to significant root fresh mass increases at harvest albeit only when applied together with the standard fertilizer rate. Under field conditions during the 2007 growing season, although not statistically significant, foliar application of ComCat® contributed to a 7 ton ha^{-1} yield increase in combination with standard fertilizer and both Kelpak® and ComCat® led to a 4 ton ha^{-1} yield increase in combination with 50% of the standard fertilizer. Although neither of the bio-stimulants contributed to a statistically significant yield increase, probably due to high standard deviation

between replicas, the average increases were substantial from an economic perspective

The vegetative growth response of carrot to different fertilizer levels both separately and in combination with bio-stimulants under field conditions was comprehensively addressed in separate chapters. As yield is the final outcome of a crop progressing through its vegetative growth phases, only yield will be used as parameter in this final discussion with the objective to integrate morphological and physiological data in terms of the response of carrot to the different treatments investigated in this study. Emphasis will also be placed on the 2007 field data.

Judged on the yield data obtained during 2007, foliar treatment with ComCat® seemed to have a consistent enhancing effect across different fertilizer levels compared to the Kelpak® treatment. Brassinosteroids, (Br's) , the key active compounds of ComCat®, have a high growth (Howell *et al*., 2007) and yield promoting ability (Sasse, 2003), occur widely in plants and induce resistance in plants towards environmental stress, herbicidal injury and salinity (Bajguz and Tretynb, 2003). Substantial international research on Br's has been conducted over the past twenty years in terms of signal transduction and mode of action (Steber and McCourt, 2001; Stundl and Schneider, 2001; Hayat *et al*., 2007) and, as a result, a lot is expected from Br's in terms of application in the agricultural industry. ComCat® has been commercialized worldwide in 2006 (Hüster, personal communication, Agraforum AG., 2007) and probably offers one of the first opportunities to test products of this kind under agricultural conditions. Kelpak®, on the other hand, has been well established as natural product over the past decade.

Confidence in these two commercial bio-stimulants by consumers will probably only be achieved via proof of seasonal consistency. Based on yield results obtained in this study, consistency in the response of carrots after treatment with the two products was not achieved under field conditions. This can be explained by the exceptional high precipitation during 2006 that most probably placed plants under water logging stress while also allowing for lateral movement of fertilizer in the soil that led to contamination of plots. As a result, the 2007 yield

results obtained under rain fed field conditions were regarded more trustworthy due to normal precipitation experienced in this season.

In order to better understand the growth and yield response of carrot to fertilizer and bio-stimulant application under field conditions in 2007, an investigation of selected physiological activities in the tap root followed. As production of sugars during photosynthesis, its accumulation and transport as well as its utilization via respiration collectively form the basis of a crop's productivity (Gibson, 2005), these aspects were investigated. In the following discussion only yield data obtained in 2007 under field conditions will be integrated with physiological data obtained during this season.

The sucrose content in tap roots tended to increase linearly with increased fertilization and also increased as growth proceeded to reach the highest level at later stages of development while the monosaccharide sugar levels showed the opposite trend. The latter corresponded with increased sucrose accumulation during early growth and again at harvest, especially where ComCat® was applied, as well as a significant increase in the translocation of radio-active labelled sucrose from the leaves to the roots at the higher fertilizer levels. This was also in concert with a marked increase in yield as well as a decline in the respiration rate of tap roots at harvest indicating that sucrose accumulated towards the end of the season, probably as a result of less being hydrolyzed to its monosaccharide forms (Nilsson, 1987).

Of special interest was the positive relationship between sucrose content, translocation of radio-activity from the leaves to the roots and final yield where ComCat® was applied in combination with the standard and 50% of the standard fertilizer. Enhanced radio-activity partitioning in carrot plants treated with ComCat® in combination with fertilizer suggests that the bio-stimulant had a strong enhancing effect on sucrose translocation. Although not well documented, the membrane energizing effect of ComCat®, in terms of its characteristic to improve sucrose translocation over membranes, was claimed by the manufacturers (Hüster, personal communication, Agraforum AG., 2007). To a lesser extent Kelpak® showed the same tendency to accelerate radio-active partitioning to the

roots at the higher fertilizer regimes, compared to ComCat® and the fertilizer only treatments, but no previous claim in this regard was found in literature. Nevertheless, treatment with ComCat® in combination with the standard fertilizer, and treatment with both ComCat® and Kelpak® in combination with 50% of the standard fertilizer level, contributed to a marked increase in yield concomitant with a sharp increase in sucrose content at harvest.

All three sugars, sucrose, glucose and fructose, were present throughout carrot development and the levels varied dependent on fertilization and time progression over the growing season. This was understandable based on the continuous conversion of glucose and fructose to sucrose or the hydrolysis of sucrose to the monosaccharide forms dependent on the energy requirements at a specific stage and this is finely regulated in plants (Krook *et al*., 2000). The observed variation in the total sugar content and composition in this study is similar to carrot fertilization reports in literature where sugar accumulation differed dependent on soil factors and fertilizer management practices (Hochmuth *et al*., 2006; Hailu *et al*., 2008).

Apart from sugar being a storage compound its accumulation in the carrot root is associated with taste and quality as are volatile compounds such as terpenes (Rosenfeld *et al*., 2004). The β-carotene (a terpene) content was not at all influenced by the incremental increase of fertilizer. An increase in β-carotene content was only observed where ComCat® was applied in combination with 50% of the standard fertilizer. Although not tested by a tasting panel, it is predicted that this slight increase will not have a significant influence on taste of the tap root.

Hence, in an attempt to shed light on the observed growth and yield responses of carrot to fertilizer and bio-stimulant application, selected physiological activities were used as parameters. These included the root respiration rate (in terms of both O_2 utilization and CO_2 release) as well as *in vitro* activities of regulatory enzymes of both the glycolytic (PFK and PFP) and oxidative pentose phosphate (OPP) pathway (G-6-PDH). At this point it must be kept in mind that these physiological responses were only measured 24 h after the second application of bio-stimulants at the 30% plant development stage. The

reason for this was to measure the response of carrots as close as possible to the bio-stimulant application time as the prolonged effect of neither was known. At most, this information can only be interpreted as representative of the inherent potential that fertilizer and the two bio-stimulants, either on its own or in combination, possess to manipulate physiological processes in carrots.

Where only fertilizer was applied, PFK activity increased linearly as the levels were incrementally elevated. This was, however, concomitant with an increase in both PFP activity and the respiration rate only where no fertilizer or 25% of the standard fertilizer was applied. Where the standard and 50% of the standard fertilizer level was applied on its own, the respiration rate as well as PFP activity linearly decreased as PFK activity increased linearly. Only PFP activity corresponded with the respiration rate indicating that PFP was active in the glycolysis direction. Interestingly, PFP activity was almost ten-fold higher than that of PFK where either no fertilizer or 25% of the standard was applied indicating that respiration rate in the glycolysis direction was rather regulated via PFP than PFK activity.

Twenty four hours after foliar application of ComCat®, PFK activity remained at a steady state at all fertilizer levels while PFP activity increased linearly as the fertilizer level was steadily elevated. Kelpak® had more or less the same effect in terms of PFK activity but, at the standard fertilizer level, it did not have an increasing effect on PFP activity as did ComCat®. At least this confirmed the claims made by manufacturers that both bio-stimulants used in this study increase the respiration rate in plants following foliar application. Interestingly, although difficult to interpret as authentic or incidental, ComCat® contributed to a marked yield increase in combination with both the standard and 50% of the standard fertilizer level while PFP activity remained high in both instances at the early growth stage where it was measured. Kelpak®, on the other hand, contributed to a yield increase in combination with 50% of the standard only, where PFP activity was high, while it had no effect in combination with the standard fertilizer where PFP activity dropped to the same low level as that of the fertilizer only control during early plant development. Whether the observed

relationship between PFP activity and yield was sustained at the time of harvest is arguable and needs to be verified. What complicates this matter further is the fact that PFP activity remained high under the influence of bio-stimulants at fertilizer levels where the respiration rate decreased.

Respiration rate increase, concomitant with an increase in the *in vitro* activities of respiratory enzymes, has been associated with abiotic stress in the past (Nielsen *et al*., 2004; Suzuki *et al*., 2005). In the present study carrot plants placed under nutrient deficiency stress reacted in a similar fashion by increasing the respiration rate. Higher fertilization levels, on the other hand, possibly reduced or eliminated nutrient acquisition stress to the level that carrot respiration, as reflected by the oxygen consumption rate, was reduced.

An active oxidative pentose phosphate (OPP) pathway, widely accepted as an alternative respiratory route, has also been associated with resistance towards cold stress in the past (Maciejewska and Bogatek, 2002). Glucose-6-phosphate dehydrogenase (G-6-PDH) is the first and only regulatory enzyme of the pathway. In this study, measured G-6-PDH activity confirmed an operative OPP-pathway at least during early plant development. As was the case with the two regulatory glycolytic enzymes, G-6-PDH activity increased significantly when the fertilizer level was increased from zero to 25% of the standard but decreased to a two-fold lower level at the standard and 50% of the standard level. Although combination treatments of fertilizer with bio-stimulants had an increasing effect on G-6-PDH activity, the level of activity remained below that observed in the fertilizer only treatments. This might indicate that the nutrient stress treatments did not necessarily lead to a situation where up scaling of the OPP-pathway was required in order to address stress conditions. However, what was interesting from this study is that foliar application of both bio-stimulants, but especially ComCat®, either induced or maintained PFP and G-6-PDH activity where fertilizer was applied at three different levels and this correlated positively with marked yield increases at both the standard and 50% of the standard fertilizer level.

In summary, during pilot trials under greenhouse conditions a general trend of vegetative growth enhancement in carrot, as fertilizer levels were incrementally

elevated, was observed. However, of special interest was that 50% of the standard fertilizer seemed to be sufficient to satisfy the growth requirements of carrots up to maturity and that additional application of bio-stimulants had the potential to contribute to increased tap root fresh mass at harvest under greenhouse conditions. These observations were confirmed under field conditions although only in 2007 where a positive relationship existed between yield, sugar translocation, sugar content and dry mass of tap roots at harvest, especially where ComCat® was applied in combination with the standard fertilizer level. Although the two bio-stimulants increased the respiration rate and the activities of regulatory glycolytic and OPP-pathway enzymes during early development, especially where zero or 25% of the standard fertilizer were applied, the respiration rate remained at a steady state at harvest for all fertilizer levels. The latter might indicate that respiratory metabolism, including OPP-pathway activity, is only enhanced under nutrient stress conditions during early root development but, that a simultaneous down scaling of nutrient and energy requirement occurs as roots mature.

In conclusion, the collective interpretation of growth and physiological data acquired during this study at different fertilizer levels, especially when considered in relationship with final yield data at harvest, does not supply a sufficient rationale to recommend either the use of sub-optimal levels on their own or in combination with the two bio-stimulants included in this study for the cultivation of carrots. However, it is recommended that this study be repeated under irrigation conditions but with plots far enough apart to prevent sideways movement of fertilizer and possible contamination of plots

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SUMMARY

Concerns regarding environmental pollution, emanating from agricultural effluent due to abusive use, have led to a paradigm shift in production technology. On the other hand, subsistence farmers in developing countries cannot afford fertilizer at the current prizes. Farmers, therefore, have to reconcile agronomic, economic, agricultural product quality and environmental aspects of crop. In view of these concerns this study evaluated the response of carrot (*Daucus carota* L. cv. Karina) to varying fertilizer levels, applied singly and in combination with two commercial bio-stimulants, ComCat® and Kelpak®. Both bio-stimulants are plant extracts, containing natural active compounds, and are applied exogenously to manipulate crop growth and yield. No reports on the use of either of the two products on carrot production could be found in literature. During both seasons of greenhouse studies, the incremental increase of NPK fertilizer contributed to increased vegetative growth and root fresh mass, though not significantly at all times. The application of bio-stimulants had an erratic effect on carrot growth under greenhouse conditions. However, both bio-stimulants in combination with the standard fertilizer level enhanced root fresh mass at harvest (Chapter 3).

Under field conditions, especially during the 2007 growing season, foliar application of both bio-stimulants enhanced yield. Although the ensuing increases were not significant, a higher increase of 7 ton ha^{-1} was attained due to application of a combination of ComCat® with the standard fertilizer. A lower increase of 4 ton ha^{-1} was achieved with combinations of ComCat® and Kelpak® with the half-standard fertilizer level (Chapter 4).

Growth and yield response of tap roots due to application of different fertilizer levels separately and in combination with bio-stimulants was verified through determination of selected physiological activities (Chapter 5). Sucrose content tended to increase, in concert with yield, as the fertilizer levels were incrementally elevated. Conversely, glucose and fructose content decreased in tap roots as maturity was attained, probably due to less sucrose being hydrolyzed at this development stage. ComCat® had a strong enhancing effect on sucrose translocation as evidenced by the positive relationship between sucrose content, radio-active translocation to the roots and final carrot root yield. The latter applied in both instances where the standard and half the

standard fertilizer levels were applied in combination with ComCat®. This effect could be related to ComCat® enabling improved sucrose transport across membranes. The effect of Kelpak® on sucrose accumulation and translocation was less evident but, in combination with half of the standard fertilizer, its application led to a slight increase in yield. The accumulation of β-carotene, a terpene associated with taste and quality of carrots, was not influenced by fertilizer application. The level, however, increased where ComCat® was combined with the half-standard fertilizer level.

To further comprehend the manipulative effects of fertilizer and bio-stimulants on physiological processes influencing growth and yield of carrots, root respiration as well as activities of glycolytic and oxidative pentose phosphate pathway regulatory enzymes was determined. In all cases the activities were only measured at 30% plant development and 24 hours after second bio-stimulant application. Both ComCat® and Kelpak® increased the respiration rate as well as the activity of glycolytic and oxidative pentose phosphate pathway key enzymes during early carrot development under 25% and zero fertilization. The latter was probably due to nutrient stress during early development. However, as carrots matured nutrient acquisition and energy needs were probably reduced as indicated by the respiration rate remaining at a steady state during later development stages and at harvest across all fertilizer levels. This correlated positively with increased sugar levels at maturity and the final yield.

In conclusion, the collective interpretation of growth and physiological data acquired during this study at different fertilizer levels, especially when considered in relationship with final yield data at harvest, does not supply a sufficient rationale to recommend either the use of sub-optimal levels on their own or in combination with the two bio-stimulants included in this study for the cultivation of carrots. However, it is recommended that this study be repeated under irrigation conditions but with plots far enough apart to prevent sideways movement of fertilizer and possible contamination of plots

OPSOMMING

Besorgdheid met betrekking tot omgewingsbesoedeling deur landbou afval as gevolg van oormatige gebruik het aanleiding gegee tot 'n paradigmaskuif in produksietegnologie. Aan die anderkant kan bestaansboere in ontwikkellende lande eenvoudig nie bemesting teen die huidige pryse bekostig nie. Boere is dus verplig om agronomiese-, ekonomiese-, produkkwaliteit- en omgewingsaspekte te versoen in hulle produksiestelsel keuse. Hierdie studie is in die lig van hierdie besorgdheid uitgevoer ten einde die respons van geelwortels (*Daucus carota* L. cv. Karina) op verskillende bemestingspeile, afsonderlik en in kombinasie met twee kommersiële bio-stimulante ComCat® and Kelpak®, te evalueer. Beide bio-stimulante is plantekstrakte met natuurlike aktiewe komponente wat as blaarbespuitings toegedien word om gewasgroei en opbrengs te manipuleer. Geen gepubliseerde verslae oor die gebruik van enige van die twee produkte op geelwortels kon in die literatuur opgespoor word nie. Gedurende beide seisoene van glashuisstudies, het die inkrementele verhoging van NPK bemesting aanleiding gegee tot 'n verhoging in groeitempo en wortelvarsmassa, alhoewel nie in alle gevalle statisties betekenisvol nie. Alhoewel blaarbespuitings met die bio-stimulante 'n wisselvallige invloed op plantgroei in die glashuis gehad het, het dit in kombinasie met die aanbevole standaard bemesting tot 'n verhoging in oesopbrengs onder glshuistoestande aanleiding gegee (Hoofstuk 3).

Onder veldtoestande, veral gedurende die 2007 groeiseisoen, het blaarbespuitings met beide bio-stimulante oesopbrengs verhoog. Alhoewel hierdie verbetering nie statisties betekenisvol was nie, is 'n oesopbrengsverhoging van 7 ton ha^{-1} gemeet waar ComCat® in kombinasie met die aanbevole standaard bemesting aangewend is. 'n Laer opbrengsverhoging van 4 ton ha^{-1} is gemeet waar beide ComCat® en Kelpak® in kombinasie met die helfte van die aanbevole bemestingstandaard toegedien is (Hoofstuk 4).

Die groei- en oesopbrengsrespons van wortels op verskillende bemestingspeile, afsonderlik en in kombinasie met bio-stimulante, is geverifieer by wyse van geselekteerde fisiologiese aktiwiteite (Hoofstuk 5). Sukrose-inhoud het, net soos oesopbrengs, verhoog namate die bemestingspeile in inkremente verhoog is. Die glukose- en fruktose-inhoud het in teenstelling skerp afgeneem namate die wortels wasdom bereik het, waarskynlik omdat minder sukrose op hierdie onwikkelingstadium gehidroliseer is. Soos aangedui

deur die positiewe verwantskap tussen sukrose-inhoud, radio-aktiewe translokasie van suiker na die wortels en die finale oesopbrengs, het blaarbespuitings met ComCat® ‘n sterk verhogingseffek op die translokasie van sukrose gehad. Laasgenoemde is in beide gevalle, waar die standaard en die helfte van die standaard bemesting in kombinasie met ComCat® aangewend is, waargeneem. Hierdie invloed van ComCat® is toegeskryf aan die vermoë daarvan om sukrose translokasie oor membrane te manipuleer. Die invloed van Kelpak® op sukrose akkumulasie en translokasie was minder opvallend, maar in kombinasie met die helfte van die aanbevole standaard bemestingspeil het dit tot ’n redelike verhoging in oesopbrengs aanleiding gegee. Die akkumulasie van β-karoteen, ‘n terpeen geassosieer met smaak en kwaliteit van geelwortels, was nie verskillend by verskillende bemestingspeile nie. Maar, waar ComCat® in kombinasie met die helfte van die aanbevole bemestingspeil toegedien is, is die β-karoteen vlak aansienlik verhoog.

Om die manipuleringseffekte van verskillende bemestingspeile en bio-stimulante op fisiologiese prosesse wat met groei en opbrengs verband hou verder wetenskaplik te begrond, is wortelrespirasie sowel as die aktiwiteite van glikolitiese en oksidatiewe pentosefosfaatweg reguleringsensieme gekwantifiseer. In alle gevalle is die aktiwiteite slegs by die 30% plantontwikkelingstadium gemeet en wel 24 uur na die tweede bio-stimulant blaarbespuiting. Beide ComCat® and Kelpak® het bygedra tot ‘n verhoging in die respirasietempo sowel as die aktiwiteite van sleutel respiratoriese en oksidatiewe pentosefosfaat ensieme gedurende hierdie ontwikkelingstadium waar geen of 25% van die standaard bemesting toegedien is. Laasgenoemde was waarskynlik die gevolg van ’n stremmingstoestand wat gedurende vroeë wortelontwikkeling geskep is. Namate wortels wasdom bereik het, het die bemesting- en energiebehoeftes van wortels waarskynlik afgeneem soos aangedui deur die bestendige respirasietempo gedurende latere ontwikkelingstadia en by finale oes in die geval van al vier verskillende bemestingspeile. Laasgenoemde het positief gekorrelleer met verhoogde sukrosevlakke en oesopbrengs.

Deur hierdie studie is die aanbevole bemestingspeil vir geelwortelverbouing in Suid-Afrika as betroubaar bevestig. Maar, waar 50% van die aanbevole standaard bemesting in kombinasie met beide bio-stimulante toegedien is, is finale oesopbrengste gemeet wat nie betekenisvol verskil het van dit wat met die standaard peil alleen behaal is nie. Vanuit ’n ekonomiese perspektief is tot die gevolgtrekking gekom dat die toediening van laasgenoemde kombinasiebehandeling vir beide bestaans- en kommersiële boere aanbeveel kan word.

Ten slotte, die kollektiewe interpretasie van groei- en fisiologiese data wat tydens hierdie studie met verskillende bemestingspeile bekom is, veral in die lig van finale oesopbrengs data, verskaf nie genoegsame rasionaal om die gebruik van suboptimale bemestingspeile, alleen of in kombinase met die twee bio-stimulante wat getoets is, vir geelwortelverbouing aan te beveel nie. Maar, dit word aanbeveel dat hierdie studie onder besproeiingstoestande herhaal word terwyl plotte sodanig gespasieer word dat sydelingse beweging van bemestingstowwe nie tot kontaminasie aanleiding sal gee nie.