YIELD AND FRUIT QUALITY ASSESSMENT OF CACTUS PEAR (*Opuntia ficus-indica* (L.) Mill.) TREATED WITH NATURAL BIO-STIMULANTS

by

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Submitted in partial fulfillment of the requirements for the degree Magister Scientiae Agriculturae (Agronomy)

THE FACULTY OF NATURAL AND AGRICULTURAL SCIENCES

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UNIVERSITY OF THE FREE STATE

BLOEMFONTEIN

SOUTH AFRICA

NOVEMBER 2004

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CHAPTER 1

INTRODUCTION AND RATIONALE

In agriculture, due to continuous shifts in the supply and demand for specific food commodities, farmers are currently experiencing increased economic pressure. Over a short period of one season the over production of staple food such as maize or wheat in the world can have a tremendous influence on the planning of farmers specializing in these crops. As a result, price fluctuations that are strictly manipulated by agricultural organizations have a direct impact on the profit margin of farmers and many either lose interest in producing these staple crops or simply stop farming. However, the dedicated farmer tends to pursue the possibility to cultivate alternative crops. In light of the fact that there are not many alternative crops to fall back on, the bottom-line requirement is that there must be consumer demand for the alternative crop in terms of food, energy, cosmetic or medical uses and therefore continued research in this regard has become imperative.

In South Africa, being a semi-arid region with limited production potential per hectare, subsistence farmers are not able to make a living on small areas of land using traditional staple crops such as maize and wheat. Alternative crops, with reasonable economic potential, might be the only way to establish small scale farming enterprises in this country. The cactus pear (*Opuntia ficus-indica* L. Mill.) is such a potential alternative crop that was introduced to South Africa in 1772 (Barbera, 1995) and is well suited for cultivation in this country. A prerequisite for an alternative crop to either be introduced or expanded in both commercial and small scale farming enterprises is that it must be well adapted to South African conditions, which *O. ficus-indica* fulfills (Barbera & Inglese, 1993; as cited by Barbera, 1995). According to Barbera (1995) opuntias and their products serve various purposes ranging from food and forage to cosmetics and medicinal applications. Further, opuntia fruit also fetch a good price relative to staple food crops (Rand ton^{-1}) in South Africa (Table 1.1) and can contribute to the financial income of the farmer either as an extra crop or a main crop (Wessels, 2004).

Table 1.1: The rank position of cactus pear fruit in Rand ton^{-1} on South African Municipal markets in different areas during the 2003/2004 season (Wessels, 2004)

MARKETS	**RAND TON^{-1}**
Klerksdorp	5332
Durban	5154
Cape town	4612
Witbank	3749
Johannesburg	3571
Springs	3327
Pretoria	3070
Nelspruit 1	2809
Kimberley	2809
East Londen	2702
Pietermaritzburg	2057
Vereeniging	2021
Welkom	1664
Bloemfontein	1658
Port Elizabeth	1447
Nelspruit 2	500
AVERAGE	**3380**

Commercial plantations have been established in South Africa during the past decades and an elevated interest in the production of fruit has been experienced (Pimienta *et al*., 1993). Although the demand for young cladodes (production of napolitos) is much higher in Mexico, the utilization of cladodes for the production of jams, jelly and chutney in this country has increased marginally. In light of these demands, a substantial contribution to the cactus pear industry could be made if ways could be found to increase either fruit or cladode yields or both depending on the objectives of specific farmers. Additionally, any research project involved in pursuing the latter should include an evaluation of the effect of treatments on fruit quality if possible.

The aim of this study was mainly to quantify the yield response of *O. ficus-indica* in terms of fruit and young cladodes, as well as certain fruit quality factors, to treatment

with natural bio-stimulants. A new generation of natural bio-stimulants currently available in the market, namely *ComCat*® (Agraforum, Germany) and Kelpak® (Qwemico, South Africa) was used as well as the prototype SS (University of the Free State, South Africa) that is still in a developmental phase. The manufacturers of the two commercial natural products have, *inter alia*, claimed an enhancing effect on crop yield via metabolic processes such as photosynthesis, the translocation of photosynthate, source/sink metabolism and chlorophyll synthesis. Additionally, Clouse (1996) and Schnabl *et al*. (2001) reported on the role these two natural products play in both vegetative growth and development via root growth, cell elongation and division, as well as in reproductive development leading to enhanced yield and quality in a variety of crops. In the case of *ComCat*® a further advantage of its application is increased resistance to abiotic stress conditions such as heat, cold, drought and flooding, as well as against biotic factors such as fungal infection (Zurek & Clouse, 1994; Takatsuto *et al*., 1996).

Only a few reports on the influence of natural bio-stimulants on yield and quality of *O. ficus-indica* could be traced in the literature and this prompted the underlying study. The literature review in Chapter 2 deals with the cactus pear plant and its economic potential in the agricultural industry, cultivation practices, available natural bio-stimulants and their application potential as tools to manipulate yield and quality in crops as well as secondary metabolites associated with fruit quality. In Chapter 3 the potential of these bio-stimulants to increase fruit and cladode yields as well as its effect on morphological fruit characteristics are reported. The influence of the above-mentioned bio-stimulants on fruit quality using sugar, β-carotene, lycopene, vitamin C and water-soluble protein content in fruit pulp as parameters is reported in Chapter 4.

According to Inglese (1995) soil management practices, together with fruit thinning, are among the most important manipulation techniques followed by farmers cultivating cactus pear for obtaining reasonable and sustainable yields. According to the author fertilization of cactus pear orchards is sometimes neglected and the importance of this standard manipulation technique in terms of fruit yield and quality is often overlooked.

Further, although cactus pear is a drought-resistant species, irrigation contributes to increased yields of both fruit and cladodes (Nobel, 1988). Pruning and fruit thinning are also two standard practices currently applied following reports on its effect on yield and quality improvement (Wessels, 1988). In light of the preceding, it was therefore necessary to adhere to the standard cultivation practices followed by cactus pear farmers and the application of bio-stimulants was merely an additional means to pursue the set objectives.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Barbera (1995) has reviewed the history, economic and agro-ecological importance of the cacatus pear. Opuntias are now part of the natural landscape and the agricultural systems of many regions of the world. Some species are even naturalized weeds in countries such as South Africa and Australia where the environmental conditions are particularly favourable. Opuntias seem to interest crop producers in areas where the wet season coincides with high temperature (Wessels, 1988). It is difficult to find better exploited and wide spread plants, particularly in the subsistence economy of arid and semi-arid zones such as South Africa, where farmers must look to those few species that can profitably survive and produce (Barbera, 1995).

In the eighteenth century, the European settlers first introduced opuntias to the Cape region of South Africa but only about 50 years earlier these intruder plants had infested approximately 900 000 ha in the Eastern Cape and the Karoo (Brutsch & Zimmermann, 1993). According to Prescott (1988) South Africa provided the example for carrying out research and development programs on these intruder plants, instead of exterminating them. In this way cactus pear was introduced to farmers as a possible alternative crop as well as to consumers (as cited by Barbera, 1995). As a result, production of opuntia fruits became particularly relevant to town markets in South Africa already during the 1960's and the traditional fruit business, based on harvests from wild plant received an injection from the increased production in cultivated plantations (Brutsch, 1984). Since 1980, intensive cultivation of opuntias became more popular and the number of plantations increased mostly in the former Transvaal and Ciskei regions, to cover about 1 500 ha (Barbera, 1995).

Agronomic cultivation of opuntias has since 1980 taken off in other parts of the world and during the past century the economic interest in fruits on the world market as well as the number of growing areas has increased considerably (Pimienta *et al*., 1993). Barbera (1995) reported that opuntia plantations covered about 50 000 ha in Mexico, 1000 ha in Chile and 40 000 ha in Brazil. The reason for this increase is probably the realization of the potential role opuntias could play in sustainable agricultural systems in especially arid and semi-arid zones due to their high degree of resistance to drought and high temperatures, their productivity even in non-fertile soils and the economic advantage they can have for low-income farmers (Barbera, 1995).

It is safe to say that the *O. ficus-indica* is currently well established as an alternative crop, although there is still potential to increase the production and quality of its fruits or cladodes (used in the production of nopalitos in Mexico) or both. In this light the underlying study was undertaken in an attempt to investigate the potential of increasing cladode and fruit yields as well as of improving quality by manipulating the plants chemically with the aid of natural bio-stimulants, combined with the correct agronomic practices.

2.2 Fruit yield enhancement

Yields of opuntia fruit through controlled cultivation are extremely variable. In Italy (Barbera, 1995) reported yields of 15 to 25 ton ha^{-1}, while lower yields of 4 to 10 ton ha^{-1} were reported in Mexico (Nerd & Mizrahi, 1995). Wessels (1988) recorded yields of 10 to 30 ton ha^{-1} in South Africa with the highest yield of 33 ton ha^{-1} obtained on an experimental farm.

Fruiting is also dependent on vegetative growth and management practices. The plant starts to produce economically viable yields two to three years after planting, depending on the cultivar, but the retention of fruit during this establishment phase is not advisable for further canopy development. Eventually a mature plant can produce 30 to 70 kg of

export-size fruits. Plant productivity in terms of fruit yield is also a function of the number of mature cladodes and cladode fertility, as well as management practices such as fruit and cladode thinning (Brutsch, 1979). According to the author, six to eight fruits per cladode can produce an annual fruit yield of 20 ton ha^{-1}, with an average fruit weight of 120 g, while 20 000 to 28 000 fertile cladodes are needed to produce this yield per hectare. An to obtain further yield increases (Brutsch, 1979). Further, natural or artificially induced re-flowering makes it possible to obtain more crop yields per annum that might have a profound effect on fruit yield (Barbera *et al*., 1991; Brutsch & Scott, 1991; Nerd & Mizrahi, 1993).

A number of plant growth regulating products applied in agriculture to manipulate plants either by means of hormones or other chemical mechanisms in order to enhance crop yields and quality, is currently available on the market. These include bio-stimulatory products such as *ComCat*® (Agraforum, Germany) and Kelpak® (Qwemico, South Africa). Current research at the University of the Free State revealed the presence of triglycerides in seeds of specific plants that show similar bio-stimulatory activities (Van der Watt, 2004; personal communication). Enhancement of both yield and quality in different crops is claimed by the producers of *ComCat*® and Kalpak®, as well as researchers in the case of the triglycerides.

2.2.1 *ComCat*®

ComCat® is manufactured by a German company, Agraforum, from the seed material of twelve different European plants and is commercially available in Europe, Asia and South America. *ComCat*® is registered by the European Union as a plant-strengthening agent and has also been approved by BCS (Bio-product Control Standards) Öko-Garantie, GmbH for application in organic farming. The product, applied either as a seed treatment or as a foliar spray on vegetables, cut flowers and agricultural crops, is not an organic fertilizer but a natural bio-stimulant that enables the crop plant to utilize nutrients more effectively through increased root growth. A stimulating effect on growth and development of crop plants, increased yields and elevation of resistance to abiotic stress

conditions in treated crop plants is claimed by the manufacturers. Brassinosteroids (BRs) were indicated as the main active substance responsible for the bio-stimulatory activity of *ComCat*® and the two main BRs responsible for the actions in plants have been identified as 24-epi-Castasterone and 24-epi-Secasterone (Schnabl *et al*., 2001).

Mitchell *et al*. (1970) first isolated BRs from the pollen of *Brassica napus* L. Their chemical structures were identified nine years later (Grove *et al*., 1979). According to Fujioka (1999) BRs are considered by some as a new class of phytohormones and more than forty have been identified, 37 in angiosperms and five in gymnosperms. It seems that these phytohormones are widely distributed in the plant kingdom and are natural growth- promoting substances also involved in the translocation of photosynthate in plants and the build up of photosynthate in seeds, as well as the induction of root growth and flower bud formation (Schnabl *et al*., 2001). Claims have also been made that BRs induce the natural resistance of crop plants to abiotic and biotic stress conditions (Zurek & Clouse, 1994; Takatsuto *et al*., 1996).

According to Yang *et al*. (1999) BRs are a group of steroidal lactones derived from 5-alpha-cholestone that have high plant physiological activity. Further functional aspects that have been reported for BRs are their direct role in cell elongation and division, source/sink metabolism, chlorophyll synthesis as well as reproductive and vascular development (Clouse, 1996). Both Sasse *et al*. (1995) (as cited by Schnabl *et al*., 2001) and Takatsuto *et al*. (1996) reported that BRs enhanced the nutrient content of fruits, providing better shape and taste of fruits, as well as having beneficial effects on germination, growth and seed quality.

From an agricultural perspective one of the most promising features of brassinosteroids is their ability to increase not only yield but also the quality of crops (Prusakova *et al*., 1999). The application of 24-epi-brassinolide and 28-homo-brassinolide to potato plants at a dosage of 10 to 20 mg ha^{-1} resulted in enhanced starch and vitamin C content, a 20% yield increase and improved quality even at lower nitrogen application rates to the soil at planting (Khripach *et al*., 1996; as cited by Schnabl *et al*., 2001).

According to Sagar (1998) 50 000 ha were under cactus pear cultivation in Mexico five years ago while more than 6 000 ha were for the production of cactus pear vegetables (nopalitos). Although there is a huge demand for nopalitos, the current problem is limited supply of the product due to low cladode yields by the plant (Flores-Valdez, 1995). As a result, there is an elevated interest by producers in promoting earlier production of cladodes to supply the early market when prices are high (Cortes *et al*., 2003). According to Cortes *et al.* (2003) the application of BRs to the plant can supply a solution to this problem as it was shown to not only initiate additional vegetative buds in *O. ficus-indica* 7 days earlier than the control, but also to increase the growing rate of the cladodes.

However, when the application of BRs in the agricultural industry is considered, care must be taken of the fact that the promoting effects of BRs are strongly influenced by environmental conditions. Pirogovskaya *et al*. (1996) reported that a more pronounced effect on plant growth after treatment with BRs was observed in crops under non-optimal conditions such as unfavourable temperature, light and soil composition as well as pathogenic infections. Both Kamuro *et al*. and Khripach *et al*. (as cited by Schnabl *et al*., 2001) confirmed the above in 1997. The latter author concluded that the physiological properties of the environmentally friendly BRs make them strong contenders to be applied as natural plant growth regulators in the agricultural industry.

2.2.2 Kelpak®

Kelpak®, a commercial bio-stimulant produced in South Africa from seaweed extracts, contains mostly natural compounds. These compounds, responsible for the bio-stimulatory actions, include: natural auxins (2.2 mg L^{-1}) and cytokinins (0.0062 mg L^{-1}) extracted from the seaweed *Ecklonia maxima*. Kelpak® is manufactured using a unique "cold cell-burst" technique that requires no heat or dehydration, thereby retaining the beneficial substances present in fresh seaweed in original form. It is applied as a foliar spray over the plant till run off or directly to the soil as a drench. The active substances in Kelpak®, as mentioned above are claimed by the producers that Kelpak®, to improve

plant performance through increased root growth, more efficient use and uptake of applied nutrients and enhanced flower formation in vegetables and ornamental plants (Anonymous, 2003).

2.2.3 Triglycerides (SS)

Unpublished data indicated that triglycerides (SS), extracted from a plant belonging to the Fabaceae family, showed significant yield increases and quality improvement of crops under both rain-fed and irrigation conditions (Van der Watt, 2004; personal communication). When SS was applied as a foliar spray or drench to various crops the latter was observed. Taiz and Zeiger (1998) reported that certain triglycerides play an important role in the induction of proteinase inhibitor biosynthesis in wounded plants. According to the previous authors triglycerides might also enhance the formation of jasmonic acid, which is essential for the activation of certain mechanisms in plants and compounds related to the synthesis of prostaglandins, which have hormonal effects in plants.

2.3 Quality improvement of fruits

2.3.1 Carbohydrates

Carbohydrates are the most abundant group of biological molecules in nature. A carbohydrate is a simple sugar or a molecule composed of two or more sugar units that are responsible for the sweet taste of fruits. All cells use them as structural materials, stored forms of energy or transportable packets of energy (Starr & Taggart, 1995). The majority of sugars in the cactus pear fruit are of the reducing type, about 53% being glucose and the remainder fructose and a little sucrose (Sawaya *et al.*, 1983; Russel & Felker, 1987). In cactus pear fruit glucose is a free sugar and directly absorbable by the human body.

2.3.1.1 Glucose and Fructose

Various sugars in a class have the same chemical formula but different atomic arrangements (Mauseth, 1995). Glucose and fructose are isomers, both with the empirical formula $C_6H_{12}O_6$, but with different chemical structures (Figure 2.1). Not much difference in chemistry exists between glucose and fructose, but the difference in molecular shape is extremely important as this relates to the specific activities of the enzymes involved in the metabolism of the two monosaccharides by both plants and animals (Mauseth, 1995).

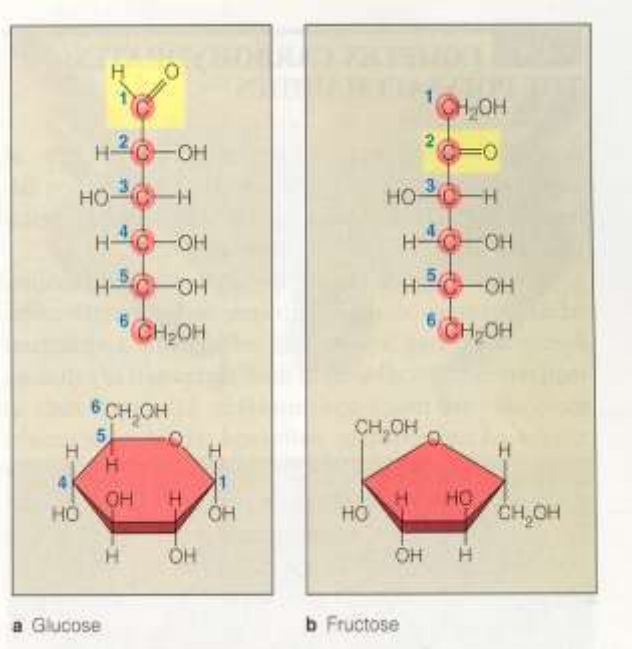


Figure 2.1: Straight chain (above) and ring (below) forms of glucose (a) and fructose (b) (Starr & Taggart, 1995).

Because of the ring formation glucose and fructose are rather unreactive, relatively inert molecules, which are ideal for physiological functions such as construction, transport of molecules and energy storage, while translocation of both is in the form of the disaccharide sucrose in plants (Mauseth, 1995). From a nutritional perspective glucose is the sole energetic metabolite in the brain and nerve cells of humans and animals that

explains the importance of its intake in this specific form (Saenz-Hernandez, 1995). According to the latter author fructose is also easily absorbed by the human body and contributes to fruit flavour and taste on account of its greater sweetness compared to that of glucose and sucrose.

2.3.1.2 Sucrose

Sucrose falls under the class oligosaccharides, which are short chains of two or more covalently bonded sugar units. Sucrose is a disaccharide, composed of two sugars, glucose and fructose (Figure 2.2; Starr & Taggart, 1995).

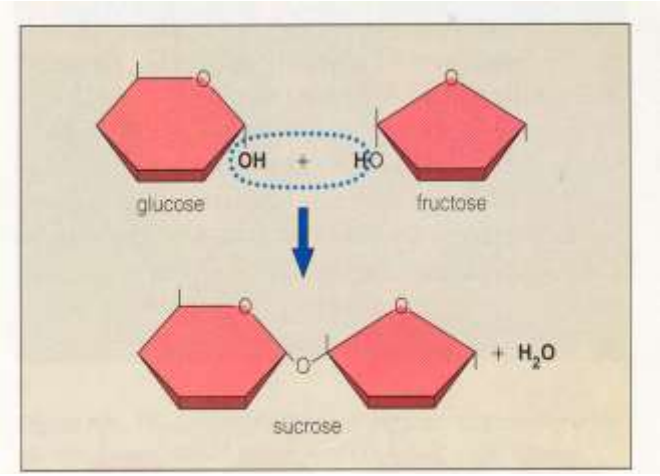


Figure 2.2: Condensation of two monosaccharides (glucose and fructose) to form the disaccharide sucrose (Starr & Taggart, 1995).

Leafy plants continually convert carbohydrates to sucrose, which is easily transported through the fluid-filled pipe lines that service all living cells in leaves, stems, fruits and roots. Sucrose is the most plentiful sugar in nature and crystallized forms are used on our tables as table sugar (Mauseth, 1995).

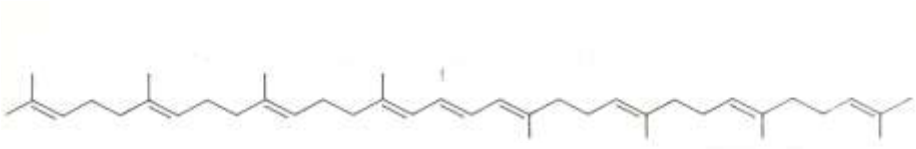
2.3.2 Carotenoids

2.3.2.1. Background

The yellow, orange and red colours of many fruits are due to the presence of carotenoids (Gross, 1987). Carotenoids derived their name from the main representative of their group β-carotene, which was isolated from carrots by Wackenroder in 1831 (as cited by Gross, 1987). According to Gross (1987) carotenoids are among the most widespread and important natural pigments found in nature, with importance not only to plants, but also to humans.

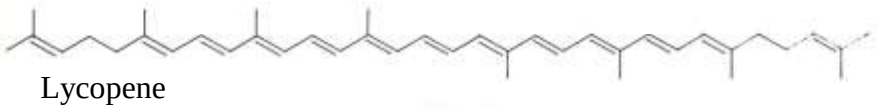
2.3.2.2 Definition, structure and classification

Carotenoids are isoprenoid polyenes formed by the joining together of eight C_5-isoprene units. These isoprene units are linked in a regular head to tail manner, except in the center of the molecule where the order is inverted tail to tail so that the molecule is symmetrical (Gross, 1987). Carotenoids can be divided into acyclic-, monocyclic- and bicyclic forms. The acyclic carotenes are phytoene, the first C_{40}-compound in carotenoid biosynthesis with three conjugated double bonds followed by more unsaturated compounds named phytofluene, neurosporene and lycopene. β-carotene is the most wide spread bicyclic carotene among them all (Gross, 1987). The difference in structure between acyclic and bicyclic carotene can be seen in Figure 2.3.

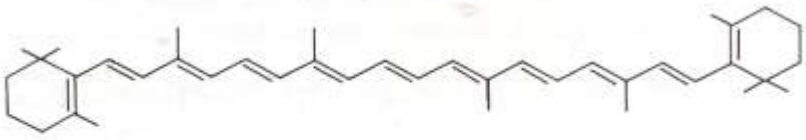


Phytoene

Phytofluene



Lycopene



β-carotene

Figure 2.3: Structures of the most important acyclic and bicyclic carotenes (Gross, 1987).

2.3.2.3 Localisation of carotenoids

Carotenoids are located in plastids, chloroplasts (in leaves and unripe fruit) and chromoplasts (in flowers and unripe fruit) with colours ranging from yellow to orange to red. Chloroplast carotenoids in plants and their approximate levels present are: lutein (40 to 50 %), β-carotene (20 to 30 %), violaxanthin (20 %) and neoxanthin (10 to 20 %). Chromoplasts on the other hand are plastids with an envelope consisting of two membranes and a stroma matrix containing ribosomes and filaments of DNA (Sitte, 1977; as cited by Gross, 1987). Laval-Martin (1974) (as cited by Gross, 1987) observed two kinds of chromoplasts in 'cherry' tomatoes: in the inner part of the pericarp globular chromoplasts containing mainly β-carotene and in the outer part chromoplasts mainly lycopene.

2.3.2.4 Function and uses of carotenoids

A variety of functions have been attributed to carotenoid pigments, but two important photo functions have been clearly established for carotenoids (Britton, 1976). The first important function of carotenoids, especially β-carotene, is in the greater absorption of

different wavelengths of light from chlorophyll for maximum light utilization and photosynthesis (Stefermann-Harmes, 1981). Britton (1976) reported enhanced chlorophyll fluorescence via the illumination of light of the wavelengths absorbed by carotenoids, which provided evidence that carotenoids transferred energy to chlorophylls.

The second most important role of carotenoids, is to alter damage that is being caused by visible radiation. Carotenoids protect living organisms from harmful photochemical reactions initiated by excited chlorophyll in the triplet state, for example that of free radicals and highly reactive singlet oxygen, by quenching the excessive energy or singlet oxygen that causes the damage (Britton, 1976). The preconditions of photo protection are related to the length of the chromophore and the amount of conjugated double bonds that must be more than nine for full protection (Mathews-Roth *et al.*, 1974), as illustrated in Table 2.1 (Davies, 1976).

Table 2.1: Absorption maxima of some carotenoids found in fruits of plants (Davies, 1976)

	Conjugated double bonds				
			Absorbance maxima		
Carotenoid	**In chain**	**In ring**		**(nm)**	
Phytofluene	5		331	348	367
Lycopene	11		447	472	504
Alpha-carotene	9	1	423	444	473
Beta-carotene	9	2	425	450	478

Another important role of carotenoids is that they act as vitamin A precursors. β-carotene, with its β-ionone rings, is the provitamin with the highest activity for the formation of vitamin A. Vitamin A activity depends on the amount and nature of the active carotenoids, their stability, digestibility and state of isomerization (Britton, 1976). From a medical perspective, both β-carotene and lycopene are amongst the most active carotenoids that are involved in preventive actions against degenerative disorders like prostate and lung cancers (Bruneton, 1995).

2.3.2.5 Lycopene

According to Bouvier *et al.* (1998) and Akhtar *et al.* (1999) lycopene is the natural red pigment exclusively synthesized by plants and localized in the chromoplasts of the pericarp tissue of ripe tomatoes. The bio-availability of lycopene for human consumption is strongly influenced by its stereochemistry. Lycopene is a highly unsaturated molecule containing thirteen double bonds, eleven of which are conjugated. The all-*trans* isomers of lycopene are the most predominant geometrical isomers found in fresh tomatoes. Lycopene undergoes *trans* to *cis* isomerization during tomato processing and storage, with the latter form more bio-available to humans (Shi & Lemaguer, 2000).

Environmental and agronomic factors were found by many researchers to have an influence on the lycopene content of fruits. In the case of temperature, Dumas *et al.* (2002) and Hamauzu *et al.* (1998) stated that temperatures below 12 °C and above 32 °C strongly reduce lycopene biosynthesis, with higher temperatures (35 °C) inhibiting the accumulation of lycopene in fruit because of the conversion of lycopene to β-carotene. Sunlight also stimulated lycopene production, except for direct excessive sunlight, which led to increased temperatures and overheating in irradiated tissues (Dumas *et al.*, 2002). Naphade (1993) found that fruit lycopene content was reduced by water stress, although Zushi and Matsuzoe (1998) discovered that the amount of carotenoids in fruit was increased by soil water deficits. Studies conducted by Dumas *et al.* (2002) revealed that mineral nutrition (nitrogen, phosphorus, potassium) had a stimulating effect towards higher lycopene concentrations.

Concerning the application of plant growth regulators for the manipulation of fruit quality, Rabinowitch and Rudish (1972) dipped full size green tomato fruit into CPTA (2-4-chlorophenylthio triethylamine hydrochloride), a bio-regulator considered as carotenoid inducer, as well as ethephon (2-chloroethyl phosphonic acid), a plant growth and development regulator, and noted faster and higher lycopene accumulation (red colour). Hsu and Yokohama (1991) applied foliar applications of DCPTA (2-(4, 4-dichlorophenoxy triethylamine hydrochloride) on tomatoes and found a 28% increase in

lycopene content in contrast to the untreated control. Rabinowitch *et al.* (1975) reported that lycopene and its colorless precursors, phytoene and phytofluene, began to accumulate in tomato fruits following the breaker stage in colour from green to red. The latter author concluded that at red-ripe stage lycopene constituted 95% of the coloured carotenoids or 73% of the total carotenoids, including phytoene and phytofluene. Giovanelli *et al.* (1999) found that lycopene in post-harvest-ripened tomatoes was almost twice the value reached in vine-ripened tomatoes.

From a medical perspective Wayne (1996), Koo (1997) and Rao and Agarwal (1999) concluded that the greater the concentration and consumption of lycopene in consumed fruit the lower the risk of developing cancer and cardio-vascular diseases due to the strong antioxidant properties it contains.

2.3.2.6 β-carotene

Although β-carotene is one of the chloroplast carotenoids, both Sitte (1977) and Laval-Martin (1974) observed β-carotene in chromoplasts isolated from the inner part of the pericarp of 'cherry' tomatoes (as cited by Gross, 1987).

Environmental and agronomic factors do not have such a significant effect on β-carotene as they have on lycopene. Koskitalo and Ormrod (1972) discovered that lower night- and day temperatures decreased lycopene content while an increase in β-carotene content was noted. Baqar and Lee (1978) reported that a temperature of 30 °C dramatically reduced the synthesis of all the carotenes in tomatoes, except for β-carotene. Cabinel and Ferry (1980) (as cited by Gross, 1987) concluded that the level of β-carotene was lower in tomatoes under lower levels of light interception, compared to that in open field tomatoes and thus they concluded that β-carotene content in fruits might be influenced by light.

Cultivation techniques practiced by farmers under rain fed or irrigation conditions had no effect on the amount and distribution of β-carotene in pink-red tomatoes (Zushi &

Matsuzoe, 1998). Fertilization on the other hand, especially higher levels of potassium, decreased β-carotene content in tomatoes (Trudel & Ozbun, 1970). Keithly *et al.* (1990) applied plant growth regulators on tomatoes as a seed treatment and the authors found that at 24/18°C day/night temperatures the concentration of β-carotene in ripe fruits increased. Further, both temperature and physiological ripening stages had an effect on β-carotene content (Rabinowitch *et al.*, 1975). The authors observed a rapid fall in chlorophyll content with the onset of the breaker stage in tomatoes, while the β-carotene content doubled during the same period. Giovanelli *et al.* (1999) observed that the β-carotene content was almost half the amount in vine-ripened tomatoes compared to post-harvest-ripened tomatoes.

From a nutritional perspective β-carotene shows the highest degree of provitamin A activity, especially when the isomer is in the *trans* form (Sweeny & Marsh, 1971). Bauernfeind (1972) concluded that the vitamin A activity in fruits and vegetables was due to the presence of carotenoids (provitamin A compounds) like β-carotene.

2.3.2.7 Vitamin C

An increased interest in the cultivation, consumption and use of *O. ficus-indica* was noted as the nutritional possibilities became known. The fact that this plant may be grown in areas unsuitable for other vegetables is also of prime importance (Saenz-Hernandez, 1995). Cactus pear has high levels of ascorbic acid with concentrations of vitamin C found higher in cactus pear fruit than in apple, pear, grape or banana (Cheftel *et al.*, 1983, as cited by Saenz-Hernandez, 1995). According to Pimienta (1990) the vitamin C concentration in cactus pear pulp may differ between fruits, ranging from 4.6 to 41 mg 100 g^{-1} (as cited by Nerd & Mizrahi, 1995).

2.3.2.8 Water-soluble proteins

Certain proteins, especially short chain polypeptides and free amino acids, are only present in fruits and the consumption thereof is of vital importance for normal

functioning of the human body. Protein concentrations in cactus pear are similar to those found in other fruits, while the total free amino acid content (257 mg 100 g^{-1} fruit pulp) is greater than the average reported in other fruits (Saenz-Hernandez, 1995). The latter author observed a relatively high content of glutamine, proline, arginine, histidine, serine and methionine in cactus pear fruits, while Sawaya *et al.* (1983) found that cactus pear fruit contained about 0.21 g protein 100 g^{-1} fruit pulp. Galizzi *et al.* (2004) reported that protein content was not correlated to fruit yield, but it was nevertheless important to maintain adequate proteins for regulatory processes and photosynthesis.

2.4 Cultural methods used to increase yield and quality of Opuntia *ficus-indica*

2.4.1 Soil management

During the soil management process tillage should be restricted to a minimum in order to avoid damage done to the plant's superficial root system. In the case of weed control, weeds must be removed manually in bush-type trained plants and left as a mulch on the soil to retain water and smother weed re-growth (Inglese, 1995). Chemical control of weeds with paraquat and glyphosate (20 g l^{-1}) is being used with success and more often than mechanical control. The soil sterilants, tebuthiuron and hexazinone, have the greatest potential to reduce weed competition for several years after treatment (Felker & Russel, 1988).

2.4.2 Fertilization

From an agricultural perspective, although farmers all over the world commonly apply both manure and inorganic fertilizers, research on the fertilization of cactus pear has been largely neglected resulting in the limited availability of scientific and technical information (Inglese, 1995). According to the author a fruiting plant must be supplied with 15 kg of manure, 350 g ammonium sulphate, 300 g super phosphate and 200 g potassium sulphate. Pimienta (1990) (as cited by Nerd en Mizrahi, 1995) recommended lower quantities of nutrients: 150 g ammonium sulphate per plant or 60 kg N ha^{-1}, 100 g

super phosphate per plant or 20 kg P_2O_5 ha^{-1}, 100 g potassium sulphate per plant or 20 kg K_2O ha^{-1} and 6 kg manure per plant or 6 t ha^{-1}. Claassens and Wessels (1997) recommended nitrogen applications of between 30 to 60 kg N ha^{-1}. Cladode N content of 0.96% can be expected under optimum conditions. Phosphate application of at least 16 kg P ha^{-1}, coinciding with a cladode P value of 15%, is required for optimum yields.

Optimum yields were obtained with a soil K content of 60 mg kg^{-1} and a cladode K content of 1.5%. Dolomitic lime, applied as a top dressing, had a beneficial effect on yield, according to Claassens and Wessels (1997). Nerd *et al.* (1991) found that 120 kg N ha^{-1} applied soon after the summer crop harvest, promotes additional budding in autumn, although it did not result in any further increases in the main fruit harvest the following summer. In this study neither potassium nor phosphorus was correlated with the occurrence of the autumn flush of flower buds, although there was a positive correlation with nitrogen.

Galizzi *et al.* (2004) determined the effect of micro nutrient applications on yield in *O. ficus-indica.* The results revealed that yield was significantly negative correlated with cladode Mn and Zn concentrations. However, the concentrations of N in the cladodes were highly significantly correlated with cladode Ca, Cu, Mn and Zn concentrations. According to the authors highly significant correlations were observed between fruit yield and quality and exchangeable soil Ca, while higher cladode K concentrations stimulated fruit firmness.

Nerd *et al.* (1991) evaluated continuous fertigation of water and nutrients through dripper lines during the year and found a decrease in the number of floral buds per plant to a much greater extent in the winter than in the summer gestation period. Fertilization on the other hand increased the production of floral buds in both crops, but to a greater extent in the winter crop. The increased floral bud production in fertilized plants was associated with an increase in NO_3-N content of the cladodes. According to the author, suspension of fertigation for four to eight weeks immediately after the summer harvest decreased cladode water content while delaying and reducing floral bud emergence the next season.

The mean fresh weight and peel to pulp ratio (w/w) was lower in fruits that ripened in the summer than in fruits that ripened in the spring (winter crop) (Nerd *et al.*, 1991).

2.4.3 Irrigation

Cactus pear is a drought-tolerant species that has very high water-use efficiencies. Nevertheless, irrigation is a common practice in areas with a dry summer season and where opuntias are intensively grown for fruit production (Nobel, 1988).

Wessels (1988) revealed that the main production areas in South Africa are situated in the summer-rainfall areas. Problems experienced in these areas are dry winter conditions, late rains, fluctuating rainfall patterns and dry spells even during the rainy season which may result in late and poor flower induction and may lead to lower yields and fruit quality. Areas with summer rainfall between 300 and 600 mm ensure high yields and regular fruit development and the need for irrigation during this period is unnecessary. Nerd *et al.* (1991) found reduced cladode fertilities and delays in spring burst in areas where the annual rainfall was lower than 300 mm. Drip irrigation with daily low volumes of 1 to 2 mm day^{-1} ensured high yields and regular fruit development (Nerd *et al.*, 1991). According to the latter authors, irrigation of about 100 mm was essential for re-flowering after the summer fruit harvest. Barbera (1984) (as cited by Inglese, 1995) reported that two to three irrigations of 60 to 100 mm applied during fruit development increased yield, fruit size and flesh percentage significantly.

However, Mulas and D'Hallewing (1997) measured higher yields on irrigated plots as a result of higher fruit numbers per cladode, although fruit weight was not influenced. An increase in fruit peel thickness and seed weight and a decrease in the juice percentage and pulp sugar content were also noted by the authors on irrigated plots.

2.4.4 Pruning

Pruning is a practice annually performed by farmers on cactus pear trees to regulate plant shape and size and to reduce the density of cladodes in the inner part of the plant canopy (Barbera *et al.*, 1991). Cladodes that develop in the inner shaded part of the canopy were found to be less productive and, therefore, production pruning is necessary to expose as many cladodes as possible to direct sunlight (Inglese, 1995). The latter author also noted that dense cladode canopies and cladodes touching the ground were easily parasitized with cochineal. Another important reason for summer pruning is to reduce the number of new cladodes, which may compete with developing fruits, and result in a sharp alternate bearing behavior (Barbera *et al.*, 1991). According to the authors, 85% to 95% of one-year-old cladodes bare fruit the year after formation and, as a rule of thumb, no more than two daughter cladodes should be retained on a parent cladode to reduce damage caused by wind. Wessels (1988) suggested pruning from May to July in South Africa, just after summer fruit harvest, when the plant is no longer actively growing and it is feasible in regions with dry winters where temperatures are high enough to dry the cut area.

2.4.5 Fruit thinning

Fruit thinning is a very important cultivation practice that is applied by farmers after fruit set. According to Barone *et al*. (1994) and Monselise & Goldschmidt (1982) the main reason for fruit thinning is to regulate crop load. According to the authors, fruit thinning practices are necessary to increase fruit size, advance fruit ripening and control alternative bearing in all fruiting trees. Inglese *et al.* (1995) showed that the time of thinning of cactus pear did not affect fruit growth and fruit weight. Fruit weight and flesh weight increased with thinning, while a fruit weight of 120 g was only obtained in cladodes with no more than six fruits. Quality parameters, for example total soluble solids, seed content and flesh percentage were not affected by thinning. However, the seed to flesh ratio decreased.

According to Inglese *et al.* (1994), photosynthesis in fruits did not contribute much to fruit size and mass but most of the needed assimilates are obtained from the mother cladode. Further, sub-terminal cladodes contribute to the daily gain in fruit dry weight during the phase of rapid flesh development of a cladode bearing ten to fifteen fruits. Wessels (1988) recommended that no more than nine to twelve fruits should be retained per cladode to obtain sufficient increase in fruit size. Wessels (1988) suggested that plants should be fruit thinned two weeks before bloom to two weeks after fruit set, with no more than 10 fruits left per cladode to diminish irregular and delayed ripening.

2.5 ASPECTS DEALT WITH IN THIS STUDY

The first priority a cactus pear farmer has in the cultivation of prickly pear is to increase yield of a good quality fruit. Some farmers need to obtain a higher fruit yield and others a better cladode yield (nopalitos) depending on the markets in their regions. The aims of this study were in essence to apply the natural bio-stimulants discussed in this chapter as foliar sprays and to follow their possible yield-increasing effect on both fruit and cladodes as edible products as well as their effect on fruit quality and morphological growth characteristics. In order to comply with the cultivation methods traditionally applied by farmers, chicken manure was used as fertilizer and fruit thinning was applied. These aspects were considered when yield data was quantified.

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CHAPTER 3

Vegetative and reproductive growth responses of *Opuntia ficus-indica* to treatment with natural bio-stimulants

Abstract

The influence of a natural commercial bio-stimulant, *ComCat*®, alone and in combination with a prototype natural product (SS) in the developmental phase, was investigated by means of two trials in a six-year-old *Opuntia ficus-indica* (L.) Mill. orchard during the 2003/2004 growing season. The main aim was to quantify the effect on cladode initiation, fruit yield and fruit characteristics. *ComCat*® showed a positive effect on fruit production while SS induced the development of new cladodes but had no stimulatory effect on fruit yield. A combination of *ComCat*® and SS, in a 50:50 ratio, increased both fruit and cladode formation while Kelpak®, a commercial seaweed extract used as a positive control, enhanced fruit development but had no significant effect on cladode initiation. *ComCat*® and SS applied in combination also produced the highest percentage of medium fruits in both trials. Statistical analysis showed that fruit length and fruit diameter were correlated with fruit mass but this was not affected by the treatments. Similarly, fruit mass and peel mass were correlated with pulp mass and this was also not influenced by any of the treatments. Two statistical models were developed to predict the influence of a) fruit length and diameter on fruit mass and b) peel mass and fruit mass on pulp mass, and showed a >50% accuracy, confirming the application potential of these models in the prickly pear industry.

Keywords: *Opuntia ficus-indica,* fruit yield, cladode yield, fruit characteristics, bio-stimulants

3.1 Introduction

Opuntia ficus-indica is well adapted to arid and semi-arid climatic zones where water is one of the major constraints for cultivation (Benson, 1982). According to Barbera and

Inglese (1993) opuntias play an important role in the economy of these climatic zones where farmers are compelled to adapt by choosing crops that can be cultivated in a sustainable manner (as cited by Barbera, 1995). However, very little is known about the manipulation of opuntias with chemicals, additional to the use of either inorganic or organic fertilizer or both, in an attempt to increase its productivity. Despite this, Pimienta *et al*. (1993) already reported a decade ago that the number of growing areas as well as the presence of opuntia fruits on world markets has increased substantially.

Barbera and Inglese 1993 (as cited by Barbera, 1995) reported prickly pear yields of 15 to 25 tons ha^{-1} in Italy while much lower yields of 4 to 10 ton ha^{-1} seemed to be obtained in Mexico (Pimienta, 1990; as cited by Nerd and Mizrahi, 1995). In South Africa the yield was also very erratic during the late eighties and varied between 10 to 30 ton ha^{-1} (Wessels, 1988) under experimental conditions. From a production point of view a mature plant is believed to produce 30 to 70 kg of export-size fruits. The productivity of the plant is also seen as a function of the number of one-year old fertile cladodes, fruit thinning and fruit size (Brutsch, 1979). According to the latter author six to eight fruits per cladode with an average weight of 120 g can extrapolate to an annual yield of 20 t ha^{-1}. Further, 20 000 to 28 000 fertile cladodes are needed for a 20 ton ha^{-1} yield and manipulation to increase fruit mass must be attempted rather than attempting to increase cladode fertility. The standard practice of fruit thinning is applied to achieve this goal. According to Van der Walt (2004) prickly pear fruit are graded into four sizes namely small (100 g), medium (120 g), large (145 g) and extra-large (180 g per fruit). Of these the medium and large categories are the most popular from a consumer perspective.

Products available on the market that have the potential to manipulate the growth and yield of plants either hormonally or by means of other metabolic mechanisms include bio-stimulants such as *ComCat*® (Agraforum, Germany) and Kelpak® (Qwemico, South Africa). However, these have not been tested (extensively) on prickly pear. This study was undertaken in an attempt to improve both the fruit and young cladode yield of prickly pear by treating adult plants with these two bio-stimulants. Current research at the University of the Free State revealed the presence of triglycerides in a seed suspension of

specific plants that show similar bio-stimulatory activities (Van der Watt, 2004; personal communication). The latter is currently tested as a prototype bio-stimulant under the acronym SS. In all cases enhancement of both yield and quality in different crops is claimed by the producers of *ComCat*® and Kelpak® as well as the researchers in the case of SS.

The main objectives of this study were to increase yield of both fruit and young cladodes (used in the production of edible nopalitos in Mexico) as well as to follow the effect of the bio-stimulants on morphological fruit parameters such as fruit length, fruit diameter and fruit mass by adhering to the normal cultivation practices in South Africa. An attempt has also been made to develop a simple model for predicting the yield outcome of the fruit by correlating morphological parameters.

3.2 Materials and methods

3.2.1 Experimental layout and treatments

Trials were conducted during the 2003/2004 growing season in a six-year-old commercial prickly pear (cultivar Skinners Court) orchard located at Bainsvlei, Bloemfontein, South Africa. The orchard was established on a 1 m deep sandy soil, classified as a Hutton soil form belonging to the Stella family (Soil classification working group, 1991). The experimental plots were located on an upper fort slope terrain unit with a straight 1% slope in a northerly direction. Plant rows were in a westerly direction, plants 2 m apart and 6 m between rows (plant population is 835 plants ha^{-1}). Two identical trials were simultaneously conducted on two sites of the orchard, referred to as the West and East blocks, which were 300 m apart. A complete randomized design was used in both trials. Twenty-five plants, more or less uniform in size, were selected in each trial. Five treatments, replicated five times, were applied and one plant represented a replicate.

Five treatments were applied:

1. *ComCat*® (*CC*), a commercial bio-stimulant with brassinosteroids as active substance and showing growth and yield-enhancing effects on other crops, was

applied as two foliar spray treatments: first spray at a concentration of 30 mg L^{-1} and the second spray at a concentration of 10 mg L^{-1}.

2. SS, a prototype natural bio-stimulant with triglyceride as active substance purified from a plant belonging to the Fabaceae family and showing plant growth regulatory properties on other crops, was applied as two foliar spray treatments: first spray at a concentration of 6 mg L^{-1} and the second spray at a concentration of 3 mg L^{-1}.

3. A combination of CC and SS in a 50:50 ratio were applied as two foliar sprays: first spray at a concentration of 30 mg L^{-1} CC + 6 mg L^{-1} SS and the second spray at a concentration of 10 mg L^{-1} CC + 3 mg L^{-1} SS.

4. Negative control (No treatment. Standard agronomic practices, including the use of chicken manure, were applied).

5. Positive control (Kelpak, a commercial seaweed extract that improves plant performance, was applied as two foliar sprays at the recommended rate of the manufacturers: first and second spray at a concentration of 10 ml L^{-1}.

Each plant was sprayed with 5 L of the different natural products, at optimal concentrations according to the manufacturers recommendations, until run-off. Treatments were applied in April after the summer harvest in March 2004 and repeated in spring (September 2004) when the plant started forming flower and cladode buds.

3.2.2 Orchard practices

Weeds were controlled chemically using Roundup at a concentration of 12 ml L^{-1} water. Approximately 15 kg dry chicken manure per plant were broadcasted in the plant row, just after the summer harvest (April 2003) in the form of mulch according to the standard procedures applied by the farmer. Soil samples were taken to a depth of 20 cm, in row

and between rows at the beginning of the trial, and were analyzed to determine the soil fertility status. After interpretation of the data the results were pooled and the means of the various fertility indicators are listed in Table 3.1. From the listed norms it is clear that the general fertility status of both blocks was adequate to supply the plants with the necessary nutrients.

Table 3.1: The average soil fertility status before fertilizer application for the west and east blocks at the beginning of the trials

Parameter	Unit	Block West	Block East	Norm Low	Norm High
Clay (top soil)	%	17	15		
Sand (top soil)	%	83	85		
Conductivity	MS m^{-1}	222	99	0-300	>400
pH (KCl)		5.4	6	<5.5	>6.5
Calcium	mg kg^{-1}	609	658	0-300	300-3000
Magnesium	mg kg^{-1}	178	170	0-150	150-300
Potassium	mg kg^{-1}	428	440	0-80	80-250
Sodium	mg kg^{-1}	38	65	0-100	100-500
Phosphorus(Olsen)	mg kg^{-1}	77	69	0-5	>10
Zinc (0.1mol/l HCl)	mg kg^{-1}	2.7	4	0-2	>5
CEC	me $100g^{-1}$	6.5	6		>5:1

The long-term climate of the area can be described as semi-arid, with an annual rainfall of 450 mm per year. Rainfall peaks in February to April but is still too low to obtain high yields. Consequently, it was decided to prevent production risk through the application of supplemental irrigation. Two dripper lines (2 L h^{-1}) were installed at each side of the plant row and 1 m apart with four drippers serving a plant. The plants were irrigated once a month using a 24-hour irrigation cycle. The orchard received 160 m^3 water ha^{-1} (16 mm) per irrigation cycle. In total the plants received 557 mm water, 365 mm from rain and 192 mm from irrigation during the trial season.

Plant pruning was done just after the summer harvest (April 2003, before the application of treatments) before the trials commenced, in order to remove all shaded and excessive cladodes. Fruit thinning was performed in spring (September 2003, just after fruit set) to obtain the correct spacing of fruits per cladode. According to standard practices applied

by the farmer, a fruit spacing of approximately 8 cm was applied. Parathion, a standard chemical used to control cochineal, was applied as a corrective treatment when necessary at a concentration of 1 ml L^{-1} water.

3.2.3 Quantification of the effects of bio-stimulants on vegetative growth of *Opuntia ficus-indica* using morphological parameters

3.2.3.1 Cladode counts

At the beginning of the trial, after the plants had been pruned and before any treatments were applied, all the old cladodes were counted on each plant in order to determine the size of each plant. It was considered necessary to note differences in size between plants at the onset. During spring and summer, when all new cladodes had formed, they were counted for each plant in the experiment, to determine the number of new cladodes formed per number of old cladode.

3.2.3.2 Fruit counts and yield characteristics

During spring (September 2003), all flower buds and established fruit were counted. Thereafter fruit were thinned to establish the correct distance between fruits. The first harvesting of fruit commenced when 50% of the fruit in the orchard reached the first stage of ripeness (colour break).

All the fruit from each treatment and its replicates were counted and weighed separately, while the fruit diameter and fruit length were also measured, using slide calipers. For the second and third harvests, the total amount and weight of fruits were determined per treatment/replicate and the averages calculated. However, for fruit diameter and length measurements during the second and third harvests, twenty fruits per replicate were picked randomly for each treatment and the averages calculated. Fruits were individually measured for pulp mass, peel thickness, peel mass, diameter and length. Fruit yield was expressed as: 1) the number of fruits before and after fruit thinning per number of old

cladodes, 2) the final fruit yield (kg per plant) per number of old cladodes at harvest and 3) ton ha^{-1} fresh mass for each treatment separately.

3.2.4 Statistical analysis

All data presented were means of five replicates along with standard deviations of means. Data were subjected to analysis of variance using the NCSS 2000 statistical program, and means were compared using the Tukey-Kramer Multiple-Comparison Test at a 5% significant level. Multiple regression and correlation analyses were performed for specific parameters using the same statistical program and tested at the 95% significance level. From these analyses linear equation models were obtained for predicting yield and quality.

3.3 Results

3.3.1 The effect of natural bio-stimulants on the fruit yield of *O. ficus-indica* expressed as kilogram per plant and ton per hectare

As indicated in Figure 3.1A, treatment with CC, CC+SS and Kelpak showed a higher average fruit yield for the west block compared to the negative control, while the SS treatment had a reducing effect. The same tendency was observed for CC and SS treatments in the east block (Figure 3.1B) but the CC+SS and Kelpak treatments did not have the same marked effect as was observed in the west block. Interestingly, where CC was applied on its own, a constant average fruit yield of 40 kg fruit $plant^{-1}$ or 34.5 ton ha^{-1} was maintained in both trials. Statistical analysis was not performed on this specific data, as only differences between plants were calculated while differences in plant size were not taken into consideration.

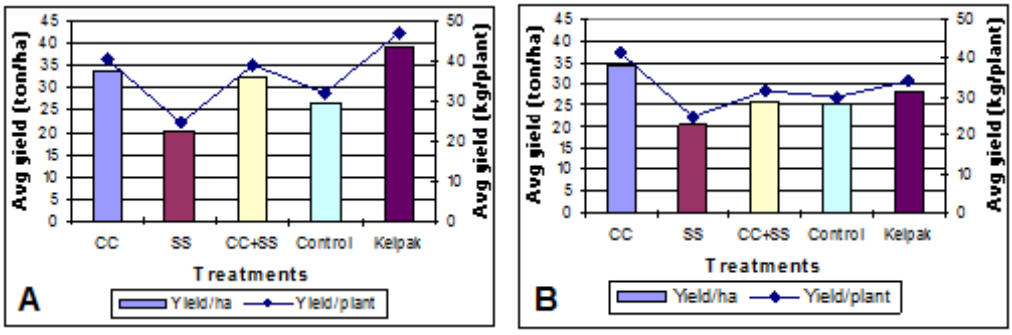

Figure 3.1: The effect of natural bio-stimulants on the average fruit yield in kilogram per plant as well as ton ha^{-1} for **A)** the west block and **B)** the east block trial.

3.3.2 The relative effect of natural bio-stimulants on average fruit yield of *O. ficus-indica* as expressed per number of old cladodes

As indicated in Figure 3.2A, the CC and Kelpak treatments as well as the untreated control were significantly better than the SS and CC+SS treatments in terms of fruit yield in the west block trial when expressed per old cladode. However, differences between the former two treatments (CC and Kelpak) as well as the untreated control were not statistically significant ($P<0.05$). Clearly, when plant size in terms of cladode number (Figure 3.2B) was considered in calculating fruit yield, a different picture emerged compared to that illustrated in Figure 3.1A. Interestingly, in both cases only CC and Kelpak tended to contribute to elevated fruit yields. Although treatment with SS either separately or together with CC resulted in a low average fruit yield in the west block trial, it stimulated new cladode formation (Figure 3.2B).

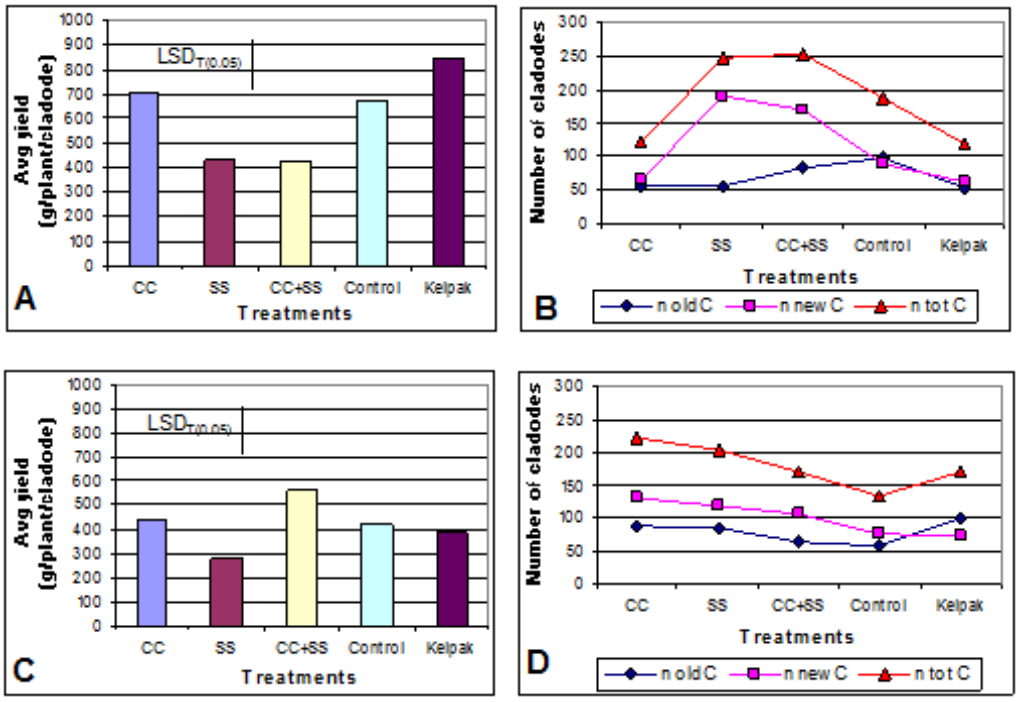

Figure 3.2: The effect of natural bio-stimulants on **A)** the fruit yield per plant as expressed per number of old cladodes (n old C) for the west block, **B)** number of old cladodes, new cladodes (n new C) and total cladodes (n tot C) for the west block, **C)** fruit yield as expressed per number of old cladodes for the east block and **D)** number of old cladodes, new cladodes and total cladodes for the east block. LSD values are indicated in figures A and C but no statistical analysis was performed on data presented in figures B and D as plant size was not taken into account and cladode number is only shown to support the discussion that will follow – see ANOVA for Fig. 3.2 in appendix A as well as discussion).

The tendencies observed in the west block trial were not repeated in the east block trial for neither fruit yield (Figure 3.2C) nor new cladode number (Figure 3.2D). In fact in the east block trial the results seemed to be in some cases the direct opposite of those observed in the west block due to differences in both plant size and the number of old cladodes per plant in the two trials. The tendency to form new cladodes seemed to have been influenced by both the number of old cladodes as well as fruit formation. Further, both fruit and new cladode yield per number of old cladodes were markedly lower in the east than in the west block trial and no significant differences existed between the different treatments. Due to the cultivation practice of fruit thinning traditionally followed by the farmer, it seemed necessary to compare the yield data both before and after fruit thinning.

3.3.3 The effect of natural bio-stimulants on new cladode formation as well as fruit yield before and after fruit thinning in *O. ficus-indica,* expressed per number of old cladodes

The results in Figure 3.3A showed that only treatment with SS had a statistically significant enhancing effect on new cladode production in the west block trial, when expressed per number of old cladodes and compared to the untreated control. Although not statistically significant, the CC+SS treatment showed the same tendency. In the same trial, although the average number of new cladodes produced by plants treated with either CC or Kelpak were lower than for the SS treatment, the former tended to stimulate total fruit set to a greater extent, albeit statistically non-significant, when expressed per number of old cladodes before thinning (Figure 3.3A). However, none of the treatments had a significant effect on the number of harvested fruit after fruit thinning in the west block trial when expressed per number old cladodes (Figure 3.3A).

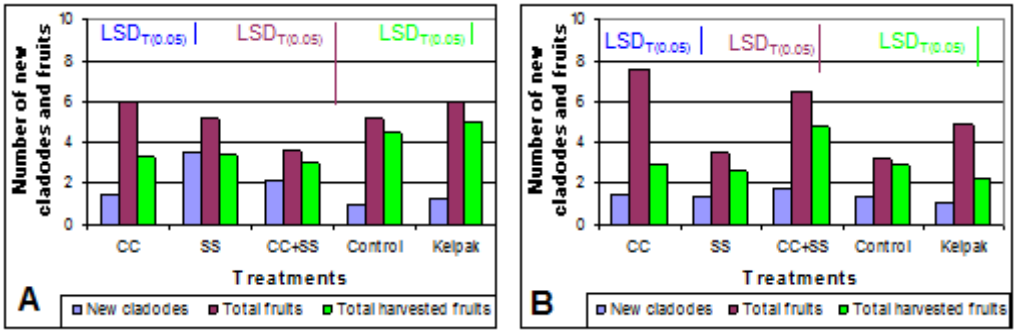

Figure 3.3 The effect of bio-stimulants on the average number of new cladodes, total number of fruits before thinning and total harvested fruits after thinning expressed per number of old cladodes for **A)** the west block trial and **B)** the east block trial. LSD values are indicated for each parameter (corresponding colours) in the figures (see ANOVA for Fig. 3.3 in appendix A as well as discussion).

In the east block trial (Figure 3.3B) the tendency of SS to enhance new cladode production, as was observed in the west block, was not repeated. However, compared to the untreated control, both the CC and Kelpak treatments showed the same tendency to increase the total amount of harvested fruit in this trial before fruit thinning while the observed difference was statistically significant for CC but not for Kelpak. Interestingly, the CC+SS treatment also significantly increased the total number of harvested fruit in the east block trial and was the only treatment that positively influenced the harvested fruit yield after thinning. In the latter case, however, the observed difference was statistically significant compared to the Kelpak treatment but not the untreated control. Subsequently, the average mass of a single fruit was measured in order to determine the relationship with other yield parameters.

3.3.4 The effect of natural bio-stimulants on the average mass of a single *O. ficus-indica* fruit at the final harvest after fruit thinning

Although no significant differences between treatments in terms of the average mass of a single fruit were observed in the west block trial (Figure 3.4A), an interesting relationship between single fruit mass and the total number of harvested fruit (after fruit thinning; Figure 3.3A; green bars) as well as average yield per old cladode (Figure 3.2A) was observed (see discussion). A similar relationship between fruit mass (Figure 3.4B) and

the total number of harvested fruits (Figure 3.3B; green bars) as well as average yield per old cladode (Figure 3.2C) was observed for the east bock trial.

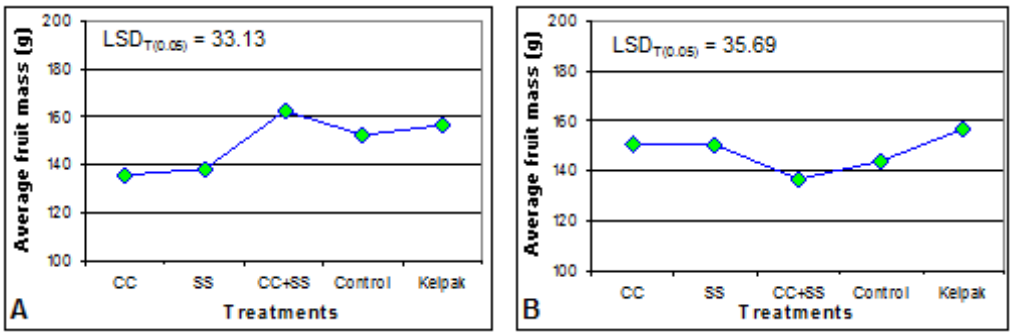

Figure 3.4: The effect of bio-stimulants on the average mass of a single fruit after thinning for A) the west block and B) the east block trial. LSD values are indicated in the figures (see ANOVA for Fig. 3.4 in appendix A as well as discussion).

3.3.5 The effect of natural bio-stimulants on *O. ficus-indica* fruit size classification

None of the treatments had a significant effect on fruit size compared to the untreated control in the west block trial (Table 3.2), as calculated only at the first harvest. However, compared to the Kelpak treatment and the untreated control, SS relatively increased the number of small fruit and strongly reduced the number of large fruit. For all treatments the amount of extra large fruit was significantly lower compared to all other fruit sizes.

Table 3.2: The influence of natural bio-stimulants on the average percentage small, medium, large and extra-large fruit in the west block trial as calculated at the first harvest (see ANOVA for Table 3.2 in appendix A as well as discussion)

Treatment	Small	Medium	Large	X-Large	Between classes
	%	%	%	%	$LSD_{T(0.05)}$
CC	40.21	41.03	18.00	0.01	6.82
SS	48.14	31.29	19.39	0.02	6.82
CC+SS	34.49	40.17	24.82	0.01	6.82
Control	29.53	33.87	34.15	0.02	6.82
Kelpak	23.88	32.87	35.48	0.08	6.82
Average	35.25	35.85	26.37	0.03	
$LSD_{T(0.05)}$	20.72	16.06	24.20	1.47	

The results in Table 3.3 showed no significant differences in a specific fruit size class between the different treatments in the east block trial. However, the tendency of SS to decrease the number of large fruits as was observed in the west block trial did not repeat itself and the opposite occurred. The reason why SS had a greater average percentage large and X-large fruits in the east block compared to the west block, was probably because of the lower average number of fruits at harvest in the east block. As in the west block trial, the Kelpak treatment showed some consistency in the ratio between small, medium and large fruits produced. Although the percentage extra-large fruits were significantly lower than the rest of the classes in the east block trial it was substantially higher than in the west block trial (Table 3.2). The only other consistency between the west and east block trials was to be found in the percentage medium fruits produced and this was especially true for the CC treatment.

Table 3.3: The influence of natural bio-stimulants on the average percentage small, medium, large and extra-large fruit in the east block trial as calculated at the first harvest (see ANOVA for Table. 3.3 in appendix A as well as discussion)

Treatment	Small %	Medium %	Large %	X-Large %	Between classes $LSD_{T(0.05)}$
CC	28.10	41.27	26.76	3.87	12.25
SS	18.28	27.16	43.67	10.88	12.25
CC+SS	38.60	31.26	22.81	7.32	12.25
Control	30.69	33.89	28.86	6.57	12.25
Kelpak	27.15	30.73	40.57	1.55	12.25
Average	28.56	32.86	32.54	6.04	
$LSD_{T(0.05)}$	41.62	20.4	41.6	14.84	

3.3.6 The effect of natural bio-stimulants on the fruit length, fruit diameter and length:diameter ratio of *O. ficus-indica* as compared to fruit mass and as calculated at the first harvest

Significant differences in the average fruit length between the CC and SS treatments grouped on the one side and the CC+SS and Kelpak treatments as well as the untreated control grouped on the other side were observed in the west block trial, with the fruit

length of the former two treatments being significantly lower than that of the latter three treatments (Figure 3.5A). Almost the same tendency was observed for fruit diameter (Figure 3.5B) except that only the SS treatment resulted in a significantly lower fruit diameter compared to the rest of the treatments that did not differ at all. However, fruit length:diameter ratio calculations again grouped the CC and SS treatments together because of significant differences between these two treatments and the other three treatments (Figure 3.5C). The calculated fruit length:diameter ratio values corresponded positively with the average fruit mass (Figure 3.5D) for the different treatments although no significant differences in the average mass of a single fruit were observed between treatments.

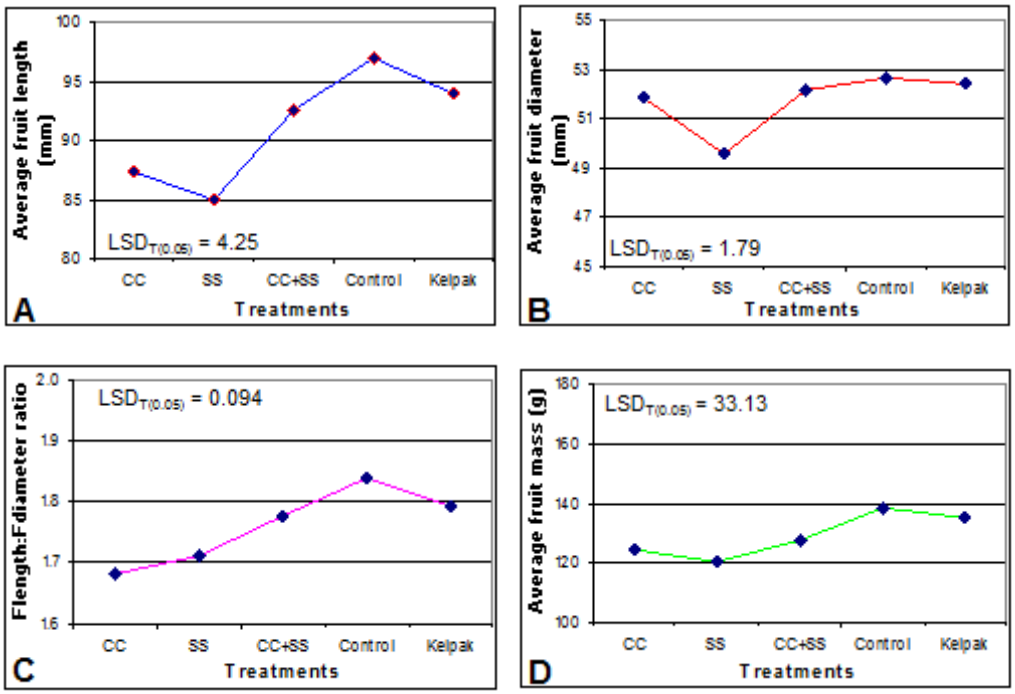

Figure 3.5 The influence of bio-stimulants on the average **A)** fruit length, **B)** fruit diameter, **C)** fruit length to fruit diameter ratio and **D)** fruit mass calculated at first harvest for each treatment in the west block trial (see ANOVA for Fig.3.5 in appendix A as well as discussion).

In comparison, the illustrated line graph patterns for fruit length (Figure 3.6A) and fruit diameter (Figure 3.6B) as well as for the fruit length:diameter ratio values (Figure 3.6C) and the average mass of a single fruit (Figure 3.6D) was almost identical in the west and east block trials except that the low values measured for the CC and SS treatments for all

of these parameters in the west block did not repeat itself in the east block. In effect, all differences were non-significant.

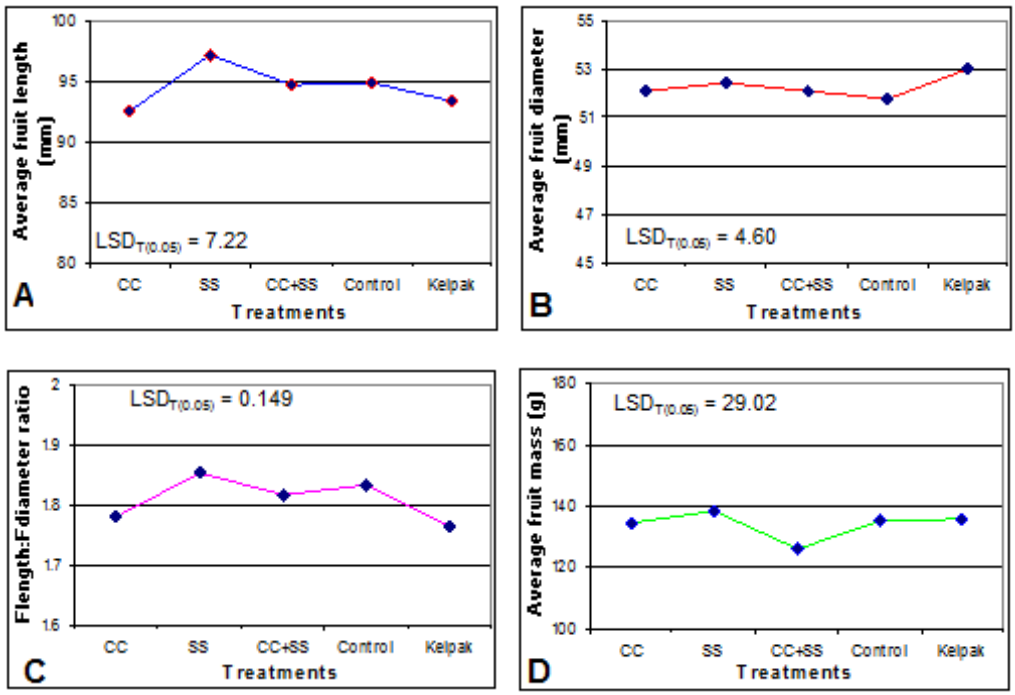

Figure 3.6 The influence of bio-stimulants on the average **A)** fruit length, **B)** fruit diameter, **C)** fruit length to fruit diameter ratio and **D)** fruit mass calculated at first harvest for each treatment in the east block trial (see ANOVA for Fig. 3.6 in appendix A as well as discussion).

3.3.7 Multiple regression and correlation analyses between fruit length, fruit diameter and fruit mass

From the possible interactions noted between fruit length, fruit diameter and fruit mass in figures 3.5 and 3.6, a linear regression equation model (Equation 3.1) was developed through multiple regression and correlation analyses of data obtained from 4000 fruits. This model showed a significant accuracy of 50.66% for the prediction of fruit mass using fruit length and fruit diameter as parameters (see discussion; Equation 3.1 and Table 3.4 in appendix A):

Equation 3.1: Fruit mass(g) = 1.9787*Fruit diameter(mm) + 1.0830*Fruit length (mm) - 71.4242

3.3.8 The effect of natural bio-stimulants on fruit mass, pulp mass, peel mass and peel thickness of *O. ficus-indica*

Compared to the untreated control, the CC treatment significantly reduced the peel mass of fruit measured during the second harvest in the west block trial while no significant differences were observed for pulp mass and fruit mass (Figure 3.7 A; LSD values indicated in the figure legend). However, this CC effect was not observed in the east block trial (Figure 3.7C) where no significant differences occurred between any of the parameters measured. Although not statistically significant, both the SS and Kelpak treatments tended to increase the peel thickness of fruit compared to the untreated controls in both the west (Figure 3.7B) and east (Figure 3.7D) block trials.

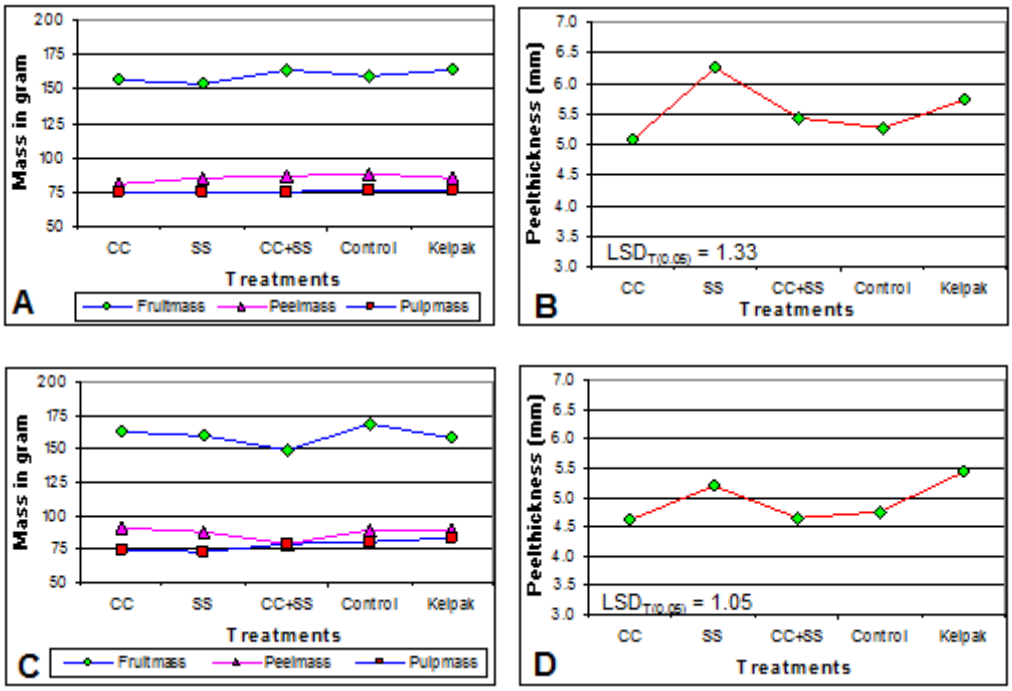

Figure 3.7 The effect of natural bio-stimulants on **A)** the average fruit mass ($LSD_{T(0.05)}$ = 19.52), pulp mass ($LSD_{T(0.05)}$ = 17.94) and peel mass ($LSD_{T(0.05)}$ = 7.54) at second harvest for the west block, **B)** average peel thickness at second harvest for the west block **C)** average fruit mass ($LSD_{T(0.05)}$ = 24.84), pulp mass ($LSD_{T(0.05)}$ = 15.39) and peel mass ($LSD_{T(0.05)}$ = 15.14) at second harvest for the east block and **D)** average peel thickness at second harvest for the east block (see ANOVA for Fig. 3.7 in appendix A as well as discussion).

3.3.9 Multiple regression and correlation analyses between fruit mass, peel mass, pulp mass and peel thickness

The data in figure 3.7 was used to perform multiple regression and correlation analyses at the 95% significance level between fruit mass, peel mass, pulp mass and peel thickness using the NCSS statistical program. From these analyses positive correlations were calculated between fruit mass and peel mass (76.03% significance at the 95% level) as well as fruit mass and pulp mass (76.97% significance) while a non-significant negative correlation was found between fruit mass and peel thickness. From the 1000 fruits used to calculate possible interactions between these parameters, fruit mass and peel mass together contributed significantly (91.92%) towards the prediction of pulp mass. A linear regression equation model (Equation 3.2) using fruit mass and peel mass for the prediction of pulp mass was developed and tested for significance (see discussion; Equation 3.2 and Table 3.5 in appendix A):

Equation 3.2: Pulp mass (g) = 1.3607+ 0.9073*Fruit mass - 0.8485*Peel mass

3.4 Discussion

When a farmer, either through irrigation, fertilization or bio-stimulants, considers manipulation of crops it is important that the pursued objectives are well defined from the onset. The implied objectives may include the improvement of either yield or quality or both. In the case of the prickly pear industry, yield improvement may further need to be defined as either an envisaged increase in fruit or young cladode production as both are used to add value by means of further processing while farmers specialize in either product and seldom in both. Opuntia fruits are consumed directly or the pulp is processed into jams, juices or alcoholic beverages while young cladodes are processed and sweetened into edible delicacies, referred to as nopalitos in Mexico. A further objective may be to enhance cladode formation in one season with the aim to increase fruit production in the following season.

In this study standard irrigation and fertilization practices were applied while the vegetative growth and yield responses of *Opuntia ficus-indica* to foliar applications of different bio-stimulants were measured as a possible means to improve either the fruit or the young cladode yield or both. The effect of SS, a prototype bio-stimulant still in the developmental phase, was tested both separately and in combination with a commercially available natural bio-stimulant, *ComCat*® (*CC*), while a natural seaweed extract trading as Kelpak® was used as a positive control. The results from both the west and east block trials indicated that foliar applications of *CC* alone contributed to a marked increase in fruit yield from about 30 kg plant^{-1} or 25 ton ha^{-1} (for the control) to about 40 kg plant^{-1} or 34.5 ton ha^{-1} when fruit fresh weight was considered directly and the fruit yield not expressed as a factor of the number of old cladodes per plant. According to Brutsch (1979), although it may differ from cultivar to cultivar, a mature plant eventually produces 30 to 70 kg of export-size fruits. The yields obtained in this study were in the range found by other researchers. Results expressed in this manner also revealed the tendency of Kelpak® as well as SS in combination with *ComCat*® (CC+SS) to contribute towards elevated fruit yield when compared to the untreated control. The latter indicated a possible synergistic effect between the active components of CC (brassinosteroids) and SS (a triglyceride) in enhancing the total fruit yield. Brassinosteroids have been shown to induce changes in plasmalemma energization, carbohydrate translocation and assimilate uptake (Arteca, 1995) that can lead to yield increases in various crops.

However, when SS was applied separately the total fruit yield (ton ha^{-1} fresh weight) was markedly lower than that of the untreated control and much lower than that obtained with the other treatments in both trials. This phenomenon is difficult to explain as little is known about the mechanism of bio-stimulatory action of SS at this stage. The active compound of SS has been identified as a triglyceride containing linoleic acid as the fatty acid moiety of the molecule. Fatty acids are intermediates of the jasmonic acid (JA) biosynthesis pathway and JA is known to elicit a variety of plant responses when applied exogenously (Staswick, 1995). These responses may include endogenous signaling towards plant growth and development. Indications are that some fatty acids can play a plant growth regulating role in crops where specific concentrations are important as

diversions from the stimulatory optimum can inhibit plant growth and development (Seigler, 1998).

Due to differences in plant size in the west and east block trials, it was necessary to also express fruit yield in terms of the number of old cladodes per plant in order to observe possible differences by comparison. This was further necessary as the standard practice of fruit thinning was followed. As could be expected, expression of fruit yield as a factor of the number of old cladodes gave a different picture than the direct illustration of total fruit fresh mass. Plants that had the least old cladodes showed the highest fruit yield as was the case for the CC and Kelpak® treatments in the west block trial. The opposite was true for the SS and CC+SS treatments where the plants that were included in the trials were larger in size and had more old cladodes. Interestingly, when fruit yield was expressed as total fresh mass and not as a factor of the number of old cladodes, the results from both the west and east block trials were similar. However, when expressed as fruit yield per number of old cladodes the results from the two trials were completely different and this can be ascribed to the differences in plant size between the two trials. In the case of prickly pear research, it seems imperative that plants included in a trial should not be selected at random but should be selected subjectively by using cladode number and general size as selection criteria in order to be able to compare results from different trials.

An important question follows namely, how does one interpret the effect of the different treatments on fruit and young cladode yield in light of the different data expression methods and in light of the fact that fruit thinning is a standard practice? Because of the difficulty to interpret yield data as a result of determining factors such as plant size and cultivation practices, results were initially expressed as total yield. However, it soon became clear that this expression method could give a distorted picture and subsequently other expression methods were employed in order to interpret results from different perspectives. Of these the most important expression method seemed to be yield per number of old cladodes as this would exclude plant size as determining factor. This method was supported by Brutsch (1979) who maintained that an increase in cladode

number seemed to be more important than an increase in cladode fertility, to obtain increased yields. The author also noted that 20 000 to 28 000 fertile cladodes with six to eight fruits per cladode (120 g each) were necessary to produce an annual fruit yield of 20 ton ha^{-1}. With this approach the SS treatment showed a statistically significant difference in the west block trial compared to the untreated control in terms of new cladode production as expressed per number of old cladodes per plant. However, probably because of the greater original plant size, this tendency was not repeated in the east block trial for the SS treatment. Interestingly, the CC+SS treatment also showed a tendency to increase the number of new cladodes and this was repeated in both trials.

If the average number of old cladodes per plant for the CC+SS treatment in both trials is taken as an example, and multiplied by the plants per hectare, a calculated average of 58 450 (64 558 for the control) cladodes per hectare had the potential to produce fruits. However, when the average number of total cladodes that could produce fruits during the following season (old plus new cladodes) is calculated for the CC+SS treatment, about 177 437 cladodes (135 363 for the control) had the potential to produce fruit before thinning practices. This example illustrates the accumulating effect this treatment could have on cladode formation (118 987 new cladodes compared to 70 804 new cladodes for the control) and indirectly fruit yield, during the following season if new cladodes are not harvested.

According to Brutsch (1979) fruit yield is a function of the number of mature cladodes, cladode fertility and management practices such as fruit and cladode thinning. The author reported that six to eight fruits per cladode have the potential to produce an annual fruit yield of 20 ton ha^{-1} and it is therefore important to try and obtain the correct number of fruit per cladode. For this reason both fruit and cladodes were counted before and after thinning and pruning and expressed per number of old cladodes in this study. By using both expression methods, it was possible to exclude differences in plant size and to observe the direct effect of treatments more clearly. When expressed per number of old cladodes, only the CC and Kelpak foliar treatments tended to increase the fruit number before thinning in both trials. After fruit thinning only Kelpak showed a tendency to

increase the number of fruit in the west block trial when expressed in the same manner. This tendency was not repeated in the east block trial, where the highest number of fruit after thinning was obtained from plants treated with CC+SS.

In the light of the different pictures emerging when different expression methods were employed, the mass of a single fruit was also measured in order to determine the relationship with other yield parameters. A clear inverted relationship was found between the average mass of a single fruit and the total number of harvested fruit after fruit thinning, as well as the average fruit yield expressed per number of old cladodes, in both trials. The lower the average mass of a single fruit the greater the number of harvested fruits after fruit thinning, as well as the average yield expressed per number of old cladodes in both trials.

In this study there was also a need to ascertain whether the different treatments had any effect on fruit size classification. From the experience of the farmer where these trials were conducted, it became clear that consumers preferred medium and large fruit to the smaller or extra large categories. For this reason the search for manipulation techniques that contribute towards a relatively constant amount of fruit in these classes is a priority. The *ComCat*® (*CC*) treatment was found to produce the highest average percentage of fruit in the medium class compared to the negative control in both the west (+21%) and east block (+22%) trials.

As no information was found in the literature with regards to the relationship between fruit diameter or fruit length with fruit yield, this aspect was investigated by means of multiple regression and correlation analyses. By applying this statistical methodology on 4000 fruits that were measured separately, a significant positive correlation between the mentioned parameters was found and a mathematical equation (model) resulted. The model showed a significant ($P<0.05$) accuracy of 50.7% for predicting fruit mass by using fruit diameter and fruit length, as parameters while deviations were not more than 5%. To test the application potential of the model from a practical perspective, ten fruits were chosen at random while fruit diameter and length values were replaced in the

equation. Calculated deviations were not more than 7% indicating that the model can be a handy tool for the prickly pear farmer to predict the average mass of a single fruit. From this spot check counts of the number of fruit per plant after thinning can supply an average that, on multiplying with the calculated mass of a single fruit, can supply an estimated fruit yield in kg plant^{-1}. Multiplication of this value with the number of plants per hectare can supply an estimated yield per hectare for the season.

Due to the importance of fruit pulp to the beverage and cosmetic industries, as well as the observation that some of the treatments had an effect on peel thickness, peel mass, fruit mass and pulp mass, the above statistical analyses were taken a step further. Pulp mass is especially important to those farmers intending to add value to their product by using the pulp only to make juices and jams. In this light multiple regression and correlation analyses were performed to test for interactions between the latter parameters. A linear equation model resulted that showed the capability of predicting pulp mass at 91.92 accuracy, using only fruit mass and peel mass as parameters. The model was tested to evaluate its application potential in predicting pulp mass. This proved to be an excellent tool as the highest deviations were found to be just more than 2% between the real values and the calculated values. Ten fruits were harvested at random and determined fruit as well as peel mass replaced in the equation. Deviations were not more than 5.5% between the real and calculated values.

In conclusion, and considering all parameters measured, the CC+SS treatment was the best general treatment from a yield perspective as it resulted in elevated cladode production when necessary (when plants were small in size) as well as elevated fruit production when plants were full grown. This treatment also contributed towards the most medium size fruits while an increase in peel thickness and a decrease in pulp mass as was seen with the SS treatment, was not observed. The two models that resulted from multiple regression and correlation analyses showed application potential for the prediction of fruit yield. Surely more research is necessary to refine these models but, in light of the fact that no such models is currently operative, its potential contribution to the prickly pear industry should be further investigated.

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CHAPTER 4

The effect of natural bio-stimulants on fruit quality in *Opuntia ficus-indica*

Abstract

The influence of a natural commercial bio-stimulant, *ComCat*®, alone and in combination with a prototype natural product (SS) still in the developmental phase, on fruit quality was investigated by means of two trials in a six-year-old *Opuntia ficus-indica* (L) Mill. orchard during the 2003/2004 growing season. Kelpak®, a commercial seaweed extract was used as a positive control. The main aim was to quantify the effect of treatments on certain fruit quality aspects. Although the tendency was not repeated in the west block trial, CC and SS significantly increased the fruit glucose content and decreased the fructose and sucrose content in the east block trial, when applied separately. Although the SS treatment tended to increase the β-carotene and water-soluble protein content in fruit, this was strongly related to fruit fresh mass. The vitamin C content in fruit, on the other hand, was not significantly affected by the different treatments and showed no correlation to fruit mass but rather to fruit size in terms of length and diameter. Four statistical models were developed, by using quantified morphological parameters, which showed a significant or near significant potential to predict a) glucose content using fruit length as parameter, b) β-carotene content using fruit diameter as parameter, c) vitamin C content using fruit diameter and fruit length as parameters and d) protein content using fruit mass as parameter. The latter models might have application potential in the prickly pear industry to predict certain outcomes, but more work is necessary to confirm their accuracy.

Keywords: *Opuntia ficus-indica*, prickly pear, sugars, β-carotene, vitamin C, water-soluble proteins, brassinosteriods, bio-stimulants

4.1 Introduction

Opuntias are part of the natural landscape and the agricultural systems of farmers in many regions of the world and contribute in various ways to sustainable farming practices

(Wessels, 1988). Irrespective of the economic potential of fruit and cladodes for food, cactus pear and their products serve various purposes in many different countries (Table 1). In this regard consumers are well aware of the nutritional aspects of the plant as well as the medicinal characteristics *O. focus-indica* possess (Barbera, 1995).

Table 4.1 Main traditional, actual and potential uses of opuntias (Barbera & Inglese, 1993, as cited by Barbera, 1995)

USAGES	PART OF PLANT AND ITS USE
Food	Fruits and fruit peel (fresh, dried, canned, frozen candied). Juice; pulp; alcoholic beverages (wine, spirits, liqueur) Jam and syrup Candies; jellies; pastries; liquid sweetener. Seed oil. Stems (fresh, precooked, frozen, jam and candies
Forage	Stems, fruits, seeds. Fodder
Energy	Biogas (stems, fruits); ethanol (stems, fruits); firewood.
Medicine	Diarrhoea (stems); diuretic (flowers, roots); amoebic dysentery (flowers); Diabetes mellitus (stems); hyperlypidemy (stems); obesity (fibers) Anti-inflammatory (stems).
Cosmetic	Shampoo; soaps; astringent and body lotions (stems).
Agronomic	Soil production; hedges and fences; mulching; windbreak (plants, stems); Organic manure.
Other	Adhesives and glues; pectin; fibers for handcrafts; paper (stems). Dyes (fruits, rearing of *Dactylopius coccus* on stems); mucillages for food Industry (stems); ornamental.

From a nutritional perspective, *O. ficus-indica* possesses inherent qualities in terms of the sugar content in fruit. Carbohydrates are the most abundant biological molecules in nature and are responsible for the sweet taste of fruits (Starr & Taggart, 1995). According to the authors all cells utilize carbohydrates either as structural materials or in stored forms as transportable packets of energy. The majority of sugars in the fruits of *O. ficus-indica* are of the reducing type of which approximately 53% is in the form of glucose and the remainder in the form of fructose (Sawaya *et al*., 1983; Russel & Felker, 1987). Glucose is directly absorbable by the human body and is the sole energetic metabolite utilized by brain and nerve cells. Fructose is also easily absorbed by the human body and contributes to fruit flavour on account of it's greater sweetness compared to that of glucose and sucrose (Cheftel *et al*., 1983; as cited by Saenz-Hernandez, 1995).

Carotenoids are among the most important natural pigments found in plants, due to various functions, and are present in the cactus pear in rather large quantities (Gross, 1987). Functions of carotenoids in plants include protection of the chloroplasts against excessive radiation and harmful photochemical reactions by preventing chlorophyll to remain in the triplet state as well as its free radical scavenging role by quenching excessive energy or singlet oxygen (Britton, 1976). Importantly, carotenoids act as a vitamin A precursor making it the pro-vitamin with the highest activity in man. Vitamin A activity depends on the amount and nature of the active carotenoids as well as its stability, digestibility and state of isomerization to prevent vitamin A deficiency in man (Britton, 1976). In addition carotenoids such as lycopene have been shown to have a preventive action against degenerative disorders like prostate and lung cancers (Bruneton, 1995). According to Britton (1976), β-carotene and lycopene are two of the most active anti-oxidants quenching free radicals and preventing membrane damage.

Vitamin C is another important compound found in cactus pear at concentrations exceeding that found in apples, pears, grapes and bananas (Cheftel *et al.*, 1983; as cited by Saenz-Hernandez, 1995). According to Pimienta (1990), the vitamin C concentration in cactus pear fruit pulp may be as high as 41 mg 100 g^{-1} fruit pulp (as cited by Nerd &

Mizrahi, 1995). Also, protein concentrations in cactus pear were found to be similar to those found in other fruits, while the total content of free amino acids (257.24 mg 100 g^{-1}) was greater than the average of other fruits (Askar and El-Samahy, 1981; as cited by Saenz-Hernandez, 1995). Sawaya *et al.* (1983) reported that cactus pear fruit contained about 0.21g proteins per 100 g fruit pulp. Galizzi *et al*. (2004) observed that protein content in cactus pear fruit was not correlated to fruit yield, as the content remained constant even when the yield was increased by means of improved management practices.

An elevated interest in expanding the cultivation of *O. ficus-indica* due to its nutritional and health attributes has been shown over the last two decades. The fact that this plant may be grown in areas unsuitable for other crops is of prime importance and its uses can contribute to the existence of people in these regions (Saenz-Hernandez, 1995). In this light the search for ways and means to increase the yield as well as the nutritional and health properties of the prickly pear fruit and increase the economical income of the farmer, must be seen as a priority. Products are currently available on the market that have the potential to manipulate plants either hormonally or by other chemical mechanisms, that makes it possible to obtain the latter. These include bio-stimulatory products such as ComCat® (Agraforum, Germany) and Kelpak® (Qwemico, South Africa). Current research at the University of the Free State revealed the presence of triglycerides in seeds of specific plants that show similar bio-stimulatory activities (Van der Watt, 2004; personal communication). In all cases enhancement of both yield and quality in different crops is claimed by the producers of *ComCat*® and Kelpak®, as well as the researchers in the case of the triglycerides. The aim of this study was, therefore, to quantify the extent to which treatment with the mentioned bio-stimulants could increase some specific quality aspects of *O. ficus-indica* fruit.

4.2. Materials and methods

4.2.1 Experimental layout, treatments and materials

For experimental layout and treatments used during the trials, see chapter 3. All materials and chemicals used for the quality assessment of fruit were purchased either from Sigma (Germany) or Merck (Germany) and were of the purest quality available.

4.2.2 Orchard practices

See Chapter 3.

4.2.3 Quantification of the effects of bio-stimulants on fruit quality

4.2.3.1 Carbohydrate sugar levels

The content of two monosaccharide (glucose and fructose) and one oligosaccharide (sucrose) sugar was determined in the fruit pulp after extraction with 80% ethanol and applying the Boehringer Mannheim / R-Biopharm enzymatic technique.

4.2.3.1.1 Extraction procedure for carbohydrates

One fruit from each replicate for each treatment was harvested during first harvest and the fruit mass measured. Eight grams of fresh pulp was removed from each fruit by separating it from the seed and subsequently placed it in a test-tube and covered with 16 ml 80% ethanol. The ethanol was pre-heated to 80°C in a water bath for 15 minutes in order to stop all enzyme reactions. Ethanol that evaporated in the process was replaced and the original 16 ml volume restored.

Subsequently, the fruit pulp was homogenized in a known volume of 80% ethanol pre-heated to 80°C, in a mortar with a pestle and centrifuged at 1200 rpm for 10 minutes at 25°C. To get rid of the ethanol, a 1 ml aliquot of each replicate was transferred to an Eppendorf vial and heated overnight at 70°C in an oven. One milliliter of distilled water was added to each Eppendorf vial to replace the original ethanol volume and to dissolve

the sugars. A 50 µl aliquot of a four times diluted sample for each replicate was taken to determine the sucrose, D-glucose and D-fructose content.

4.2.3.1.2 Determination of the sugar content: Principle of the Boehringer Mannheim enzymatic procedure

The methodology outlined by Boehringer Mannheim (Cat. Nr. 10716 260 035) after Bergmeyer and Brent (1974) was used to determine the sugar content. The D-glucose concentration was determined before and after the enzymatic hydrolysis of sucrose. D-fructose was determined subsequently to the determination of D-glucose.

Determination of D-glucose before inversion:

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

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

In the presence of *glucose-6-phosphate dehydrogenase* (G-6-PDH) the glucose-6-phosphate (G-6-P) formed is specifically oxidized by NADP to gluconate-6-phosphate with the formation of NADPH [2]:

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

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

Determination of D-Fructose:

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

$$\text{D-Fructose} + \text{ATP} \xrightarrow{HK} \text{F-6-P} + \text{ADP} \qquad [3]$$

4.2.3.1.3 Calculations of sucrose, D-glucose and D-fructose concentrations

The reducing sugar contents of fruit pulp extracts were calculated according to the method of the suppliers of the test kits (Boehringer Mannheim, 2004).

4.2.3.2 β-carotene and lycopene

β-carotene and lycopene were determined by means of High Pressure Liquid Chromatography (HPLC) separation using external standards. The extraction procedure followed for both lycopene and β-carotene were according to the method described by Sadler *et al*. (1990).

4.2.3.2.1 Chromatography

A Waters high-performance liquid chromatograph (HPLC) with a Waters 490 E detector was used for lycopene and β-carotene measurements. Isocratic separation was achieved on a Phenomenex C-18 (5µ) column (4.6 mm x 25 cm). The mobile phase used was methanol:THF:water (67:27:6), with a flow rate of 2.5 ml/min and a sample injection volume of 50 µl. Detection was done at 435 nm, the absorption maximum for β-carotene in the mobile phase. Run time of the HPLC was 30 minutes at ambient temperature.

4.2.3.2.2 Preparation of standards

An external β-carotene standard was used and obtained from Sigma Chemical Co, Germany. A stock solution for β-carotene was prepared by dissolving 0.022 g of the

compound into 25 ml tetrahydrofuran. The standard was stored at -20°C, protected from light by covering the vial with aluminium foil and protected from oxidation by passing nitrogen gas through the solution after it was opened. Before the standard was injected into the HPLC, 10 µl of standard was taken and diluted with 990 µL tetrahydrofyran (THF). Fifty microliter of the diluted standard, with a concentration of 4.4×10^{-7} g β-carotene, was injected six times into the HPLC and the average calculated (see Figure 4.10A in appendix B). It was found that 4.4×10^{-7} g β-carotene was equal to a peak area of 1563.2533. Peak area was used to quantify the β-carotene concentration. No lycopene data is supplied as lycopene was not detected in freshly harvested fruit of the cactus pear cultivar under scrutiny during the 2004 season. However, overripe fruit harvested at the end of the 2003 growing season and used to calibrate the HPLC, contained lycopene (see Figure 4.9A and 4.9B in appendix B).

4.2.3.2.3 Sample preparation

One fruit from each replicate for every treatment was taken during first harvest and its fruit mass determined. Subsequently, the fruit pulp was separated from the seeds, a 6 g sample sliced with a sharp knife into a fine mush and transferred to a 100 ml glass bottle. The pulp was covered with 25 ml of a hexane-acetone-ethanol (50:25:25) mixture and nitrogen gas was immediately bubbled through the mixture to limit oxidation. The bottle was sealed with a stopper and shaken mechanically for ten minutes (Sadler *et al*., 1990). After ten minutes 15 ml of distilled water was added and the mixture shaken for an additional five minutes. The crude pulp extract was separated into two distinct polar and non-polar layers. The upper hexane layer contained the β-carotene and 50 µl was injected into the HPLC without further treatment (see Figure 4.10B in appendix B).

4.2.3.3 Vitamin C

Vitamin C (ascorbic acid) content in the pulp was measured by means of HPLC. The extraction procedure followed for vitamin C was according to the method described by

the Association of Official Analytical Chemists (AOAC) (1970), with slight modification where the titration method was replaced with a HPLC method.

4.2.3.3.1 Chromatography

A Volkswagen-Stiftung LC-10AT(VP) Shimadzu HPLC with a SPD-10A(VP) Shimadzu UV-Vis detector was used for vitamin C measurement. A Shimadzu C-R6A Chromatopac printer was used to print the graphs. Isocratic separation was achieved on a Phenomenex Synergi (4µ) Hydro-RP 80A (250 mm x 4.60 mm) column. The mobile phase was 0.05 M KH_2PO_4 at a pH of 2.5. The flow rate was 0.8 ml min^{-1} and the injected sample volume was 10 µl. Routine detection was done at 265 nm, at a sensitivity of 0.005 AUFS. The running time was 20 minutes at ambient temperature.

4.2.3.3.2 Preparation of standards

The external vitamin C standard used was obtained from Merck Chemicals, S.A. A stock solution was prepared by dissolving 1 mg of the standard in 100 ml distilled water. The standard was stored at -20°C, protected from light by covering the vial with aluminium foil while nitrogen gas was bubbled through the solution every time after opening in order to minimize oxidation. Ten micro liters containing 1 x 10^{-4} mg vitamin C, was injected into the HPLC several times and the average area was calculated as 3188220 (see Figure 4.11A in appendix B).

4.2.3.3.3 Sample preparation

One fruit from each replicate for every treatment was taken during first harvest and its fruit and pulp mass measured. Subsequently, the fruit pulp was homogenized in a Waring blender, the volume measured using a measuring cylinder and centrifuged for one minute. The homogenized fruit pulp separated in three distinct layers with mainly seeds forming the bottom layer. A clear green fluid formed the middle layer while mainly waxes formed the top layer. Only the middle layer was removed by means of a Gilson

pipette, the volume measured again and syringed through a 0.45 micron filter into a 1 ml Eppendorf vial that was stored at 0°C in a dark place before 10 µl was injected into the HPLC (see Figure 4.11B in appendix B).

4.2.3.4 Water-soluble proteins

The total water-soluble proteins were determined in the fruit pulp, using the Biorad method with γ-globulin as standard (Bradford, 1976). A Biorad micro plate reader (spectrophotometer) was used to determine the protein content in the fruit at 595 nm.

4.2.3.4.1 Extraction of protein from fruit pulp

One fruit from each replicate per treatment was taken during first harvest and it's mass determined. Six grams of pulp were homogenized in 24 ml extraction buffer containing 12.5 mM Tris HCl, 2 mM EDTA, 10 mM Mercapto-ethanol (added just before use) and 2 mM PMSF (made up beforehand in 1 ml ethanol). One milliliter aliquots were subsequently transferred to Eppendorf vials and sentrifuged at 12 000 rpm for 10 minutes at room temperature. The supernatant was transferred to clean Eppendorf vials and kept on ice until protein determinations commenced. Ten microliter aliquots of the supernatant from each replicate were used for protein determinations.

4.2.3.4.2 Determination of the protein content in the fruit pulp

An Elisa-plate was prepared with four replicates for each of the blank (water), the standard (0.4 µg μl^{-1}) and the treatments. Biorad reagent was added last and the contents stirred for five minutes before the absorbance was read at 595 nm using a micro-plate reader.

4.2.3.4.3 Calculations for protein content in total enzyme extract

The following equation was used for calculating the water-soluble protein content in the total volume of the pulp extract:

Equation 4: water-soluble protein content in the total volume of the pulp extract =

$$\frac{\text{OD reading per replicate}}{\text{OD reading for blank}} \times \frac{\text{0.4 µg standard}}{1000} \times \frac{\text{total volume extract (µl)}}{\text{10 µl aliquot}}$$

= mg protein x 1000

= µg protein in total volume extract

4.2.3.5 Statistical analysis

All data presented were means of five replicates. Data were subjected to analysis of variance using the NCSS 2000 statistical program and means were compared using the Tukey-Kramer Multiple-Comparison Test at $p<0.05$. Differences at $P<0.05$ were considered significant. Multiple regression and correlation tests were performed with the same statistical program and tested at both the 90% ($P<0.10$) and 95% ($P<0.05$) significance level in order to ascertain relationships between parameters.

4.3 Results

4.3.1 The effect of natural bio-stimulants on the glucose, fructose and sucrose content in the fruit pulp of *O. ficus-indica*

Although no statistically significant differences were observed between treatments in terms of the glucose content (Figure 4.1A) in the fruit pulp, it was at least 40-fold higher than that of fructose (Figure 4.1B) and sucrose (Figure 4.1C) in the west block trial. The fructose content was second highest in the fruit pulp and the CC+SS treatment contributed to a statistically significant elevation compared to the rest of the treatments (Figure 4.1B). The sucrose content in the pulp of fruit harvested from the untreated control was significantly higher compared to that of fruit harvested from all of the other treated plots, except to that for CC+SS (Figure 4.1C). No relationship between the sugar

concentration in fruit pulp and the yield parameters fruit mass, fruit length and fruit diameter (Figure 4.1D) was observed.

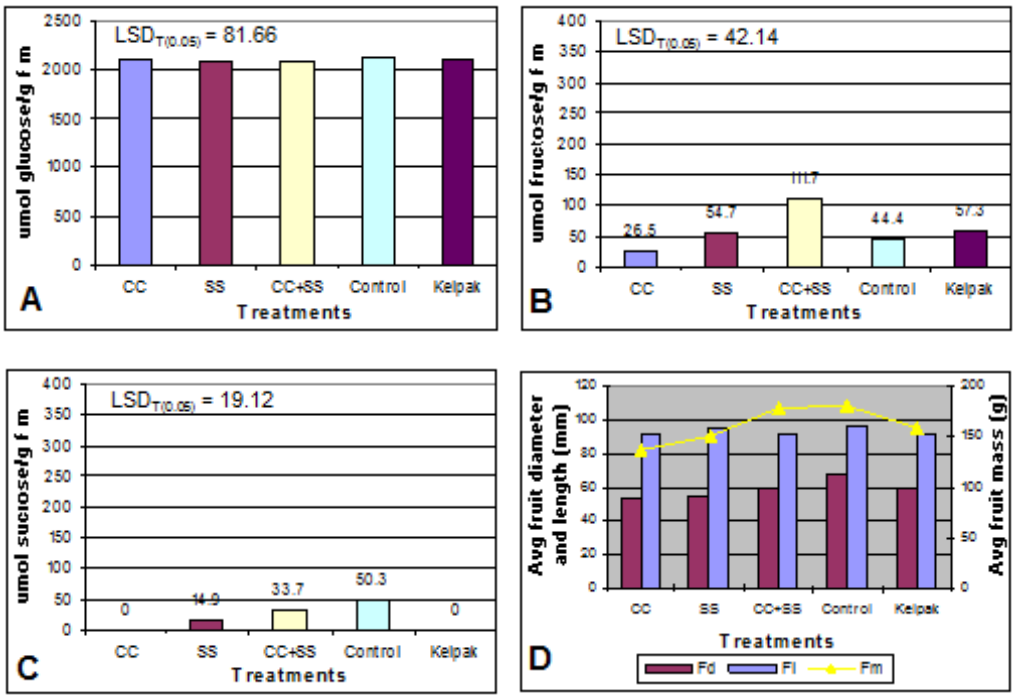

Figure 4.1: The effect of natural bio-stimulants on the average **A**) glucose, **B**) fructose and **C**) sucrose content in the fruit pulp of *O. ficus-indica* cultivated in the west block trial. The average diameter (Fd), length (Fl) and mass (Fm) of fruit used to extract the sugars are supplied in graph **D** (no statistical data as the information is only supplied to ascertain possible relationships between the sugar content in fruit pulp and the yield parameters fruit mass, fruit diameter and fruit length, see ANOVA for Fig. 4.1 in appendix B as well as discussion).

In the east block trial the glucose content measured in the fruit pulp of CC and SS treated plants was significantly higher compared to that of the untreated control as well as the combination (CC+SS) and the Kelpak treatments (Figure 4.2A). Interestingly, fruit pulp from treatments with the lowest glucose content showed a significantly higher fructose content (Figure 4.2B). Although not in exactly the same pattern, the latter was also observed in the west block trial (Figure 4.1B). Again no significant differences between treatments were observed in terms of the sucrose content in the fruit pulp but the sucrose was far less abundant compared to the two monosaccharides as was the case in the west

block trial. Again no interactions between sugar content and the yield parameters fruit mass, fruit length and fruit diameter were observed (Figure 4.2D).

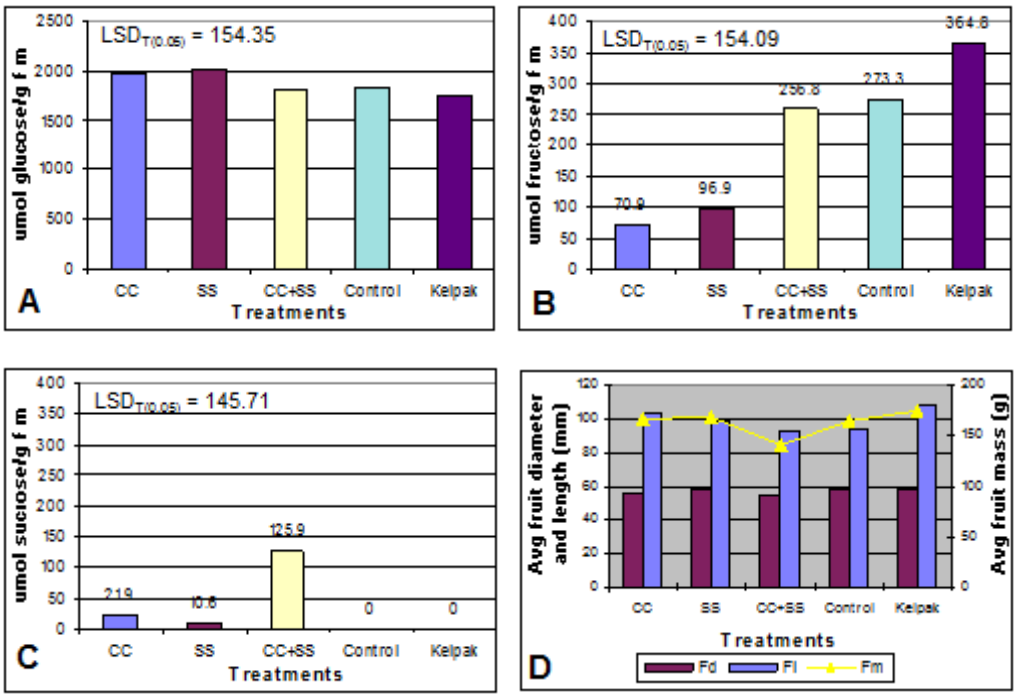

Figure 4.2 The effect of natural bio-stimulants on the average **A**) glucose, **B**) fructose and **C**) sucrose content in the fruit pulp of *O. ficus-indica* cultivated in the east block trial. The average diameter (Fd), length (Fl) and mass (Fm) of fruit used to extract the sugars are supplied in graph **D**. (no statistical data as the information is only supplied to ascertain possible relationships between the sugar content in fruit pulp and the yield parameters fruit mass, fruit diameter and fruit length, see ANOVA for Fig. 4.2 in appendix B as well as discussion).

4.3.2 Multiple regression and correlation analyses between the glucose content in fruit pulp and the length, diameter and mass of whole fruit

From the data presented in Figures 4.1 and 4.2 a linear regression equation model (Equation 4.1) was developed through multiple regression and correlation analyses to assess the possible interactions between the glucose content in fruit pulp and the length, diameter and mass of the fruit from which the sugars were extracted.

Equation 4.1: µmol glucose per g fresh mass^{-1} = 2525.774 - 5.5520 * Fruit length

No correlation between the glucose content and both fruit diameter and fruit mass was observed. However, although a non-significant negative correlation between fruit length and glucose content was calculated at the 95% probability level (0.0710) it was significant at the 90% probability level. For this reason only fruit length was included into the linear equation model (equation 4.1) for predicting the glucose content per gram fresh pulp mass (see discussion and linear equation model 4.1 in appendix B).

4.3.3 The effect of natural bio-stimulants on the β-carotene content in the pulp of *O. ficus-indica* fruit

No significant differences between treatments were noted in terms of the β-carotene content in fruit pulp, but CC and SS treatments tended to contribute to an increase of 21% and 16% respectively compared to the untreated control (Figure 4.3A). Interestingly, the highest β-carotene content was measured in pulp obtained from fruit with the lowest fresh mass, which incidentally was fruit harvested from CC and SS treated plants (Figure 4.3B). The opposite was true for the other two treatments as well as the untreated control where the higher and very similar fruit fresh mass coincided with the lower and very similar β-carotene content. The latter relationship is clearly illustrated by the β-carotene:100 g fruit mass ratio (Figure 4.3D) although a constant fresh mass of 6 g pulp was used to extract the β-carotene. Further, almost the same relationship between β-carotene content and fruit length and diameter was observed (Figure 4.3C) where the β-carotene content in fruit pulp was lowest when the fruit length and diameter were the greatest (compare figures 4.3A and 4.3C).

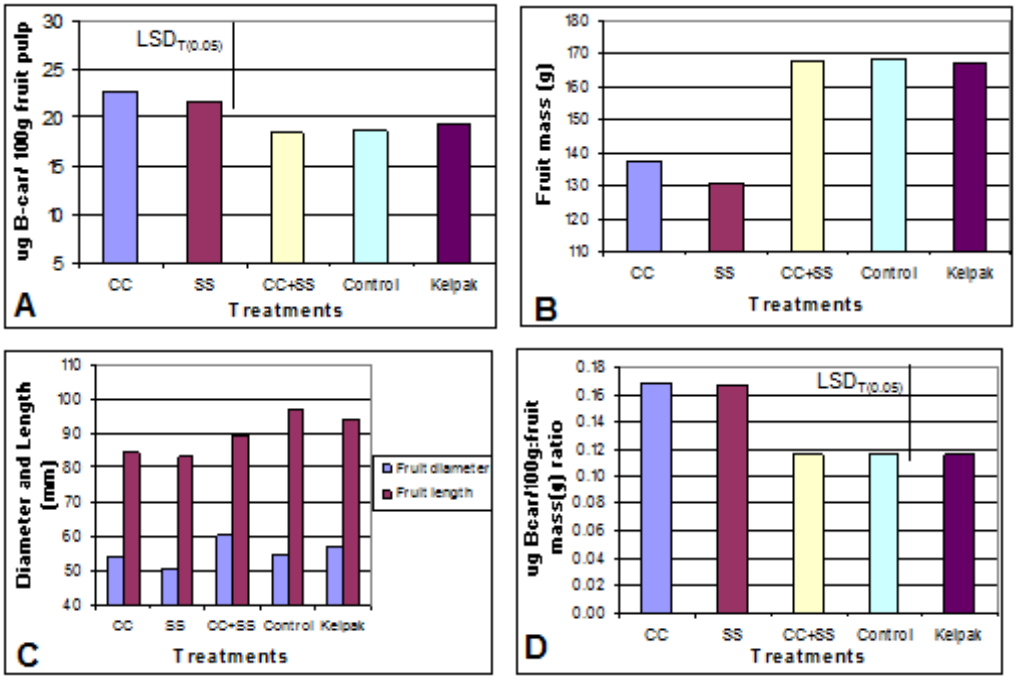

Figure 4.3: The influence of natural bio-stimulants on the average **A**) β-carotene content of fruit pulp, **B**) fruit mass, **C**) fruit diameter and fruit length and **D**) the β-carotene:fruit mass ratio in the west block trial (see ANOVA for Fig. 4.3 in appendix B as well as discussion).

Although not as distinct as in the west block trial, the same relationships between β-carotene content in the fruit pulp (Figure 4.4A) and fruit mass (Figure 4.4B and 4.4D), as well as fruit length and diameter (Figure 4.4C) was observed in the east block trial. Further, compared to the untreated control, the tendency of CC and SS treatments to enhance the β-carotene content in the fruit pulp (Figure 4.4A) was confirmed in the east block trial.

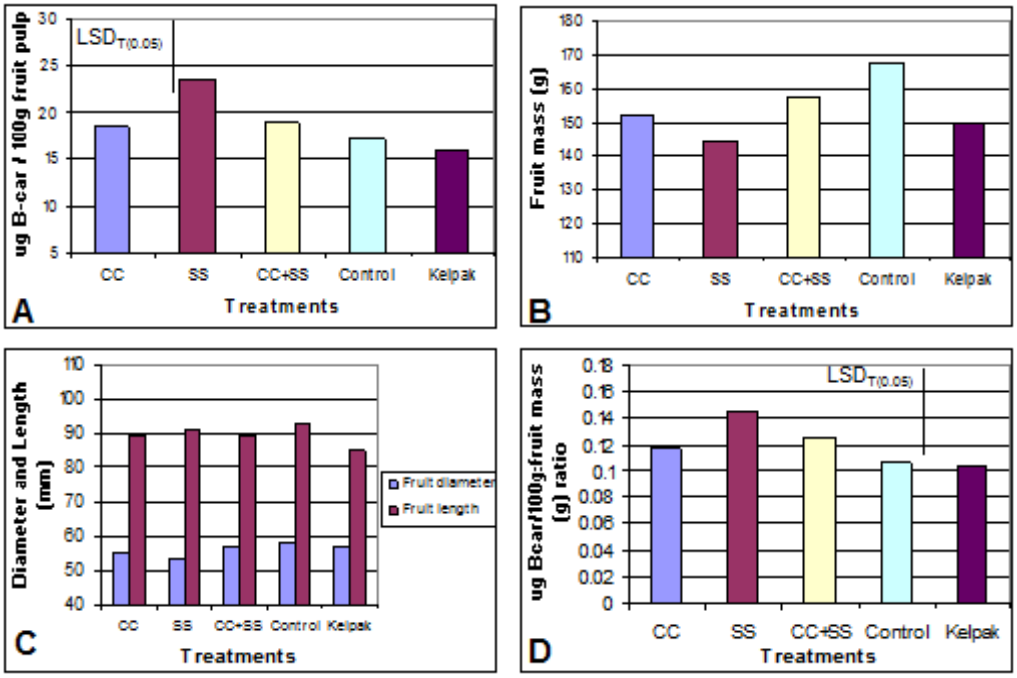

Figure 4.4: The influence of natural bio-stimulants on the average **A)** β-carotene content in fruit pulp, **B**) fruit mass, **C**) fruit diameter and fruit length and **D**) the β-carotene:fruit mass ratio in the east block trial (see ANOVA for Fig. 4.4 in appendix B as well as discussion).

4.3.4 Multiple regression and correlation analyses between the β-carotene content in fruit pulp and the length, diameter and mass of whole fruit

From the possible interactions noted between the β-carotene content in fruit pulp and fruit length, fruit diameter and fruit mass in Figures 4.3 and 4.4, a linear regression equation model (Equation 4.2) was developed through multiple regression and correlation analyses of data obtained from one fruit per replicate. The importance of this model was to ascertain whether the differences in β-carotene content observed between treatments were due to the treatments per se or the differences in morphological data obtained for fruit mass, length and diameter. In other words, to ascertain whether the treatments had a direct effect on the β-carotene content in fruit pulp or whether the effects were indirectly via treatment effects on fruit growth.

Equation 4.2: µg β-carotene $100g^{-1}$ fruit pulp = 31.9535 - 0.2291* Fruit diameter

Although the relationships discussed under section 4.3.3 were clearly visible on interpretation of the illustrated graphs, non-significant correlations between the β-carotene content in fruit pulp and fruit mass, length and diameter were calculated by means of multiple regression and correlation analyses at the 95% probability level ($P<0.05$). However, at the 90% probability level ($P<0.1$) a negative correlation (-0.0687) between fruit diameter and β-carotene content was calculated. Therefore, only fruit diameter was included in the linear equation model (4.2) for the prediction of β-carotene in 100 g fruit pulp (see discussion and linear equation model 4.2 in appendix B).

4.3.5 The effect of natural bio-stimulants on the vitamin C content in the pulp of *O. ficus-indica* fruit

Although no significant differences in the vitamin C content of fruit pulp were observed between the different treatments (Figure 4.5A), the CC, SS and Kelpak treatments contributed to slightly higher vitamin C contents compared to the untreated control in the west block trial. No relationship between the vitamin C content and either fruit mass or pulp mass was observed (Figure 4.5B). However, comparing the line graph patterns of vitamin C content (Figure 4.5A) and fruit length (Figure 4.5C) a converse relationship seemed apparent. The lower the vitamin C content in the pulp the greater the fruit length and vice versa.

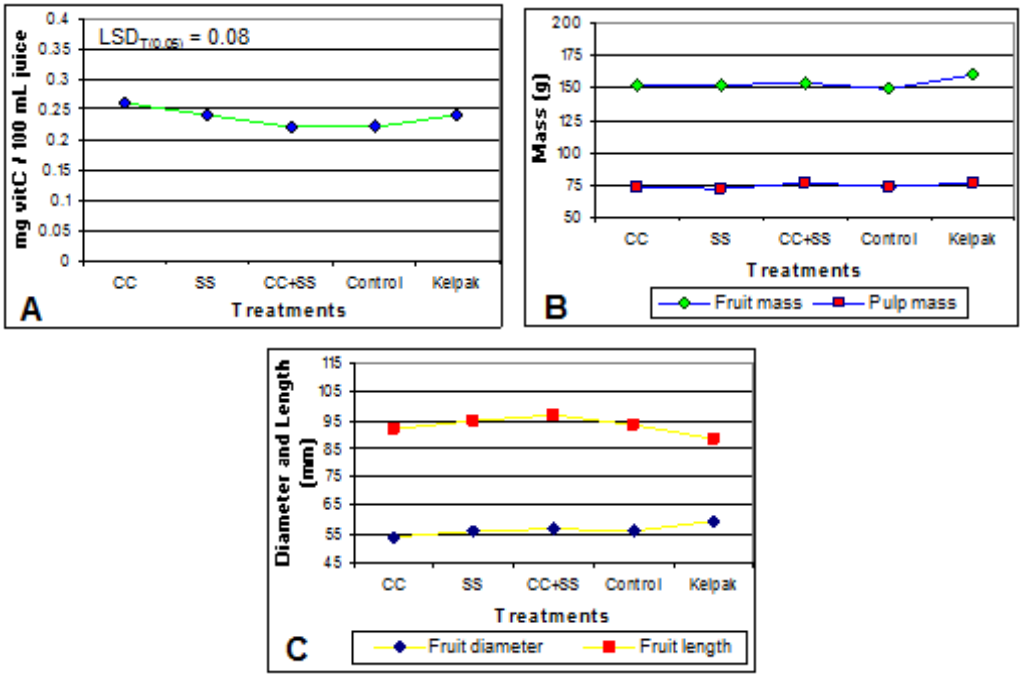

Figure 4.5: The influence of natural bio-stimulants on the average **A**) vitamin C content in fruit pulp, **B**) fruit mass and pulp mass and **C**) fruit diameter and length in the west block trial (see ANOVA for Fig. 4.5 in appendix B as well as discussion).

In the east block trial (Figure 4.6A) results were rather erratic in terms of the vitamin C content in fruit pulp and no significant differences were observed. However, although not as distinct, the converse relationship between vitamin C content in the pulp and fruit length (Figure 4.6C), as was observed in the west block trial, seemed to repeat itself in the east block trial if the line graph patterns are compared. Again no significant differences between treatments in terms of fruit mass and pulp mass (Figure 4.6C) were observed.

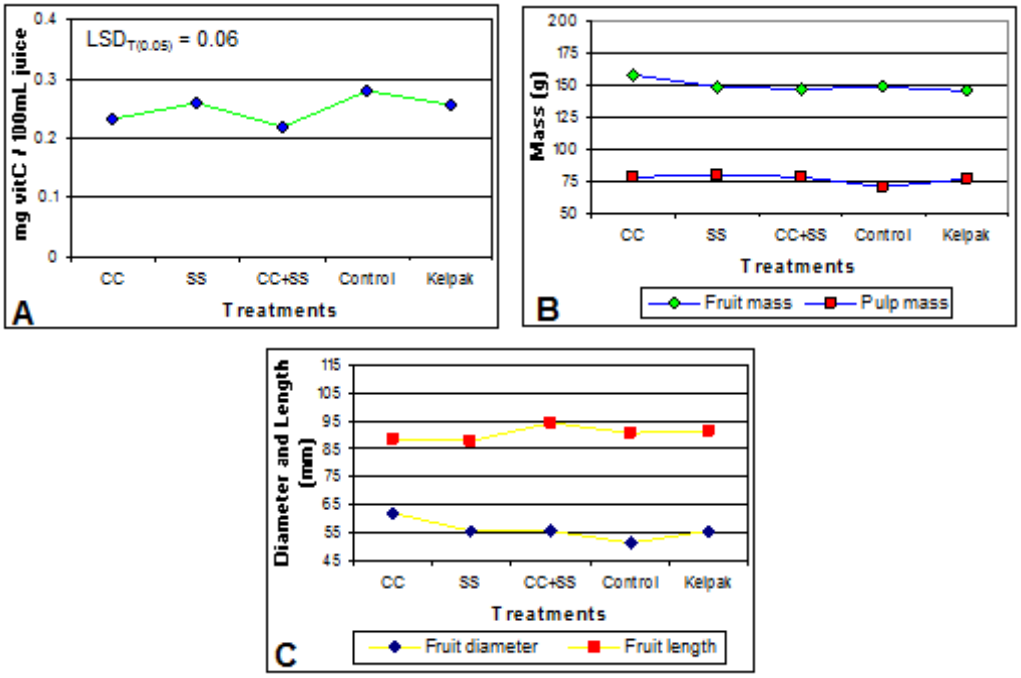

Figure 4.6: The influence of natural bio-stimulants on the average **A)** vitamin C content in the fruit pulp, **B**) fruit and pulp mass and **C**) fruit diameter and fruit length in the east block trial (see ANOVA for Fig. 4.6 in appendix B as well as discussion).

4.3.6 Multiple regression and correlation analyses between the vitamin C content in fruit pulp and the length, diameter and mass of whole fruit

From the interactions noted above, multiple regression and correlation analyses indicated a significant negative correlation between fruit diameter and vitamin C content. Although a non-significant negative correlation was calculated between fruit length and vitamin C content, this was at the 95% probability level. However, the latter was significant at the 90% probability level and therefore included in the linear equation model (see discussion and linear equation model 4.3 in appendix B):

Equation 4.3: mg vitamin C $100g^{-1}$ fruit pulp = 0.4545 - 2.0588E-03 * Fruit diameter – 1.0634E-03 * Fruit length

4.3.7 The effect of natural bio-stimulants on the water-soluble protein content in the fruit pulp of *O. ficus-indica*

The SS and Kelpak treatments contributed to a higher and the CC treatment to a much lower protein content in the fruit pulp compared to the untreated control (Figure 4.7A) while an inverse relationship between fruit mass and protein content was again observed in the west block trial (Figure 4.7B). The higher the fruit mass the lower the pulp protein content. Although not as distinctly, the same relationship between protein content and fruit diameter as well as fruit length was observed (Figure 4.7C). The greater the fruit diameter and fruit length the lower the protein content, although only 6 g fruit pulp was extracted for treatments. Protein:fruit mass ratio calculations were higher for all treatments compared to the untreated control but differences were not statistically significant (Figure 4.7D).

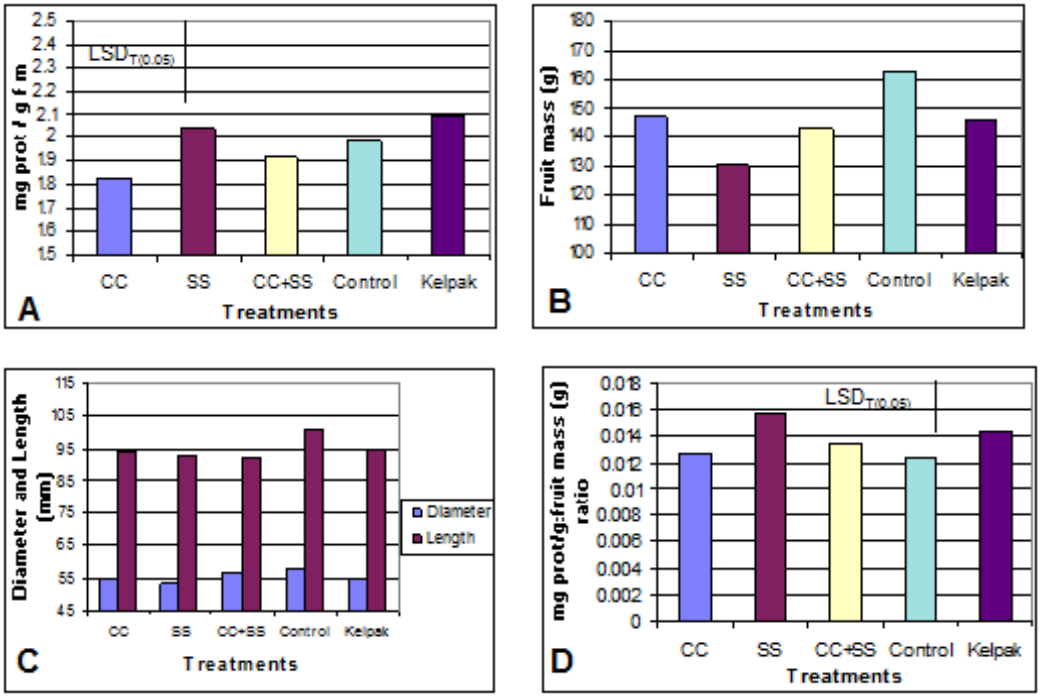

Figure 4.7: The influence of natural bio-stimulants on the average **A**) total soluble protein content in fresh fruit pulp, **B**) fruit mass, **C**) fruit diameter and fruit length and **D)** protein content:fruit mass ratio in the west block trial (see ANOVA for Fig. 4.7 in appendix B as well as discussion).

In the east block trial the tendency of the Kelpak treatment to contribute to a higher and the CC treatment to significantly lower protein content compared to the control in the fruit pulp was repeated but this was not observed for the SS treatment, as was the case in the west block trial (Figure 4.8A). Further, the same inverse relationship between protein content and morphological parameters as was seen in the west block trial was also observed in the east block (Figures 4.8B and 4.8C). No significant differences between treatments in terms of the protein:fruit mass ratio was observed in the east block trial (Figure 4.8D).

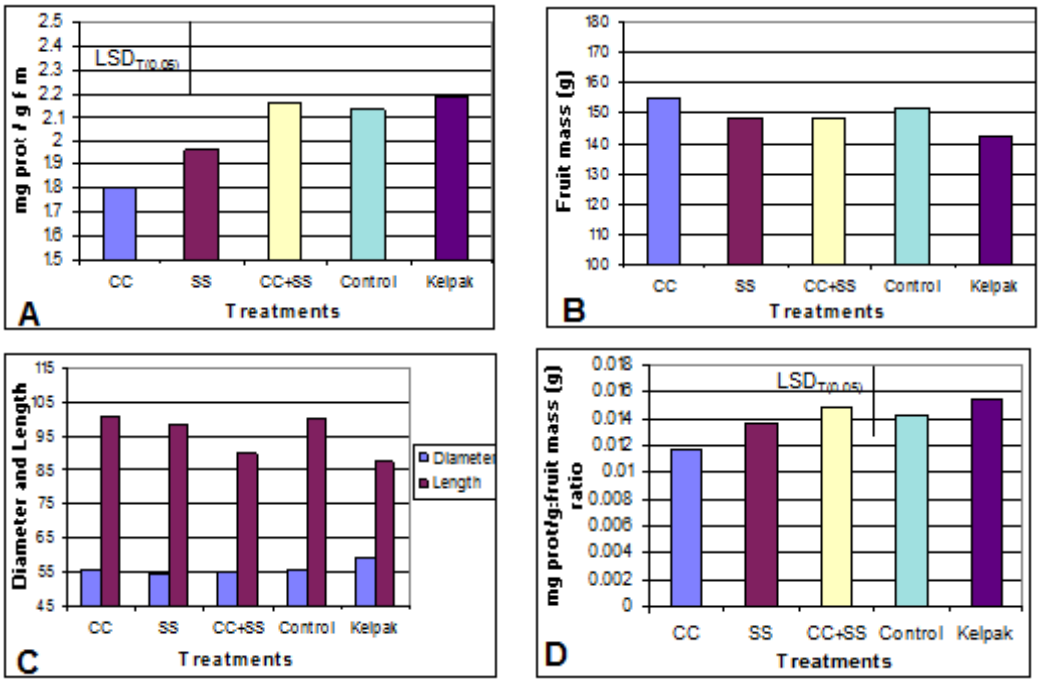

Figure 4.8: The influence of natural bio-stimulants on the average **A**) total water soluble protein content in fresh fruit pulp, **B**) fruit mass, **C**) fruit diameter and fruit length and **D)** protein:fruit mass ratio in the east block (see ANOVA for Fig. 4.8 in appendix B as well as discussion).

4.3.8 Multiple regression and correlation analyses between the water-soluble protein content in fruit pulp and the length, diameter and mass of whole fruit

Multiple regression and correlation analyses revealed a non-significant relationship between protein content in fruit pulp and both fruit diameter and fruit length but a

significant negative correlation with fruit mass. A linear equation model (Equation 4.4) was subsequently developed using only fruit mass as a parameter (see discussion and linear equation model 4.4 in appendix B):

Equation 4.4: mg protein g^{-1} fresh pulp mass = 2.5013 - 3.3376E-03 * Fruit mass

4.4 Discussion

When yield improvement strategies on any crop are pursued, it is important to also quantify possible effects on the quality of harvestable parts, as yield improvement concomitant with a decline in quality might be contra productive. In this study the potential of improving both fruit and cladode yield in *O. ficus-indica* by foliar applications of natural bio-stimulants was investigated while standard cultivation practices were followed. Additionally, the possible influence of exogenously applied bio-stimulants on fruit quality was measured. These quality parameters included sugar, β-carotene, vitamin C and total water-soluble protein content in fruit pulp.

Soluble carbohydrates contribute to the taste and nutritional value of prickly pear fruit. Sweetness is an important determining factor from a consumer perspective. Glucose, fructose and sucrose content were quantified as each sugar specifically contributes towards fruit quality. According to Cheftel *et al.* 1983 (as cited by Saenz-Hernandez, 1995) glucose is the sole energetic metabolite in the brain and nerve cells of humans and is easily absorbed by the human body. Further, according to the authors, fructose contributes to fruit flavour and taste on accounts for greater sweetness compared to glucose and sucrose.

Glucose was detected in the fruit pulp at much higher amounts than fructose and sucrose. This corresponded with the findings of Sawaya *et al.* (1983) as well as Russel and Felker (1987) indicating that the majority of sugars in the cactus pear fruit were of the reducing type namely glucose. Although significant differences between treatments were statistically calculated for all three sugars, the results obtained from the west and east

block trials were not exactly the same. However, a similar tendency observed in both trials was a much higher content of the monosaccharide reducing sugar glucose, compared to fructose, and an extremely low content of the non-reducing sugar sucrose. Further, multiple regression and correlation analyses of the results from both trials revealed that fruit length significantly ($P<0.1$) contributed to the prediction of glucose content. The resulting linear equation model was tested using the results from ten randomly picked fruit and the calculated percentage deviation was as low as 0.85% in some replicates confirming a rather high prediction accuracy of sugar content by using a linear model developed from morphological data. It seems that especially fruit size is correlated with glucose content and this might be explained by increased sucrose translocation from storage organs as fruit swell during the fruit filling stage. Glucose being the predominant monosaccharide is probably genetically determined.

Currently the anti-oxidant content in fruit is quite an issue in the media due to alleged health benefits for humans and this has also become a marketing tool. In this light two anti-oxidants namely lycopene and β-carotene were included as additional quality parameters in this study. Although lycopene was detected in overripe fruit of the green fruit prickly pear cultivar Skinner's Court during preliminary screening tests the previous season, no lycopene was detected in freshly harvested fruit in the 2003/2004 season (results not shown).

Although differences in the total β-carotene content were noted between treatments in the west block, the results were not exactly the same in the east block. In the west block, compared to the untreated control, both CC and SS treatments contributed to an increased β-carotene content in fruit pulp of 21% and 16% respectively while in the east block trial only the marked effect of SS was repeated. From this it is difficult to generalize in terms of the role the bio-stimulants played in β-carotene synthesis *in vivo*. Further, β-carotene:fruit mass ratio calculations showed that CC, SS and CC+SS in both the west and east block trials tended to have a decreasing effect on fruit mass with a concomitant increase in β-carotene content. The reverse effect was observed for Kelpak indicating that morphological parameters (fruit size and/or mass) might be determining factors rather

than the bio-stimulant effect or that bio-stimulants had an indirect effect via fruit development. Subsequently, multiple regression and correlation analyses were conducted to quantify the observed relationship between β-carotene content and fruit diameter and length as well as fruit mass. Fruit diameter was found to correlate the best with the β-carotene content in fruit pulp but was only significant at the 90% ($P<0.1$) probability level. The resulting linear equation model was tested using fruit diameter data from ten randomly picked fruit but a rather high percentage deviation (between 18% and 26%) indicated that the prediction accuracy of the model was not high enough to link fruit diameter per se to β-carotene content or other morphological parameters for that matter. The only reasonable deduction that can be made from this is that differences in β-carotene content in fruit pulp could neither be linked to treatment with bio-stimulants nor growth responses.

Vitamin C (L-ascorbic acid), synthesized from carbohydrate reserves, is an important component of many fruits and is important in human nutrition (Sneader, 1985, as cited by Seigler, 1998). Besides the nutritional value of vitamin C, it is *de novo* involved in the removal of active oxygen during photosynthesis, activation and deactivation of enzymes and growth regulation confirming its importance as a secondary metabolite (Seigler, 1998). In this study exogenously applied CC and SS contributed to elevated amounts of vitamin C in fruit pulp in both trials. Multiple regression and correlation analyses showed that vitamin C content in fruit pulp was neither correlated to fruit mass nor pulp mass but in contrast, significantly ($P<0.05$) correlated to fruit diameter. The resulting linear equation model for the prediction of vitamin C in fruit pulp, which included both fruit diameter and fruit length (significant at $P<0.1$) as parameters, was tested using data from ten randomly picked fruit. The percentage deviation was again rather high (11.38%) indicating that the prediction accuracy of the model was not suitable from a practical perspective. However, from this it could be concluded that the elevated amounts of vitamin C measured in fruit pulp was a direct result of metabolic stimulation by CC and SS and not indirectly via fruit growth.

The occurrence of protein in edible plant material is always regarded as a bonus from a nutritional perspective due to its importance in the daily human diet. Total water-soluble protein content was measured in fruit pulp to ascertain whether exogenously applied bio-stimulants had an effect on its production. Although not consistently in both the west and east block trials, the CC treatment significantly reduced the water-soluble protein content in fruit pulp in some cases. No significant differences were observed between the other treatments. Multiple regression and correlation analyses revealed a significant negative correlation between protein content and fruit mass but testing of the resulting linear equation model showed poor prediction accuracy. This was in agreement with the findings of Sawaya *et al.* (1983) as well as Galizzi *et al.* (2004) who reported that protein content was not correlated to fruit yield.

It can be concluded that foliar treatments of adult *O. ficus-indica* plants with different bio-stimulants did not have such a significant effect on quality parameters as it had on yield. Although prediction models developed through multiple regression and correlation analyses were not accurate enough to predict the content of all the compounds used as quality parameters, this was sufficient for predicting both glucose and β-carotene content indicating its possible application potential under farming conditions. More research is necessary to confirm this assumption.

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CHAPTER 5

GENERAL DISCUSSION

The set objectives of this study were to quantify the fruit yield and quality responses of *Opuntia ficus-indica,* as well new cladode production by treatment with natural bio-stimulants, while standard soil and orchard practices were followed. *ComCat*® (CC), a commercially available natural bio-stimulant known for its stimulating role in the growth and development of a variety of crops, along with SS both separately and in combination with SS, a prototype product still in the developmental stage, was applied as foliar sprays on six year old cactus pear plants. Kelpak®, an additional commercial bio-stimulant known for its growth stimulation properties, was used as a positive control.

Compared to the untreated control, foliar sprays on *O. ficus-indica* plants with the above mentioned natural bio-stimulants in two different trials produced varying results in terms of the total fruit yield. However, the *ComCat*® (CC) treatment consistently contributed to a increase (+36%) in fruit yield while the tendency of the SS treatment to reduce the fruit yield (-22%) repeated itself in both trials when expressed as total yield (ton ha^{-1}). Where CC and SS were applied in combination a marked increase in fruit yield was observed in one trial but this was not repeated in the other, as was the case in the positive control Kelpak®. From this it is difficult to decide whether the mechanism of action of the active substances in CC (brassinosteroids) and SS (triglycerides) acted in a synergistic or antagonistic fashion in the case of the cactus pear. Similar conflicting results have been obtained with other crops treated with CC and SS in combination (Van der Watt, 2004; personal communication) indicating that different crops react in different ways to this particular treatment. Interestingly, in the case of the cactus pear, the presence of CC in the combination treatment (CC+SS) seemed to have nullified the negative effect SS had on the total fruit yield indicating that the biochemical pathway that forms part of the mechanism of action of CC might have an overriding effect on the biochemical route triggered by SS.

In plants, according to Seigler (1998), triglycerides act as precursors for the production of jasmonic acid (JA), known for a wide range of physiological functions including growth-inhibiting capabilities (Dathe *et al*., 1991) and the promotion of senescence. From this it has been argued that JA should be recognized as a representative of a unique class of phytohormones (Hamberg & Gardner, 1992). On the other hand, brassinosteroids (BS) are recognized as a new class of phytohormones due to their synergistic action with indole acetic acid (IAA) in promoting root growth, shoot elongation, flower bud formation and crop yield (Arteca, 1995). Conflicting physiological activities of JA and BS might serve as an explanation for the fact that the CC+SS combination spray decreased the positive effect of CC and improved the negative effect of SS on fruit yield when applied separately. The fruit yield of 34.5 ton ha^{-1} that was obtained with the CC treatment was slightly higher than the 33 ton ha^{-1} average reported by Wessels (1988) on a experimental farm in South Africa.

Despite its negative effect on fruit yield, the SS treatment contributed to a considerable increase in new cladode formation of over 200% for the west block trial when expressed per number of old cladodes. Probably due to rather large standard deviations between replicates encountered with this crop, the calculated difference in new cladode formation between the untreated control and the SS treatment, although statistically significant in the west block trial, the tendency was not repeated in the east block trial. Interestingly, the larger the plants were at the beginning of the trials (more old cladodes) the lower was new cladode production and the greater was the fruit yield. Alternatively phrased, in cases where specific treatments (SS and CC+SS) stimulated cladode production the fruit yield was not stimulated and vice versa. Overall, the positive control Kelpak® showed an irregular tendency towards fruit yield enhancement and a consistent tendency in inhibiting new cladode production.

When the average fruit mass (of single fruits) was determined for both trials, an extremely important observation for prickly pear farmers was made namely the higher the number of harvested fruits the lower the average fruit mass and vice versa. This confirmed the need for the standard practice of fruit thinning in an attempt to avoid the

latter and to ensure a fair amount of marketable fruit. In this regard the *ComCat*® treatment contributed to the highest number of medium-sized fruits at final harvest and according to the farmer on whose farm the trials were conducted, the greatest local consumer demand is for medium class fruits.

In light of the inverse relationship between fruit number and fruit size highlighted above, morphological characteristics such as fruit mass, fruit length and fruit diameter were used in multiple regression and correlation analyses in an attempt to develop models to predict fruit yield. As could be expected, the linear equation model that resulted for predicting fruit mass by using fruit diameter and fruit length data as parameters was significantly accurate. As fruit size is related to fruit mass it is fair to assume that this prediction model (equation 3.1; chapter 3), with some modification, can also be applied to predict the average size and the category it would be classified in. The practical application of this model for the farmer is debatable as there is little one can do to change the appearance of fruit at the end of a season just before harvesting. However, this information could be put to some use for the following season and for a specific cultivar as management practices might be employed to either increase or decrease fruit size. Of these the extent of fruit thinning as well as fertilization and irrigation can be employed to either increase or decrease fruit number and size. From this study it became clear that natural bio-stimulants offer an additional technique to manipulate fruit number and size (*ComCat*®) as well as new cladode formation (SS).

There are two aspects to the stimulation of new cladode growth that need consideration. Firstly, the advantage of a higher yield in the case where the objective is to add value by processing young cladodes to marketable commodities such as nopalitos in Mexico, jam and chutney from fruit and secondly, the advantage of having more cladodes during the following season where the objective is to produce more or better fruit. This study provided a strong indication that a foliar spray with SS can contribute to either of the two stated objectives.

Another aspect of the prickly pear industry that needs consideration is the processing of fruit pulp into beverages, fruit cocktails or plain fruit juice. In the case of a producer specializing in this aspect, it is safe to say that neither fruit number, size or mass will be regarded as more important than the fruit pulp. In this study the influence of bio-stimulants on the fruit pulp content was investigated in an attempt to manipulate it. A close relationship between fruit mass, peel mass and pulp mass was observed namely the higher the fruit mass the greater the peel mass, but the lower the pulp mass and vice versa. Multiple regression and correlation analyses resulted in a linear equation model (equation 3.2; chapter 3) that significantly predicted fruit pulp mass by using both fruit and peel mass as parameters. The significance of this relationship is rather difficult to contemplate as well as the manipulation methodology to control it from a management perspective. Further, neither of the bio-stimulant treatments had a significant effect on either peel thickness or fruit pulp. It is suggested that, in the event where fruit pulp is the main product the farmer is after, future research should concentrate on known manipulation techniques and/or should be conducted with an entrepreneurial approach.

The influence of natural bio-stimulant treatments on the quality of prickly pear fruit was additionally investigated. Sugar, β-carotene, vitamin C and total water-soluble protein content in fruit pulp were taken as indicators of fruit quality. The rationale for this choice of parameters is that much has been written on the nutritional value of sugars and soluble proteins as well as its contribution to the taste of fruit. The advantages of β-carotene and vitamin C have been dealt with in chapter two. Further, all these attributes have become marketing tools lately. The results obtained in this study showed that glucose was the predominant sugar and that both the *ComCat*® and SS treatments contributed to marked increases in the glucose (up to +8%) and β-carotene (up to +28%) content in fruit pulp, compared to the untreated control. Although these differences were not statistically significant (probably due to large standard deviations) it is suggested that future research should pursue this aspect further. Multiple regression and correlation analyses using fruit morphological parameters to predict the glucose content in fruit pulp showed no significance at the 95% probability level.

Interestingly, in general the glucose content in fruit pulp was 20 fold higher than that of fructose and 40 fold higher than sucrose. To supply a reason for this was outside of the scope of this study. However, it can be speculated that the elevated glucose content under the influence of *ComCat*®, as was especially seen in the east block trial, must have been due to induced translocation of sucrose from storage organs to fruit during the fruit filling stage as sucrose is the form in which carbohydrate is translocated. According to Arteca (1995) brassinosteroids, the active substance of *ComCat*®, have been shown to induce changes in plasmalemma energization, sucrose transport and assimilate uptake. The disaccharide sucrose is probably hydrolyzed to the two monosaccharides glucose and fructose in maturing fruit after it has been offloaded while fructose is probably converted to glucose. The latter is most probably genetically controlled in this crop.

The increasing effect both *ComCat*® and SS treatments had on the β-carotene content in fruit pulp while the latter correlated significantly with fruit size (diameter), is an aspect that needs to be considered. Do these two bio-stimulants had a direct effect on β-carotene content via its metabolic synthesizing route or indirectly via its effect on fruit growth and/or development? It was observed in this study that the lower the fruit mass the higher the β-carotene content in the fruit pulp. However, multiple regression and correlation analyses revealed that only fruit diameter and not fruit mass correlated with β-carotene content in the fruit pulp and also only at the 90% probability level. From this it is deduced that the effect of *ComCat*® and SS treatments was rather via a metabolic influence than via a growth influence.

No significant differences between different treatments in terms of either vitamin C or the water-soluble protein content in fruit pulp, were observed in this study. The linear model equations (equation 4.3 and 4.4; chapter 4) resulting from multiple regression and correlation analyses were also not sufficiently accurate in predicting the content of these two compounds in fruit pulp at the 95% probability level.

In conclusion, when yield was expressed as fruit mass per number of old cladodes the yield increase under the influence of *ComCat*® was not spectacular. Further, as fruit size

is a vital determining factor as far as consumer demand is concerned, *ComCat*® contributed to a marked increase in the more saught of medium class fruit compared to the untreated control. In the case where new cladode formation is the principle objective due to its economic value, the SS treatment showed possibilities as an additional manipulation treatment. Although Kelpak® showed a tendency to increase the total fruit yield in one of the trials it had no effect on new cladode formation. In this sense both the *ComCat*® and the SS treatments outperformed Kelpak®. Not much could be deduced from this study in terms of the manipulation potential of bio-stimulants in increasing the quality of fruit. Finally, prediction models resulting from multiple regression and correlation analyses of morphological, yield and quality data strongly indicated that this approach might become handy tools for farmers assisting them in managing their orchards not only in a specific growing season but especially for the following season. More research is necessary to pursue this aspect.

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APPENDIX A

ANOVA tables are numbered according to corresponding figure numbers.

Figure 3.2A: ANOVA of the average fruit yield expressed as fresh mass per number of old cladodes for each treatment of the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	545703.4	136425.9	14.59	0.000047*	0.999892
Error	15	140262.4	9350.825			
Total	19	685965.8				

* Term significant at alpha = 0.05

Figure 3.2C: ANOVA of the average fruit yield expressed as fresh mass per number of old cladodes for each treatment of the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	163360.8	40840.21	4.82	0.010592*	0.868346
Error	15	127063.6	8470.907			
Total	19	290424.4				

* Term significant at alpha = 0.05

Figure 3.3A: ANOVA of the average number of new cladodes expressed per number of old cladodes for the west block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	15.78197	3.945494	15.09	0.000039*	0.999928
Error	15	3.921412	0.261427			
Total	19	19.70339				

* Term significant at alpha = 0.05

Figure 3.3A: ANOVA of the average number of total fruit before thinning expressed per number of old cladodes for the west block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	15.78388	3.94597	1.16	0.367207	0.277793
Error	15	51.02649	3.401766			
Total	19	66.81036				

* Term significant at alpha = 0.05

Figure 3.3A: ANOVA of the average number of total harvested fruits after thinning expressed per number of old cladodes for the west block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	11.21397	2.803492	7.35	0.001739*	0.973485
Error	15	5.719088	0.381273			
Total	19	16.93306				

* Term significant at alpha = 0.05

Figure 3.3B: ANOVA of the average number of new cladodes expressed per number of old cladodes for the east block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	0.997564	0.249391	0.49	0.742827	0.135088
Error	15	7.627187	0.508479			
Total	19	8.624751				

* Term significant at alpha = 0.05

Figure 3.3B: ANOVA of the average number of total fruit before thinning expressed per number of old cladodes for the west block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	56.63216	14.15804	10.85	0.000246*	0.99789
Error	15	19.57308	1.304872			
Total	19	76.20524				

* Term significant at alpha = 0.05

Figure 3.3B: ANOVA of the average number of total harvested fruits after thinning expressed per number of old cladodes for the west block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	15.3896	3.847399	4.36	0.015445*	0.828935
Error	15	13.23194	0.88213			
Total	19	28.62154				

* Term significant at alpha = 0.05

Figure 3.4A: ANOVA of the average mass of a single fruit at the final harvest after fruit thinning in the west block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	2706.445	676.6112	2.21	0.104875	0.540856
Error	15	6130.081	306.5041			
Total	19	8836.525				

* Term significant at alpha = 0.05

Figure 3.4B: ANOVA of the average mass of a single fruit at the final harvest after fruit thinning in the east block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	547.2014	136.8004	0.58	0.67934	0.161765
Error	20	4702.791	235.1395			
Total	24	5249.992				

* Term significant at alpha = 0.05

Table 3.2: ANOVA of the average percentage small fruits for each treatment at the first harvest for the west block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	1413.322	353.3304	3.92	0.022489*	0.78277
Error	15	1350.645	90.04299			
Total	19	2763.967				

* Term significant at alpha = 0.05

Table 3.2: ANOVA of the average percentage medium fruits for each treatment at the first harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	316.5983	79.14958	1.46	0.262614	0.34574
Error	15	811.7469	54.11646			
Total	19	1128.345				

* Term significant at alpha = 0.05

Table 3.2: ANOVA of the average percentage large fruits for each treatment at the first harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	1058.557	264.6393	2.15	0.124073	0.495976
Error	15	1842.147	122.8098			
Total	19	2900.704				

* Term significant at alpha = 0.05

Table 3.2: ANOVA of the average percentage X-large fruits for each treatment at the first harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	1.6666	0.41665	0.92	0.475679	0.225605
Error	15	6.7611	0.45074			
Total	19	8.4277				

* Term significant at alpha = 0.05

Table 3.2: ANOVA of the fruit classes at first harvest for the west block trial

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	39.13779	9.784449	0.15	0.963773	0.072952
Class	3	16797.97	5599.324	83.95	0.000000*	1
TC	12	2763.23	230.2692	3.45	0.000683*	0.984302
Error	60	4001.796	66.69661			
Total	79	23602.13				

* Term significant at alpha = 0.05

Table 3.3: ANOVA of the average percentage small fruit for each treatment at the first harvest for the east block trial

Analysis of Variance Table						
Source Term	**DF**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob Level**	**Power (Alpha=0.05)**
Treatment	4	852.3608	213.0902	0.59	0.677389	0.154202
Error	15	5450.161	363.3441			
Total	19	6302.521				

* Term significant at alpha = 0.05

Table 3.3: ANOVA of the average percentage medium fruit for each treatment at the first harvest for the east block

Analysis of Variance Table						
Source Term	**DF**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob Level**	**Power (Alpha=0.05)**
Treatment	4	445.2526	111.3131	1.27	0.323348	0.303541
Error	15	1309.805	87.32034			
Total	19	1755.058				

* Term significant at alpha = 0.05

Table 3.3: ANOVA of the average percentage large fruit for each treatment at the first harvest for the east block

Analysis of Variance Table						
Source Term	**DF**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob Level**	**Power (Alpha=0.05)**
Treatment	4	1320.072	330.0181	0.91	0.483482	0.222332
Error	15	5443.614	362.9076			
Total	19	6763.687				

* Term significant at alpha = 0.05

Table 3.3: ANOVA of the average percentage extra-large fruits for each treatment at the first harvest for the east block

Analysis of Variance Table						
Source Term	**DF**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob Level**	**Power (Alpha=0.05)**
Treatment	4	201.2697	50.31742	1.09	0.396748	0.262161
Error	15	692.47	46.16467			
Total	19	893.7397				

* Term significant at alpha = 0.05

Figure 3.3: ANOVA of the fruit classes at first harvest for the east block trial

Analysis of Variance Table						
Source Term	**DF**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob Level**	**Power (Alpha=0.05)**
Treatment	4	0.00002	0.000005	0	1	0.05
Class	3	9816.308	3272.103	15.22	0.000000*	0.99951
TC	12	2818.955	234.9129	1.09	0.382546	0.512833
Error	60	12896.05	214.9342			
Total	79	25531.31				

* Term significant at alpha = 0.05

Figure 3.5A: ANOVA of the average fruit length at first harvest for the west block

Analysis of Variance Table						
Source Term	**DF**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob Level**	**Power (Alpha=0.05)**
Treatment	4	486.7054	121.6764	24.18	0.000000*	1
Error	15	100.6349	5.031744			
Total	19	587.3403				

* Term significant at alpha = 0.05

Figure 3.5B: ANOVA of the average fruit diameter at first harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	29.82434	7.456086	8.32	0.000403*	0.991907
Error	15	17.93036	0.896518			
Total	19	47.7547				

* Term significant at alpha = 0.05

Figure 3.5C: ANOVA of the average fruit length:fruit diameter ratio at first harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	0.079736	0.019934	8.04	0.000491*	0.989963
Error	20	0.04956	0.002478			
Total	24	0.129296				

* Term significant at alpha = 0.05

Figure 3.5D: ANOVA of the average fruit mass at first harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	1084.947	271.2366	17.37	0.000003*	0.999998
Error	15	312.2313	15.61157			
Total	19	1397.178				

Term significant at alpha = 0.05

Figure 3.6A: ANOVA of the average fruit length at first harvest for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	59.63278	14.9082	1.02	0.418864	0.264329
Error	20	291.1077	14.55538			
Total	24	350.7405				

* Term significant at alpha = 0.05

Figure 3.6B: ANOVA of the average fruit diameter at first harvest for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	3.973671	0.993418	0.17	0.952242	0.078326
Error	20	118.361	5.918049			
Total	24	122.3347				

* Term significant at alpha = 0.05

Figure 3.6C: ANOVA of the average fruit length:fruit diameter ratio at first harvest for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	2.42E-02	6.04E-03	0.97	0.446003	0.251205
Error	20	0.124573	6.23E-03			
Total	24	0.148725				

* Term significant at alpha = 0.05

Figure 3.6D: ANOVA of the average fruit mass at first harvest for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	412.2026	103.0507	0.73	0.579467	0.196012
Error	20	2807.552	140.3776			
Total	24	3219.755				

* Term significant at alpha = 0.05

Linear equation model 3.1: Multiple regression analysis of data for predicting the average fruit mass using fruit diameter and fruit length as parameters.

Independent	Regression	Standard	T-Value	Prob	Decision	Power
Variable	Coefficient	Error	(Ho: B=0)	Level	5%	5%
Intercept	-71.42419	3.111612	-22.9541	0	Reject Ho	1
Fruit diameter	1.978693	0.043621	45.3613	0	Reject Ho	1
Fruit length	1.082957	2.65E-02	40.9354	0	Reject Ho	1
R-Squared	0.506611					

*Reject Ho = Significant at the 95% probability level

ANOVA of equation model 3.1 to test the significance of the model

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Intercept	1	7.36E+07	7.36E+07			
Model	2	1839051	919525.5	2174.243	0	1
Error	4235	1791056	422.9175			
Total	4237	3630107	856.7634			

*Probability level < 0.05 = Significant at the 95% probability

Figure 3.7A: ANOVA of the average fruit mass at second harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	1478.646	369.6614	3.36	0.037349*	0.709711
Error	15	1648.574	109.9049			
Total	19	3127.22				

* Term significant at alpha = 0.05

Figure 3.7B ANOVA of the average pulp mass at second harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	9.36183	2.340457	0.03	0.998568	0.054144
Error	20	1796.988	89.84941			
Total	24	1806.35				

* Term significant at alpha = 0.05

Figure 3.7A: ANOVA of the average peel mass at second harvest for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	183.3956	45.84889	2.89	0.048652*	0.672576
Error	20	317.183	15.85915			
Total	24	500.5786				

* Term significant at alpha = 0.05

Figure 3.7B: ANOVA of the average peel thickness at second harvest for west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	4.183078	1.04577	2.11	0.117146	0.520225
Error	20	9.904932	0.495247			
Total	24	14.08801				

* Term significant at alpha = 0.05

Figure 3.7C: ANOVA of the average fruit mass at second harvest for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	469.6646	117.4162	0.75	0.572344	0.188384
Error	15	2343.759	156.2506			
Total	19	2813.423				

* Term significant at alpha = 0.05

Figure 3.7C: ANOVA of the average pulp mass at second harvest for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	277.008	69.252	1.39	0.283119	0.330485
Error	15	744.8423	49.65615			
Total	19	1021.85				

* Term significant at alpha = 0.05

Figure 3.7C: ANOVA of the average peel mass at second harvest for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	339.9204	84.98011	1.77	0.187888	0.41349
Error	15	721.1221	48.07481			
Total	19	1061.042				

* Term significant at alpha = 0.05

Figure 3.7D: ANOVA of the average peel thickness at second harvest for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	2.150598	0.53765	2.31	0.105151	0.528032
Error	15	3.48675	0.23245			
Total	19	5.637349				

Term significant at alpha = 0.05

Linear equation model 3.2: Multiple regression analysis of data for predicting the average pulp mass using fruit length and peel mass as parameters.

Independent	**Regression**	**Standard**	**T-Value**	**Prob**	**Decision**	**Power**
Variable	**Coefficient**	**Error**	**(Ho: B=0)**	**Level**	**5%**	**5%**
Intercept	1.360693	0.888672	1.5312	0.126045	Accept Ho	0.334271
Fruit length	0.907281	1.06E-02	85.6486	0	Reject Ho	1
Peel mass	-0.8484701	0.019643	-43.1956	0	Reject Ho	1
R-Squared	0.919228					

*Reject Ho = Significant at the 95% probability level

ANOVA of equation model 3.2 to test the significance of the model

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Intercept	1	5917079	5917079			
Model	2	362739.8	181369.9	5735.82	0	1
Error	1008	31873.54	31.62057			
Total	1010	394613.3	390.7063			

Term significant at alpha = 0.05

Table 3.4: A test run to quantify the prediction accuracy of regression model 3.1 in predicting the fruit mass (calculated fruit mass) using fruit diameter and fruit length as parameters. The average fruit mass was calculated for all harvested fruit in five replicates and compared with the average mass for 10 fruits randomly picked.

Fruit diameter	Fruit length	Fruit mass	Calculated fruit mass
56.90	96.16	175.59	145.31
50.32	91.11	119.74	126.82
46.86	104.10	124.58	134.04
51.44	105.91	153.24	145.06
50.01	102.17	129.14	138.18
53.52	108.41	156.05	151.88
48.92	93.02	125.63	126.11
49.35	82.18	108.35	115.23
42.18	91.50	94.78	111.13
51.37	96.00	139.29	134.19
56.93	109.37	170.89	159.67
53.71	94.90	147.01	137.63
52.66	94.96	132.11	135.62
50.65	97.41	143.97	134.29
54.11	99.83	138.25	143.76
50.26	96.18	134.05	132.19
53.92	106.72	137.93	150.85
42.28	88.81	81.65	108.42
50.02	99.79	123.48	135.62
55.32	114.58	175.27	162.13
53.66	100.61	158.77	143.71
48.86	94.80	131.82	127.92
49.43	86.89	120.79	120.48
52.43	101.45	150.47	142.19
51.25	88.61	105.53	125.95
55.15	106.06	156.75	152.56
52.10	107.00	150.03	147.55
56.94	102.71	173.86	152.48

53.39	98.68	153.25	141.09
53.67	102.80	147.61	146.11
55.04	106.78	167.18	153.13
55.80	106.32	164.88	154.13
58.69	84.92	152.57	136.67
56.54	102.69	160.45	151.66
47.62	92.25	133.47	122.71
55.42	92.52	146.37	138.43
55.30	117.51	159.88	165.26
52.05	88.72	130.95	127.65
44.09	97.28	96.25	121.17
52.51	97.29	137.44	137.84
60.83	102.96	178.69	160.45
51.96	103.61	133.91	143.60
59.27	103.26	182.64	157.68
55.75	98.25	157.90	145.29
54.52	98.96	155.10	143.63
57.37	96.04	169.23	146.11
43.23	92.33	92.97	114.11
53.87	100.92	142.97	144.46
56.75	101.93	170.30	151.26
42.15	96.14	97.01	116.10
55.45	110.56	172.99	158.03
53.08	53.08	99.97	91.09
49.61	92.24	130.01	126.64
55.22	110.29	177.32	157.28
55.55	106.49	163.40	153.82
53.33	110.48	166.39	153.75
54.84	93.37	145.94	138.21
50.10	93.13	125.41	128.57
52.04	99.94	131.13	139.78
54.00	107.30	167.26	151.63
55.27	103.49	157.20	150.02
55.06	94.44	155.35	139.80
53.20	93.72	136.85	135.34
42.02	90.92	82.43	110.19
44.38	104.96	126.22	130.06
55.57	108.45	184.46	155.98
48.26	95.49	116.64	127.48
51.28	103.80	135.42	142.46
56.13	103.75	162.02	152.00
53.95	91.20	155.83	134.10
58.40	103.98	172.65	156.74
52.89	100.48	146.84	142.05
53.50	92.07	138.64	134.15
49.06	103.16	113.14	137.37
49.11	97.55	110.25	131.40

56.98	102.61	170.48	152.45
57.28	105.40	167.44	156.06
	Average for replicate 1	**142.88**	**139.56**
	Average of 10 fruits randomly picked in this replicate	**143.62**	**137.68**
	% deviation (between replicate average and calculated average)	**2.38**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**4.31**	
54.45	97.00	157.91	141.37
50.32	91.56	129.45	127.30
52.82	94.50	133.29	135.43
50.19	91.42	113.75	126.89
55.71	93.10	153.88	139.64
47.13	89.42	99.61	118.67
50.10	97.91	124.39	133.75
54.15	96.94	142.76	140.71
54.39	94.34	145.47	138.37
55.56	91.48	156.21	137.59
46.44	88.82	89.24	116.66
51.30	90.92	137.61	128.55
52.50	81.22	134.58	120.42
55.60	97.52	166.53	144.21
45.74	98.49	103.55	125.75
54.87	97.04	147.15	142.24
46.55	82.21	92.49	109.72
52.95	106.77	153.65	148.98
55.42	97.26	151.85	143.57
56.36	90.65	164.70	138.27
48.20	86.18	99.68	117.28
53.19	90.16	137.10	131.47
55.06	90.43	147.70	135.46
52.33	95.51	134.57	135.56
52.81	89.88	143.67	130.41
52.83	76.15	129.07	115.58
51.54	95.24	126.31	133.70
55.22	93.34	156.68	138.93
53.12	90.85	141.83	132.07
51.90	97.22	138.04	136.56
54.13	92.61	149.33	135.98
52.11	91.31	139.92	130.57
153.80	90.22	118.72	330.61
121.26	56.83	96.24	230.06
95.74	54.40	80.12	176.93
46.60	93.63	93.12	122.18

51.18	87.38	93.63	124.48
48.71	83.60	135.31	115.50
52.28	83.02	101.96	121.93
48.15	94.32	127.82	126.00
47.47	86.67	110.17	116.37
49.55	90.01	122.95	124.10
46.92	87.02	121.88	115.66
55.53	95.10	143.25	141.45
55.61	98.26	152.77	145.03
51.21	81.07	111.32	117.70
48.42	91.51	125.69	123.49
53.50	83.04	135.93	124.37
48.22	88.43	110.46	119.76
49.55	81.52	104.22	114.91
50.12	110.43	131.06	147.34
43.36	90.16	81.90	112.02
55.56	100.29	161.54	147.13
44.84	53.43	90.98	75.17
54.24	86.68	86.68	129.77
52.67	85.86	124.31	125.78
56.79	101.19	154.78	150.53
51.04	98.70	132.76	136.46
50.68	90.09	124.56	126.42
54.11	93.72	136.14	137.14
53.25	88.09	124.50	129.34
58.07	95.38	154.90	146.78
98.96	82.26	107.51	213.48
54.70	111.14	166.76	157.18
36.36	88.96	66.47	96.87
56.56	92.70	157.12	140.89
55.41	93.02	148.19	138.96
50.37	111.06	121.62	148.52
53.60	89.24	153.98	131.28
50.96	100.85	129.13	138.63
46.75	90.07	100.64	118.63
50.02	83.46	109.00	117.94
45.48	104.92	116.17	132.20
50.15	92.00	128.62	127.44
46.43	83.60	101.92	110.99
50.16	92.43	138.90	127.93
57.39	107.33	156.13	158.37
51.74	88.75	119.80	127.07
50.63	88.02	119.79	124.08
50.44	88.34	110.59	124.05
50.82	78.45	105.49	114.09
51.89	87.86	123.32	126.40
45.36	93.52	114.92	119.61

51.65	78.83	116.03	116.15
51.28	103.38	128.72	142.00
50.60	91.52	132.41	127.81
51.32	94.52	138.81	132.49
54.52	102.23	150.75	147.17
52.05	100.81	151.18	140.74
46.74	52.78	106.90	78.22
48.36	85.70	105.34	117.08
51.48	103.10	154.47	142.10
54.08	97.05	129.70	140.69
53.08	104.97	151.97	147.29
54.62	92.41	138.75	136.73
50.86	90.07	119.27	126.76
53.44	102.46	162.86	145.28
46.26	94.44	102.53	122.39
56.03	106.35	163.04	154.62
52.27	106.30	157.05	147.13
49.44	91.38	108.40	125.37
56.63	92.95	148.88	141.29
58.98	97.14	149.11	150.48
51.17	88.83	113.71	126.03
45.86	90.53	106.40	117.36
54.23	98.56	150.72	142.62
47.73	104.72	119.24	136.43
	Average for replicate 2	**128.35**	**134.44**
	Average of 10 fruits randomly picked in this replicate	**127.22**	**129.05**
	% deviation (between replicate average and calculated average)	**4.74**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**1.44**	
47.77	95.30	122.75	126.31
53.34	96.06	154.58	138.15
55.96	93.98	124.97	141.08
49.72	85.24	120.06	119.27
50.53	89.34	132.34	125.31
51.73	99.95	132.26	139.18
48.93	81.60	107.43	113.77
50.72	86.22	125.63	122.31
43.50	81.93	83.52	103.38
51.45	85.93	137.64	123.44
55.61	96.88	157.91	143.53
45.95	92.36	110.00	119.52
53.76	91.26	151.35	133.79
96.97	97.49	122.51	226.03

52.97	101.28	167.21	143.07
51.17	96.13	130.38	133.93
51.60	83.40	111.83	121.00
53.81	90.97	140.57	133.57
97.30	82.09	99.97	210.01
49.07	91.22	110.92	124.46
45.91	89.27	94.33	116.10
49.47	99.40	117.86	134.11
51.18	82.84	119.60	119.56
43.67	81.14	84.95	102.86
48.57	96.95	132.24	129.68
47.51	96.31	127.00	126.89
51.88	86.69	124.17	125.12
100.54	46.44	124.22	177.81
54.00	101.30	151.58	145.13
48.12	87.01	115.40	118.02
50.09	92.08	131.21	127.41
50.42	102.85	141.95	139.73
47.34	74.69	96.11	103.14
49.57	86.03	109.84	119.83
51.60	84.92	127.73	122.65
47.63	89.83	118.69	120.11
52.90	87.60	134.11	128.12
54.47	91.91	147.98	135.89
49.93	78.84	109.94	112.76
47.96	80.43	107.59	110.58
52.12	87.99	122.73	127.00
48.58	90.24	118.99	122.43
49.35	99.80	121.26	134.31
51.63	85.32	115.17	123.14
47.19	79.40	102.47	107.94
50.69	107.67	136.87	145.48
42.94	97.08	98.88	118.68
50.95	82.90	134.50	119.17
52.61	88.58	136.10	128.61
50.05	78.56	108.74	112.69
50.79	88.50	127.03	124.92
45.11	75.05	86.55	99.11
48.70	79.07	109.76	110.57
44.80	86.89	92.61	111.32
46.18	93.59	102.56	121.31
47.60	88.15	110.54	118.23
50.12	87.00	120.15	121.97
51.45	83.21	108.99	120.50
51.46	96.53	138.00	134.94
46.96	89.47	110.65	118.39
53.23	79.28	155.75	119.76

50.03	95.59	133.92	131.09
48.50	77.31	105.46	108.27
46.77	77.74	103.80	105.31
49.84	77.98	110.13	111.65
50.99	100.30	130.36	138.09
49.80	92.03	130.79	126.78
50.86	89.61	133.83	126.26
48.72	90.02	125.53	122.47
52.02	85.60	152.65	124.21
53.43	87.31	150.51	128.85
46.90	82.46	104.26	110.68
51.24	94.10	136.74	131.87
	Average for replicate 3	**122.42**	**126.89**
	Average of 10 fruits randomly picked in this replicate	**110.68**	**118.13**
	% deviation (between replicate average and calculated average)	**3.65**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**6.72**	
49.20	76.93	107.70	109.24
57.75	96.18	147.30	147.01
58.30	104.41	188.00	157.01
60.04	89.89	175.40	144.73
61.30	106.15	194.00	164.83
47.58	110.18	118.20	142.05
51.18	97.29	118.00	135.21
54.13	94.37	139.80	137.89
54.72	111.55	154.80	157.66
55.37	113.46	170.30	161.01
48.97	102.24	130.90	136.20
56.02	99.44	159.60	147.12
51.49	99.94	121.00	138.69
50.59	98.49	112.00	135.34
47.90	91.66	122.00	122.62
54.97	88.70	124.30	133.41
52.99	95.93	151.40	137.32
49.87	97.71	129.90	133.07
60.18	111.59	205.40	168.51
50.11	90.08	131.40	125.29
58.63	111.92	191.30	165.80
52.54	111.28	150.70	153.05
55.08	109.10	151.10	155.72
57.03	102.81	168.50	152.76
51.48	108.65	129.10	148.11
52.47	101.16	146.80	141.95

52.56	91.87	125.30	132.07
51.52	96.61	131.00	135.15
53.95	86.66	132.40	129.18
51.30	97.78	135.30	135.98
47.50	119.65	146.10	152.15
54.69	94.04	146.20	138.64
49.94	85.35	98.60	119.83
52.01	105.43	142.10	145.67
47.47	85.35	100.20	114.94
47.95	97.09	102.20	128.60
46.98	96.63	102.10	126.19
48.74	84.51	91.40	116.54
47.84	89.94	87.60	120.64
43.46	83.65	83.90	105.16
36.71	89.98	65.90	98.66
55.46	93.95	143.00	140.06
53.52	96.91	154.10	139.43
56.80	100.04	160.20	149.31
54.67	104.18	152.20	149.58
48.74	111.18	122.90	145.43
52.61	111.61	157.90	153.55
59.57	106.34	188.30	161.61
57.02	103.23	183.00	153.20
47.01	94.24	115.80	123.66
47.71	103.91	118.70	135.51
50.64	92.16	119.90	128.59
43.19	96.99	104.50	119.08
59.50	104.56	164.90	159.55
49.07	100.07	133.70	134.05
58.17	106.39	180.50	158.90
52.39	92.33	137.00	132.23
52.76	98.15	140.70	139.27
53.79	115.16	162.20	159.73
56.01	85.33	148.30	131.82
53.84	96.90	149.60	140.05
48.47	104.52	128.20	137.68
49.05	93.59	132.60	126.99
47.25	99.08	116.50	129.37
51.03	85.98	119.60	122.67
55.65	87.33	139.20	133.27
45.43	76.81	98.40	101.65
53.99	108.98	166.60	153.43
52.17	99.32	130.00	139.37
44.77	98.87	113.30	124.24
45.70	100.61	115.50	127.96
48.42	82.93	101.70	114.20
56.01	104.89	158.20	153.00

51.24	90.22	116.10	127.67
49.75	102.73	125.80	138.27
56.33	102.68	154.60	151.24
45.63	89.59	95.50	115.89
46.65	82.76	121.40	110.51
55.08	115.01	157.80	162.12
49.74	93.42	151.10	128.17
49.30	94.72	120.10	128.71
50.05	81.10	116.60	115.44
46.42	105.76	112.30	134.97
39.82	71.29	65.00	84.57
57.78	120.45	178.30	173.35
64.16	99.40	240.10	163.18
59.02	113.90	191.90	168.71
46.69	121.17	147.80	152.19
54.64	96.70	144.60	141.42
49.39	88.94	134.60	122.63
40.79	94.35	102.50	111.47
55.66	88.75	144.40	134.83
49.57	84.34	112.30	118.00
47.18	100.33	111.30	130.59
49.02	102.56	133.40	136.64
48.72	88.52	127.00	120.85
48.49	94.71	99.90	127.09
46.88	84.26	96.70	112.59
50.16	96.35	118.90	132.17
43.61	93.00	104.40	115.59
46.42	89.76	99.60	117.64
43.61	98.02	83.50	121.02
44.16	98.69	92.90	122.84
39.76	66.88	54.30	79.68
52.87	112.51	168.70	155.04
54.71	107.80	180.50	153.58
55.40	102.18	172.70	148.86
53.27	102.70	155.30	145.21
49.93	98.05	124.70	133.56
55.13	94.13	155.40	139.60
52.13	87.51	137.00	126.50
51.53	96.21	141.20	134.73
56.07	96.18	158.10	143.68
47.49	101.65	112.80	132.63
46.67	96.19	111.60	125.10
52.94	108.82	155.30	151.18
49.32	90.41	117.40	124.08
46.28	94.31	105.30	122.29
53.21	96.62	146.60	138.50
56.53	96.28	142.90	144.70

46.87	107.49	133.10	137.73
55.57	99.27	160.40	146.04
51.98	103.62	153.40	143.65
56.48	98.49	146.70	147.00
59.11	102.32	175.40	156.35
58.04	97.67	168.60	149.20
50.80	92.37	128.40	129.13
56.42	100.13	178.40	148.65
42.96	87.22	83.30	108.04
48.05	98.80	100.00	130.65
49.92	103.01	133.70	138.91
47.56	96.32	135.10	127.00
51.12	89.88	138.60	127.07
52.68	95.31	151.90	136.03
51.81	102.34	162.10	141.93
54.43	110.98	163.50	156.47
50.71	91.53	126.70	128.04
54.83	101.38	146.50	146.86
55.42	107.87	158.40	155.06
58.53	108.32	184.20	161.70
58.59	101.78	166.90	154.74
50.48	95.78	119.00	132.19
42.85	98.00	99.00	119.50
55.42	98.07	153.20	144.45
54.42	92.86	132.50	136.82
52.12	94.41	138.20	133.95
49.43	92.50	128.40	126.56
47.53	99.85	137.70	130.76
42.91	99.31	103.30	121.03
52.22	82.34	130.80	121.08
45.46	90.81	93.10	116.87
43.05	89.56	102.00	110.75
51.73	95.51	142.70	134.37
43.63	101.37	116.10	124.69
44.61	92.99	105.70	117.55
54.71	100.11	155.20	145.25
61.23	103.19	214.50	161.49
52.71	102.79	138.40	144.19
49.22	86.79	109.30	119.96
56.38	101.10	162.20	149.63
47.32	98.72	138.50	129.12
58.32	115.44	204.60	169.00
47.10	96.42	114.60	126.20
47.57	96.55	103.80	127.27
48.58	101.47	131.11	134.59
52.24	101.28	121.90	141.63
49.52	86.63	110.90	120.38

56.01	94.01	160.20	141.22
47.15	86.17	98.50	115.19
49.22	97.97	124.10	132.07
46.86	90.47	86.50	119.28
54.58	112.67	164.80	158.59
55.58	111.30	168.90	159.09
43.96	92.46	133.70	115.69
50.61	87.53	133.60	123.51
53.97	98.64	152.20	142.19
52.18	109.89	149.20	150.84
54.45	99.43	142.80	144.00
56.20	93.67	155.70	141.22
50.06	107.67	132.10	144.24
51.18	91.48	123.00	128.92
46.36	93.95	104.00	122.06
48.77	109.23	129.60	143.37
51.09	101.88	133.50	140.00
47.17	87.83	98.80	117.03
49.83	87.57	106.40	122.01
50.27	105.26	116.10	142.04
56.28	92.71	123.80	140.34
46.73	111.65	118.40	141.96
47.81	98.32	122.10	129.66
38.60	67.18	53.40	77.71
36.54	91.40	70.50	99.86
40.12	87.15	83.20	102.34
39.95	102.65	80.20	118.79
46.44	93.40	91.50	121.62
44.52	74.04	72.90	96.85
42.69	70.72	65.40	89.64
33.83	61.53	42.40	62.15
	Average for replicate 4	**132.15**	**134.25**
	Average of 10 fruits randomly picked in this replicate	**132.09**	**137.39**
	% deviation (between replicate average and calculated average)	**1.59**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**4.01**	
58.66	94.27	158.10	146.74
46.60	93.65	119.60	122.21
47.35	88.40	102.70	118.00
54.11	92.24	141.00	135.54
58.46	95.23	171.60	147.38
50.41	87.34	130.80	122.91
46.31	82.39	103.80	109.44

49.86	79.98	114.50	113.85
53.70	97.73	149.20	140.67
50.13	85.71	120.50	120.59
46.21	88.71	105.70	116.08
52.57	93.32	140.20	133.66
46.70	94.51	111.10	123.34
54.52	97.32	153.90	141.85
53.39	93.05	150.40	134.99
55.09	91.89	155.40	137.10
55.48	91.65	155.30	137.61
53.39	94.60	127.90	136.67
52.19	106.65	131.40	147.35
51.27	101.81	139.50	140.28
60.06	96.66	175.60	152.10
50.76	94.52	129.10	131.38
57.06	92.84	146.70	142.03
57.32	109.82	162.60	160.93
51.46	91.32	117.90	129.30
48.21	92.05	108.20	123.66
45.06	84.56	95.00	109.31
44.73	104.02	99.10	129.74
56.50	91.94	127.00	139.94
47.55	90.17	117.30	120.32
42.84	96.01	90.50	117.32
49.30	95.60	105.00	129.66
47.46	73.81	104.00	102.42
43.07	71.71	69.14	91.46
61.46	110.83	216.20	170.22
53.19	99.57	157.20	141.66
54.24	84.18	140.80	127.07
59.42	89.78	154.30	143.38
55.47	100.67	169.20	147.36
54.24	81.21	129.70	123.85
55.76	86.55	143.80	132.64
52.26	98.71	133.00	138.89
52.13	89.00	136.20	128.11
99.13	162.10	196.12	300.28
53.87	94.22	137.12	137.21
50.88	84.93	126.30	121.23
54.13	92.51	146.60	135.87
53.61	79.88	125.20	121.16
54.04	87.68	146.30	130.46
50.27	80.66	118.20	115.40
55.74	89.29	143.90	135.57
53.47	89.81	151.20	131.64
55.86	100.58	164.60	148.03
58.14	92.81	156.90	144.13

50.62	81.47	108.80	116.97
46.97	78.88	101.00	106.94
54.22	80.45	147.50	122.99
57.23	89.45	169.40	138.69
46.96	84.80	119.20	113.33
53.96	83.75	137.60	126.05
57.86	84.63	143.80	134.72
55.67	82.39	151.10	127.96
55.27	82.58	145.90	127.37
50.09	91.20	121.20	126.46
53.28	103.72	154.10	146.33
49.35	87.19	101.60	120.65
55.70	92.26	132.40	138.71
52.91	104.46	132.20	146.40
55.87	97.34	146.80	144.54
46.83	83.88	105.40	112.08
51.75	91.06	143.30	129.59
48.42	83.05	119.01	114.33
48.89	87.18	98.10	119.73
40.10	65.26	60.30	78.60
45.94	75.18	80.40	100.90
45.71	85.16	92.70	111.25
56.90	94.79	148.00	143.82
52.73	88.75	125.10	129.03
50.24	82.95	120.30	117.82
52.73	96.21	131.10	137.11
54.87	88.01	138.80	132.46
54.16	86.02	139.10	128.90
47.57	87.52	104.00	117.49
57.18	91.74	155.80	141.07
45.65	77.35	89.10	102.67
49.10	88.47	117.40	121.54
55.29	79.13	127.50	123.68
49.12	90.11	120.70	123.36
52.89	87.56	127.80	128.06
49.85	86.50	111.20	120.89
48.36	79.77	113.70	110.66
59.61	95.71	167.10	150.18
55.51	98.31	148.20	144.88
55.91	79.85	111.70	125.68
57.71	98.23	185.30	149.15
48.08	84.13	104.30	114.82
50.39	88.97	118.90	124.64
54.91	82.88	137.10	126.99
52.71	101.28	140.90	142.56
47.03	84.17	90.70	112.79
48.09	84.65	104.40	115.41

49.29	85.54	107.10	118.75
50.78	97.89	112.01	135.07
48.24	81.34	104.70	112.12
94.05	151.84	128.60	279.12
57.93	100.68	157.20	152.24
52.73	96.72	144.80	137.66
50.63	93.96	137.10	130.52
58.05	98.40	163.50	150.01
51.24	91.87	125.00	129.46
50.51	91.95	114.60	128.10
48.15	99.65	119.90	131.77
52.58	72.12	115.30	110.72
52.56	92.05	126.70	132.27
44.67	89.98	89.90	114.41
54.42	87.50	133.80	131.02
49.32	79.48	107.50	112.24
49.91	88.92	113.10	123.63
50.43	80.31	109.70	115.34
48.49	98.27	110.00	130.95
39.81	66.10	56.70	78.93
37.05	57.51	40.80	64.17
55.51	93.41	138.80	139.58
45.65	96.40	93.70	123.30
52.17	83.21	106.30	121.92
61.05	93.39	156.10	150.52
53.26	84.43	134.09	125.40
57.34	93.35	162.20	143.13
95.49	99.27	151.80	225.03
49.77	106.40	123.00	142.29
52.65	79.60	134.10	118.96
56.71	96.05	154.00	144.81
51.96	93.33	139.40	132.47
54.04	106.84	163.90	151.21
51.74	85.42	124.30	123.46
54.34	88.94	143.90	132.42
55.12	89.16	145.80	134.20
48.57	90.73	104.30	122.94
50.54	90.84	121.20	126.96
50.69	85.62	115.30	121.60
52.44	95.96	137.00	136.26
50.55	95.64	112.30	132.18
53.02	83.58	120.10	124.00
43.67	76.63	80.30	97.98
41.37	66.42	58.50	82.37
	Average for replicate 5	**127.94**	**130.63**
	Average of 10 fruits randomly picked in this replicate	**124.40**	**126.16**

	% deviation (between replicate average and calculated average)	**2.11**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**1.42**	

Table 3.5: A test run to quantify the prediction accuracy of the regression model 3.2 for predicting the pulp mass (calculated pulp mass) using fruit mass and peel mass as parameters. The average fruit mass was calculated for all harvested fruit in ten replicates and compared with the average mass for 10 fruits randomly picked.

Fruit mass	Peel mass	Pulp mass	Calculated pulp mass
162.70	80.48	81.78	80.69
152.02	84.19	66.76	67.85
183.26	97.65	84.92	84.78
136.42	76.62	59.44	60.12
170.46	93.89	76.16	76.35
163.17	105.76	57.58	59.67
196.12	96.26	98.18	97.62
141.83	82.30	58.65	60.21
134.69	71.60	62.81	62.81
153.98	76.82	75.50	75.88
163.35	81.48	81.06	80.43
149.26	80.26	68.86	68.68
185.16	75.00	87.44	105.72
152.23	85.42	66.47	67.00
152.70	87.12	80.14	65.98
158.07	68.47	89.30	86.68
151.64	80.55	70.40	70.60
156.69	84.06	72.39	72.20
169.80	97.65	73.27	72.56
174.50	97.65	75.05	76.83
	Average for replicate 1	**74.31**	**74.63**
	Average of 10 fruits randomly picked in this replicate	**72.18**	**72.60**
	% deviation (between replicate average and calculated average)	**0.44**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**0.58**	
178.86	95.51	82.06	82.60
167.48	77.76	89.06	87.34
195.64	94.52	100.61	98.66

148.90	70.29	77.93	76.82
152.88	70.26	82.15	80.45
226.53	128.58	97.32	97.79
280.49	144.78	135.36	133.00
137.30	67.76	69.20	68.44
116.29	59.27	56.60	56.58
140.36	61.94	78.12	76.15
138.69	59.87	78.52	76.39
130.05	68.13	61.53	61.55
177.34	84.73	92.16	90.37
166.55	81.06	84.98	83.69
256.68	154.55	100.78	103.11
160.57	74.81	85.49	83.57
139.24	69.37	69.54	68.83
143.61	75.66	67.73	67.46
202.38	114.46	87.47	87.86
150.90	80.24	70.28	70.19
	Average for replicate 2	**83.34**	**82.54**
	Average of 10 fruits randomly picked in this replicate	**86.84**	**85.78**
	% deviation (between replicate average and calculated average)	**0.97**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**1.23**	
194.72	99.95	94.28	93.22
217.74	112.04	104.85	103.85
200.65	98.79	100.87	99.59
196.68	90.34	106.07	103.15
185.80	78.21	106.95	103.58
123.09	71.52	51.35	52.36
204.56	109.43	94.13	94.11
136.50	66.13	69.97	69.10
137.48	73.31	63.70	63.89
161.86	81.13	80.25	79.38
218.42	120.95	96.77	96.91
174.60	78.21	94.92	93.41
147.60	84.54	62.80	63.55
161.26	79.98	80.89	79.81
207.10	90.01	116.61	112.89
136.18	71.23	64.37	64.48
145.30	72.17	72.90	71.96
188.45	91.53	96.61	94.68
135.42	70.84	64.00	64.12
	Average for replicate 3	**85.38**	**84.42**
	Average of 10 fruits	**87.24**	**86.22**

	randomly picked in this replicate		
	% deviation (between replicate average and calculated average)	**1.14**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**1.18**	
147.30	74.30	71.90	71.96
196.00	73.50	97.40	116.83
205.60	108.00	84.70	96.26
169.20	85.00	83.80	82.75
170.40	89.70	80.40	79.85
160.60	87.70	72.40	72.66
126.90	67.10	59.50	59.56
168.00	85.10	82.50	81.58
155.20	57.40	77.80	93.47
147.90	73.30	74.30	73.36
162.80	77.40	84.19	83.40
122.30	70.90	50.70	52.16
165.30	104.20	60.50	62.92
168.10	82.70	85.10	83.71
154.80	78.00	76.40	75.63
129.20	63.10	65.70	65.04
149.10	84.60	64.20	64.86
132.80	72.30	65.70	60.50
136.60	71.50	64.90	64.63
	Average for replicate 4	**73.79**	**75.85**
	Average of 10 fruits randomly picked in this replicate	**78.47**	**82.83**
	% deviation (between replicate average and calculated average)	**2.79**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**5.55**	
153.00	93.40	59.00	60.93
174.20	96.40	77.40	77.62
136.50	71.50	64.40	64.54
168.70	95.60	72.70	73.31
189.30	112.60	75.20	77.57
201.20	116.10	79.40	85.40
155.10	85.70	68.30	69.37
149.30	77.87	70.50	70.75
161.10	101.10	59.00	61.74
109.10	78.90	49.70	33.40
161.90	78.00	83.50	82.07

145.00	84.80	60.00	60.97
154.80	85.50	69.10	69.26
147.60	79.60	67.90	67.74
171.30	88.40	82.60	81.77
134.90	76.90	57.90	58.51
123.70	73.80	49.90	50.97
136.90	77.40	59.40	59.90
149.70	91.60	58.00	59.46
142.30	89.80	52.40	54.27
	Average for replicate 5	**65.82**	**65.98**
	Average of 10 fruits randomly picked in this replicate	**67.56**	**67.46**
	% deviation (between replicate average and calculated average)	**0.25**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**0.15**	
Fruit mass	**Peel mass**	**Pulp mass**	**Calculated pulp mass**
218.36	131.10	86.08	88.24
188.90	91.96	96.50	94.72
160.71	83.60	76.33	76.24
142.61	77.80	64.50	64.74
168.82	86.70	81.50	80.97
158.49	87.80	70.00	70.66
138.13	65.80	71.70	70.85
171.92	97.80	73.30	74.36
163.90	89.80	73.65	73.87
216.89	114.20	102.30	101.25
242.42	138.60	103.30	103.71
185.40	99.50	85.40	85.15
228.90	129.10	99.10	99.50
177.30	102.00	74.70	75.68
133.34	60.30	67.68	71.18
134.40	68.40	65.31	65.26
128.08	62.50	64.90	64.54
124.34	72.40	51.50	52.74
132.30	68.20	63.70	63.53
120.30	73.00	46.75	48.57
	Average for replicate 6	**75.91**	**76.29**
	Average of 10 fruits randomly picked in this replicate	**79.59**	**79.59**
	% deviation (between replicate average and calculated average)	**0.50**	
	% deviation (between average for 10 fruits and	**0.00**	

	calculated average for 10 fruits)		
199.97	112.42	87.10	87.41
183.21	99.30	83.31	83.33
153.06	82.02	70.50	70.64
167.30	96.90	70.06	70.93
153.93	87.70	65.83	66.61
180.10	92.38	87.20	86.38
154.41	75.43	78.49	77.45
159.26	78.34	80.60	79.39
175.24	96.68	78.12	78.33
149.90	78.60	70.90	70.67
152.20	83.00	68.70	69.03
194.00	98.00	95.40	94.22
204.60	116.10	88.00	88.48
182.80	106.90	75.10	76.51
184.30	103.80	79.80	80.50
186.30	84.80	101.00	98.44
156.40	90.20	65.80	66.73
169.80	85.20	83.50	83.13
178.97	107.50	71.00	72.53
143.66	80.45	62.66	63.44
	Average for replicate 7	**78.15**	**78.21**
	Average of 10 fruits randomly picked in this replicate	**77.21**	**77.11**
	% deviation (between replicate average and calculated average)	**0.07**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**0.13**	
174.20	81.50	91.60	90.26
198.81	97.02	101.20	99.42
199.20	121.10	73.40	79.34
182.10	117.40	63.80	66.97
138.50	79.60	58.00	59.48
167.20	87.20	79.50	79.07
214.40	117.80	96.00	95.93
212.90	107.30	105.30	103.48
199.60	109.90	89.00	89.21
170.30	94.70	75.10	75.52
183.30	110.90	71.90	73.57
170.00	76.00	93.00	91.12
199.50	117.80	81.10	82.41
170.50	87.70	82.00	81.64
184.90	108.10	75.90	77.40
141.70	72.00	69.20	68.83

181.10	92.40	88.20	87.27
161.30	104.90	55.60	58.70
172.80	111.80	60.20	63.28
174.50	92.40	81.20	81.28
	Average for replicate 8	**79.56**	**80.21**
	Average of 10 fruits randomly picked in this replicate	**83.29**	**83.87**
	% deviation (between replicate average and calculated average)	**0.82**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**0.69**	
130.90	74.50	55.50	56.91
140.50	70.70	69.30	68.85
135.10	64.30	70.00	69.38
145.80	72.80	72.50	71.87
178.50	102.80	73.60	76.09
148.70	77.00	71.50	70.94
146.00	79.70	65.40	66.20
130.20	75.10	53.70	55.77
155.50	89.20	65.50	66.76
133.50	65.20	67.60	67.16
151.30	90.00	60.80	62.27
136.10	69.10	66.70	66.21
140.00	70.90	67.80	68.22
128.50	73.20	54.80	55.84
136.80	78.40	57.30	58.96
107.80	57.40	48.30	50.46
114.90	65.50	48.20	50.03
115.70	61.70	53.20	53.98
122.40	78.30	43.30	45.98
94.50	57.40	41.90	38.40
	Average for replicate 9	**60.35**	**61.01**
	Average of 10 fruits randomly picked in this replicate	**66.46**	**66.99**
	% deviation (between replicate average and calculated average)	**1.11**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**0.80**	
192.50	109.10	83.20	83.44
186.30	115.38	70.40	72.49
171.50	91.30	79.60	79.49
176.40	103.90	71.60	73.25

127.60	61.40	65.50	65.03
179.70	108.80	69.90	72.09
157.30	90.90	65.60	66.95
136.30	71.39	64.10	64.45
96.90	45.93	47.10	50.31
120.70	69.20	51.00	52.16
173.20	89.30	83.40	82.73
165.83	99.60	65.30	67.31
163.70	79.80	83.90	82.18
146.00	92.80	52.90	55.09
158.90	80.80	77.40	76.97
206.10	122.90	82.70	84.07
146.10	77.70	67.50	67.99
150.20	76.90	60.77	72.39
181.10	104.80	75.80	76.75
83.00	45.60	36.80	37.98
	Average for replicate 10	**67.72**	**69.16**
	Average of 10 fruits randomly picked in this replicate	**66.80**	**67.97**
	% deviation (between replicate average and calculated average)	**2.11**	
	% deviation (between average for 10 fruits and calculated average for 10 fruits)	**1.75**	

APPENDIX B

ANOVA tables are numbered according to corresponding figure numbers.

Figure 4.1A: ANOVA of the glucose content in fruit pulp (µmol g fresh mass^{-1}) for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	6194.533	1548.633	1.11	0.389202	0.266038
Error	15	20977.48	1398.499			
Total	19	27172.02				

* Term significant at alpha = 0.05

Figure 4.1B: ANOVA of the fructose content in fruit pulp (µmol g fresh mass^{-1}) for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	16280.69	4070.172	10.93	0.000236*	0.998011
Error	15	5587.052	372.4701			
Total	19	21867.74				

* Term significant at alpha = 0.05

Figure 4.1C: ANOVA of the sucrose content in fruit pulp (µmol g fresh mass^{-1}) for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	5799.769	1449.942	28.63	0.000019*	1
Error	10	506.5025	50.65025			
Total	14	6306.271				

* Term significant at alpha = 0.05

Figure 4.2A: ANOVA of the glucose content in fruit pulp (µmol g fresh mass^{-1}) for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	187892.2	46973.05	9.4	0.000521*	0.993757
Error	15	74956.45	4997.096			
Total	19	262848.7				

* Term significant at alpha = 0.05

Figure 4.2B: ANOVA of the fructose content in fruit pulp (µmol g fresh mass^{-1}) for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	249058.8	62264.69	13.98	0.000060*	0.999822
Error	15	66800.17	4453.345			
Total	19	315858.9				

* Term significant at alpha = 0.05

Figure 4.2C: ANOVA of the sucrose content in fruit pulp (µmol g fresh mass^{-1}) for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	34268.27	8567.067	2.61	0.100011	0.51613
Error	10	32884.4	3288.44			
Total	14	67152.67				

Linear equation model 4.1: Multiple regression and correlation analyses of data for the prediction of the amount of glucose (µmol g fresh mass^{-1}) using fruit length as parameter

Independent	**Regression**	**Standard**	**T-Value**	**Prob**	**Decision**	**Power**
Variable	**Coefficient**	**Error**	**(Ho: B=0)**	**Level**	**5%**	**5%**
Intercept	2525.774	288.7995	8.7458	0.000000	Reject Ho	1
Fruit length	-5.551996	2.988744	-1.8576	0.070983	Accept Ho	0.440733
R-Squared	0.083251					

*Reject Ho = Significant at the 95% probability level

ANOVA of equation model 4.1 to test the significance of the model

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Intercept	1	1.59E+08	1.59E+08			
Model	1	68119.71	68119.71	3.4508	0.070983	0.440733
Error	38	750128.3	19740.22			
Total	39	818247.9	20980.72			

* Term significant at alpha = 0.05

Figure 4.3A: ANOVA of the β-carotene content (µg $100g^{-1}$ fruit pulp) for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	77.66566	19.41642	0.78	0.550924	0.206741
Error	20	497.4511	24.87256			
Total	24	575.1168				

* Term significant at alpha = 0.05

Figure 4.3D: ANOVA of the β-carotene:fruit mass ratio for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	1.62E-02	4.04E-03	2.9	0.048215*	0.673992
Error	20	2.79E-02	1.39E-03			
Total	24	4.40E-02				

* Term significant at alpha = 0.05

Figure 4.4A: ANOVA of the β-carotene content (µg $100g^{-1}$ fruit pulp) for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	89.4219	22.35547	1.55	0.225072	0.392272
Error	20	287.6859	14.3843			
Total	24	377.1078				

* Term significant at alpha = 0.05

Figure 4.4D: ANOVA of the β-carotene:fruit mass ratio for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	5.74E-03	1.44E-03	1.05	0.406203	0.270734
Error	20	2.73E-02	1.37E-03			
Total	24	3.31E-02				

* Term significant at alpha = 0.05

Linear equation model 4.2: Multiple regression and correlation analyses of data for the prediction of the amount of β-carotene (µmol 100 g fruit pulp^{-1}) using fruit diameter as parameter

Independent	**Regression**	**Standard**	**T-Value**	**Prob**	**Decision**	**Power**
Variable	**Coefficient**	**Error**	**(Ho: B=0)**	**Level**	**5%**	**5%**
Intercept	31.95354	6.930527	4.6105	0.00003	Reject Ho	0.994715
Fruit length	-0.2290789	0.123035	-1.8619	0.068746	Accept Ho	0.446245
R-Squared	0.067357					

*Reject Ho = Significant at the 95% probability level

ANOVA of equation model 4.2 to test the significance of the model

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Intercept	1	18245.08	18245.08			
Model	1	67.97219	67.97219	3.4667	0.068746	0.446245
Error	48	941.1555	19.60741			
Total	49	1009.128	20.59444			

* Term significant at alpha = 0.05

Figure 4.5A: ANOVA of the vitamin C content in fruit pulp for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	6.09E-03	1.52E-03	0.98	0.442205	0.252995
Error	20	3.12E-02	1.56E-03			
Total	24	3.73E-02				

* Term significant at alpha = 0.05

Figure 4.6A: ANOVA of the vitamin C content in fruit pulp for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	0.010457	2.61E-03	3.01	0.042739*	0.692551
Error	20	1.74E-02	8.69E-04			
Total	24	2.78E-02				

* Term significant at alpha = 0.05

Linear equation model 4.3: Multiple regression and correlation analyses of data for the prediction of vitamin C content (µmol 100 ml fruit pulp^{-1}) using fruit diameter and fruit length as parameters.

Independent	**Regression**	**Standard**	**T-Value**	**Prob**	**Decision**	**Power**
Variable	**Coefficient**	**Error**	**(Ho: B=0)**	**Level**	**5%**	**5%**
Intercept	0.4545022	8.11E-02	5.6076	0.000001	Reject Ho	0.999792
Fruit diameter	-2.06E-03	8.61E-04	-2.3912	0.020847	Reject Ho	0.648855
Fruit length	-1.06E-03	5.53E-04	-1.9247	0.060336	Accept Ho	0.470298
R-Squared	0.132142					

*Reject Ho = Significant at the 95% probability level

ANOVA of equation model 4.3 to test the significance of the model

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Intercept	1	2.906551	2.906551			
Model	2	8.97E-03	4.49E-03	3.5782	0.035772	0.355518
Error	47	5.89E-02	1.25E-03			
Total	49	6.79E-02	1.39E-03			

* Term significant at alpha = 0.05

Figure 4.7A: ANOVA of total water-soluble protein content in fresh fruit pulp for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	0.217101	5.43E-02	1.51	0.236053	0.382614
Error	20	0.717067	3.59E-02			
Total	24	0.934167				

* Term significant at alpha = 0.05

Figure 4.7D: ANOVA of total water-soluble protein:fruit mass ratio for the west block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	3.87E-05	9.68E-06	2.1	0.118794	0.517595
Error	20	9.22E-05	4.61E-06			
Total	24	1.31E-04				

* Term significant at alpha = 0.05

Figure 4.8A: ANOVA of total water-soluble protein content in fresh fruit pulp for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	0.540142	0.135036	4.75	0.007385*	0.890936
Error	20	0.56854				
Total	24	1.108683				

* Term significant at alpha = 0.05

Figure 4.8D: ANOVA of total water-soluble protein:fruit mass ratio for the east block

Analysis of Variance Table						
Source		**Sum of**	**Mean**		**Prob**	**Power**
Term	**DF**	**Squares**	**Square**	**F-Ratio**	**Level**	**(Alpha=0.05)**
Treatment	4	3.57E-05	8.92E-06	1.48	0.24633	0.373948
Error	20	1.21E-04	6.04E-06			
Total	24	1.56E-04				

* Term significant at alpha = 0.05

Linear equation model 4.4: Multiple regression and correlation analyses of data for the prediction of the total water-soluble protein content (mg g^{-1} fresh mass) in fresh fruit pulp using fruit mass as parameter

Independent Variable	Regression Coefficient	Standard Error	T-Value (Ho: B=0)	Prob Level	Decision 5%	Power 5%
Intercept	2.501314	0.240108	10.4175	0	Reject Ho	1
Fruit length	-3.34E-03	1.62E-03	-2.0616	0.044683	Reject Ho	0.524079
R-Squared	0.081344					

*Reject Ho = Significant at the 95% probability level

ANOVA of equation model 4.4 to test the significance of the model

Analysis of Variance Table						
Source Term	**DF**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob Level**	**Power (Alpha=0.05)**
Intercept	1	201.9648	201.9648			
Model	1	0.172347	0.172347	4.2502	0.044683	0.524079
Error	48	1.946398	4.05E-02			
Total	49	2.118744	4.32E-02			

* Term significant at alpha = 0.05

Figure 4.9A: Spectrum of a 5 µg external lycopene standard injected into the HPLC before the trial commenced

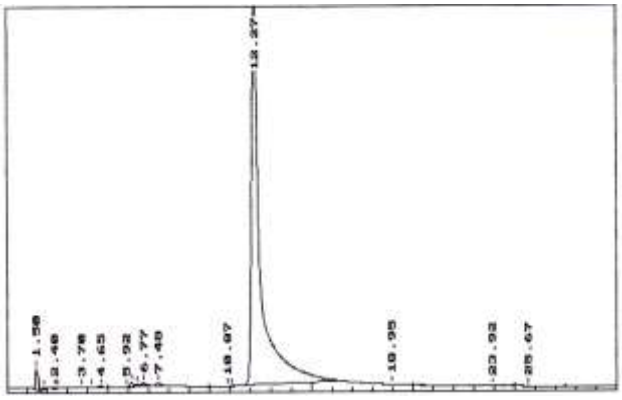
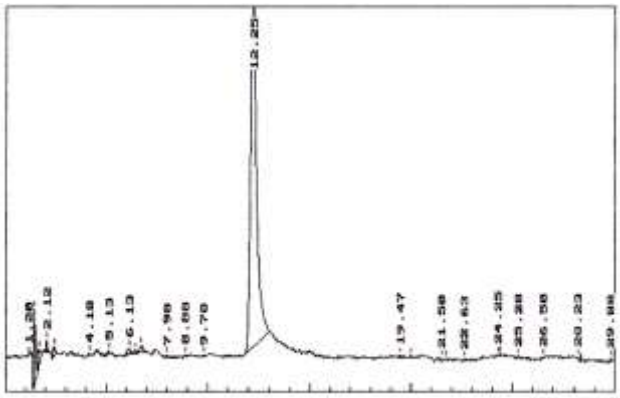


Figure 4.9B: Spectrum of 7.5 µg fruit pulp extracted from a green fruit and injected into the HPLC to screen for lycopene



Note: Lycopene was found in overripe green fruit only and not investigated further.

Figure 4.10A: Spectrum of a 50 µL external ß-carotene standard injected into the HPLC

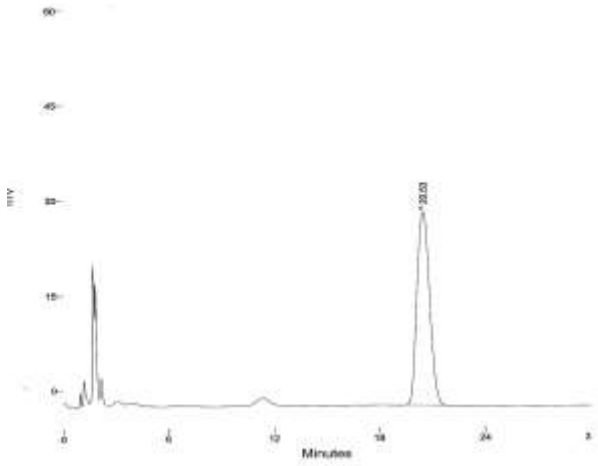


Figure 4.10B: Spectrum of a 50 µL fresh fruit pulp extract injected into the HPLC for ß-carotene detection

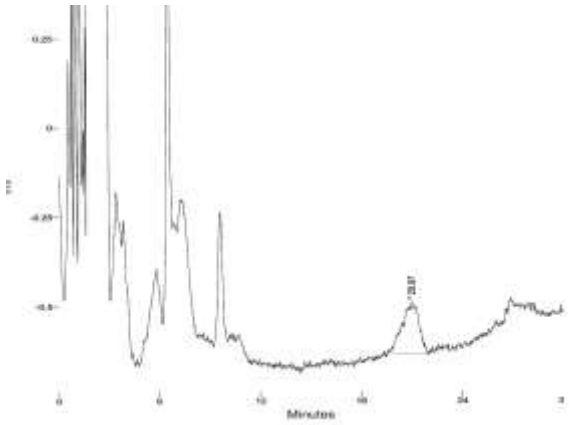


Figure 4.11A: Spectrum of a 10 µL external vitamin C standard injected into the HPLC for identification

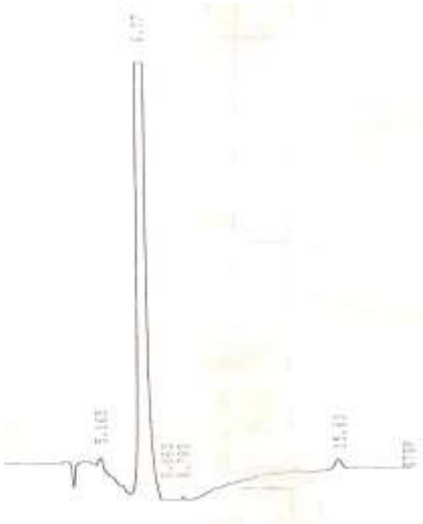


Figure 4.11B: Spectrum of a 10 µL fresh fruit pulp extract injected into the HPLC for vitamin C detection

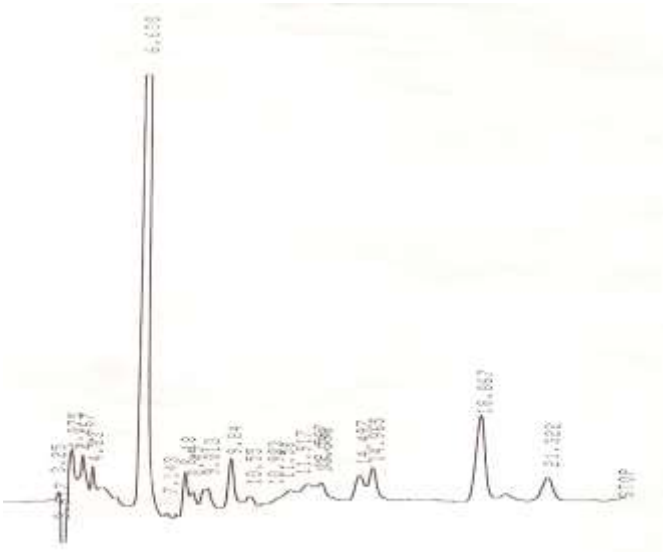


Table 4.2: A test run to quantify the prediction accuracy of regression model 4.1 for predicting the glucose content (calculated glucose) using fruit length as parameter. The average glucose content was calculated for ten treatments and its five replicates selected in the west and east block trials.

Treatment	Replicate	Fruit length	[Glucose]	[Calculated Glucose]
1	1	93.5700	2102.8730	2006.2734
	2	92.8500	2133.9610	2010.2708
	3	85.9400	2121.6420	2048.6351
	4	95.8600	2113.2740	1993.5593
	5	92.0550	2117.9375	2014.6846
		Average for 5 replicates	**2117.9375**	**2014.6846**
		% deviation between real and calculated average values	**5.13**	
2	1	97.5900	2097.0040	1983.9543
	2	93.2300	2067.3690	2008.1610
	3	95.8400	2108.2770	1993.6703
	4	92.2700	2094.9120	2013.4910
	5	94.7325	2091.8905	1999.8192
		Average for 5 replicates	**2091.8905**	**1999.8192**
		% deviation between real and calculated average values	**4.60**	
3	1	94.9600	2023.5550	1998.5561
	2	89.2300	2034.5380	2030.3690
	3	92.8000	2126.4650	2010.5484
	4	91.2700	2139.5390	2019.0430
	5	92.0650	2081.0243	2014.6291
		Average for 5 replicates	**2081.0243**	**2014.6291**
		% deviation between real and calculated average values	**3.30**	
4	1	99.2200	2149.0690	1974.9046
	2	95.9000	2122.3970	1993.3372
	3	102.7700	2145.5820	1955.1950
	4	88.6000	2103.2210	2033.8668
	5	96.6225	2130.0673	1989.3259
		Average for 5 replicates	**2130.0673**	**1989.3259**
		% deviation between real and calculated average values	**7.07**	
5	1	86.0800	2132.3340	2047.8578
	2	88.2100	2150.9860	2036.0321
	3	96.6300	2110.0200	1989.2842
	4	95.7700	2038.8960	1994.0590
	5	91.6725	2108.0590	2016.8083
		Average for 5 replicates	**2108.0590**	**2016.8083**
		% deviation between real and calculated average values	**4.52**	
		East block trial		
		Fruit length	**[Glucose]**	**[Calculated Glucose]**
6	1	97.6100	1982.4730	1983.8433

	2	105.3200	1981.1950	1941.0374
	3	108.1700	1949.4680	1925.2142
	4	101.3400	1966.3770	1963.1343
	5	103.1100	1969.8783	1953.3073
		Average for 5 replicates	**1969.8783**	**1953.3073**
		% deviation between real and calculated average values	**0.85**	
7	1	98.6000	2013.9670	1978.3468
	2	92.9400	1992.8740	2009.7711
	3	93.3100	2003.1590	2007.7169
	4	110.7700	2021.8700	1910.7790
	5	98.9050	2007.9675	1976.6534
		Average for 5 replicates	**2007.9675**	**1976.6534**
		% deviation between real and calculated average values	**1.58**	
8	1	101.9700	1909.8960	1959.6366
	2	96.6500	1857.0760	1989.1732
	3	85.6800	1739.9880	2050.0786
	4	85.4300	1760.9070	2051.4666
	5	92.4325	1816.9668	2012.5888
		Average for 5 replicates	**1816.9668**	**2012.5888**
		% deviation between real and calculated average values	**10.77**	
9	1	89.5700	1887.2340	2028.4814
	2	99.5600	1713.0840	1973.0169
	3	88.8000	1838.1910	2032.7564
	4	98.0200	1894.3810	1981.5670
	5	93.9875	1833.2225	2003.9554
		Average for 5 replicates	**1833.2225**	**2003.9554**
		% deviation between real and calculated average values	**9.31**	
10	1	108.2100	1864.2810	1924.9921
	2	119.7800	1665.3770	1860.7554
	3	95.6900	1658.5200	1994.5031
	4	107.7000	1818.9570	1927.8236
	5	107.8450	1751.7838	1927.0186
		Average for 5 replicates	**1751.7838**	**1927.0186**
		% deviation between real and calculated average values	**10.00**	

Table 4.3: A test run to quantify the prediction accuracy of regression model 4.2 for predicting the β-carotene content (calculated β-carotene) using fruit diameter as parameter for ten treatments and its five replicates selected in the west and east block trials

Treatment	Replicate	Fruit diameter	[B-carotene]	[Calculated B-carotene]
1	1	51.5700	19.9605	20.1388
	2	51.3500	22.7700	20.1892
	3	56.1700	30.4450	19.0850
	4	49.7200	24.0417	20.5626
	5	60.5300	16.6298	18.0861
		Average for 5 replicates	**22.7694**	**19.6123**
		% deviation between real and calculated average values	**16.10**	
2	1	51.4100	22.2357	20.1755
	2	53.2200	17.1693	19.7608
	3	49.8000	20.8752	20.5443
	4	48.2000	26.7860	20.9109
	5	50.9200	21.7700	20.2877
		Average for 5 replicates	**21.7672**	**20.3358**
		% deviation between real and calculated average values	**7.04**	
3	1	53.9600	18.4300	19.5913
	2	63.0700	20.2185	17.5042
	3	65.3400	14.3781	16.9841
	4	59.5800	21.3209	18.3037
	5	60.8100	17.7791	18.0219
		Average for 5 replicates	**18.4253**	**18.0810**
		% deviation between real and calculated average values	**1.90**	
4	1	52.8300	18.6700	19.8501
	2	52.7700	25.6132	19.8639
	3	52.9100	14.9410	19.8318
	4	54.8500	23.1270	19.3874
	5	58.9300	11.0005	18.4526
		Average for 5 replicates	**18.6704**	**19.4772**
		% deviation between real and calculated average values	**4.32**	
5	1	52.0400	25.7070	20.0311
	2	67.4000	19.2100	16.5122
	3	56.3400	13.3226	19.0460
	4	54.2500	25.8947	19.5248
	5	55.5300	11.9153	19.2316
		Average for 5 replicates	**19.2099**	**18.8691**
		% deviation between real and calculated average values	**1.81**	
		East block trial		
		Fruit diameter	**[B-carotene]**	**[Calculated B-carotene]**

6	1	55.8500	18.2800	19.1583
	2	54.9200	16.4188	19.3713
	3	58.8000	14.4016	18.4824
	4	51.5200	24.6750	20.1503
	5	72.4500	17.6150	15.3552
		Average for 5 replicates	**18.2781**	**18.5035**
		% deviation between real and calculated average values	**1.23**	
7	1	57.7000	25.4022	18.7344
	2	56.3300	25.0738	19.0483
	3	50.1900	20.9300	20.4550
	4	50.0700	22.6813	20.4825
	5	56.6200	10.5784	18.9819
		Average for 5 replicates	**20.9331**	**19.5404**
		% deviation between real and calculated average values	**7.13**	
8	1	65.5000	20.3827	16.9475
	2	57.5200	18.3400	18.7757
	3	54.8600	16.1842	19.3851
	4	50.9200	20.5469	20.2877
	5	54.1300	16.2311	19.5523
		Average for 5 replicates	**18.3370**	**18.9896**
		% deviation between real and calculated average values	**3.56**	
9	1	57.2000	17.6300	18.8490
	2	53.1000	14.3312	19.7883
	3	61.3900	20.7814	17.8891
	4	60.9200	15.8793	17.9967
	5	59.5800	19.5149	18.3037
		Average for 5 replicates	**17.6274**	**18.5654**
		% deviation between real and calculated average values	**5.32**	
10	1	56.5300	17.4508	19.0025
	2	50.6000	15.0100	20.3610
	3	63.9900	18.3421	17.2934
	4	57.5300	12.8535	18.7734
	5	53.2400	11.3758	19.7562
		Average for 5 replicates	**15.0065**	**19.0373**
		% deviation between real and calculated average values	**26.86**	

Table 4.4: A test run to quantify the prediction accuracy of regression model 4.3 for predicting the vitamin C content (calculated vitamin C) using fruit diameter and fruit length as parameter for ten treatments and its five replicates selected in the west and east block

Treatment	Replicate	Fruit length	[Vitamin C]	[Calculated Vitamin C]
1	1	96.4600	0.1928	0.2337
	2	93.1900	0.2388	0.2431
	3	96.9900	0.2323	0.2401
	4	90.7000	0.2836	0.2590
	5	81.2300	0.3479	0.2501
		Average for 5 replicates	**0.2591**	**0.2452**
		% deviation between real and calculated average values	**5.66**	
2	1	86.1200	0.2241	0.2394
	2	95.2500	0.2545	0.2442
	3	96.7400	0.2226	0.2455
	4	94.3700	0.2553	0.2351
	5	102.2300	0.2376	0.2253
		Average for 5 replicates	**0.2388**	**0.2379**
		% deviation between real and calculated average values	**0.39**	
3	1	93.1600	0.2618	0.2441
	2	95.7800	0.2433	0.2424
	3	96.6300	0.1770	0.2339
	4	102.3700	0.2213	0.2229
	5	95.2000	0.1915	0.2264
		Average for 5 replicates	**0.2190**	**0.2339**
		% deviation between real and calculated average values	**6.85**	
4	1	92.3500	0.2266	0.2442
	2	100.3200	0.1909	0.2265
	3	81.6300	0.2369	0.2579
	4	99.5500	0.1678	0.2319
	5	92.6800	0.2558	0.2402
		Average for 5 replicates	**0.2156**	**0.2401**
		% deviation between real and calculated average values	**11.38**	
5	1	90.6100	0.2525	0.2490
	2	76.1000	0.1682	0.2391
	3	108.1300	0.2362	0.2188
	4	73.6200	0.2658	0.2504
	5	91.8800	0.2556	0.2357
		Average for 5 replicates	**0.2356**	**0.2386**
		% deviation between real and calculated average values	**1.24**	
		East block trial		
		Fruit length	**[Vitamin C]**	**[Calculated Vitamin C]**

6	1	97.0900	0.2432	0.2402
	2	85.8400	0.2319	0.2433
	3	107.0700	0.1919	0.2277
	4	93.1800	0.2857	0.2347
	5	58.3900	0.2058	0.2165
		Average for 5 replicates	**0.2317**	**0.2325**
		% deviation between real and calculated average values	**0.34**	
7	1	101.3000	0.2465	0.2303
	2	99.3500	0.2515	0.2339
	3	63.6900	0.2950	0.2725
	4	92.2700	0.2476	0.2416
	5	82.1400	0.2583	0.2561
		Average for 5 replicates	**0.2598**	**0.2469**
		% deviation between real and calculated average values	**5.23**	
8	1	107.1600	0.2354	0.2183
	2	88.6900	0.1936	0.2580
	3	85.8400	0.2262	0.2373
	4	95.9100	0.2370	0.2502
	5	94.4400	0.2076	0.2344
		Average for 5 replicates	**0.2200**	**0.2396**
		% deviation between real and calculated average values	**8.93**	
9	1	87.1700	0.2493	0.2923
	2	91.4100	0.2507	0.2377
	3	85.4900	0.2580	0.2403
	4	90.4000	0.3111	0.2529
	5	97.5200	0.3182	0.2413
		Average for 5 replicates	**0.2775**	**0.2529**
		% deviation between real and calculated average values	**9.71**	
10	1	90.2800	0.1980	0.2443
	2	84.9700	0.2531	0.2543
	3	91.1700	0.2694	0.2434
	4	87.3100	0.2678	0.2467
	5	102.1100	0.2818	0.2282
		Average for 5 replicates	**0.2540**	**0.2434**
		% deviation between real and calculated average values	**4.37**	

Table 4.5: A test run to quantify the prediction accuracy of regression model 4.4 for predicting the protein content (calculated protein) using fruit mass as parameter for ten treatments and its five replicates selected in the west and east block

Treatment	Replicate	Fruit mass	[Protein]	[Calculated Protein]
1	1	144.6500	1.7490	2.0185
	2	113.5800	1.8140	2.1222
	3	133.8000	1.8590	2.0547
	4	161.8500	1.8240	1.9611
	5	182.1000	1.8720	1.8935
		Average for 5 replicates	**1.8236**	**2.0100**
		% deviation between real and calculated average values	**10.22**	
2	1	136.4400	2.0730	2.0459
	2	126.2500	2.0020	2.0799
	3	122.4500	2.3580	2.0926
	4	136.4800	1.7360	2.0458
	5	128.7100	2.0240	2.0717
		Average for 5 replicates	**2.0386**	**2.0672**
		% deviation between real and calculated average values	**1.40**	
3	1	133.3500	1.8980	2.0562
	2	146.1100	2.0280	2.0136
	3	142.7400	1.7680	2.0249
	4	130.9900	2.0920	2.0641
	5	160.5100	1.7940	1.9656
		Average for 5 replicates	**1.9160**	**2.0249**
		% deviation between real and calculated average values	**5.68**	
4	1	147.4600	2.3840	2.0091
	2	181.0600	1.6970	1.8970
	3	168.0100	2.2610	1.9405
	4	160.6200	1.7550	1.9652
	5	155.1000	1.8400	1.9836
		Average for 5 replicates	**1.9874**	**1.9591**
		% deviation between real and calculated average values	**1.44**	
5	1	146.1300	2.0890	2.0136
	2	147.5900	2.1440	2.0087
	3	141.6200	2.0580	2.0286
	4	155.6200	1.9450	1.9819
	5	136.2400	2.2070	2.0466
		Average for 5 replicates	**2.0886**	**2.0159**
		% deviation between real and calculated average values	**3.61**	
		East block trial		
		Fruit mass	**[Protein]**	**[Calculated Protein]**
6	1	131.0000	1.8250	2.0641

	2	167.3000	1.7680	1.9429
	3	165.9000	1.8080	1.9476
	4	154.2000	1.8080	1.9866
	5	154.6000	1.8020	1.9853
		Average for 5 replicates	**1.8022**	**1.9853**
		% deviation between real and calculated average values	**10.16**	
7	1	145.4000	1.9600	2.0160
	2	191.3000	1.8530	1.8628
	3	119.1000	2.0720	2.1038
	4	127.2000	2.0270	2.0768
	5	159.0000	1.8860	1.9706
		Average for 5 replicates	**1.9596**	**2.0060**
		% deviation between real and calculated average values	**2.37**	
8	1	175.8000	2.0490	1.9145
	2	131.9000	2.1840	2.0611
	3	146.7000	2.1670	2.0117
	4	124.9000	2.2460	2.0844
	5	160.5000	2.1610	1.9656
		Average for 5 replicates	**2.1614**	**2.0075**
		% deviation between real and calculated average values	**7.67**	
9	1	150.5000	2.0940	1.9990
	2	155.0000	1.6450	1.9840
	3	139.8000	2.4480	2.0347
	4	178.6000	2.0320	1.9052
	5	134.2000	2.4360	2.0534
		Average for 5 replicates	**2.1310**	**1.9953**
		% deviation between real and calculated average values	**6.80**	
10	1	146.7000	2.1280	2.0117
	2	120.5000	2.0720	2.0991
	3	134.9000	2.0990	2.0511
	4	150.7000	2.2560	1.9983
	5	158.1000	2.3930	1.9736
		Average for 5 replicates	**2.1896**	**2.0268**
		% deviation between real and calculated average values	**8.03**	

SUMMARY

In arid and semi-arid regions of South Africa, both subsistence and commercial farmers experience economic pressure due to varying prices of staple crops such as maize and wheat as well as weather uncertainties. The prickly pear, *Opuntia ficus-indica*, is an alternative crop that can partially offer a solution to these problems as an extra income. Further, it is capable to produce both fruit and young cladodes under rather extreme weather conditions, including severe drought. Further, its economic potential per hectare far exceeds that of maize and wheat. This supplied a rationale for investigating new ways for manipulating the crop with the aim to increase either fruit yield and quality or new cladode production or both, by applying natural bio-stimulants as foliar sprays to adult plants while standard management practices were adhered to.

ComCat® (CC), a commercially available natural bio-stimulant known for its potential to stimulate yield, growth and development in some crop plants, along with SS, a prototype natural bio-stimulant still in the developmental phase, was used in this study in an attempt to reach the set objectives. Kelpak®, a commercially available bio-stimulant also known for its growth stimulating properties, was used as a positive control. Although, in the two trials conducted, the results were not consistent as far as all measured parameters are concerned and were not statistically significant in all cases, *ComCat*® (CC) consistently contributed to a increase in the total fruit yield expressed in ton ha^{-1} while the SS treatment consistently contributed to elevated new cladode production in both trials.

When fruit and new cladode yield data was expressed per old cladode, a different picture arose. This prompted the need to correlate the relationship between morphological and yield data. Subsequently, multiple regression and correlation analyses were performed using morphological parameters such as fruit-, peel- and pulp mass as well as fruit diameter and length to predict fruit yield and quality. Although the linear equation models resulting from this statistical calculation did not consistently show significant prediction accuracy at the 95% probability level, it was a worthwhile exercise as definite

correlations were observed at least at the 90% probability level. Prediction models resulting from multiple regression and correlation analyses of morphological, yield and quality data strongly indicated that this approach might become a handy tool for farmers assisting them in managing their orchards not only in a specific growing season but especially for the following season. More research is necessary to pursue this aspect.

Finally, manipulation of fruit yield in *O. ficus-indica* by foliar sprays of adult plants with *ComCat*® and manipulation of new cladode production by treatment with SS, can become additional techniques to optimize the productivity of this crop plant.

OPSOMMING

In ariede en semi-ariede streke van Suid Afrika beleef beide kommersiële en bestaansboere tans ekonomiese druk weens wisselende pryse van stapelgewasse soos mielies en koring, asook die onsekerheid van klimaatsomstandighede. Die turksvy, *Opuntia ficus-indica*, is ‘n alternatiewe gewas wat gedeeltelik ‘n bydrae kan lewer om hierdie probleme te oorbrug, aangesien vrugpryse nie so wisselvallig is as mielie- en koringpryse nie en aangesien die gewas steeds vrugte en nuwe kladodes kan produseer onder uiterste klimaatsomstandighede, soos droogte. Verder, oortref die ekonomiese potensiaal van turksvyvrugte per hektaar dié van mielies en koring by verre. Laasgenoemde het die rasionaal verskaf om die moontlikheid van nuwe tegnieke te ondersoek ten einde die gewas só te manipuleer dat dit tot verhoogde vrugopbrengs en –kwaliteit, asook verhoogde nuwe kladode-produksie aanleiding kan gee. Ten einde hierdie doelwitte te bereik, is natuurlike bio-stimulante as blaarbespuitings op volwasse plante toegedien, terwyl standaard bestuurspraktyke gevolg is.

ComCat® (CC), ‘n kommersieël beskikbare natuurlike bio-stimulant bekend vir sy potensiaal om oesopbrengs, kwaliteit asook groei en ontwikkeling in gewasse te stimuleer, tesame met SS, ‘n prototipe natuurlike bio-stimulant tans in die ontwikkelingsfase, was in hierdie studie gebruik. Kelpak®, ‘n kommersieel beskikbare bio-stimulant ook bekend vir groeistimuleringseienskappe, was as positiewe kontrole

gebruik. Alhoewel die resultate in beide proewe nie deurgaans dieselfde was in terme van al die gemete parameters nie en ook nie altyd statisties betekenisvol was nie, het *ComCat*® (CC) bygedra tot betekenisvolle verhoging van vrug opbrengs, uitgedruk as ton ha^{-1} terwyl die SS behandeling deurgaans tot verhoogde kladode-produksie aanleiding gegee het.

Wanneer nuwe kladode-produksie as ‘n waarde per ou kladode uitgedruk was, het ‘n nuwe tendens tevoorskyn gekom. Laasgenoemde het aanleiding gegee tot die behoefte om die verwantskap tussen morfologiese en oesopbrengsdata te korreleer. Gevolglik is meervoudige regressie en korrelasie-analises uitgevoer deur gebruik te maak van morfologiese parameters soos vrug-, skil- en vlees (“pulp”) massa asook vrugdeursnee en -lengte, om vrug-oesopbrengs en -kwaliteit te voorspel. Alhoewel die linieêre model vergelykings wat hieruit voortgespruit het nie deurgaans statisties betekenisvolle voorspellingsakkuraatheid by 95% waarskynlikheid getoon het nie, was die oefening betekenisvol in die sin dat definitiewe korrelasies op die 90% waarskynlikheidsvlak waargeneem is. Die voorspellingsmodelle wat hieruit voortgespruit het, het sterk daarop gedui dat hierdie benadering ‘n handige instrument vir turksvy-boere kan word in die bestuur van hulle boorde, nie net alleen vir ‘n spesifieke seisoen nie, maar ook met die oog op beplanning vir die opvolgende seisoen. Meer navorsing is egter nodig om hierdie aspek op te volg.

Ten slotte moet beklemtoon word dat die manipulering van turksvy-plante deur blaarbespuiting van volwasse plante met *ComCat*®, ten einde vrugopbrengs te verhoog en manipulering van kladode-produksie deur behandeling met SS, die potensiaal besit om as addisionele tegnieke vir die optimalisering van hierdie alternatiewe gewas se produktiwiteit toegepas kan word.

ACKNOWLEDGEMENTS

First and foremost I thank Professor J.C. Pretorius of the Department of Soil-, Crop and Climate Sciences, University of the Free State, for his ever-ready encouragement, advice and guidance.

Thanks are also extended to Professor L. Van Rensburg, co-study leader, of the Department of Soil-, Crop- and Climate Sciences, University of the Free State. Gratitude is also expressed to Professor Van Rensburg and his family for the help given during the experiment in so many ways and the opportunity to use their farm for the experiment. I am obliged to Anelien Bothma-Schmidt for her friendly support throughout the study.

I am particularly indebted to my colleagues and friends Elmarie Van der Watt, Louise Zietsman, Charlotte Maree, Philip Swart, Thian de Schmidt, Stefan Rost, as well to the workers who helped during the trials. Piet Botes of the Department of Biochemistry for his assistance and for making available the HPLC and all the equipment needed throughout the trials.

For their patience, reassurance, help and support in many ways I thank my family, Riaan and Stiene Buitendag, Este Buitendag and especially Madele Kock.

Last but not least I want to thank our Father for giving me the insight, knowledge and opportunity to do this degree as best as possible.