EVALUATION OF FRUIT GROWTH AND DEVELOPMENT OVER A VERY EXTENDED HARVESTING PERIOD OF 'HASS', 'FUERTE', 'GEM' AND 'RYAN' AVOCADO FRUIT

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The research work reported in this thesis was as a result of experiments carried out in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, from April 2016 to October 2017, under the supervision of Prof. Isa Bertling and Dr Samson Z. Tesfay (School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal; South Africa)

By submitting this thesis electronically, I hereby declare that the entirety of the research was because of my own investigations. It therefore represents my original work except where otherwise stated and due acknowledgments are accorded.

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DEDICATION

I dedicate this Thesis to my parents/Guardians Thokozile, Zamokwakhe and Thathezakhe Mbhele, who saw the least potential in me from a young age, believed in my pipe dreams and supported me from the very beginning of my University life until today. Without any doubt and lack of faith in my capabilities. Today, your compromises, genuine love, and generosity has allowed me to discover my intellect capacity as a young lady from a deprived background, without your support I don't know what of me would have become in this life, Thank you.

PREFACE

This thesis is a compilation of manuscripts where an individual chapter is an independent article introduced disjointedly. Hence, some repetition between individual chapters has been inevitable. Each chapter in this thesis is formatted to the requirements of Elsevier BV Publishers of Postharvest Biology and Technology.

ABSTRACT

Assessing avocado fruit growth and development by measuring fruit diameter during ontogeny may, therefore, offer clues to better understand whole plant behaviour. Plant sampling was carried out over different developmental stages from early to an extended growing season on four cultivars (‘Hass’, ‘Fuerte’, ‘Gem’ and ‘Ryan’). Mesocarp, exocarp and seed fruit tissues were used to determine internal parameters such as sugars, antioxidant, oil content, dry matter, and calcium). The sugars were extracted and analysed by isocratic HPLC. D-Mannoheptulose in mesocarp+exocarp tissues was found in significant amounts (‘Hass’ = 16.47±1.140 mg/g DM, ‘Fuerte’ = 11.92±1.780 mg/g DM, ‘Gem’ = 9.35±1.410 mg/g DM, ‘Ryan’ = 7.52±1.271 mg/g DM), with perseitol also being significant for all cultivar (‘Hass’ = 4.87±0.662 mg/g DM, ‘Fuerte’ = 5.77±0.650 mg/g DM, ‘Gem’ = 5.09±0.577 mg/g DM, ‘Ryan’ = 3.86±0.227 mg/g DM). D-Mannoheptulose was found in high levels in the mesocarp and exocarp compared to the seed. Perseitol was predominantly found in the seed for all cultivars (‘Hass’ = 7.31±0.486 mg/g DM, ‘Fuerte’ = 6.71±0.842 mg/g DM, ‘Gem’ = 6.76±0.224 mg/g DM, ‘Ryan’ = 8.62±0.473 mg/g DM). The C6 common sugars sucrose and glucose were detected in low concentrations in the mesocarp+exocarp fruit tissue, with sucrose being dominantly present in the seed. Calcium was determined by fruit ashing using HC*l*/HNO_3 for digestion and strontium buffer solution for calcium extraction. Calcium concentration was significantly different during the ontogeny of each cultivar (‘Hass’ $p = 0.007$, ‘Fuerte’ $p < .001$, ‘Gem’ $p < .001$, and ‘Ryan’ $p < .001$). The calcium uptake peak is mostly reached during early fruit set stages of avocado fruit, followed by a decline and constant continuous low concentrations as approaching maturity. When fertilizer is applied during maturity calcium uptake in the avocado fruit tends to increase. Maturity indicators such as oil content, dry matter and fruit are significantly different across all fruit developmental stages. Oil content percentage ($p < .001$ all cultivars), dry matter ($p < .001$ all cultivars) and fruit size for both low and high tree fruit load ($p < .001$ all cultivars, except ‘Hass’ with $p = 0.812$ for high tree load fruits). During the extended hanging period maturity indices accumulation had a continually increased per cultivar, Oil% (‘Hass’ = 18.1%, ‘Fuerte’ = 12.74%, ‘Gem’ = 13.41%, and ‘Ryan’ = 17.41%), dry matter (‘Hass’ = 40.37 mg/g DM, ‘Fuerte’ = 24.01 mg/g DM, ‘Gem’ = 44.29 mg/g DM, and ‘Ryan’ = 35.39 mg/g DM), and size (‘Hass’ = 69.73mm, ‘Fuerte’ = 68.46mm, ‘Gem’ = 75.34mm, and ‘Ryan’ = 76.75mm), all significantly increased.

In Overall this study revealed that avocado fruit development does not necessarily end at the commercial harvesting period, but continues on fruits still attached to the tree after the single sigmoidal growth curve. When fruit harvesting is prolonged, the internal parameter for fruit growth, and C7 sugars, content contributes significantly throughout fruit ontogeny but varies in levels between cultivars. Calcium concentration uptake is in higher demands at early fruit set, where peak accumulation is reached almost at similar period with C7 sugars per cultivar. Therefore, C7 sugars and calcium in avocado are correlated during fruit growth and development. By extending fruit harvesting it allows the avocado fruit to mature by accumulating higher concentrations of sugars and, calcium immature harvest which result in negative market outcomes. This is especially true for late maturing cultivars which are less susceptible to poor postharvest quality. Therefore, avocado fruit development does not only follow a single sigmoidal growth curve but a double sigmoidal one.

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TABLE 2: C7 sugar concentrations in (mg/g DM), from mesocarp+exocarp fruit tissues harvested at early maturity stage in August 2017 at Everdon Estate, stored under cold (8^{o}C) and room (21^{o}C) temperatures.

CHAPTER 1

GENERAL INTRODUCTION

Avocado (*Persea americana*. Mill.) is a climacteric, evergreen, subtropical fruit originating from an area including the current countries Guatemala, Mexico as well as other parts of Central America. Avocado belongs to the family Lauraceae, a family containing mostly woody species distributed worldwide, from tropical to subtropical areas (Rohwer, 1993). Avocado is characterized by a distinctive maturation and ripening process. It is difficult to identify whether an avocado fruit has reached harvest maturity, as the external appearance of the fruit gives no clue to the stage of maturity. Also there are no precise, easily measured internal qualities of avocado fruit (Lee, 1981). Avocado differs from other horticultural or climacteric fruit, as the avocado fruit only ripens or softens after its removal from the tree and subsequent storage at room temperature (20 - 25ºC) for several days (Lee et al., 1983).

The avocado fruit exhibits several unusual characteristics of physiological and morphological nature; this results in the determination of avocado maturity being subjective. The fruit does not exhibit physical characteristics that simply demonstrate precise maturity stage, which result in the hardship of growers to identify whether the fruit is commercially ready for harvest (Omoniyi, 2014). Watada et al (1984) defined fruit maturation as a phase of growth and development preceding the completion of physiological or horticultural maturity. Physiological maturity is defined as a phase of development of a fruit when final growth has been achieved and the fruit has matured to such an extent that the following phase of development can be attained successfully (Watada et al., 1984; Omoniyi, 2014). However horticultural maturity is concerned with the time of harvest as related to an end-use that can be translated into market requirement which often bears little relation to physiological maturity (Wills et al., 2007).

Avocado fruit does not reach a certain stage of physiological maturity that is related to its horticultural maturity, as avocado undergoes several alterations in fruit skin, flesh and seed during maturation, where different maturity indices are used such as oil content and dry matter which tends to increase during maturation (Johnston et al., 2006). However, these maturity indices tend to lack relation towards the physiological changes required for fruit ripening initiation, and just reflect a storage product accumulated during growth that is more affected by environmental and cultural conditions (Johnston et al., 2006). In addition these indices have a vast variation in accumulation for all the avocado cultivars that are commercially cultivated. Numerous studies of chemical and physiological evaluations have been conducted to identify

one or more parameters or components which would alter significantly during maturation and be consistent within all avocado cultivars, such as oil content, dry matter, total soluble solids, pectinmethylesterase enzyme, taste, shriveling of the seed coat, moisture content, (Barmore, 1977; Lee et al., 1983; Zauberman and Schiffman-Nadel, 1972). However, this aim has not been possible to be accomplished, because avocado currently lack a harvest indicator that relates to the physiological state of being competent to ripen (Lee et al., 1983). Avocado fruit is classified as a climacteric fruit due to its respiration pattern during ripening, which requires a peak in CO_2 and ethylene accumulation to ripen under room temperatures (Biale, 1954), which is a bit odd from other climacteric fruit. Avocado does not ripen while attached to the tree (Schroeder, 1953), requires removal from the tree to initiate the ripening process (Barmore, 1977). Lee et al (1983), postulated that immature avocado fruit are known to undergo shrinkage, become rubbery, watery, overly soft and undesirable to the consumers.

The growth pattern of the avocado fruit follows a single sigmoidal growth curve (Robertson, 1971), with the early period of fruit growth characterized by rapid cell division (Barmore, 1977). Avocado fruit development is uniquely different from that of most other fruit in that cell division in the mesocarp tissue extends beyond the initial fruit growth period (Cutting and Bower, 1988). Fruit growth continues even after harvest maturity has been reached for as long as the fruit remains attached to the tree (van den Dool and Wolstenholme, 1983). ‘‘Hass’’ in particular is an avocado cultivar that has a high tendency to remain attached to the tree much longer after commercial maturity has been reached. Eventually avocado fruit drop occurs after a certain period during late maturity stage which might be due to unsuitable environmental conditions such as, drought stress, elevated temperatures above 35 ºC that cause sunburn and induced transpiration rate. Increases in ethylene production and abscission hormones during the late hang period might be another reason for avocado fruit drop. Previous studies have shown that late hang avocado fruit seed tends to germinate with a few centimeters-long radicle, and this might be a cause of fruit drop as new organs have high sink strength of carbohydrates and energy sources. This continued fruit growth is due to the formation of new cells in the mesocarp made possible by cytokinin activity (Gazit and Blumenfeld, 1970); this growing process is balanced by the presence of abscisic acid (ABA) (Cowan et al., 1997). Cell expansion is known to stops when about 50% fruit size is attained at full maturity, while cell division accounts for the continued growth (Cummings and Schroeder, 1942; Barmore, 1977; Cutting and Bower, 1988). In avocado seeds a certain cytokinin to abscisic acid (ABA) ratio is the most likely trigger of the seed coat sustained viability, which, in turn, facilitates the

movement and supply of the necessary resources, such as building blocks, hormones and energy sources, to the mesocarp, thereby permitting further mesocarp expansion (Cutting and Bower, 1988).

The relationship between avocado fruit maturity and oil content in particular, but also with size and dry matter has led to various investigations trying to relate changes in these parameters to fruit development, with the intent to use one or more of these features as an avocado maturity standard or index (Barmore, 1977). Oil content has been recognized to have a close relation with avocado fruit development, as in avocado the mesocarp oil concentration increases rapidly with fruit maturity, although there is a large difference in oil concentration among different cultivars (Lee, 1981). The 8% oil criterion for avocado was established in 1925 to insure minimum maturity and quality (Lee, 1981). This estimate is, however, too low for many cultivars, as some can possibly reach an oil percentage of 12% or more during the maturation period (Lee et al., 1983). The increase in percent dry weight during maturation is mainly due to the increase in percent oil. However, it is far easier to analyze percent dry weight than percent oil content, and new regulations based on dry weight were formulated in New South Wales, Australia. The regulation defined a minimum maturity of avocado fruit as 21% dry weight (Lee, 1981), although Clifford (1991) found that some avocado varieties can reach 24% or more dry matter, depending on the location where the fruit is grown. The increase in fruit size is correlated to dry matter accumulation along with fruit development and maturation.

Plants capture sunlight energy and convert it into carbohydrates; in which form the carbohydrate is stored differs between plants, although starch is the most common storage carbohydrate (Taiz and Zeiger, 2006). The accumulated carbohydrates can be further converted to high energy storage molecules, such as oils (Liu et al., 2002). The development of an acceptable size of high quality avocado fruit is dependent on the availability and distribution of carbohydrates (Bertling and Bower, 2005). Sugars in avocado are found in various forms such as major non-structural carbohydrates which are heptoses, D-Mannoheptulose and perseitol (Liu et al., 1999, 2002 and Tesfay et al., 2012). Isomers of the heptoses volemitol, perseitol, as well as its hydrogenated form D-Mannoheptulose, have been reported to be produced in substantial amounts in avocado (Liu et al., 1999). However, avocado seeds also contain the common carbohydrate storage forms sucrose and starch (Liu et al., 2002), which is commonly the case in plants producing rare carbohydrates (Häfliger et al., 1999 and Tesfay et al., 2012). Häfliger et al. (1999) proposed that the heptose formation in avocado is catalysed by three

known enzymatic reactions that will form C7 intermediates, the heptose pathway formation requires NADPH/NADP+ as redox power (Häfliger et al., 1999; Tesfay et al., 2012). The C7 sugars are an exceptional group of carbohydrates that are predominantly produced in avocado; they have been postulated to fulfil a variety of functions, such as energy storage, and as antioxidant (Tesfay et al., 2010). Davenport and Ellis (1959) suggested that the C7 sugars may play a significant role in the metabolism of the avocado fruit and that the lipid material presumably contributes to respiration. Tesfay et al. (2012) postulated that D-Mannoheptulose is the energy source sustaining plant growth, while perseitol is the storage carbohydrate in adult tissue.

Antioxidants availability in avocado has been reported on lipophilic versus hydrophilic radical scavenging activity of avocado (Vinokur and Rodov 2006; Bertling et al., 2007). However avocado C7 sugars, D-Mannoheptulose and perseitol has been suggested to form an important part of the antioxidant pool in avocado, by protecting the developing fruit from oxidative stress and, therefore, playing an important role in the development of healthy fruit (Bertling et al., 2007). Antioxidants are substances which when present at low concentration compared to those of an oxidizable substrate significantly delay or prevent the oxidation of that substrate (Hamzah et al., 2013). Antioxidants are known to neutralize free radicals by donating one of their own electrons, ending the electron-stealing reaction (Kaur and Kapoor, 2001), also suggested to have a well-defined role as preservatives. “Free radicals” are formed through a highly reactive atom (Oxygen) that is capable of becoming part of potentially damaging molecules capable of attacking healthy cells causing them to lose their structure and function (Percival, 1998).

The free radicals are extremely reactive compounds also called reactive oxygen species (ROS). Types of ROS include free radicals such as “hydroxyl, superoxide, nitric oxide, nitrogen dioxide, peroxyl, and non-free radicals such as hydrogen peroxide and singlet oxygen play an important role in the development of several pathological conditions such as lipid peroxidation, protein oxidation, DNA damage and cellular degeneration” (Hamzah et al., 2013). The removal of reactive oxygen species (ROS) from cells can be accomplished by scavenging enzymes (e.g. superoxide dismutase, ascorbate peroxidase and catalase) which will inactivate the ROS or by small scavenging molecules (e.g. ascorbic acid, glutathione, and polyphenols) which combine with the ROS to form non-toxic compounds (Bertling et al., 2007). Kaur and Kapoor (2001) reported that some of the vital synthetic antioxidant are butylated hydroxyanisole (BHA),

butylated hydroxy toluene (BHT), tert- butylhydroquinone (TBHQ), propyl gallate (PG) and tocopherols. However, reducing agents that function by transferring hydrogen atoms are categorized as oxygen scavengers such as ascorbyl palmitate, sulphites, ascorbic acid, glucose oxidase and erythorbic acid (Kaur and Kapoor, 2001).

Avocado sugars that are found in predominant quantity especially (Cowan, 2004) the C7 sugar Mannoheptulose acts as a hexokinase inhibitor (Pego et al., 1999); ions, such as calcium $^{2+}$ play a vital role in mediating endogenous development programs, perceiving and transcribing extra cellular signals and so regulating and optimizing plant growth (Marschner, 1995) . The sugar alcohol perseitol has been suggested to be mostly a reserve carbohydrate in avocado (Cowan, 2004), supported by the fact that perseitol is predominantly stored in avocado seed tissue (Tesfay et al., 2012). Previous studies have postulated that, sucrose and starch only seem to act as energy sources in seed tissue (Bertling and Bower, 2005; Tesfay et al., 2012).

Avocado seed has been found to store C7 sugars (particularly perseitol) in greater abundance than sucrose. Solutes provision to the fruit mesocarp and exocarp tissues comes from the seed and is dependent on the ability to transport the solute through the seed coat (Steyn et al., 1993). Tesfay et al. (2012) suggested that solutes, such as mineral nutrients, hormones and carbohydrates are transferred through seed coat via the plasma membrane and plasmodesmata. Due to these structures, the avocado seed can supply the required energy and the required building blocks of structural carbohydrates to the mesocarp. Avocado leaves are known to have a high storage capability of C7 sugars, with Mannoheptulose dominant over perseitol and the C6 sugars, sucrose, glucose and fructose (Bertling and Bower, 2006). Previous studies indicate that a high fruit load affects the accumulation of carbohydrates negatively, resulting in a lower concentration of C7 sugars in such fruit compared with a fruit from a tree with a lower fruit load (Bertling and Bower, 2006).

The variation between and within avocado fruit characteristics can range from anatomical features to fruit quality – related ones, such as the alteration in carbohydrates accumulation (Bertling and Bower, 2005). C7 sugars decrease in the avocado mesocarp as the harvesting season progresses (Tesfay et al., 2012; Tesfay et al., 2011). When the fruit reaches commercial maturity the concentration of C6 sugars tends to the lower than the C7 concentration (Bertling and Bower, 2005). C7 sugars has been established as major contributors to the antioxidant activity of the mesocarp (Tesfay et al., 2010), a measure, likely to be related to the susceptibility of the fruit to mesocarp disorders. These occur during the early and late

harvesting season, mainly as discolouration and vascular browning. As the avocado fruit approaches harvesting time, the sugar alcohol perseitol tends to increase in concentration, indicating that the availability of this sugar could be related to fruit storability (Bertling and Bower, 2005). C7 sugars are mostly known to be important storage reserves in the tree (Tesfay et al., 2012), but Liu et al. (2002) found that these compounds are also phloem-mobile products of primary CO_2 fixation. These sugars seem to contribute to the carbon balance in the plant more so than the conventional C6 sugars (Liu et al., 2002; Bertling and Bower, 2005). It has been postulated that avocado plants use D-Mannoheptulose for a variety of purposes, ranging from energy sources to antioxidant and transport sugars (Liu et al., 2002; Tesfay et al., 2010; Tesfay et al., 2012), and that perseitol, the reduced form of D-Mannoheptulose, may also function as storage and/or as transport carbohydrate. This confirms that C7 sugars plays a monumental role in avocado fruit tissue and indicates that these sugars make and important contribution to fruit growth an development.

Wilkinson (1968) postulated that calcium uptake during avocado fruit growth tends to be rapid during the cell division stage of fruit development, followed by a slower or even no uptake during cell expansion. Although avocado is characterized by unrestricted cell division throughout its growth and development phase, maximum cell division takes place during early fruit growth (Barmore, 1977), thereafter slowing down with cell expansion becoming predominant. The calcium content tends to be lower later in the growing season, during the fruit maturation period (Ginsberg, 1985). Roux and Slocum (1982) indicated that calcium also acts as a second messenger in response to various stimuli, which affect and result into plant growth alterations. Previous studies have reported that calcium transport in plants occurs mainly via the xylem vessels and in calcium exchange sites along xylem walls (Hanson, 1984). Bangerth (1979) suggested that transpiration might play a vital role in calcium uptake and distribution throughout the plant. Calcium availability in the plants is vital, therefore, continuous supply of calcium is essential, as little or no redistribution occurs to the new growth zones after accumulation in one site (Poovaiah, 1985). As calcium uptake and translocation occur via xylem vessels, Bower (1985) postulated that the operation of irrigation systems during the avocado fruit-growth season will affect fruit calcium concentration at a critical period during fruit development.

Research by Cutting and Bower (1989) indicated that a certain proportion of calcium distributed to the different plant structures appears to be aligned with basipetal auxin

movement while Felle (1988) reported evidence of a strong interaction between calcium and IAA (lndole-3-acelic acid) at the cellular levels. Cutting and Bower (1989) found that as calcium-uptake levels increase in fruit, a decrease in the vegetative flush results. The same authors postulated that avocado fruit are weak calcium-accumulators during fruit growth, due to the reduced availability of auxin in fruitlets. Leopold and Kriedemann (1975) reported that the more vigorous the vegetative flush growth, the more calcium gets directed into that flush and therefore less calcium goes into the fruit, the stronger the basipetal IAA transport (Banuelos et al., 1987) and the stronger the vegetative sink strength and the higher the calcium-allocation to that vegetative sink. Therefore, vegetative flush vigour management during, and shortly after, avocado flowering and fruit set holds the potential to direct calcium towards fruit; consequently better postharvest quality can be achieved (Cutting and Bower, 1989). In apples (Ferguson, 2001) found that tree crop load has a significant influence on fruit quality that is not necessary related to fruit size, but to light cropping, as apples has been associated with large fruit prone to calcium-related disorders.

As a climacteric fruit, avocado is known to have a relatively short storage life under ambient temperature; the fruit can also not be stored under low temperatures, such as below ($0\,^{o}C$) for a long period due to susceptibility to chilling injury (Pantastico et al., 1975). Tingwa and Young (1974) found that ripening and ethylene production is delayed in avocados with higher endogenous calcium levels. It seems that avocado respiration is more inhibited by the presence of calcium than by ethylene production. Previous studies on apples found that fruit with high calcium levels tend to have reduced respiration rates regardless of whether the alteration in calcium are endogenous (Bramlage et al., 1974; Faust and Shear, 1972) or due to applied calcium (Bangerth et al., 1972). Other authors found that vacuum infiltration of whole fruit with calcium solutions, particularly calciumCl_2 infiltrations (Wills and Tirmazi, 1982) resulted in a reduction of the peak size of CO_2 accumulation and of ethylene production, thereby effectively delaying the ripening process; the time to reach the climacteric was, however, not markedly affected.

Calcium has been reported to affect a vast number of physiological processes in plants (Jones and Lunt, 1967; Wills and Tirmazi, 1982; Bower, 1985) and to inhibit aspects of abnormal senescence in many fruit and vegetables. Bower (1985) implied that calcium has a role in avocado fruit physiology which begins during early fruit growth and lasts to late harvesting dates, making, the mesocarp calcium concentration a vital parameter for the early prediction of physiological disorders. Bower (1985) indicated that avocado fruit with low calcium levels

have a greater susceptibility towards physiological disorders and poor post-harvest quality. Rapid softening after harvest and chilling injury is likely associated with low fruit calcium levels (Chaplin and Scott, 1980). A limitation to the use of calcium to minimize fruit respiration and other metabolic activity of whole fruit is the relatively low rate of calcium uptake from a dipping-solution by the fruit (Wills and Tirmazi, 1982). Scott and Wills (1977) researching apples found a greater uptake of the calcium solution when a vacuum was applied than by dipping the fruit into a calcium solution. The calcium application resulted in a retardation of senescence. Poovaiah (1979) found that calcium has a potential to slow down senescence in ripening tomatoes and attributed this effect to a prolonged membrane integrity following calcium application. Therefore, avocado fruit development from fruit set to late maturity period is dependent on various aspects which determines the performance of accumulated physiological and morphological characteristics of the fruit. Unrestricted cell division, maturity indices accumulation correlation with prolonged harvest, avocado fruit inability to ripen on the tree, high calcium $^{2+}$ levels at maturity and harvest, has led to the postulation that avocado fruit growth following a double sigmoidal curve when attached on the tree after commercial maturity until eventual fruit drop.

Research Hypothesis

This study endeavours to investigate fruit growth and development in relation to maturity indices from the early to the late avocado growing season. The hypothesis is that avocado fruit does not only follow a single sigmoidal growth pattern but a double sigmoidal growth curve, since avocado has a unique unrestricted fruit growth resulting from continuous cell division, while the fruit is still attached on the tree. Furthermore, calcium, antioxidant and C7 sugars contribute significantly to the ability of avocado fruit to hang on the tree, even after commercial maturity is attained.

Research Aim

To find a compatible concomitant relation between the availability and performance of calcium in the fruit mesocarp+exocarp along with the sugars from early to prolonged fruit development. To find what happens to the C7 avocado sugars during prolonged fruit attachment in relation to antioxidant amount, fruit quality and storage longevity. Also, to find the relation on what the fruit depends on as an energy source during late harvesting period, which can lead to the possibility of avocado fruit following a double sigmoidal growth curve.

Research Objectives

- To evaluate and probe maturity parameters (oil, dry matter and size) during fruit development of (‘Hass’, ‘Fuerte’, ‘Ryan’ and ‘Gem’).
- To investigate the role and contribution of non-structural and structural avocado sugars during development at pre-harvest and prolonged hanging fruit in four avocado cultivars.
- To investigate the amount and contribution of antioxidant on ‘Gem’ and ‘Ryan’ at pre- and post-harvest treated with $8^{o}C$ and $21^{o}C$ temperatures.
- To evaluate the relation of calcium accumulation in mesocarp and exocarp in all four avocado fruit during fruit development.

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CHAPTER 2

EVALUATION OF AVOCADO FRUIT DEVELOPMENT - A REVIEW

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ABSTRACT

Avocado (*Persea americana* Mill.) fruit tree development under anatomical, morphological and physiological aspects has been studied intensively, which resulted to the discovery of avocado fruit development being unique. Avocado exhibits unrestricted cell division which continues past maturity as long as the fruit is attached on the tree, while cell enlargement is reported to stops at about 50% of fruit development stage. Avocado does not ripen while attached to the tree, it requires detachment from the tree and stored under ambient temperatures for several days to allow increase in ethylene, CO_2 and respiration rate for softening initiation. Avocado fruit growth is known to follow a single sigmoidal pattern, with an annual fruit growth cycle. In addition, it has been reported that when the fruit is late hang on the tree it tends to utilize most of the stored energy resources for the following fruit growth season, resulting in alternate bearing. Maturity in avocado is complex, due to the lack of consistent relationship between towards physiological stage of the fruit and external appearance. Maturity stage is not easily detected in avocado, destructive methods have to be conducted in order to identify if the fruit is ready for harvest and consumer attractive. Maturity indicators such as oil content, and dry matter are used as major maturity indices as are found to have a correlation with fruit growth and development. Although Oil content and dry matter lack consistence between different avocado cultivars. ‘‘Hass’’ tends to possess high oil content up to 35% when is late harvested. Avocado cultivars are found in large quantities per growing country which are breed in respective to environment suitability, and consumer expectations. Three horticultural races of avocado plant are traced within every cultivar developed, which mostly define the performance of the cultivar; namely Guatemalan race, West Indian race and Mexican race. Various hybrids of crosses between the races are found mostly in all commercially growing regions, including ‘Hass’, ‘Fuerte’, ‘Ryan’, ‘Gem’, ’Pinkerton’, ‘Edranol’, etc. These cultivars have been reported to have a noticeable lack of correlation in terms of maturity indices, growing environment, availability in the market, postharvest storage life, differs per cultivar. However, fruit growth and development is dependent to various factors which contribute towards healthy, consumer desired avocado fruit, such as endogenous, cultural practices, environment and pathogen free environment. Plant growth hormones such as Abscisic acid (ABA), Cytokinins, and Ethylene have been reported to be of monumental role during early and late avocado fruit growth and developmental stages. Temperature is also one of the major factor, high temperatures such as 35^{o}C and low (0^{o}C or negative) temperatures are of threat to the fruit due to susceptibility to sunburn and chilling injury. Therefore, avocado

fruit physiological development complexity, uniqueness of unrestricted cell division, ability to hang on the tree after commercial harvest, and to not ripen when attached in the tree, has resulted to the postulation of the fruit growth curve not only following single sigmoidal curve but a double sigmoidal curve on fruits under prolonged development. Although commercially it might not be favorable for some avocado cultivars, it might be ideal for late maturing cultivars. As postharvest quality might be reduced and susceptible to disorders on early maturing cultivars such as ‘Fuerte’ on late maturing cultivars the fruits will be in possession of high oil content, attractive consumer quality, reduced shrinkage, watery, rubbery and market availability might be increased.

1. INTRODUCTION

Avocado (*Persea americana* Mill.) is an evergreen subtropical fruit tree, native to Central America and Mexico. It is grown commercially in the warm and cool northern and eastern parts of South Africa. Approximately 40 000 t of the crop production is exported to Europe and the UK. 'Hass' and 'Fuerte' are the major cultivars in SA, each accounting for 37% of the area under avocados, although approximately 70 % of ''Hass'' is being produced and the remaining 30% is comprised mostly of 'Fuerte', 'Ryan', 'Pinkerton' and other cultivars such as 'Gem' (SAAGA 2017). Numerous studies of avocado fruit development have been conducted (e.g. Schroeder, 1958; Cutting and Bower, 1988). These researchers have discovered that the avocado fruit exhibits several unusual characteristics, of both, physiological and morphological nature. Another rather unusual aspect of avocado is, that the fruit does not ripen, while attached to the tree. It requires harvesting (severance from the mother tree) before it can soften. Avocado fruit growth and development is divided into three phases. The initial period is characterized by slow fruit growth (phase I) and an increase in cell multiplication, while phase II is a period of exponential growth, where cells expand rapidly and resources accumulate in both, the seed and fruit pericarp. The third and final period is the one of declining growth which is characterized by the alterations associated with fruit softening (Schroeder, 1953; Martens et al., 1994).

Avocado fruit growth and development commences with fruit set, one of major important stages of ovary development. After pollination has occurred under favourable conditions, the pollen germinates to form a pollen tube, which grows through the stigma, into the style and ovary tissues to the ovule which contains an egg cell (Schroeder, 1953). After the pollen tube, has transported the sperm nucleus to the egg cell for fertilization, the fusion of reproductive organs follows, forming an embryo. Stimulation of cell division in the ovary wall is initiated at the period of embryo formation (Schroeder, 1958). The produced embryo grows into the juvenile avocado seedling, and the ovule transforms and develops into seed within the ovary, which grows into the avocado fruit (Bain and Robertson, 1951).

The avocado species, *Persea americana*, is divided into three horticultural races, with three distinguished botanical or "varieties" according to origin in the sub region (Bergh, 1987): the West Indian race (*Persea americana* var. *americana*), the Guatemalan race (*Persea. americana* var. *guatemalensis*) and the Mexican race (*Persea*. *americana* var. *drymifolia*). These races are

cultivated throughout the world's avocado growing regions and are, often as hybrids of the races, commercially used cultivars (Wood, 1984).

Trees and fruit of the Mexican race bear the following characteristics: fruit are small in size with a waxy surface, delicate, thin skin that is easily damaged during shipping or handling, fruit colour varies from dark green to deep purple. The larges seed is often used as a 'nurse seed' due to its high tolerance to *Phytophthora* (Bender, 2005). The fruit takes about six months to reach maturity, has a high oil content that gives it a rich flavour and a pleasant creamy texture (Wood, 1984; Bender, 2005). There are no pure commercially used Mexican cultivars; Mexican genes have, however, been included in Mexican-Guatemalan hybrids, such as 'Hass' and 'Fuerte'. The race conveys cold hardiness to the tree, which allows Mexican hybrid cultivars to be grown in cool areas, thereby allowing to prolongation the harvesting season by up to half a year (Bender, 2005).

Trees of the Guatemalan race are subtropical, moderately cold- and salinity-tolerant compared with the Mexican race; leaves are less hairy often with a very distinct feature of red colour of the new foliage (Bender, 2005). The fruit is medium in size with thick, rough skin with nutty pulp and small seed. The fruit takes longer to mature than the Mexican varieties (Wood, 1984). The oil content of the flesh is generally high, giving the fruit a pleasant flavour and texture. Fruit generally keeps well under refrigeration and can be 'stored on the tree' (late-hung) (Griesbach, 2005). The cultivars, for example 'Hass', 'Reed', and 'Nabal, mature late. 'Fuerte' is considered to be a natural Mexican x Guatemalan hybrid (Bender, 2005).

Various commercially cultivated avocado trees have been selected from the three races, (Mexican, Guatemalan and West Indian) to form hybrids which are adapted to various climatic conditions. Cultivars that are predominantly Mexican include 'Bacon', 'Zutano', 'Shepard', 'Santana' and 'Rincon' (Bender, 2005). Mexican-Guatemalan hybrids include 'Fuerte', 'Albyoce' and 'Ryan'; predominately Guatemalan cultivars are 'Hass', 'Gwen', 'Pinkerton', 'Edranol', 'Gem', 'Hazzard', 'Sharwill', 'Whitsell' 'Lamb 'Hass'' and 'Wurtz' (Wood, 1984).

'Hass', a Mexican-Guatemalan hybrid (mostly Guatemalan) seedling (Figs 1: A), has become one of the most-planted avocados cultivars worldwide. From the marketing standpoint 'Hass' appears to 'have everything': popular size, small seed, change in colour from green to black

when ripe, good shipping and storage life requirements and long season complimenting ‘Fuerte’ (Figs 1B) (Griswold, 1945). ‘Hass’ has been identified as the avocado with the best overall quality from a consumer’s perspective. It has the longest harvest season of all commercial avocado cultivars; this cultivar season varies with country and location. Commercial harvesting in South Africa commences in June in the warmer parts of the country and in cooler area in August, lasting until August and October, respectively (SAAGA, 2017). ‘Hass’ is harvested while still green, but turns black when the fruit is ready to eat, a useful indicator for the consumer. When fruit are, however, stored on the tree (late-hung), the exocarp tends to turn black, while still attached on the tree (Griswold, 1945). There rarely is the danger of picking immature fruit, as there is no market advantage, which is experienced when the fruit is harvested with an oil percentage below 12 to 25% (FM) and less than 25% dry matter. The cultivar ‘Fuerte’ originated from a natural Mexican x Guatemalan hybrid (Figs 1: B) and is known for its excellent quality and has a rich creamy texture, although it has a short picking season and less ideal market storage longevity (Rounds, 1946) compared to ‘‘Hass’’. Wood (1984) postulated that in South Africa ‘Fuerte’ is still used as the standard by which all other avocado varieties on the market are judged, considering the colour and texture of the skin, fruit shape and size. ‘Fuerte’ fruit has both, good internal and external qualities, and characteristics that have been largely responsible for its popularity with nurserymen, growers, shippers and consumers (Rounds, 1946; Bergh, 1984). However, ‘Fuerte’ has worldwide fallen out of favour, it neither stores very well nor does it ship easily, due to the fragile thin skin of ‘Fuerte’ susceptible to post-harvest handling damages, compared with ‘Hass’ being the major contributor to that difference between these cultivars. The fruit flesh is attractive, of excellent quality and has a rich nutty flavour with no fiber, and an oil content when mature from 8 to 18%.

‘Ryan’, a Guatemalan cultivar (Figs 2: C), has the advantage to reach maturity when no other avocado cultivars are available on the market, making the fruit in high demand when available and fetching good prices for the producer and marketer (Wood, 1984). ‘Ryan’ contributes about 12% to the cultivars produced by the South African avocado industry (SAAGA, 2017). ‘Ryan’ maturity and harvesting period in South Africa is around September to December in warm areas and November to February in cooler areas. Only few (SAAGA, 2017) studies have been carried out investigating ‘Ryan’ fruit growth and postharvest behavior, as it is not a major cultivar worldwide. Wood (1984) investigates ‘Ryan’ fruit development and reported that the cultivar has a large seed, which tends to germinate inside the fruit while still attached to the

tree; this can lead to mesocarp rot and sometimes failure to ripen satisfactorily. ‘Gem’ is a newer ‘Hass’-type avocado cultivars that currently contributes only 2% to the South African avocado industry (SAAGA, 2017).

‘Gem’ (Figs 2: D) is characterised by its green skin that turns blackish-purple when ripe, (Bergh, 1984) perhaps the best characteristics of ‘Gem’ is that the fruit, when cut in half, has a much slower oxidation rate (the flesh stays greener longer) than does ‘Hass’. In addition, ‘Gem’ fruit can hang late on the tree, exceeding the period over which ‘Hass’ can be stored, without the compromise in fruit quality, which results in the fruit quality excellence of ‘Gem’. However, the ‘Gem’ cultivar scarcity in the market has depleted due to increase in production as it is a recommended substitute of ‘Hass’ cultivar, because it tends to have maximum late season hung period, accumulate high level of dry matter without off flavour, has pest tolerance, and good eating quality for longer than ‘Hass’. However, it matures later than ‘Hass’ California with less oil accumulation.

Figure 1: The two major, commercially cultivated avocado cultivars in South Africa and worldwide: ‘Hass’ A (34 mm,) and ‘Fuerte’ B (41 mm).

Figure 2: ‘Ryan’ C (54 mm) and ‘Gem’ D (51 mm) hybrid cultivars contribute less significantly towards the major cultivated and exported avocado cultivars in South Africa.

2. FRUIT GROWTH AND DEVELOPMENT

Avocado fruit growth is known to follow a single sigmoidal curve (Valmayer, 1964; Robertson, 1971). The fruit growth is determined by the occurrence of cell division and cell enlargement (Martens et al., 1994). However, in most fruits cell division is only experienced for a minimal period after fruit set (Nitsch, 1965). The fruit growth after this period is due to cell expansion (Martens et al., 1994). In avocado it is unique, in that cell division continues as long as the fruit is attached to the tree (Schroeder, 1958). Cell division in the avocado fruit mesocarp is continuous from the initial growth stage towards the developmental period and even occurs in the mature fruits attached on the tree after commercial maturity and harvest has been reached (Schroeder, 1958). Cummings and Schroeder (1942) reported that in some fruit species, cell expansion stops when 50% of the fruit diameter or size is achieved at full maturity, while continuous cell division accounts for the continued growth. Existence of avocado fruit size variation among same cultivar fruit is due to environment, cultural practices, and yield and water relations. Moore-Gordon (1997), stated that during early stages of fruit development cell division occurs at high rates and then is widespread throughout the fruit tissues. During this period only a moderate, constant increase in size of individual cells is observed (Schroeder, 1958). By the time the fruit has attained about half of its full size, most of the individual fruit cells throughout the tissues have achieved their maximum dimensions (McOnie and Wolstenholme, 1982). Schroeder (1953), found that cell number increases, as indicated by the number of cells by a radius in a given fruit. As the size of fruit increases, the number of cells along the radius also increases (Figs 3). During the later period of fruit

development after physiological maturity is reached, cell division continues to increase the cell number within the tissues, even as horticultural maturity is approached (Schroeder, 1958). Continuous expansion in fruit size is due to on-going cell division, which is unrestricted throughout the growing season and even occurs in mature fruit attached to the tree (Letham and Williams, 1969).

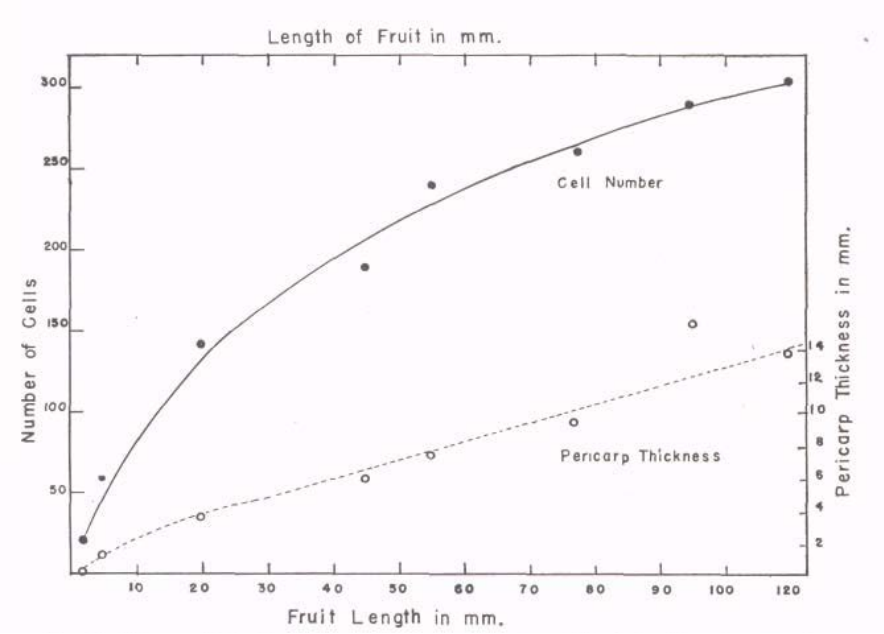

Figure 3: Cell number and pericarp thickness plotted against size of 'Fuerte' avocado fruit. (Source: Schroeder, 1953)

The increase in cell diameter and cell number in avocado fruit indicates, that the increase in fruit size results from both, cell division and an increase in cell size during the early period of fruit development, with cell division as the major factor contributing to the increase in fruit size in the latter phase of fruit development (Figs 4). Schroeder (1953), postulated that cell division apparently continues throughout the time the fruit remains on the tree. Avocado fruit size variation exists amongst fruit of the same cultivar in a certain region because of genetic variation, racial origin, cultural practices, yield, water relations, and climactic conditions. Previous studies on fruit size determination discovered that fruit growth does not follow a smooth curve but consists diurnal fluctuations (Schroeder, 1958). Observing fruit size over consecutive days showed that in the early morning the fruit tends to be at maximum size due to fruit turgid, followed by shrinkage that begins about two hours after sunrise and proceeds as

temperatures increase until late afternoon when temperature declines again. During the night, fruit tend to expand again (Schroeder, 1958).

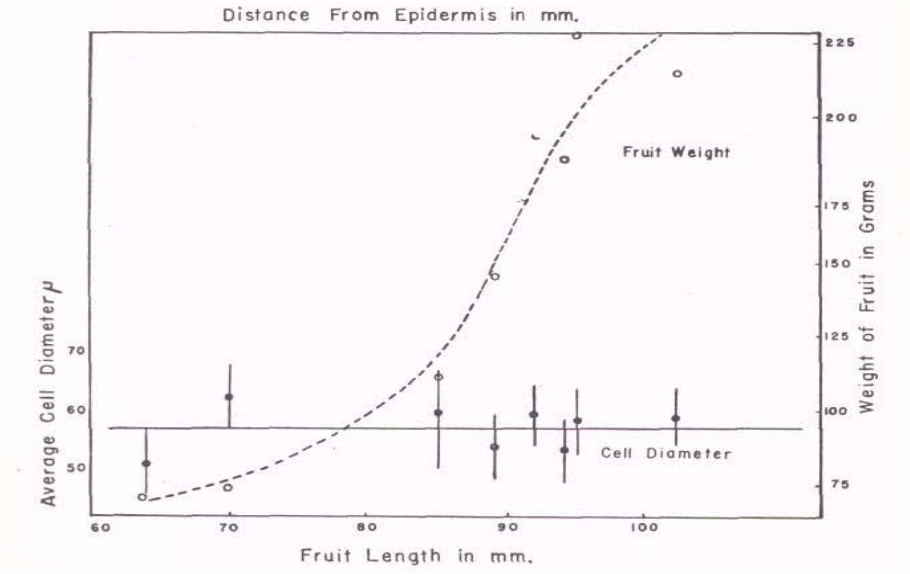

Figure 4: Relationship of average cell size to size (length and mass) of horticulturally mature 'Fuerte' avocado fruit. Vertical lines represent standard deviation of measurements. (Source: Schroeder, 1953).

Fruit growth can either follow a single sigmoidal (expolinear) growth pattern, depending on the measured parameter (Figs 5), while other fruit display a double sigmoidal growth pattern (Figs 6). Most fruit exhibit a single sigmoidal growth curve, which is characterized by an initial slow increase in size after bloom followed by a relatively rapid increase and then a decrease in growth as fruit maturity is approached (Lakso and Goffinet, 2003). Fruits that exhibits a double sigmoidal curve, firstly accumulate a single sigmoidal growth pattern during the first 50 % of growing season, then followed by a rapid increase in fruit size in a linear shape, which indicates a continuous cell enlargement of the fruit mesocarp up to 75% growing season (Figs 6), and then a slow increase in size after commercial maturity and harvest have been achieved (Lasko and Goffinet, 2003). As the ripening stage is accomplished on fruits that are still atached on the tree, this leads to constant fruit size accumulation.

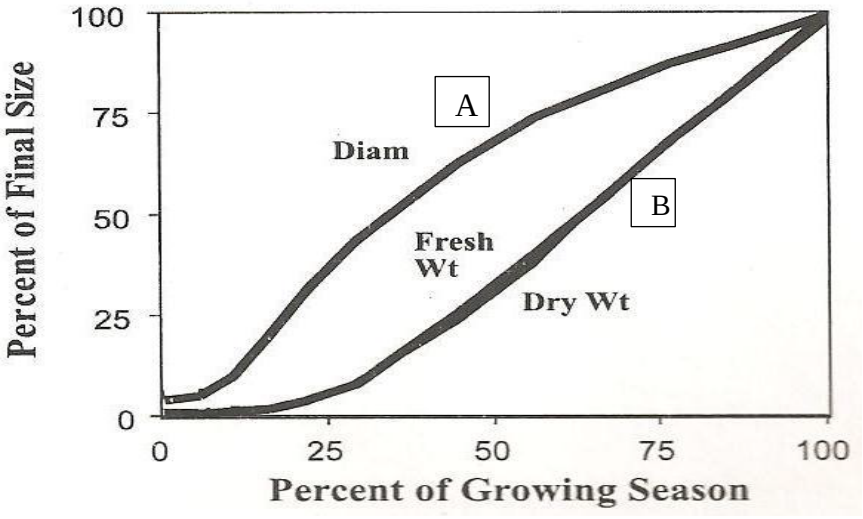

Figure 5: Patterns of horticultural fruit growth (apple, pome fruit); single sigmoidal (A) and expolinear growth pattern (B) (Source: Lakso and Goffinet, 2003).

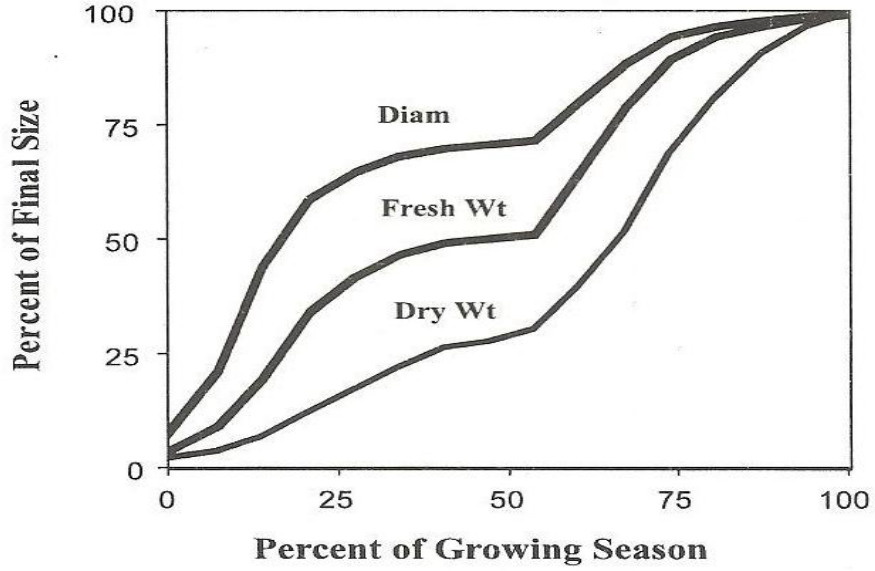

Figure 6: Double sigmoidal growth pattern of stone fruit (Source: Lakso and Goffinet, 2003).

2.1 Factors affecting fruit development

Cutting (1984) postulated that in avocado fruit exposed to stress, the abscisic acid concentration tends to increase, creating an irreversible loss in ability of cells to grow. The

interaction of increasing abscisic acid concentration with constant or decreasing cytokinin concentration reduces the rate of cell division thereby controlling size expansion (Hills, 1980). Auxins have been found to contribute to avocado fruit development by increasing the sink strength of the fruit and by regulating endosperm development (Cutting et al., 1985). Gazit and Blumenfeld (1970) discovered that fruit growth in avocado is likely promoted by high levels of cytokinin which increase sink strength for nutrients. Although cytokinin decrease in concentration as the fruit approach maturity, cytokinin content in avocado is positively related to fruit growth rates and cell division (Martens et al., 1994).

2.2 Avocado fruit maturity

Avocado fruit exhibit several unusual characteristics both, physiological and morphological in nature (Schroeder, 1958). Avocado maturity is divided into two different stages, physiological maturity and horticultural maturity. Physiological maturity in avocado is a stage where most fruit development has been achieved, but softening is still in process. In addition Dilley, (1969) and Leopold, (1971), stated that the avocado seed is important in fruit development by influencing growth, size, shape, and maturation. Martens et al., (1994), postulated that auxins, gibberellins, and abscisic acid interact with or influence production and effects of ethylene on the fruit senescence or maturity. Meaning that for maturity initiation in avocado fruit to occur, the seed has to be in a conducive state leading in ideal matured fruit with desired fruit quality. However, maturation stage is also characterized by an unusually high rate of metabolic activity compared with other fruit (Cutting and Bower, 1988). Horticultural maturity stage is a growth stage defined by the palatability of the product. A commodity is deemed as 'horticulturally mature', when it can ripen to a 'pleasant taste' after removal from the mother plant, meaning that when the fruit is picked at this stage a produce of good eating quality or high palatability will result (Lee et al., 1983). In avocado, horticultural maturity is mostly reached after fruit harvesting and storage under conducive conditions to allow ripening and palatability or consumption.

2.3 Fruit ripening

Avocado is a climacteric fruit with a marked increase in respiration rate at the beginning of the ripening process, after maximum CO_2 emission has occurred (Cutting and Bower, 1988). Rhodes (1981) defined climacteric as "a period in the ontogeny of certain fruit during which a series of biochemical alterations are initiated by the autocatalytic production of ethylene, marking the change from growth to senescence and involving an increase in respiration and

leading to ripening". During the ripening period of avocado fruit, ethylene plays a vital role, as it triggers fruit softening. Yang (1981) found initiation of ethylene formation to be the key element for normal ripening of climacteric fruit, like avocado. The rate of ethylene accumulation tends to increase dramatically at or before the time at which the respiration is climacteric and the onset of physiological alterations are aligned with this peak (Martens et al., 1994).

Avocado fruit ripening involves alteration in fruit colour, taste, texture and smell, altogether making the fruit ready for consumption. During the ripening process, a number of catabolic alterations are experienced which requires a large number of energy inputs as well as prolonged integrity of membranes (Bruinsma, 1981). Avocado softening with desired and acceptable palatability occurs only when a certain level of maturity (Spencer, 1965 and Hobson, 1979) has been achieved. Blumenfeld and Gazit (1974), stated that the "ripening characteristic of the avocado fruit is also exceptional, as even mature avocado fruits having high oil content and sometimes germinating seeds, will not ripen unless harvested". Various enzymes have been found to play a vital role in avocado fruit development. Cellulase is a major enzyme in avocado cell walls and, consequently, it is reasonable to expect cellulase to play a significant role in avocado softening (Scott et al., 1963). Pesis et al. (1978) discovered a rapid increase in cellulase activity accompanying avocado fruit ripening that was closely correlated with the respiratory climacteric and ethylene evolution. Abscisic acid levels have been found to remain more or less constant during avocado fruit maturation, but rise dramatically during ripening, because of *de-novo* synthesis, rather than release from the bound form (Cutting et al., 1985).

Fruit that is harvested before horticultural maturity is reached, tends to ripen only slightly, to a poor flavour, and will predominantly shrivel due to water loss (Barmore, 1977). Once horticultural maturity has been accomplished, the rate of ripening progressively reduces with increasing maturity (Zauberman and Schiffman-Nadel, 1972). Avocado maturity also depends on an active lipid metabolism, with fast oil accumulation commencing at about time of growth reduction and the initiation of maturity (Kikuta and Erickson, 1968). Avocado fruits do not ripen (soften), while still attached to the tree, but will soften a few days after being removed from the tree. The ability of non-stop fruit size increase may be explained by unrestricted cell-division and cell expansion, and in part this is why the fruit does not soften while still firmly attached to the tree (Schroeder, 1953). Fruit storage life after softening is generally very short at room temperature.

The unusual or uniqueness of physiological and morphological behaviours of avocado fruit compared to many species have resulted into speculation that avocado fruit growth does not only follow a single sigmoidal curve but rather a double sigmoidal curve. Schroeder (1958), found that avocado fruit during development indicate the potentialities of the tissues to maintain an active cell-division, which increase the cell number within the tissues at all stages even as horticultural maturity is achieved, with extent cell-division activity at nearly all stages of development. As evidence of cell-division in mature fruits is easily demonstrated (Schroeder, 1958), as in this study avocado fruit size measurement from early to late growth stage, demonstrated a continuous fruit diameter accumulation with unlimited increase on fruit still attached (prolonged hanging) on the tree within all four cultivars. This can be explained by the fact that fruit size increase results primarily from cell-divisions in the avocado, as it is reported to only cease after fruit being physical by detached from the stem, without any nutrients and water supply (Schroeder, 1958). Continuous cell division can be sufficiently associated with why the fruit does not soften while still firmly attached to the tree, and can also further explain the fruit exhibition of relatively higher rate of respiration in comparison with other fruit. However, the rates of cell division and cell enlargement are more rapid in young fruits than in older ones (Blumenfeld and Gazit, 1974), which explains the formation of a double sigmoidal after horticultural maturity as the fruits growing season is at a much later stage, as second fruit drop period is approached.

The monumental role of avocado seed as a strong sink organ and its effect during fruit growth, has resulted in unusual physiological processes associated with fruit development. Indeed, avocado seed tissue contains higher levels of plant growth hormones (auxins, gibberellins and cytokinin) than the pericarp, which are believed to control growth and differentiation in the young fruits (Gazit and Blumenfeld, 1970; Blumenfeld and Gazit, 1974). Martens et al. (1994), stated that hormones produced by the seed play a vital role in the resource mobilization into the developing fruit (Biale, 1954), as (Blumenfeld and Gazit, 1974) suggested that the seed tissues can synthesize growth substances. Therefore, substituted various effects of seeds including fruit set and prevention of abscission by growth regulators, partially explain the continuous cell-division and ability of avocado fruit to be attached on the tree after commercial harvest with withheld ripening. Martens et al. (1994), stated that the interrelationship between auxins and ethylene metabolism in fruit retention supports the view that auxins are important in fruit retention. Schroeder (1958) stated that healthy avocado fruits remaining on the trees stay firm and continue to grow and to accumulate oil for several months after maturation.

Banuelos et al. (1987), postulated that auxins are also vital in calcium allocation to developing tissues, which is one of this study's focus calcium concentration determination from early to late growth stage in relation to avocado fruit development and ability to hang on the tree, along with withheld ripening on the tree is critical in order to find any relationship between calcium accumulation, fruit growth development pattern during prolonged attachment on the tree. However, Cutting and Bower (1989), has postulate that reduced levels of calcium in avocado fruit are associated with high rate of softening and chilling injury susceptibility after harvest. Auxins have been suggested to be responsible for avocado fruit inability to ripen on the tree (Tingwa and Young, 1975). Endogenous levels of auxins act as inhibitors of ripening which must be deactivated before ripening (Frenkel and Dyck, 1973). Cutting (1984) found that when the plant is under stress it tends to increase (ABA) content resulting in an irreversible reduction in fruit growth which is thought to involve the interaction of abscisic acid and cytokinin reducing the rate of cell division (Hills, 1980). Therefore, during avocado maturity stage where the seed is mature, the potential for further fruit growth could be limited, although slow fruit growth certainly occurs if the fruit is firmly attached to the tree. Martens et al. (1994), postulated that minimal cell division is possible due to self-sufficiency of the flesh at this time for low concentration of promotive plant hormones, especially cytokinin.

3. Conclusion

Avocado fruit development possibly follows a double sigmoidal curve due to the continuous cell division, which allows the fruit to expand in diameter as it is attached on the tree, which has been proven to have an undeniable correlation with maturity indicators (oil content and dry matter) throughout the developmental stage of the fruit from the early to late growing period. To conclude the role that is played by unrestricted cell-division and cell number increase in avocado fruit during post-harvest developmental stage is dependent on various factors which determines the ability to hang on the tree after commercial harvest including unrestricted cell division which is the core factor. In addition, fruit growth and retention management is possible through sufficient cultural practices; timing of fertilizer application, irrigation scheduling and a healthy root system.

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CHAPTER 3

CALCIUM CONCENTRATION DURING GROWTH AND DEVELOPMENT OF 'HASS', 'FUERTE', 'GEM' AND 'RYAN' AVOCADO FRUIT

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ABSTRACT

Calcium is one of the essential macro-nutrients required in vast quantity during the fruit growth and developmental period, as it is responsible for cell membranes stabilization, for pliability and affecting normal cell division by sustaining cell cohesion, porous membranes, and cell elongation. Plant material of four avocado cultivars (‘Hass’, ‘Fuerte’, ‘Ryan’, and ‘Gem’) was collected at different developmental stages over an extended time, from fruit set to very late harvest. In avocado fruit calcium concentration differs between cultivars, ‘Hass’ tends to have a high calcium concentration, which might be related to greater tendencies of late hanging, late maturity, longevity of storage with preserved fruit quality compared with other cultivars. ‘Fuerte’ was the lowest accumulator of calcium, which could explain the early maturity, resulted poor storage quality, as low levels of in avocado are associated with reduced postharvest life. However, ‘Ryan’ and ‘Gem’ calcium concentrations are greater than ‘Fuerte’ and they perform better than ‘Fuerte’ in terms of prolonged harvest, storage life and postharvest quality. Therefore, calcium application during the early fruit initiation stage is recommended as fruit quality can be maintained much longer during postharvest.

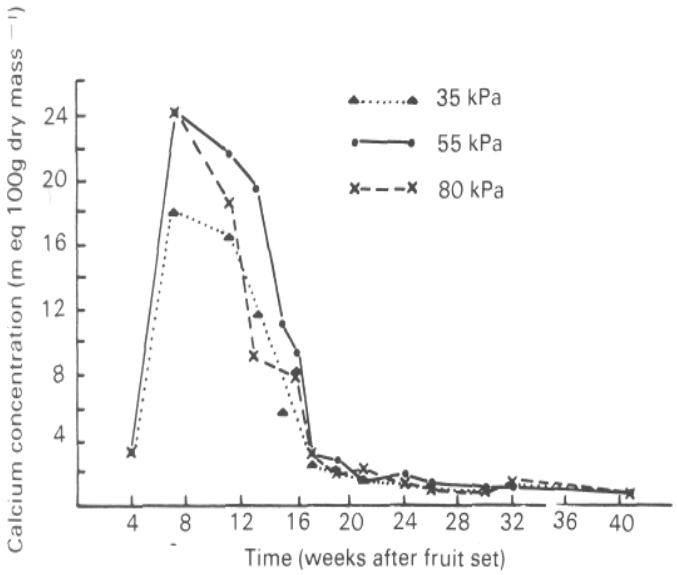
1. INTRODUCTION

Nourishment of horticultural tree crops is dependent on the availability and uptake of essential macro- and micro-nutrients incorporated in the soil. Calcium is one of the macro-nutrients essential for plant growth. Calcium is available to plants as the ionic form calcium. Calcium is immobile in plants, meaning it cannot move from old to young leaves, as it is phloem-immobile (Jones and Lunt, 1970). Calcium is essential for plant growth and fruit development (Marschner, 1983). Calcium has major functions in the plant, such as the stabilization of cell membranes and its pliability, affecting normal cell division by sustaining cell cohesion and porous membrane (Uchida, 2000), and cell elongation. Calcium is involved in the formation of lecithin, a phospholipid that is important in cell membrane permeability (Burstrom, 1968). This element also acts in mitotic cell division in the growth of meristems and the absorption of nitrate (Rodriguez, 1992). Calcium preserves the structure of plant tissue and acts as a factor that maintains cohesion of cells (Marschner, 1983). Calcium also contributes to the plant's resistance to diseases based on its ability to fortify the cell wall (Uchida, 2000). Low amounts of calcium in a plant result in cell wall collapse, so that the plant would not be able to grow upright (Marschner, 1983).

The level of calcium available during plant growth and development profoundly affects fruit and vegetable physiology (Jones and Lunt, 1970). There is increasing evidence that calcium ions act as intracellular messengers in plants (E*l* Habbasha and Ibrahim, 2015). Changes in cell wall architecture, membrane pores, and enzyme activation are known to affect various aspects of cell physiology (Poovaiah, 1985). Previous studies have emphasized that about 60-75% of the total fruit tissue calcium is found in the cell wall (Demarty et al., 1984; De Freitas et al., 2010). The essential role of calcium in cell membranes has been proven observations: Firstly, calcium deficiency conditions result in extreme membrane deterioration (Marinos, 1962); secondly, calcium alters the actual composition of membranes; its incorporation into natural (Paliyath et al., 1984) or artificial phospholipid membranes results in an extensive alteration in membrane fluidity and water permeability (Paliyath et al., 1984); thirdly, calcium can significantly change an array of physiological activities which are specially correlated with the membrane function: e.g can turn on the active transport of some ions through membranes (Hanson, 1983). Previous studies have shown that the role of calcium ions ($calcium^{2+}$) and of the calcium-binding protein calmodulin in plant development has been acknowledged (Poovaiah, 1986). Calcium appears to function as a "second messenger" in plant cells (Whitney et al., 1986). Since the discovery of calmodulin evidence has accumulated, that calcium

messages are often transferred by this ubiquitous calcium-binding protein (Cheung, 1980; Poovaiah, 1985). Previous studies have confirmed that some cell functions are modulated, at least in part, by calcium^{2+} and calmodulin. (Poovaiah, 1986).

Calcium ions (Ca^{2+}) in living cells must be maintained at low levels in the cytosol (0.1–0.2 µM concentration), (de Freitas and Mitcham, 2012) concentration or keep its concentration at the cytoplasm at millimolar levels (Poovaiah, 1985; Hapler and Wayne, 1985), as high calcium levels in the cytosol from 2 µM and more might cause toxicity and cell death (White and Broadley, 2003), considering that calcium is a signaling molecule in the cytosol (de Freitas and Mitcham, 2012). Although an elevated calcium in the cytoplasm is necessary for signal transduction, a prolonged increase in calcium is lethal (Clapham, 1995). A high sustained calcium concentration is implicated in apoptosis, both during normal development (e.g. tissue patterning and xylogenesis) (Levine et al., 1996). An increase in calcium^{2+} in the cytosolic is effected by calcium influx to the cytosol either from the apoplast, across the plasma membrane or from endoplasm reticulum or vacuole (White and Broadley, 2003). The calcium influx is mediated by calcium permeable ion channels, and their type, cellular location and abundance will influence the spatial characteristics of calcium cytoplasm perturbation (White and Broadley, 2003). Calcium as a signaling molecule its content and concentration determines cell division, the plant cell may thus actively exclude calcium from the cytoplasm by pumping the ions from the cytoplasm, and also by maintaining membrane impermeability to these ions (Whitney, 1985). Due to the phloem-immobility of calcium, only extremely low calcium levels are found inside the living phloem; this leads to the inability of phloem vessels to contribute to the calcium requirements of developing leaves and fruit (Taylor and Locascio, 2004; Ho and White, 2005). Calcium movement into leaves and fruit occurs exclusively through xylem vessels, which are non-living cells at the fruit maturity stage (Ho et al., 1993; Taylor and Locascio 2004). Calcium translocation through the xylem vessel into the fruit and leaves occurs passively and is driven by transpiration (Ferguson and Watking, 1989). The movement of calcium is affected by the transpiration rate of an affected organ and as a result calcium accumulation is much greater in leaves than in fruit (Ho and White, 2005; de Freitas et al., 2011b).



Source: (Bower, 1985)

Figure 1: Effect of irrigation regimes (replenishment at specific soil moisture tensions) on fruit calcium concentration during the fruit growing season.

Partitioning of calcium into growing fruit occurs quickly during the early stages of development, after fruit set, but calcium uptake into fruit tends to be reduced due to less uptake into the fruit during the later growth stages (Ferguson, 2001). A study by Whitney et al. (1986) study indicate that non-vigorous trees are more likely to have high-calcium fruits at maturity than vigorously growing trees (Figure 1); which also shows calcium concentration accumulation and peak trend between 'Fuerte' and 'Hass', where 'Fuerte' fruits tend to have a lower calcium concentration compared with ''Hass''. However, it has been demonstrated by (Whitney et al., 1986) that the calcium concentration accumulation peak is most likely reached around 6 weeks after fruit set and followed by a decline, thereafter when the fruit approaches maturity total calcium content tends to have a steady increase, as shown in (Figure 1). Whitney et al. (1986), postulated that avocado tree vegetative: reproductive balance is easily tipped due to excessive vegetative growth mostly experienced during spring growth season, more so in 'Fuerte' with consequent poor fruit set and low fruit calcium concentration.

Calcium is possibly the most important mineral determining the quality of fruit, specifically in apples and pears, as a result of long storage periods during which calcium deficiencies can manifest (Faust, 1975), although it might not play the same role in avocado. The low levels of calcium in avocado have been correlated with the susceptibility to chilling injury and avocados

with low calcium levels tend to ripen more swiftly than avocados with higher amounts of calcium (Wills and Tirmazi, 1982; Tingwa and Young, 1974). Fruit ripening and postharvest quality are mostly affected by the mineral composition, predominantly the calcium concentration (Sorce et al., 2011). Preservation of comparatively high calcium concentration in fruit tissues results in a slow ripening rate, which is related to reduced respiration, decreased ethylene accumulation, slower fruit softening and reduced susceptibility to certain post-harvest disorders (Hewett et al., 1999; Ferguson et al., 2003; Thorp et al., 2003; Sorce et al., 2011).

The objective of this study was to investigate the influence of calcium on certain fruit quality parameters during fruit growth and development from fruit set to late harvesting stage, beyond the commercial harvesting period. It was also intended to determine if calcium has a significant contribution towards the maturity and ripening processes in avocado, by interpreting the accumulation rate during fruit growth and development, from early growing season of four cultivars 'Hass', 'Fuerte', 'Ryan' and 'Gem' to prolonged fruit growth with respective to different commercial picking times at Everdon.

2. MATERIALS AND METHODS

2.1 Plant materials

2.1.1 Fruit collection

During the 2016/2017 growing season 'Hass', 'Fuerte', 'Ryan' and 'Gem' avocado cultivars were collected from trees in Everdon Westfalia Estate (Latitude: 29°45‟S, Longitude: 30°25‟E) located in cool subtropical area of KwaZulu-Natal, with a well-managed orchard having an efficient irrigation system. Trees were marked as low and high fruit load within branches. Fruit picking for analysis commenced in February after fruit set initiation till September. As per sampling time 10 fruit were collected, consisting of 5 low and 5 high tree loads per cultivar.

2.1.2 Fruit drying

Fruit with mesocarp+exocarp combined and seed removed were stored at a -65^{o}C freezer for 24 hours, and then transported into the freeze drier for 5 days. After freeze drying, the samples were grinded into powder using a coffee grinder.

2.2 Determination of calcium in avocado mesocarp+exocarp tissues

2.2.*1 Fruit tissue ashing*

Avocado grinded tissue of 0,5 g was approximately weighed to the nearest mg, and thereafter samples were transferred into a wide-form porcelain crucible of approximately 20 mL capacity which was clearly labelled. The crucibles were placed into a cold muffle furnace set at 500ºC and the samples were ignited for 2 hours, removed and placed in a desiccator to cool for about 5 minutes. The ashed samples were moistened with water and slowly 10 mL 4M HC*l*/HNO_3 acid mixture was added, then digested on a sand bath for 20 minutes. The samples were transferred quantitatively onto filter paper with distilled water and into a 250-mL volumetric flask using a glass rod to prevent losses. The crucible was washed well with distilled water and also the precipitate on the filter paper, transferred into the volumetric flash and distilled water was added to make up to volume.

2.2.2 Calcium analysis

From the prepared solutions in 250 mL volumetric flask, 5 mL aliquot of extract was pipetted into a test tube. A 1 mL strontium buffer solution (2500 mg/L strontium in a solution of 7,61 g/L $SrCl_2$ $6H_2O$) was added into the test tubes, the solutions were mixed thoroughly. The test tubes were retained for calcium readings by AAS (atomic absorption spectroscopy): calcium amount was calculated and converted into mg/kg.

Data analysis

GenStat software was used to analyse raw data using a Randomised Complete Block Design (RCBD) model, where alpha is (0.01).

3. RESULTS

3.1 Calcium concentration in avocado fruit tissue

There was significant difference in calcium concentration during ontogeny stages of avocado fruit, as shown by the p-value (< .001) for all four cultivars (‘Hass’, ‘Fuerte’, ‘Gem’ and ‘Ryan’) (Table 1). Calcium concentrations on analyzed mesocarp + exocarp fruit tissue during early growth stage (at fruit set and past the marble size) ranged from 600 to 1000 mg/kg DM, (Fig’s 1-2). Calcium accumulation in avocado fruit tissue seemed to reach a peak concentration followed by a sharp decrease on the winter period. During the extended harvest period calcium

concentrations in avocado fruit showed an increase (from August to October), after fertilizer application of 200kg/ha in August with Atlas Micromix, and in October with Atlas 8.1.8 at 2 kg/tree, fertilizer and Kuludol lime 250 kg/ha in the orchard. The increase in calcium concentration accumulation differed per cultivar.

3.2 Calcium between the cultivars

3.2.1 'Hass' and 'Fuerte'

Results of ''Hass'' and 'Fuerte' fruit tissues between the two cultivars showed a significant difference of calcium throughout the pre-harvest fruit growth period, even though the cultivars were grown on the same topography and location. 'Hass' and 'Fuerte' had a correlation between calcium accumulated amounts and performance during the ontogeny development of the fruit (Fig .1A-1B). During on the winter period the decrease in calcium tended to be more severe on the 'Hass' compared to 'Fuerte' avocado fruit. On the late harvesting period 'Hass' responded to the fertilizer application by absorbing more calcium in the fruit. No further data was collected on 'Fuerte' due to mistakenly harvesting experimental trees during July harvest at Everdon.

3.2.2. 'Gem' and 'Ryan' calcium concentration

'Gem' and 'Ryan' avocado cultivars grown on the same topography tended to have similar amounts of calcium concentrations during early fruit development, whereas in winter period the accumulation of calcium tended to vary notably but decreasing for both cultivars. 'Gem' tended to experience more reduction in calcium than 'Ryan' (Fig 2A-2B). 'Gem' calcium performance has more similer to ''Hass'', while 'Ryan' calcium accumulation was more similar to 'Fuerte' avocado fruit. After fertilizer and lime application in the orchard, 'Ryan' accumulated a larger amount of calcium in the fruit, than 'Gem'. 'Gem' accumulation increased in August followed by a decrease in October. Figs 4 presents the temperatures and rainfall received by the orchard during the 2016/2017 growing season for avocado fruits, as temperature plays a significant role through calcium absorption and movement via the transpiration stream to the fruit and water availability.

4. DISCUSSION

Calcium concentration during the fruit growth season from fruit set to maturity followed a sigmoidal pattern for all four avocado cultivars, which confirms with other authors (Robertson, 1971, Barmore, 1977, and Bower, 1985) who postulated that calcium growth curves are sigmoidal. The pattern of calcium accumulation as explained by Wilkinson (1968) underlay's the rapid calcium uptake during the cell division stage of fruit growth, followed by reduced uptake or even loss, during cell expansion. Barmore (1977), stated that extreme cell division takes place during early ontogeny of the fruit, although avocado fruit has an unrestricted cell division throughout its growth phase, thereafter predominantly slowing down with cell growth or expansion. This which further explains the decrease in calcium concentration as the fruit approaches maturity.

The difference in calcium concentration between the cultivars at the initial sampling time could be related to the variation of cultivars growing period and stages, because at Everdon 'Hass' and 'Fuerte' were a bit behind in terms of development compared with 'Gem' and 'Ryan' in terms of fruit growing stage. At initial sampling, 'Gem' and 'Ryan' calcium uptake was almost at peak accumulation (< 1000 mg/kg 'Gem'), (>1300 mg/kg 'Ryan') (Fig 1-2) in March, with fruit diameter ranging from (60 mm 'Gem', 64 mm 'Ryan'). Whereas, for 'Hass' and 'Fuerte' uptake on first sampling in February ranged between (< 500 mg/kg 'Hass'), (>400 mg/kg 'Fuerte') (Figs 1), with fruit size of (50 mm 'Hass', 55 mm 'Fuerte'). This results confirm those of Bower (1985) and Whitney (1985) where lower calcium concentrations at initial fruit set followed by a sharp increase in calcium uptake up to six weeks, in correspondence to a period of rapid cell division occurred.

Bower (1985), explained that the maximum uptake of calcium after fruit set could be through the link between actively dividing cells and calcium accumulation, as actively dividing cells are high calcium sinks (Bangerth, 1979). Early growth stage is a vital period for fruit development and membrane integrity and other characteristics as calcium *per se* could affect fruit physiology, since events of importance occur during early fruit life. Bower (1985) stated that not only total calcium levels are vital but also calcium acting as a secondary messenger in response to various stimuli, resulting in plant growth changes (Roux and Slocum, 1982). The peak of calcium accumulation was reached mostly in April for all the four cultivars (''Hass'', 'Fuerte', 'Gem' and 'Ryan') grown at Everdon estate in KwaZulu-Natal (KZN). The

significant difference of calcium accumulated by each cultivar at peak increase confirms postulations by Bower (1985) that calcium accumulation depends on the cultivar, including tree canopy and tree vigor (Whitney, 1985).

'Hass' and 'Fuerte' were compared in this experiment regarding samples from trees grown at the same orchard topography, with lime application as calcium addition in the 2016/2017 growing season. Generally, 'Hass' had a higher calcium concentration than 'Fuerte' throughout the sampling period. 'Hass' had a lesser dense tree canopy, which makes it a non-vigorous tree with generally smaller tree size, smaller leaf size (Whitney, 1985). 'Hass' had greater concentration of calcium in comparison to 'Fuerte', which concur with findings of Bower (1985) and Whitney (1985) findings. Trees with light tree canopy tend to have fewer terminal buds, which is advantageous as terminal buds are known to cease extension growth and production of leaves which reduces calcium competition between the leaf and fruit, resulting in greater calcium uptake by the fruit. 'Fuerte' had a denser tree canopy, meaning it is a vigorous tree, with high vegetation flush observed predominantly in February to April. Which links to the larger leaf size, increased leaf: fruit ratio which makes leaves to be stronger sinks of calcium uptake, with greater growth of terminal buds and shoots formation that suppress reproductive (Whitney, 1985), resulting in intensive competition and reduced accumulated of calcium in the fruit.

Calcium uptake 'Gem' and 'Ryan' avocado cultivars, was compared. These cultivars were grown in the same topography in the orchard. 'Gem' avocado cultivar is closely related to 'Hass' by few typical characteristics such as colour change at ripening stage, while 'Ryan' is known to be in close to 'Fuerte' in terms of fruit shape and being an evergreen fruit even at ripening. However, observed 'Gem' calcium uptake concentrations were lower than 'Ryan' throughout the experiment, although they both have calcium pattern that follows a sigmoidal pattern. 'Ryan' tends to have a dense tree canopy than 'Gem', categorizing 'Ryan' as a vigorous tree and 'Gem' as non-vigorous, which confirms the speculated relationship between the four cultivars. Calcium concentration relationship is the opposite, which raises levels of curiosity and questions. 'Gem' tree canopy is light with a number of fruits observed with sun blemishes that are mostly situated on the top tree of the canopy with few leaves, which supports findings that non-vigorous fruit showed a greater evidence of sun blemishes later in the season Whitney, (1985). Trees with a dense canopy will generally provide sufficient shade for the fruit than trees with sparse leaf coverage.

Cutting and Bower (1989), postulated that avocado is known to be one of the weak calcium accumulator fruits. Since calcium is immobile, it implies that calcium enters the fruit through the xylem where water is transported in the plant. The flow of water into fruit through xylem is predominantly through transpiration, therefore fruits with greater transpiration rate should accumulate more calcium than fruits with reduced transpiration. Between these four cultivars experimented, 'Hass' and 'Ryan' were the greater calcium accumulators. 'Ryan' is categorized as a vigorous tree due to its large tree size, large leaf area and dense canopy. The greater calcium uptake in 'Ryan' is possibly related to the fruit allocation on the tree which is on the outside tree canopy with exposure to sun and resulting in elevated levels of transpiration and more calcium movements through xylem flow of water. Resulting of 'Hass' concur with findings of Bower (1985) and Whitney (1985), that non-vigorous tree would have a relatively higher calcium concentration due to smaller leaf size and less dense canopy with least fruit shading, which results in increased transpiration with high calcium transportation. 'Fuerte' and 'Gem' were observed to be the lesser calcium accumulators. The 'Fuerte' findings confirms Whitney et al., (1986) results, that dense canopy with high vegetative flush which leads to high levels of leaf and fruit competition for calcium, since new shoots and leaves are the greater sinks. However, 'Gem' fruit allocation on the tree was predominately within the lower base canopy where there was greater shading, which could be an explanation of the lower calcium concentration observed despite having a less dense canopy. Therefore, shade and reduced transpiration are possibly the proper explanations.

Cutting and Bower (1989), previous studies have showed that a particular amount of calcium allocated to the various plant structures appears to be dependent on basipetal auxin movement, which coincides with Bangerth (1979), whose postulations that calcium transport may be dependent on an oppositely directed transport of auxin. Meristematic regions that are actively growing are known to be major auxin producers and exporters which may lead to be strong sinks of calcium (Whitney, 1985). Leopold and Kriedemann (1975), found that avocado trees with vigorous vegetative flush results to greater production of (Indole-3-acetic acid) IAA. The stronger the basipetal IAA transport (Banuelos et al., 1987), the stronger the vegetative sink strength and resultant calcium allocation. This, add on the explanation that non-vigorous trees in this case 'Hass' have greater calcium concentration due to less competition by their less vigorous developing shoots with less auxin production (Whitney, 1985). High vegetation flush was experienced during the early growing stage of 'Fuerte' which could have led to lower

calcium uptake than ‘Hass’, while it was a bit ahead of ‘‘Hass’’ in terms of fruit ontogeny growth stage. This coincide with (Whitney et al., 1986), findings that fruit from non-vigorous ‘Hass’ trees accumulated nearly twice as much calcium during the first six weeks of fruit growth, a vigorous trees with high vegetative flushes (Cutting and Bower, 1989).

During the stage of intense active cell division, calcium concentration was found to increase rapidly as observed in March for ‘Hass’ and April for ‘Fuerte’, ‘Gem’ and ‘Ryan’, which in some way confirms the postulations of calcium transport being dependent on auxin. The observed steeper levels of calcium uptake after rapid increase peak period, showed that a decrease in total calcium content caused the typical sigmoidal pattern, it was probably related to growing conditions such as temperature and rainfall (Figs 2) may have been near optimal for fruit growth and probably resulted in rapid cell division, induced phloem transport into the fruit, and generally increased metabolic activity in the fruit (Whitney, 1985). As it is known that calcium translocation in the fruit is influenced by transpiration rate. Wiresum (1966), reported that calcium allocation in the tree is correlated with root healthiness, and the amount of water entering through xylem, which varies within growing season periods, where it can certainly be very small on other periods.

The extensive decrease in calcium uptake in avocado fruit is mostly related to that of apple, as Wiresum, (1966) found that apples at early fruit set stage tend to have more or less normal calcium concentration, then followed by an extreme low influx and a strong dilution period. During the fast growth, the investigated fruit organs more likely received no or hardly any water which obviously reduce transpiration rate and then resulted in calcium supply to be likely eliminated. However, generally high humidity conditions during this period (April to May), would have limited fruit transpiration, and thus xylem transport into the fruit. Also, the spring growth flush competitive effects were most likely vital in explaining the calcium uptake trend at this period. A continuous decrease in calcium uptake was observed during the experiment as the avocado fruit approaches maturity (Figs 1). This could be explained by (Wiresum, 1966) postulations that the increase in fruit size and dry matter simultaneously require an influx of water and assimilates along with mineral substances. Wiresum (1966) further clarified that these are supplied in the same period through mass flow, occurring in phloem, (Tammes and Van Die, 1964; Ziegler, 1962) taking into consideration the extremely low calcium content in the sieve tubes.

Bower (1985), conducted an irrigation experiment on the calcium uptake pattern of avocados. It showed that fruit from trees under low water stress with frequent irrigation had a severe decline in calcium concentration from 7 weeks onwards, which coincided with this experiment results as Everdon is an irrigated orchard. Whitney (1985) suggested that a possible explanation of continuous calcium uptake decline is when the roots are supplied with abundant water, then water may enter the root via a symplastic pathway, resulting in N, P, and K ions that are actively taken up from soil solution to enter the root cortical cells along with water. Due to the dependence of calcium movement on water flow through the apoplastic compartment, under very low water stress the reduction of water flow could severely reduce the calcium uptake (Whitney, 1985). Overall Bower, (1985) indicated that both very frequent but sparse as well as only occasional but heavy irrigation are likely to significantly affect the pattern of calcium accumulation.

The main focus of the study was the performance of calcium during prolonged attachment of the avocado fruit on the tree after commercial harvesting have been reached, as cell division continues. Calcium content during fruit postharvest life have been investigated in relation to ripening and shelf life. Ferguson (2001), reported that calcium is not a sole indicator of fruit quality since it must be in conjunction with other maturity indicators, as it is one of few measurable quantitative parameters that can be made before harvest to give an indication of risk and storage behavior physiological disorders. An experiment by Eaks (1985) on calcium effect on respiration, ripening and ethylene production on avocado fruit, postulated that when ‘Hass’ and ‘Fuerte’ are treated with calcium during storage by dipping and vacuum infiltration methods, avocado fruit climacteric peaks, and ripening are delayed, compared to those untreated. However, the concentrations applied should be considered carefully due to fruit internal quality and palatability. Results of Eaks (1985) showed that “fruit treated with 0.3 M calcium took about 14 days to soften but lacked acceptable palatable qualities, while those treated with 0.2 M calcium concentration had the greatest effect in delaying ripening and reducing respiratory rates and rates of ethylene evolution without interfering with normal ripening”. calcium treatment on avocado fruit reduced external quality but improved the internal quality and chilling injury symptoms, although commercial application is impractical because of calcium solution being vacuum-infiltrated into the fruit, which causes adverse effect on the fruit external appearance of the fruit at ripening and storage period. (Eaks, 1985). In addition Ferguson (2001) postulated that maximizing calcium concentrations in apple fruit,

without incurring damage, will reduce risk of disorders and help in maintaining firmness and other desirable quality properties.

5. CONCLUSION

In conclusion, maintenance high calcium levels throughout the avocado fruit development period possesses the ability to extend fruit attachment to the tree depending on a cultivar. calcium endogenous application into the orchard during early fruit growth stages is advantageous as its will manipulate fruit quality, reduce fruit chilling injury susceptibility, preserve a healthy cell wall and promote cell division consistency and, also increase disease resistance. If calcium is of high concentrations under prolonged harvesting period, it will result in favourable fruit production characteristics on both commercial grower's side and the consumer is desired quality. However, organic fertilizer and lime application at maturity stage has revealed that avocado fruit can still manage to increase calcium uptake, which is interesting as it could mean that harvest period can be further extended, with reduced chilling injury, diseases resistance with reduced susceptibility to postharvest disorders and convenient for export as storage longevity is induced.

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TABLE 1: C.V. %, LSD, S.E and P-values of analysis of variance for fruit growth and development between four avocado cultivars ‘‘Hass’’, ‘Fuerte’, ‘Gem’ and ‘Ryan’ at pre-harvest period, during 2016-2017 growing season.

Cultivar	**C.V.%**	**LSD**	**S.E**	**P-Value**
‘Hass’	147.6	1781.2	618.3	0.007
‘Fuerte’	21.1	169.2	58.0	< .001
‘Gem’	15.0	120.7	41.7	< .001
‘Ryan’	18.6	228.1	78.7	< .001

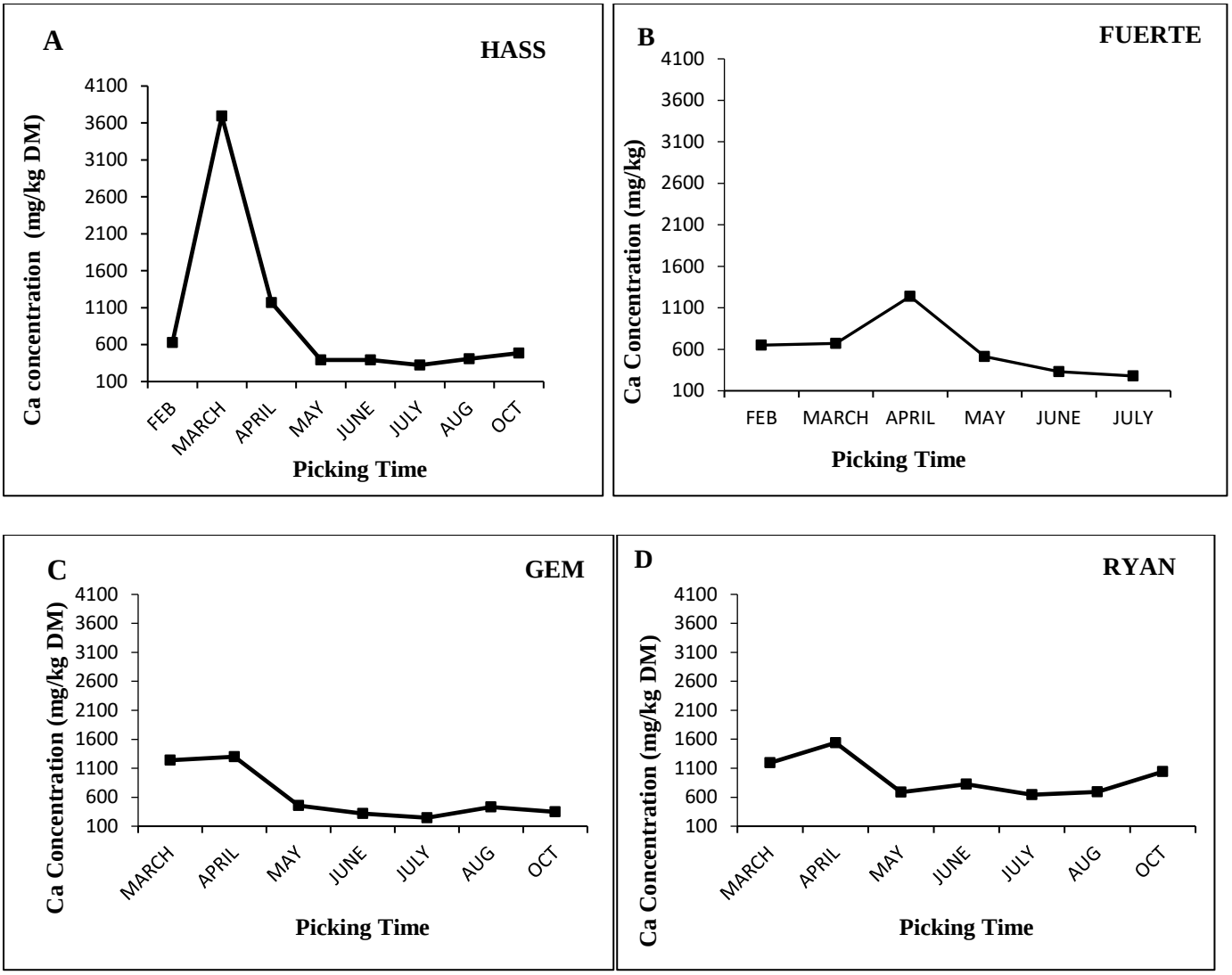
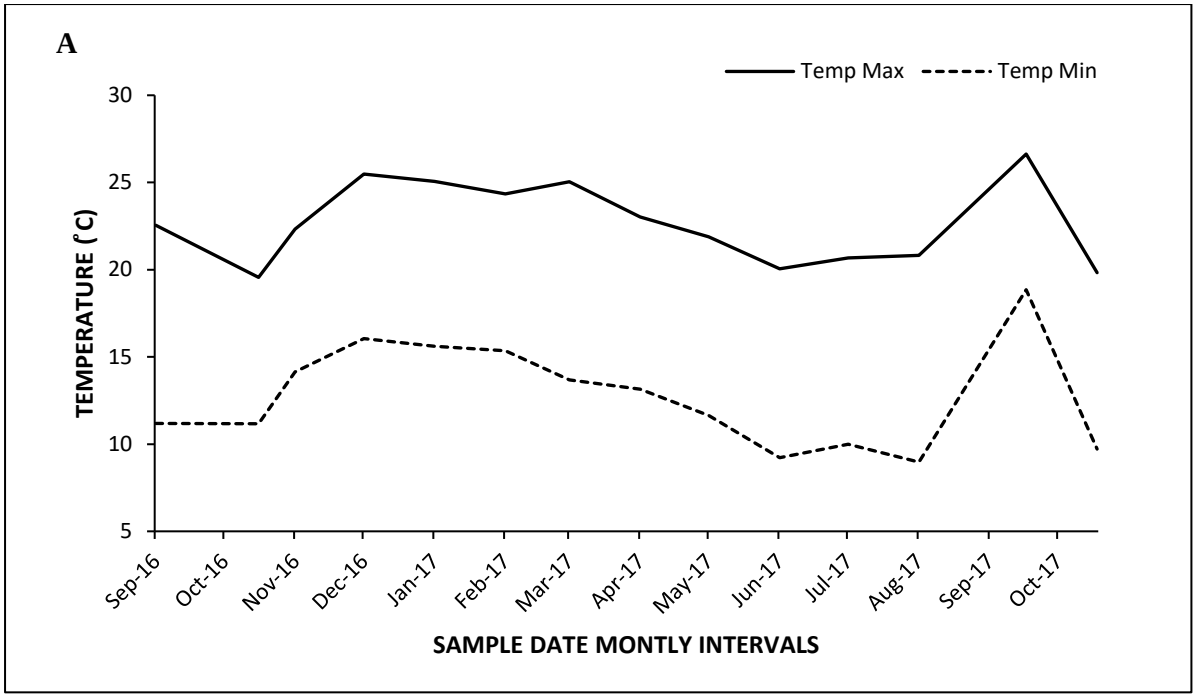


FIGURE 2: Alterations in calcium accumulation during fruit growth and development between (A) ‘Hass’, (B) ‘Fuerte’, (C) ‘Gem’ and (D) ‘Ryan’ avocado cultivars from Everdon estate 2016-2017 growing season.



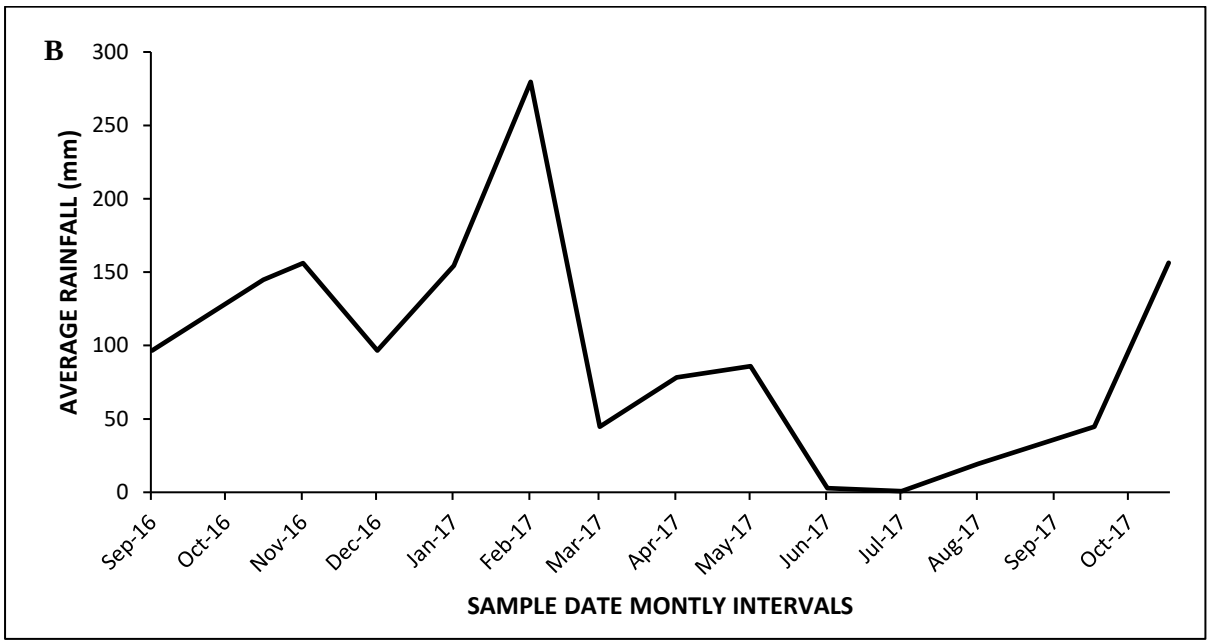


FIGURE 3: Climatic data for the experimental orchard site for the 2016/2017 growing season at Everdon Estate, showing temperature (A) and rainfall (B).

CHAPTER 4

SUGARS AND TOTAL ANTIOXIDANTS DURING THE FRUIT GROWING SEASON

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ABSTRACT

Soluble carbohydrates are found in larger amounts in avocado fruit as they are of great importance throughout fruit growth and the development period. The non-structural C7 sugars, heptoses, Mannoheptulose and perseitol are predominantly found in avocado fruit, but varies in concentrations within fruit tissues and per cultivar. These non-structural sugars (alcohol sugars) have been found to act as antioxidant in avocado fruit due to their physiological functions, including protecting the developing fruit from oxidative stress, which is vital in the development of healthy fruit. C6 common sugars such as sucrose, glucose, fructose have been identified in avocado fruit but in limited quantities compared to C7 sugars. Plant material of 'Hass', 'Fuerte', 'Gem', and 'Ryan' was collected in different ontogeny fruit growth stages over an extended period. D-Mannoheptulose is of predominant availability in avocado mesocarp+exocarp fruit tissues, followed by perseitol and C6 sugars (sucrose and glucose). In the avocado seed perseitol is the most dominant alcohol sugar followed by sucrose. Sugars accumulation (both common C6 and C7) trend showed similarities between the cultivars, with 'Hass' as a greater accumulator of the C7 sugars. During prolonged hanging of the fruits, D-Mannoheptulose levels decrease, while perseitol concentrations increases in the mesocarp+exocarp tissues, which indicated approach ripening process, as D-Mannoheptulose was in low levels to act as an ethylene inhibitor. An increase in perseitol in seed confirmed being a storage alcohol sugar. Therefore, during extended harvest period D-Mannoheptulose acts as a source of energy for fruit development throughout fruit size increase. Since during maturity photosynthesis rate is low, with limited energy produced for both late fruits, initiation of new season flowers results in alternate bearing. Perseitol as a storage sugar is in greater demand in the seed, which could provide energy for radicle protrusion in seed occurring in late hung avocado fruit.

1. INTRODUCTION

Avocado fruit are unique containing a large amount of soluble carbohydrates as the 7-carbon sugar Mannoheptulose and its sugar alcohol perseitol (Burdon et al., 2011). The biochemical and physiological functions of the avocado seven-carbon (C7) sugar heptose, Mannoheptulose and its 'sister C7 sugar alcohol', perseitol, have been debated as they are the major forms of nonstructural carbohydrates in the avocado tree and fruit (Liu et al., 1999a). This scenario is different from most fruit species, where the 6-carbon soluble sugars are predominantly present, mostly glucose, fructose and the disaccharide sucrose, as well as starch as the storage carbohydrate (Burdon et al., 2011). The avocado plant has been found to consist Mannoheptulose and perseitol in all major plant tissues, that are able to synthesize and translocate significant amounts of these C7 sugars (Liu et al., 1999), making them even importance in avocado growth and development (Tesfay et al., 2011).

Various fruit species have been reported to consist of a greater number of dietary components acting as antioxidant: vitamins A, B, C, E), fiber, polyphenols, flavonoids, calcium, isoflavanones, chlorophyllin, alipharin, sulphides, catechin, etc.) (Karakaya and Kavas, 1999), which plays vital roles in plants and animals accordingly. Percival (1998) stated that vitamin C and E, carotenoids, and polyphenols antioxidant importance is to help protect cells from free radical damage. Organisms are equipped with both endogenous (catalase, superoxide dismutase, glutathione peroxidase/reductase) and exogenous (vitamin C, E, β-carotene) antioxidant defense systems against reactions of free radicals (Hamzah et al., 2013).

In avocado plants, certain alcohol sugars have been found to act as antioxidant such as sorbitol and mannitol when added to produce (Faraji and Lindsay, 2004). Bertling et al. (2007), postulated that C7 sugar alcohols form a vital part of the antioxidant pool in avocado, which protects the developing fruit from oxidative stress and, which is vital in the development of healthy fruit. Therefore, antioxidant in consideration of their mode of action, have been identified as the terminators of free radical chain in lipid oxidation by donating electrons or hydrogen to fat containing a free radical and to the formation of a complex between the fat chain and a free radical (Kaur and Kapoor, 2001). Kaur and Kapoor (2001) suggested that antioxidant eliminate the free radical chain of oxidative reactions by donating hydrogen from the phenolic hydroxyl groups, forming stable free radicals that do not initiate further oxidation of lipids (free radical terminators).

As a result of its non-osmotic features as a sugar alcohol, perseitol might be of importance as a storage molecule in avocado (Bertling and Bower, 2005). The presence of C7 sugars in the avocado fruit flesh has been reported by La Forge (1916). The heptoses volemitol and perseitol are isomers, while the hydrogenated form of perseitol is D-Mannoheptulose; these rare sugars are produced in considerable amounts in avocado fruit (Liu et al., 1999a), and are the dominat sugars in mature avocado fruit and leaves (Tesfay et al. 2012). The sugar profile in avocado fruit differs in seed and seedlings grown under illuminated versus non-illuminated environments, the sugar quantity (free sugar and reducing sugar) as the osmotica of the cell was larger in the dark than in the light, whereas the quantity of starch was greater in the light than in the dark (Kazarma et al., 1978). In avocado fruit C7 sugars contribute over 10% of the fruit tissue dry mass and present substantial amounts of exocarp and seed dry mass (Liu et al., 1999a, 1999b; Tesfay, 2012).

Mannoheptulose (Board et al., 1995) and perseitol (Ishizu et al., 2002) have been investigated and described and are associated, or have an anti-cancer inhibitor. To append, Mannoheptulose has been related with an insulin secretion inhibitory effect (Ferrer et al., 1993). In correlation, the increase in non-structural carbohydrates occurrence based on a C6 hexose skeleton, which are glucose, sucrose and starch are present in extremely lower amounts in avocado fruit tissues (Liu et al., 2002). Previous studies have investigated the potential role of perseitol in the source-sink relationship in growing leaves and fruit, and appeared to denote that the C7 sugars plays a role in the translocation of carbohydrates during the development stage of the fruit (Bertling and Bower, 2006).

In avocado, the biosynthesis and exact purpose of C7 sugars persists in being unclear, in spite of these specific sugars being the predominant form of non-structural carbohydrates in ‘Hass’ avocado (Liu et al., 1999a). Others have proposed, Liu et al., (2002) a conceivable link between C7 sugars metabolism and fruit ripening, possibly by acting as a ripening inhibitor factor (Meyer and Terry, 2010). Liu et al., (2002); Tesfay et al., (2010) and Tesfay et al. (2012), have postulated that avocado plants use D-Mannoheptulose for a diversity of purposes, which varies from energy sources to antioxidant and transport sugars, and that perseitol may also function as storage and transport carbohydrates (Tesfay et al., 2012). Various parts of avocado fruit also contain other prevalent sugar alcohols (Koch 1996) and, common C6 sugars such as fructose and glucose. Bertling and Bower (2006) found out that the concentration of common sugars in a fruit are in limited amounts compared with to the C7 sugars which again

could denote their significance, but not necessarily as transport sugars, but as storage molecules.

Sugar translocation and sink metabolism is vital for fruit growth because it affects sink strength, sink turgor, and provides the basic building blocks for many structural and messenger molecules (Yu, 1999; Cripps and Cowan, 2000) and provides the substrates for metabolism. Sucrose serves as a compound for the long-distance transport of metabolites, to add as a stimulus in osmotic solute movement, and activates numerous genes (Koch, 1996). In addition, many plant genes are regulated by sugars including those that contribute during photosynthesis, protein accumulation, starch, lipid and nitrogen metabolism (Pego et al., 1999).

It has been discovered that the availability of sucrose to the fruit plays a crucial role in fruit development and hence affect fruit size (Cripps and Cowan, 2000). Burdon et al. (2011) found that perseitol was the predominant non-structural carbohydrate in the fruit mesocarp, with minimum amounts of Mannoheptulose, sucrose, fructose and glucose during the late maturity stage of avocado fruit development. The level of sugar accumulation both C7 and C6 during late harvest varies except for Mannoheptulose. Tesfay et al. (2012) found that the C7 Mannoheptulose predominant availability as an antioxidant could play a pivotal role in protecting the fruit mesocarp from various postharvest disorders. Furthermore, alteration in sugar distribution will affect the overall fruit physiology and growth, since sugar controls development through alterations in tissue responsiveness during fruit development (Trewavas, 1982, 1991; Firn, 1986).

The study objectives were to investigate further the role of insoluble and soluble C7 and C6 sugars during early and late fruit development, and to measure accumulation in relation to storage life, fruit quality and rate of ripening, from different cultivars that have a greater tendency to hung on the tree for a prolonged period. Furthermore, the comparison of total antioxidant available on ‘Gem’ and ‘Ryan’ cultivars during growth and storage period were investigated.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals were obtained either from Sigma-Aldrich®, Saarchem®, Fluka®, or Glycoteam GmbH.

2.2 Plant material

2.2.1 Fruit collection

During the 2016/2017 growing season ‘Hass’, ‘Fuerte’, ‘Ryan’ and ‘Gem’ avocado cultivars were collected from trees in Everdon Estate (Latitude: 29°45‟S, Longitude: 30°25‟E) located in cool subtropical area of KwaZulu-Natal, a well-managed orchard with efficient irrigation system. Trees were marked as low and high fruit load within branches. Fruit picking for analysis commenced in February after fruit set initiation till September. As per sampling time 10 fruit were collected, consisting of 5 low and 5 high tree loads per cultivar.

2.2.2 Fruit drying

Fruit with mesocarp+exocarp combined and seed removed, was stored at a -65^{o}C in a freezer for 24 hours, and then transported into freeze drier for 5 days. After freeze drying, the samples were grinded into powder using a coffee grinder.

2.3 Determination of sugar content in mesocarp+exocarp fruit tissue

According to the method described by (Bertling and Bower, 2005), 0.1g freeze dried sample was weighed and placed into test tubes, 10 mL of 80% aqueous Ethanol was added per sample for sugars extraction. The samples were homogenized and incubated at 80^{o}C for 1 hour, and then stored at 4^{o}C for 24hrs. The samples were centrifuged for 15 minutes and filtered into scintillation vials using glass wool. Samples were dried, ethanol evaporated by using an evaporator EZ2 (GenVac Ltd, Suffolk, England), 2 mL ultra-pure water was added into the dried samples and filtered through 0.4 µm nylon filters into the HPLC vials with blue caps, prior to injection into an HPLC System. Sample separated using a gel column attached to a refractive index detector. Sugar identification was carried out by comparison of retention times with standards.

2.4 Measurements of Total Antioxidants (Frap) in mesocarp+exocarp fruit tissue at pre- and postharvest

'Gem' and 'Ryan' avocado cultivar harvested in August at maturity stage, were treated under room temperature 21^{o}C and cold storage 8^{o}C, replicated four times per cultivar compared with untreated fruit. Tissues were milled, 0.1-0.2 g was weighed out, and 5 ml of 1N perchloric acid was added. The samples were vortexed and centrifuged at 12,042g for 10 min at 4^{o}C. The concentration of total antioxidant was measured using the sample supernatant (Halvorsen et al., 2002). Antioxidant levels were determined as "total antioxidant capacity" (TAOC) using the FRAP (ferric reducing ability of plasma) assay (Benzie and Strain, 1996).

Data analysis

GenStat software was used to analyse raw data using a Randomised Complete Block Design (RCBD) model, where alpha is (0.01).

3. RESULTS

3.1 Sugar profile of mesocarp + exocarp and seed on early fruit growth stage

3.1.1 'Hass'

D-Mannoheptulose is found to be most dominat sugar after fruit set initiation, followed by perseitol and Sucrose (Figs 1A). D-Mannoheptulose concentration ranges from 25-30 mg/g DM during early fruit development (Feb-April) with size expansion of 42-60 mm , tends to decrease on winter period, and show induction in amounts close to physiological maturity development stage initiation (June-July), on tree attached fruit. Glucose is not detected during early growth stages of avocado fruit (Feb-April), and is only detected during early physiological maturity stage (Figs 1A). 'Hass' seeds tend to have perseitol as the more dominant soluble sugar throughout the experimental period of fruit development, D-Mannoheptulose was present in higher concentration during early fruit growth, while sucrose was found to be greater in the seed than mesocarp+exocarp fruit tissue (Figs 3A). Also, the seedshad no glucose.

3.1.2 'Fuerte'

Soluble sugars such as D-Mannoheptulose, perseitol, and sucrose are found to be more dominant during growth and developmental stage. D-Mannoheptulose were in abundant concentrations on early fruit growth 21-19 mg/g DM with a fruit diameter of 45-62 mm from

Feb-Mar, while sucrose and glucose were barely available in mesocarp + exocarp fruit tissue (Figs 1B). As ‘Fuerte’ approaches maturity D-Mannoheptulose concentration decreases as well as sucrose and glucose, but perseitol tends to increase (Figs 1B). Glucose was the least available soluble sugar in ‘Fuerte’ mesocarp+exocarp tissues, although tends to be more detected during harvesting maturity stage. D-Mannoheptulose, perseitol and sucrose soluble sugars were detected by the HPLC in seeds. Perseitol was in high concentration in the seed compared to the mesocarp+exocarp tissue in July (Figs 3B), which was the harvesting time in Everdon Estate.

3.1.3 ‘Gem’

Analyzed mesocarp+exocarp tissue showed D-Mannoheptulose, perseitol and sucrose soluble sugars to be dominant throughout the experimental period, except for glucose which is only detected in low concentrations commencing in June to July. D-Mannoheptulose is found at maximum amounts during Mar–April on fruit with 60-67 mm average diameter, which depreciates in winter and then increase in July in correlation with fruit size. However, in winter when D-Mannoheptulose was low, perseitol was more dominant than all the available soluble sugars (Figs 2C). Perseitol was found to be more abundant in the seed followed by sucrose, while D-Mannoheptulose was present in lower concentration then in mesocarp+exocarp (Figs 4C). D-Mannoheptulose concentration decreases more during the winter period.

3.1.4 ‘Ryan’

D-Mannoheptulose was the most dominant soluble sugar with high concentrations throughout the experimental period, even in winter it did not decrease much but only reduced to 16 mg/g DM, followed by an induction as maturity stage is reached (Figs 2D). Perseitol is in lower concentrations compared with D-Mannoheptulose but has the same accumulation performance during the fruit developmental period. Sucrose is detected throughout the analysis but in lower amounts than perseitol and D-Mannoheptulose (Figs 2D). Glucose is only detected during early maturity corresponding with fruit diameter increase. Perseitol is found to be the most dominant soluble sugar in ‘Ryan’ seeds which increases in accumulation in response to fruit development stage and diameter expansion (Figs 4D). D-Mannoheptulose was found to decrease in concentration as the fruit initiate maturity. However, sucrose was in higher amounts compared with mesocarp+exocarp analysis (Figs 4D).

3.2 Total antioxidant capacity in fruit mesocarp+exocarp tissues of 'Gem' and 'Ryan' at pre- and postharvest

'Gem' seemed to have high capacity of total antioxidant (TAO) throughout the experimental period compared with 'Ryan'. In 'Gem', the antioxidant capacity variation within the picking period was high, with TAO during winter period showing a decrease in capacity (Figs 5A). However, 'Ryan', TAO capacity of 'Ryan' showed a sigmoidal trend (Figs 5B), with stability amounts between May and June, having no increase or decrease in antioxidant.

4. DISCUSSION

4.1 Total soluble sugars concentrations in mesocarp+exocarp and seed avocado fruit tissues

C7 sugars, D-Mannoheptulose and perseitol are predominantly available compared to identified common C6 sugars (sucrose and glucose), from marble size fruit to maturity within all four cultivars ('Hass', 'Fuerte', 'Gem' and 'Ryan') avocado fruits, which confirms Bertling and Bower (2006) postulations that C7 sugars are major player in fruit development. The rersults correlate with findings of Tesfay et al. (2012) that the two C7 sugars, D-Mannoheptulose and perseitol, were found to be available in different mature avocado tissues (exocarp, mesocarp and seed) (Liu et al. 2002; Landahl et al., 2009). As stated by (Daie, 1985; Dey and Dixon, 1985) starch and soluble sugars are the predominant reserve carbohydrates available for energy, growth and maintenance. (Liu et al., 2002; Tesfay et al., 2012), and it was postulated that avocado plants uses D-Mannoheptulose as an energy source, antioxidant and for sugars translocation within the plant, while the purpose of perseitol is to function as storage and/or as transport carbohydrate (Tesfay et al., 2012). Sugars are known to play a vital role in source-sink relationship initiation, (Tesfay et al., 2012) being transported via vascular tissue (Liu et al. 2002), both C7 sugars could play a role in carbon allocation and, hence, sink establishment, which explains the high concentrations found at early fruit growth as cell division is at a rapid rate with elevated demands of energy for enzymes activities and metabolism. Liu et al., (1999) stated that in avocado plant the trend of carbohydrates reserves in different storage organs is dependent on carbohydrate use and mobilization processes occurring during the growing season.

The C7 and common C6 sugar accumulation in mesocarp+exocarp fruit tissues seemed to follow a relatively similar pattern for all the cultivars. C7 sugars at initial sampling were found

at higher concentrations, with D-Mannoheptulose more dominant followed by perseitol during the sampling period Feb to April. A sharp decline in May or the winter period in all cultivars, for both C7 and C6 sugars (Figs 1-4) was detected which confirms study findings of (Tesfay et al., 2012). However, the seeds for all cultivars had perseitol and sucrose as the most dominant sugars through the experiment (Figs 3-4), while D-Mannoheptulose and glucose were the least detected sugars. Burdon et al. (2011), stated that in fruit soluble carbohydrates are not simply influenced by vegetative flush or reproductive growth, but instead are dependent on the tree overall availability of carbohydrates with fluctuations overtime i.e. during early stages of shoot growth there will be limited supply, whereas later, once that shoot growth has become productive, there will be increased carbohydrate available. Hence, two conditions of fruit carbohydrate status may be envisaged: one where they are self-supporting and the second where they are supported by the tree (Burdon et al., 2011). In avocado plant, the C7 sugars are found in various organs such as roots, stem, leaves (Liu et al., 1999) and the fruit, where they act as carbohydrates or energy sources. Meaning the explanation for high concentration at early fruit development stage could be possibly due to the fruit being the major sink of energy and environmental conditions being favorable for photosynthesis rate and carbohydrate production along with synthesis. As further explained (Daie, 1985; Cannell, 1985; Priestley, 1987; Wolstenholme and Whiley, 1989), timing and extent of phenological events are the major factors in tree growth vigor and fruit production, which are under the control of tree carbon, energy availability and partitioning in response to environmental conditions in order to promote whole tree performance (Davenport, 1982; Kaiser and Wolstenholme, 1994; Scholefield et al., 1985).

However, the decline in sugars during winter period could be related to carbohydrate competition between different avocado plant organs, as explained by (Liu et al., 1999). The authors found carbohydrates fluctuations during growth on fruit phenology, where increase in TSS (total soluble sugars) and starch concentrations in the stems during the winter were detected, in relation to the winter sugar peak which coincided with the coolest annual temperatures. Burdon et al. (2010) postulated that carbohydrate supply reduction during tree high demand track is mostly likely dependent to a limited period of low temperatures resulting in a mis-match between supply and demand. However, in this study, the fruit tended to be negatively affected when not receiving a supply of soluble carbohydrate from the tree, although growth and dry matter accumulation picked up later. Liu et al. (1999), further explained that this could suggests that sugar accumulation might be acting to enhance shoot

cold hardiness (Kramer and Kozlowski, 1979). Jackson and Sweet (1972) postulated that stem carbohydrate reserves might therefore be an important reserve for panicle development, which demands carbohydrate energy input, leading to avocado fruit being a weak carbohydrate sink in winter. However, studies of Kaiser and Wolstenholme (1994) studies indicated that high carbohydrate reserves do not necessarily result to elevated flowering, fruit set and induced fruit yield, reserve carbohydrates can be used preferentially for vegetative growth which was observed predominantly in 'Fuerte' and 'Ryan' cultivars (Liu et al., 1999).

The sugar accumulation pattern avocado fruit tissues (mesocarp+exocarp and seed tissues) after winter declined, showing a variation in performance per cultivar. As the four sampled cultivars grown at Everdon orchards were on different growing stages at the initial sampling period, ''Hass'' was the latest, followed by 'Fuerte' while 'Gem' and 'Ryan' were a bit ahead at developmental stages although 'Fuerte' reached maturity and harvested first. This explains some of the alterations in sugar concentration throughout the experiment (Figs. 1 and 2). The increase in C7 sugars in spring could be related to conducive environment conditions, promoting higher rates of photosynthesis producing carbohydrates, and the fruit being a major sink, although the increase in sugars is not as high as compared to the early fruit development stage concentrations. However, tree vigor, photosynthesis rate and tree canopy can be used as justifications for the alterations in accumulation patterns of C7 and C6 sugars during the avocado fruit growth season. Others (Cannell, 1985; Finazzo et al., 1994; Priestley, 1987; Wibbe and Blanke, 1995) have also postulated that current canopy photosynthesis and/or the fruit bearing load may control final fruit production, with regards to accumulated sugars and what the fruit depends on during prolonged attachment to the tree.

Performance of D-Mannoheptulose mesocarp+exocarp and seed fruit tissues as the fruit approached maturity showed variation in concentrations accumulated depending on the cultivar, but on overall a decrease in D-Mannoheptulose concentrations was experienced for all four cultivars (Figs 1-4). This confirms findings of Tesfay (2009) findings that mesocarp and seed D-Mannoheptulose declined as fruit approached maturity. Further decline in D-Mannoheptulose from harvest maturity to the "eat-ripe" stage confirms suggestions by Liu et al. (2002) that this carbohydrate is the respiratory substrate of the ripening fruit. (Tesfay, 2009). Perseitol concentration in mesocarp+exocarp decline was detected as the fruit approach and/or achieved maturity per cultivar (Figs 1-2), while in the seeds concentrations showed an

increase (Figs 3-4), confirming with (Tesfay, 2009) findings and (Bertling and Bower, 2006) postulations on the importance of this C7 alcohol sugar as marker for post-harvest quality. A decline in (Bertling and Bower, 2006; Tesfay, 2009) mesocarp+exocarp sucrose and glucose (C6 common sugars) was detected at maturity and prolonged harvesting time (Figs 1-2), while in the seed only sucrose was detected showing increase in concentration but in different amounts per cultivar (Figs 3-4). However, avocado seeds are known to contain a noticeable amount of sucrose and starch (common storage forms of carbohydrates) (Liu et al., 2002), which is commonly the case in plants producing rare carbohydrates (Häfliger et al., 1999). This coincides with findings of Tesfay, (2009), where the only soluble carbohydrates present in significant amounts in mature seeds of 'eat-ripe' avocado fruit were found to be perseitol and, at lower concentration, sucrose also coherent with a report by Liu et al. (1999) report. Tesfay (2009) postulated that, starch and perseitol accumulated in typical sink tissues and acted as storage carbohydrates. As storage carbohydrates typically accumulate in seeds, perseitol should be the likely reserve carbohydrate (Tesfay, 2009). However, Liu et al. (2002) postulated that the abundant amount of perseitol as a major C7 sugar in the seed, while D-Mannoheptulose was not present in adequate amounts in the seed. Tesfay et al. (2012) reported that there is a greater possibility that this alcohol sugar was released from its reduced form, perseitol, which, therefore, acted as the storage form of D-Mannoheptulose (Tesfay et al., 2012).

Thus, the quality of the fruit may be affected by how much carbohydrate the fruit is receiving from the tree (Burdon et al., 2011). Cowan (2017) postulated that in the avocado plant the predominantly available mobile C7-sugars are Mannoheptulose and perseitol and that presumably both are available for growth and development. By monitoring alterations in fruit sugar content and composition over the growing season of development until harvestable maturity, the fate of tree-derived sugars could be deduced (Cowan, 2004). Cowan (2017) postulated that after harvest, and during ripening, sucrose, glucose, fructose, and Mannoheptulose decreased, suggesting that these sugars are consumed as part of the respiratory climacteric, which occurs coincident with the accumulation of triglycerides.

Previous, studies have researched extended angles of the rarely identified C7 sugars context in avocado, which includes tree fruit bearing load as a factor for sugars availability. As Bertling and Bower (2006) described avocado leaves on non-bearing trees seemed to have higher C7 sugar concentrations than bearing trees. This could be an indication that C7 sugars play an important role in fruit growth and development, as, seemingly, leaves cannot accumulate the

same concentration of C7 sugars when the tree carries a heavier crop load. Cowan (2017) stated that, Boron (B) deficiency in higher plants, particularly during reproductive growth directly affects productivity (Cong et al. 2015), which is reported to decrease accumulation in carbohydrate production and export. However, B deficiency leads to growth inhibition which is presumably related to decrease in sink demand, and also leads to reduction in carbohydrates from source tissues (Cowan, 2017). Boron translocation through the phloem has been demonstrated for other species, typically in association with sorbitol or mannitol (Cong et al., 2015; O'Neill et al., 2001), while the recently isolated B-perseitol complex from avocado (Minchin et al., 2012) provides support for both phloem mobility of this nutrient and a role for heptitols in B translocation (Cowan, 2017).

4.2 Total antioxidant capacity in 'Gem' and 'Ryan' avocado cultivars at pre- and postharvest

Total antioxidant capacity for 'Gem' and 'Ryan' showed no significant difference between the two cultivars from pre-to postharvest sampling period (P = 0.353 'Gem') and (P = 0.667 for 'Ryan') (Table. 3). 'Gem' and 'Ryan' mesocarp+exocarp fruit tissue has an extremely low total antioxidant capacity (Figs 5) compared to ''Hass'' across all developmental stages (Bertling et al., 2007 and Tesfay et al., 2010). Results of Bertling et al. (2007) showed that total antioxidant capacity in exocarp and mesocarp can reach about 47.53mg/g DM in exocarp and 22.76 mg/g DM in mesocarp for 'Hass', which was confirmed by Tesfay et al. (2010) that exocarp and seed avocado fruit tissue has greater amounts of total antioxidant capacity. Tesfay et al. (2010), further explained these findings as mostly related to the fact that avocado fruit unique in development and ripening pattern from other fruit such as extended period of cell division past commercial harvesting Moore-Gordon et al. (1998) also Tesfay (2009) explained that high levels of abscisic acid (ABA) which is drought stress related hormone, availability quantities in fruit are physiologically unique, which is also known to increase in accumulation with maturity (Cutting and Bower, 1987). Therefore, it is possible that during this extended 6 to >12 months developmental period (Scora et al., 2002), avocado fruit will be exposed to environmental stress. Tesfay et al. (2010), in order to protect the fruit, more importantly from an evolutionary perspective, the seed from an environment of oxidative stress, tropical and subtropical avocado fruit seeds contain a variety of free-radical and reactive oxygen species (ROS) scavengers systems (Soong and Barlow, 2004). Therefore, antioxidant are more prevalent in stress-exposed fruit tissues including exocarp and seed than mesocarp avocado fruit tissue (Tesfay et al., 2010). Figs5 results show that 'Gem' and 'Ryan' antioxidant accumulation capacity has a significant difference compared ''Hass'', which arose curiosity on the reasons why there is

such variation. D-Mannoheptulose acts as one of the energy resource for avocado fruit during pre and post-harvest ontogeny growth stages, especial during the extended fruit attachment period as the photosynthesis rate is reduced, with new buds and leaves as stronger sink of photo-assimilates. Perseitol increase in the seed is more likely the source of energy for radicle protrusion in the seed while fruits are still attached to the tree. Decrease in C7 sugars avocado mesocarp+exocarp fruit tissues could be related to ripening approach. However, soluble sugars (C7 and common C6) differed between cultivars.

5. CONCLUSION

In Conclusion, C7 sugar were found in all four avocado fruit cultivars. C7 were dominantly found in the fruit mesocarp and exocarp, while the seed had low amounts. C7 availability in avocado fruit varies with development stage regards to the sugar type. D-Mannoheptulose levels are high during early fruit development stage and decrease at maturity, which could have a relation with avocado fruit ripening. Increasing amounts of perseitol in seeds have similarities for all four cultivars. The decrease of D-Mannoheptulose amounts had a correlation with total antioxidants decrease in ‘Gem’ and ‘Ryan’ cultivars. Therefore, C7 sugar have a noticeable correlation with avocado fruit developmental phases.

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TABLE 1: Comparison of soluble sugar concentration of fruit tissue (mesocarp + exocarp and seed) in four avocado cultivars ‘‘Hass’’, ‘Fuerte’, ‘Gem’ and ‘Ryan’ during fruit growth and development pre-harvest for the 2016-2017 growing season, Everdon Estate in KwaZulu-Natal, South Africa

		mg/g DM			
Cultivar	**Tissue**	D-Mannoheptulose	Perseitol	Sucrose	Glucose
‘Hass’	**Meso+Exo**	16.47±1.140	4.87±0.662	1.83±0.3	0.916±0.1369
	Seed	5.53±0.589	7.31±0.486	2.76±0.4	nd
‘Fuerte’	**Meso+Exo**	11.92±1.780	5.77±0.650	1.67±0.2	0.928±0.0965
	Seed	1.59±0.242	6.71±0.842	3.33±0.3	nd
‘Gem’	**Meso+Exo**	9.35±1.410	5.09±0.577	2.87±0.3	0.735±0.0684
	Seed	1.637±0.175	6.76±0.224	1.65±0.9	nd
‘Ryan’	**Meso+Exo**	7.52±1.271	3.86±0.227	1.94±0.5	0.794±0.1385
	Seed	2.76±0.346	8.62±0.473	2.75±0.5	nd

Mean ± SE

Nd = Not detected

TABLE 2: Comparison of soluble sugar concentration of fruit tissue (mesocarp + exocarp and seed) in four avocado cultivars ‘‘Hass’’, ‘Fuerte’, ‘Gem’ and ‘Ryan’ during fruit growth and development pre-harvest for the 2016-2017 growing season, from Everdon Estate in KwaZulu-Natal, South Africa

LSD’s		**mg/g DM**			
Cultivar	**Tissue**	D-Manno	Perseitol	Sucrose	Glucose
‘Hass’	**Meso+Exo**	3.285	1.919	1.187	0.2721
	Seed	1.815	1.456	1.171	nd
‘Fuerte’	**Meso+Exo**	5.195	1.897	0.6291	0.3148
	Seed	0.790	2.747	1.255	nd
‘Gem’	**Meso+Exo**	4.085	1.673	0.762	0.2107
	Seed	0.5542	0.878	0.3692	nd
‘Ryan’	**Meso+Exo**	2.807	0.740	0.4105	0.4268
	Seed	1.089	1.637	1.641	nd

LSDs

Nd = Not detected

TABLE 3: Total antioxidant capacity of (mesocarp + exocarp) fruit tissue for ‘Gem’ and ‘Ryan’ avocado cultivars during fruit growth and development pre-harvest for the (2016-2017) growing season, from Everdon Estate in KwaZulu-Natal, South Africa

Cultivar	**P-value**	**LSD**	**Mean ± SE**
‘Gem’	0.353	1.075	1.77±0.341
‘Ryan’	0.667	0.818	1.20±0.367

TABLE 4: : C.V. % values for the soluble and insoluble avocado sugars from analysis of variance for fruit growth and development between four avocado cultivars ‘‘Hass’’, ‘Fuerte’, ‘Gem’ and ‘Ryan’ at pre-harvest period, during 2016-2017 growing season

		C.V.%			
Cultivar	**Tissue**	**D-Manno**	**Perseitol**	**Sucrose**	**Glucose**
‘Hass’	Meso+Exo	15.5	30.4	26.0	33.4
	Seed	18.5	11.5	24.2	nd
‘Fuerte’	Meso+Exo	33.4	25.2	28.5	23.3
	Seed	26.3	21.7	20.0	nd
‘Gem’	Meso+Exo	33.7	25.4	20.4	20.8
	Seed	18.5	5.7	9.9	nd
‘Ryan’	Meso+Exo	26.5	13.1	14.5	39.0
	Seed	21.7	9.5	29.9	nd

Nd = Not detected

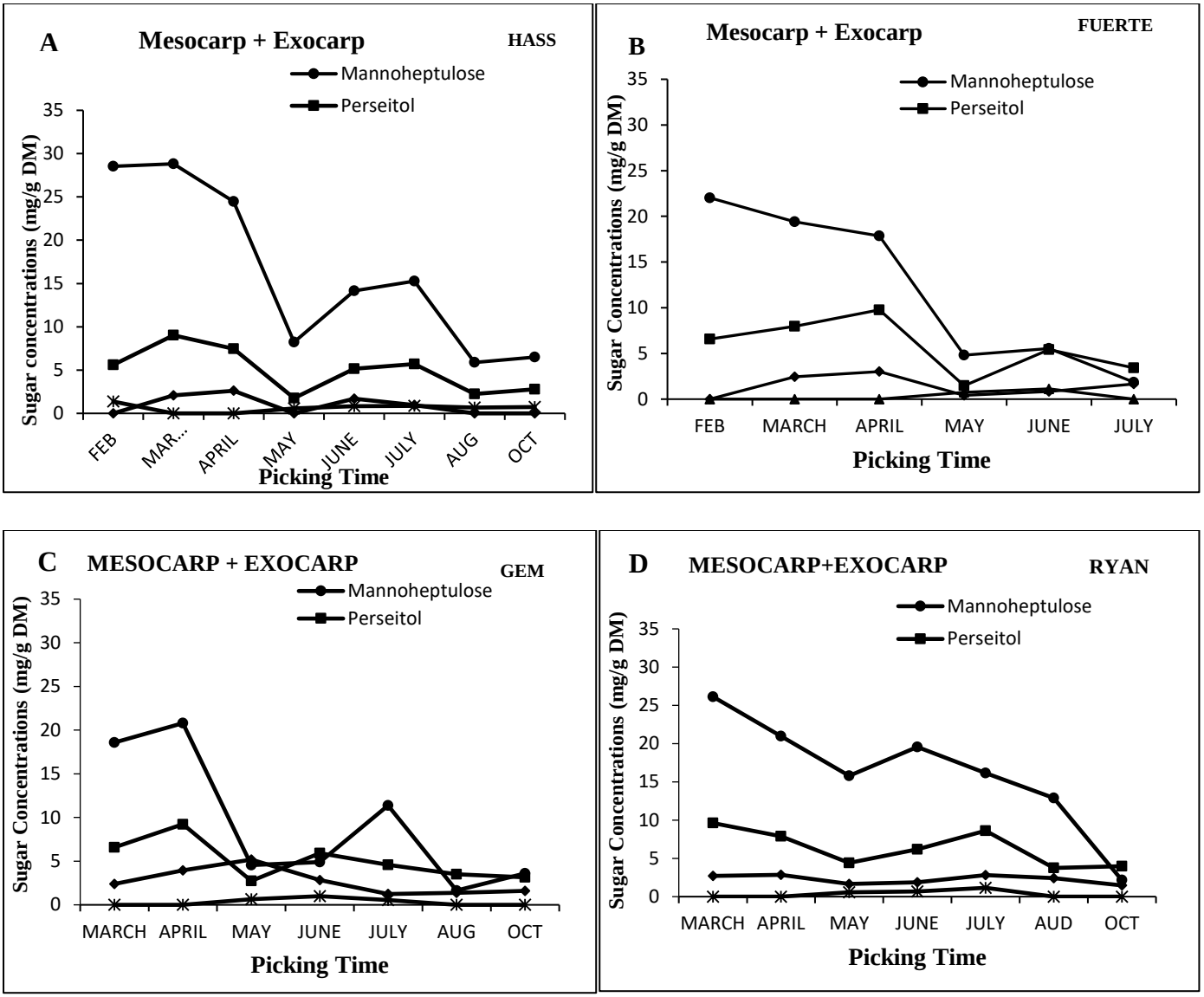


FIGURE 1: carbohydrates (% Total soluble sugars) concentration of avocado fruit tissue mesocarp + exocarp, during pre-harvest growth and development from 2016-2017 growing season, between four cultivars (A) 'Hass', (B) 'Fuerte', (C) 'Gem' and (D) 'Ryan'.

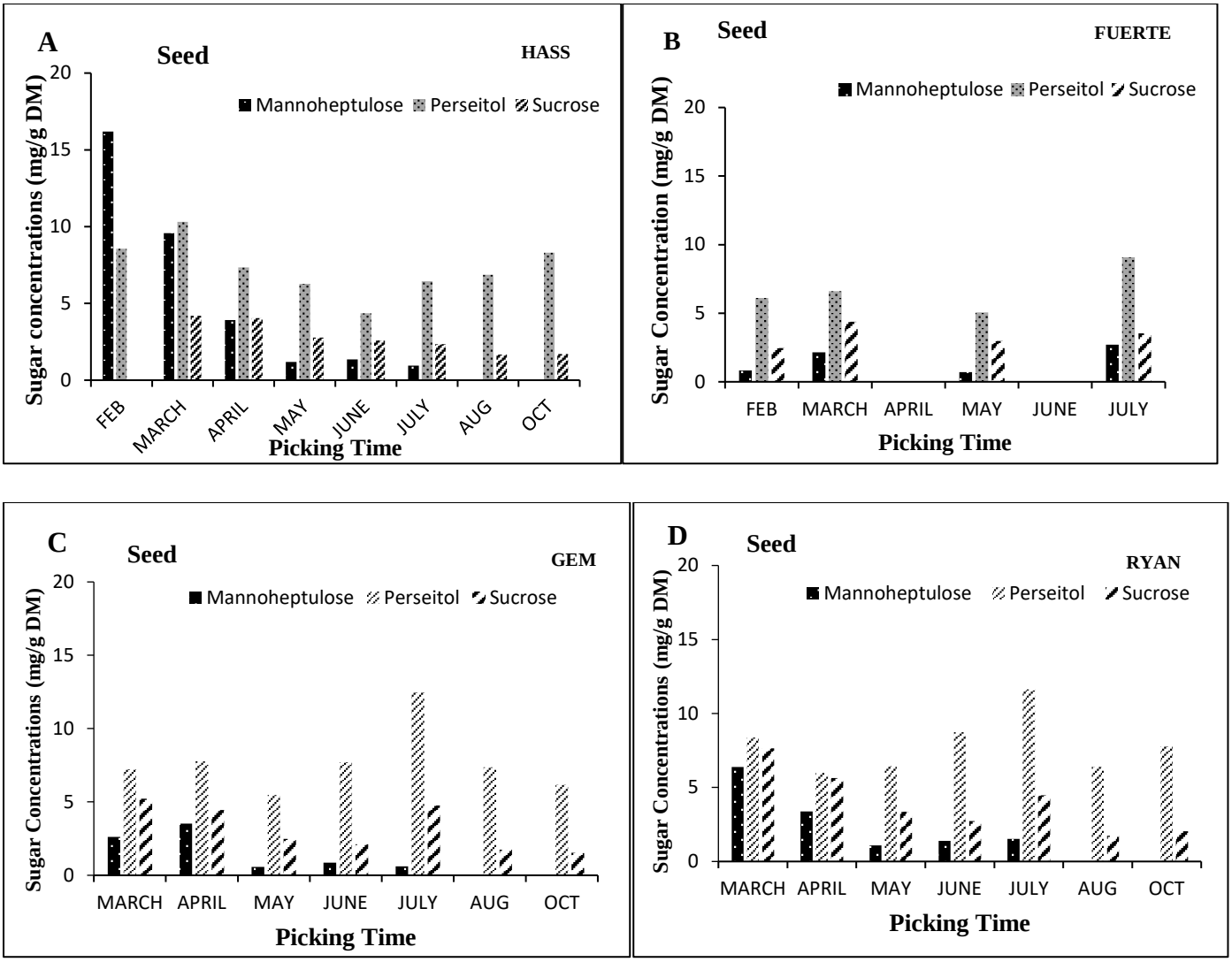
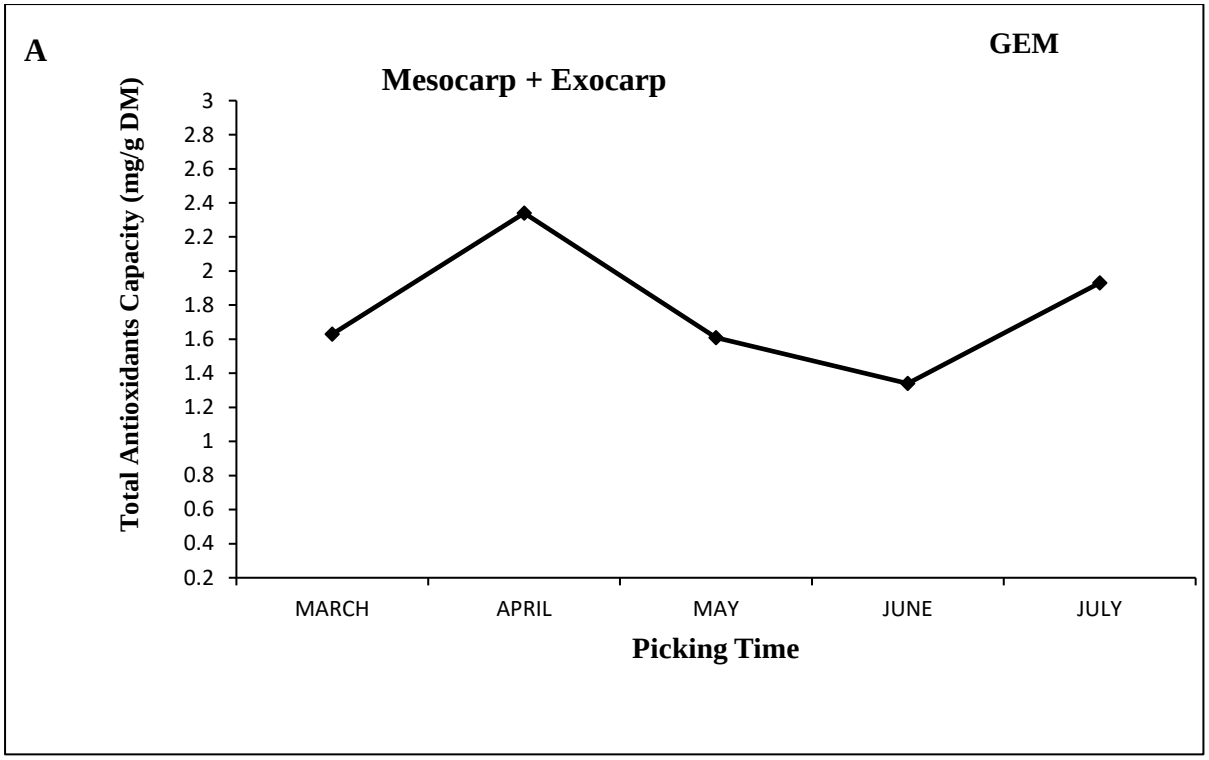


FIGURE 2: Carbohydrates (% Total soluble sugars) concentration of avocado fruit tissue seed, during pre-harvest growth and development from 2016-2017 growing season at Everdon Estate, between four cultivars (A) 'Hass', (B) 'Fuerte' (C) 'Gem' and (D) 'Ryan'.



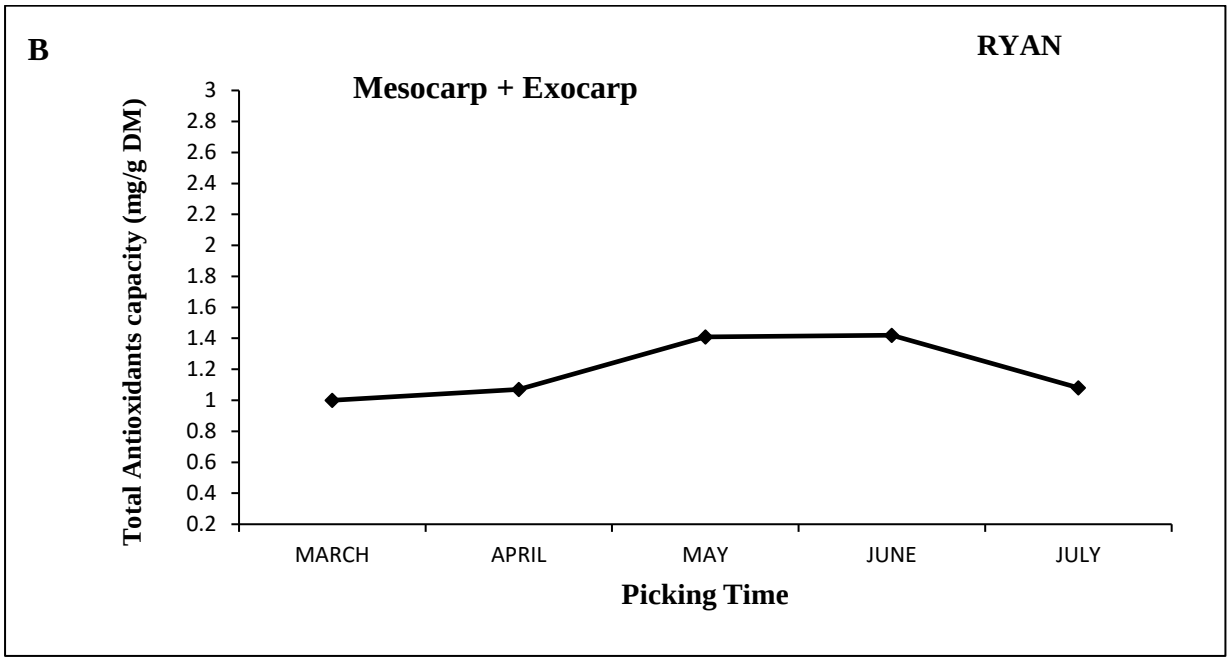


FIGURE 3: FRAP Total Antioxidants capacity on mesocarp+exocarp combined avocado fruit tissues during pre-harvest growth and development from 2016-2017 growing season at Everdon Estate, on 'Gem' (A) and 'Ryan' (B) cultivars.

CHAPTER 5

ALTERATION IN OIL CONTENT, DRY MATTER AND FRUIT SIZE DURING FRUIT DEVELOPMENT

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ABSTRACT

The subjectivity of avocado fruit maturity determination has led to the use of destructive and expensive methods to determine convenient harvest maturity time, which has resulted in immature fruit harvesting, poor postharvest quality and unattractive fruits to consumers, negatively affecting commercial grower. The commonly used avocado maturity indicators (oil content, dry matter and size) have a consistent correlation with fruit growth and developmental stages, where under very extended harvest time the maturity indicators tend to have a continuous increase in accumulation. Oil content and dry matter percentages differ in respective to different avocado cultivar. Plant material of four avocado cultivars (‘Hass’, ‘Fuerte’, ‘Ryan’, and ‘Gem’) was collected at different developmental stages over an extended time, from fruit set initiation to very late harvest ontogeny. The maturity indicators were found to have a continuous increase in accumulation if the avocado fruit is still attached to the tree past commercial harvest (oil% up to 25% and dry matter up to 50%), which justifies the reason why the commercially 8% oil content is too low for harvesting. However, maturity indices accumulation varies per avocado cultivar in relation to growing environment and growing location. ‘‘Hass’’ is known to be the predominant accumulator of oil content and dry matter, which has a continuous increase in accumulation on fruits still attached to the tree. Although the Everdon orchard environment is not sufficiently favourable for growing ‘‘Hass’’ as the fruit size measurements were smaller size than commonly expected in comparison to other growing areas. Alterations in oil content, dry matter and size of avocado fruit are mostly dependent on the growing environment, cultural practices and cultivar, regarding achieved maturity stage and the extended harvesting period.

1. INTRODUCTION

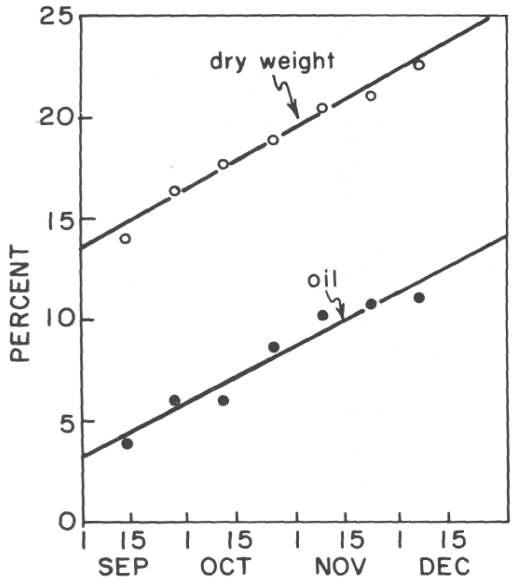
Avocado is a climacteric fruit that does not soften on the tree, so it requires to be harvested during a suitable physiological maturity stage to meet the desirable edible characteristics of taste and firmness (Gil, 2000; Gamble et al., 2010; Carvalho et al., 2014). Thus, it is very hard to usually determine the appropriate maturity stage in various avocado cultivars due to the fruit does not exhibiting any notable external changes (Kassim et al., 2013; Carvalho et al., 2014). Avocado fruit maturity and picking time has external market indicators such as (colour and size) or internal indices (oil content and dry matter percentage) determined in the fruit mesocarp.

Avocado is one of the fruit that is mostly consumed worldwide due to being an important oil fruit and a major source of edible lipids (Ozdemir and Topuz, 2003). From a nutritional aspect, it has a high content of unsaturated fatty acids (Ozdemir and Topuz, 2003) as one of its distinguishing characteristics. Furthermore, avocado contains a vast amount of vitamin E, ascorbic acid, vitamin $B_{6,}$ β-carotene and potassium (Bergh, 1992; Ozdemir and Topuz, 2003).

The use of dry matter as a maturity index for avocado fruit has become predominantly used and accepted extensively. Minimum acceptable values have been initiated as a legal standard for each cultivar in most avocado cultivating countries. The most common and acceptable minimum dry matter percentage for harvest ranges from 19% to 25%, depending on the cultivar and the country (23.0% for Mexico, South America and South Africa, 21.6-22.8% for USA, 21.1% for Australia for ‘‘Hass’’), (Hofman et al., 2002; Orhevba and Jinadu, 2011; Kassim et al., 2013; Carvalho et al., 2014), (21.1% for USA and 21.0% for South Africa for ‘Fuerte’), for ‘Ryan’ and ‘Gem’ there has not been sufficient studies on dry matter widely. As dry matter of avocado proceeds with accumulation while the fruit is still attached in the tree, (due to unusual cell division that continues as long as the fruit remains on the tree), it is expected that delayed harvesting would result in reduced availability of energy from stored sources for the following year crop (Whiley et al., 1996). Avocado fruit picked with dry matter levels below the acceptable minimum percentage tend to have an irregular ripening and the fully development of quality attributes of the fruit will be compromised (Carvalho et al., 2014). Fruit harvested during post-harvest period with a high dry matter tend to ripen faster and have a reduced storage life which is undesirable to the market (Wu et al., 2011). Whiley et al. (1996) postulated that early picking of late-maturity ‘‘Hass’’ at 25% to 30% dry matter resulted in

maximum production; but harvesting at 35% dry matter reduces yields resulting in alternate bearing for the following year.

Moisture content as a maturity index of avocado fruit has been extensively used, although it has been found to be the least reliable criterion, which led to avocado oil content to be widely accepted as the most appropriate index for maturity without actual ripening of the fruit (Halton, et al., 1957; Gazit and Spodheim, 1969). Avocado oil content is a major internal quality that leads to the interaction maturation and ripening processes. Oil content is mostly used as an avocado maturity index to determine the proper time to harvest avocado as indicated by commercial producers using 8% as acceptable percentage. Oil content accumulation varies from maturation stage to ripening. Late hanged avocado seems to continue accumulating oil content and exceed the minimum 8% (Figs 1) and increase up to 25% or more (Table 1). When the oil content increases change occurs in the oil composition (Lee et al. 1983).



Source; (Lee et al., 1983)

Figure 1: Percent oil and percent dry weight during development and maturation of 'Hass' fruit. Each point represents the mean value of 6 fruit. These data, which are representative of a large number of data sets, were collected in 1981.

In avocado fruit mesocarp, oil content increases a few weeks after the fruit set, and oil can be correlated after fruit set and during growth with the age of the fruit (Ozdemir and Topuz, 2003). Ozdemir and Topuz (2003) found that as oil content increases in the mesocarp, water content decreases by the same amount, so that the total percentage of oil and water stays

constant during the fruit life. Avocado fruit tend to have a high concentration of oil in the fruit yellowish mesocarp and seed compared to the green part next to the skin (Hofman et al., 2013). The oil content in avocado fruit depends on several factors, including cultivar, agro-ecological conditions of growth and the fruit growth stage (Carvalho et al., 2014; Kassim et al., 2013; Wedding et al., 2013, Chen et al., 2009). Oil content accumulation is also affected by the tree location in terms of inductive environmental conditions such as cool temperatures, high relative humidity, reduced water stress, nutrient availability and addition of foliar plant growth regulators.

Table 1: Seasonal changes in moisture, oil and solids content of avocado mesocarp (g/100g)

FUERTE							
	5 April	15 May	30 May	21 June	27 July	22 Aug	27 June'
Moisture	74,9	70,4	68,6	63,7	67,7	59,0	64,0
Oil	15,0	20,1	22,1	27,5	24,7	30,7	29,0
Total solids	10,1	9,5	9,3	8,8	7,6	10,3	7,0

EDRANOL				RYAN			HASS
	28 June	28 July	24 Aug	31 July	28 Aug	6 Oct	23 Aug
Moisture	76,2	66,7	67,8	71,4	65,5	64,2	64,6
Oil	14,7	24,7	23,5	19,8	24,4	22,1	23,9
Total solids	9,1	8,6	8,7	8,8	10,1	13,7	11,5

Source: (Du Plessis, 1979)

Fruit size is also used as an important maturity indicator in avocado fruit and component of yield as premium prices are often paid for larger fruit (Kaiser and Wolstenholme, 1994). 'Hass' fruits are known to be, at best, medium sized, but with a proportion of the crop being too petite for profitable market (Lahav and Adato, 1990; Kohne, 1991; Cutting, 1993; Wolstenholme and Whiley, 1995). Delay in avocado fruit delayed harvest has a potential contribute a small but commercially significant fruit growth and increase in fruit size (Kaiser and Wolstenholme, 1994), because of continued cell division in avocado fruits attached to the tree (Schroeder, 1952; Valmayor, 1967). The late maturing avocado cultivars such as 'Hass' and 'Gem', tend to stay attached on the tree for varying periods after minimum physiological maturity has been achieved, which makes it easier to take advantage of market opportunities especially in cooler growing areas (Whiley et al., 1996). Late maturity in avocado cultivars cultivated in cooler areas is mostly due to delayed flowering and fruit set. Expectations of future increase in avocado production has led to commercial growers to be delaying harvest since avocado fruit

normally does not ripen on the tree, for market delay. Delayed harvest is postulated to result in the tree simultaneously caring mature fruit while flowering and setting the next season crop (Whiley et al., 1996).

Physiological maturity in avocado fruit is the developmental stage where most growth has occurred, while ripeness suggests a readiness for consumption, and on the other hand horticultural maturity is a stage of development where harvested fruit will undergo normal softening with desired eating quality (Lee et al., 1983). For avocado physiological maturity, does not coincide with horticultural maturity. Early avocado fruit picking results in immature fruit harvested, which do not ripen properly but tends to be rubbery, flavourless, shriveled, watery and blackened (Lee et al., 1983), which is most likely to be the result of delayed marketing. Identification of horticultural maturity is complicated and not obvious in avocado fruit, because maturation is not accompanied by changes in external appearance.

The study objectives were to measure oil content, dry matter and fruit size maturity indices from early fruit set to late or past commercial harvesting period by prolonging attachment of the fruit on the tree, in four avocado cultivars with two late maturing and two early maturing cultivars, and see if is there any correlation towards reduced post-harvest disorders and increase in late market production of avocado fruit.

2. MATERIALS AND METHODS

2.1 Plant material

2.1.1 Fruit collection

During early fruit, set ‘Hass’, ‘Fuerte’, ‘Ryan’ and ‘Gem’ avocado cultivars were collected monthly from trees in Howick KwaZulu-Natal at Everdon Estate during the 2016/2017 growing season and in Midlands (Latitude: 29˚28′S; Longitude 30˚161′E), Bounty Farm in Pietermaritzburg, during (2015/2016) growing season, both cool subtropical areas, only ‘‘Hass’’ cultivar was measured.

In the 2015/2016 growing season, fruit harvesting was carried out from March to May 2016, a period beyond that of commercial harvest. During each sampling, ten fruits were collected from five randomly selected trees once per month. Harvested fruit were transported, in a

cooled container, to the University of KwaZulu-Natal (PMB) postharvest laboratory for oil content analysis and dry matter determination.

In the 2016/2017 avocado growing season, fruit harvesting began from February to September 2017, a period commencing at early fruit development until beyond commercial harvest. During sampling, the trees were marked as low and high fruit load branches. Per cultivar 10 fruit were collected from selected trees, five fruits from low and five on high load branches and transported in boxes convenient for avocado fruits for laboratory oil content and dry matter determination.

2.2 Size measurement

Starting in March, five trees were marked that displayed an average fruit load, for the 2015/2016 season at Bounty farm. On these trees, five fruits per tree were marked to measure fruit diameter during the observation period. Fruit diameter was measured once per month over a period of three months from March to May using digital calipers (Absolute Digimatic, Mitutoyo, Japan)**.** In the 2016/2017 season, 10 trees were marked per cultivar prior to high and low fruit load per branch. Fruit diameter was measured the same way as during the previous season, except that it was measured for both high and low fruit loads, size measurement commenced in February after fruit until past the commercial harvesting period and compared.

2.3 Dry matter determination

Ten harvested fruit were cut open, the peel and seed removed, so that only the fruit mesocarp was used. The tissue was cut into cubes that weighed about 20 g per fruit. Weighed samples were transported into the specimen jars, liquid nitrogen was added per sample. Samples were stored in the freezer for 10 min and then freeze-dried for five days. After freeze-drying sample were weighed again and mesocarp dry matter recorded as: Mesocarp dry matter % = dry mass (g) / fresh mass (g) X 100. The same method was followed for both seasons except that, for the 2015/2016 season the number of fruit replicates and cultivars were increased.

2.4 Oil content determination

Mesocarp oil content was determined according to Meyer and Terry (2008). Oil analysis was conducted once a month from March until May. The dried samples were ground after dry matter determination, using a coffee grinder. An amount of 300 mg of ground sample was weighed out and transferred into ten test tubes for oil analysis. Hexane (9 ml) was added to each test tube and all test tubes were then transferred into a water bath at 40^{o}C for 10 min. The

mass of ten scintillation vials was recorded. After 10 min in the water bath the samples filtrates were transferred into scintillation vials using a funnel and glass wool. Hexane (6 ml) was added to the test tubes for 5 min and then transferred into scintillation vials, a further 3 ml hexane was added to rinse the tubes and added to the liquid in the scintillation vials. The hexane was evaporated from the scintillation vials using an evaporator EZ2 (GenVac Ltd, Suffolk, England). After drying, the mass of the vials containing the extracted oil was determined and the amount of oil determined by subtracting the mass of the individual vial from the mass of the vial containing the oil. The percentage oil was calculated as: "[oil mass (g)/ dry mass (g)] x 100. The same method was followed for the 2016/2017 growing season.

Data analysis

GenStat software was used to analyse raw data using a Randomised Complete Block Design (RCBD) model, where alpha is (0.01).

3. RESULTS

3.1 Oil Content

3.1.2 'Hass' and 'Fuerte'

There was a significant difference between sampling period for both ''Hass'' and 'Fuerte' ($p < .001$), (Table. 2). 'Hass' which was a bit behind 'Fuerte' in terms of growth stage, had relatively the same percentage of oil content on the first analysis, thereafter oil accumulation showed an increase for the following sampling times. Only in May did oil content show a reduction in accumulation, followed by an increase onwards (Figs 2A). In 'Fuerte' a continuous increase in oil content accumulation was observed during the experiment period until harvesting in July (Figs 2B). No further data was collected for 'Fuerte' due to experimental trees being completely harvested by mistake. 'Hass' oil content increased in response to extended harvesting period while fruit were attached to the tree.

3.1.3 'Gem' and 'Ryan'

Significant differences in oil content were observed during the picking times for both 'Gem' and 'Ryan' through different ontogenic stages of the fruit ($p < .001$), (Table. 2). 'Gem' showed a continuous increase in oil content until June, followed by a noticeable decrease in July (Figs 3C) 'Ryan' oil content accumulation was observed during picking time with a decrease only in June (Figs 3D). Consistent increase in oil content percentage occurs in relation to fruit diameter expansion.

3.2 Dry Matter

3.2.1 'Hass' and 'Fuerte'

Between the sampling periods a significant difference was identified for both cultivars 'Hass' and 'Fuerte' (Table. 2). In 'Fuerte', a greater dry matter accumulation than in 'Hass' (Figs 4A and 4B), with a correlation in performance as both decreased in dry matter in June. However, fruit dry matter had a continuous accumulation in fruits still attached to the tree (Figs 4A).

3.2.2 'Gem' and 'Ryan'

A significant difference was observed between the picking times during growth and developmental stage of the fruit for both cultivars 'Gem' and 'Ryan' ($p < .001$) (Table. 2). Continuous dry matter accumulation was observed during the picking times of the experimental period for both 'Gem' and 'Ryan' with a minor decrease in June (Figs 5C and 5D), followed by consistent increase onwards.

3.3 Fruit diameter (Size)

3.3.1 'Hass' and 'Fuerte'

In ''Hass'' fruit, diameter measured during the experimental period showed a significant difference for low tree fruit load ($p < .001$) but was not significantly different for high ($p = 0.812$) tree fruit load (Table. 2). However, there was a significant difference between the high and low tree fruit load. 'Hass' fruit size showed continuous expansion from early fruit growth to maturity, although a decrease in size in June was noticed (Figs 6A), and further increase in fruit diameter on fruit still attached to the tree past harvesting time was observed (Figs 6A) . On 'Fuerte' the size expansion was significantly different between the high ($p < .001$) and low ($p < .001$) tree fruit load and through the measuring periods (Table. 2). Continuous increase in size accumulation was observed throughout the experimental period till harvesting in July (Figs 6B).

3.3.2 'Gem' and 'Ryan'

In 'Gem' a significant difference in fruit size was observed between the high ($p < .001$) and low ($p < .001$) tree fruit load (Table. 2). Continuous increase in fruit diameter was observed until June, followed by a steady fruit size for both high and low tree fruit load (Figs 7C).Further, 'Ryan' showed a significant difference between high ($p < .001$) and low ($p < .001$) tree fruit load (Table. 2). An increase in fruit diameter of 'Fuerte' from the first size measurement towards harvest maturity was observed, with a minor decrease in June (Figs 7D),

followed by an increase during extended fruit hanging period for both cultivars on low and high fruit load trees.

4. DISCUSSION

The maturity indices examined (oil content, dry matter and size) had a correlational accumulation pattern throughout the experimental period regardless of the cultivar. ‘‘Hass’’ tended to have the highest oil content and dry matter despite being behind in terms of developmental stage compared with ‘Fuerte’, ‘Gem’ and ‘Ryan’ growth stages at Everdon (Figs 2-7). The maturity indicators accumulation pattern follows a sigmoidal curve for all cultivars which coincide with the commonly known avocado fruit growth pattern which is sigmoidal curve. However, fruit size does not reflect how much oil content or dry matter accumulated by the fruit, and available oil content and dry matter depends mostly on the cultivar mostly. Large fruits do not necessarily mean high oil content and dry matter, and these are mostly dependent on the developmental stage and cultivar. As avocado fruit growth is known to follow a single sigmoidal curve, where the early growth stage is characterized by very rapid cell division which is in high demands of energy (Barmore, 1976). Therfore, maturity indicators which have weak sink-strength for photo assimilates and energy which strengthens with fruit development and maturity initiation are established. During the mid (March-June) and late (July- onwards) developmental stages of avocado fruit at Everdon, where the cell division rate is minimized, maturity indices such as oil content and dry matter tend accumulate more dominantly as carbohydrate availability favors maturation achievement and fruit size increases due to maximized cell enlargement.

Avocado cultivars vary in terms of maturity, where there are early and late maturity cultivars. In early-maturing cultivars such as ‘Fuerte’, the latter portion of the growth pattern is steep and fruit are still increasing in size at harvest. In the later-maturing cultivars (‘Hass’ and ‘Gem’), growth pattern is moderately steep and increases in fruit diameter which is has slowed down long before commercial maturity is attained (Barmore, 1976). Maturity in avocado fruit is defined by various parameters which require human input. A mature fruit is identified by an achieved developmental stage that when harvested, it will soften to an edible condition with acceptable flavor and texture identifiable with that cultivar. An immature fruit is one that has not yet attained this proper stage of development and, although it will soften, will not attain acceptable eating quality. In addition, an immature avocado held under ripening conditions will

often shrivel and become rubbery and discolored (Barmore, 1976). When late hanging is practiced, fruit may be present on the trees coincident with the critical flowering and fruit set periods of the following year's crop. Consequently, the trees will be required to maintain a double fruit load until harvesting or natural fruit drop takes place (Barmore, 1976).

Oil content

'Hass', 'Fuerte', 'Gem' and 'Ryan' avocado cultivars at initial sampling had oil content that exceeded the 8% oil which is commonly used for commercial harvesting readiness. 'Hass'11.08%, 'Fuerte'10.99%, 'Gem'11.15%, and 'Ryan'11.89% accumulated oil content at initial sampling (Figs2-3), which lack any relationship towards findings of oil content at maturity (Lee et al., 1983). Although the fruits were not exactly at identical developmental stage, 'Hass' was at early fruit set stage followed by 'Fuerte', 'Gem' and 'Ryan' were at similar stages (marble size). This confirms postulations, that not all avocado cultivars have comparable oil content, various cultivars of commercial importance range from low 3% to a high of 30% or greater (Barmore, 1976). The differences in oil content between cultivars grown at the same location are primarily the results of different racial origin of cultivars. Fruit of the Mexican type have the highest oil content followed by the Guatemalan and West Indian types, which explains why ''Hass'' has the highest oil content, due to its being a pure Guatemalan cultivar, 'Fuerte' being a hybrid of Guatemalan x Mexican (Hatten et al, 1964; Reeder, 1969; Barmore, 1976).

However, at initial sampling these cultivars were at early fruit developmental stage, not even close to maturity and harvesting confirming that the commercial oil content harvesting indicator is biased and does not necessary apply to all avocado growing countries worldwide. The 8% oil content is highly subjective, meaning it depends on the cultivar, growing location, environmental conditions and cultural practices. 'Hass' avocado cultivar contains the highest amount of oil compared to other measured cultivars in this experiment. As the four cultivars approach maturity, fruit oil content was ranging between 15-20% (Figs 2-3), showing slow accumulation rate. Oil content accumulation curve shows similarities towards fruit growth pattern which is sigmoidal, as Lee (1981) stated that there is a close relationship between oil content and the development of an avocado fruit. Maturation indicators accumulation coincides with fruit development stages in relation to availability of energy sources, metabolism and enzyme synthesis rate of carbohydrates present. The avocado fruit possesses a remarkable ability to synthesize oils and accumulate levels as high as 30% of fruit weight or greater, (Barmore, 1976). It has long been recognized that as the avocado fruit matures, there is

a concomitant increase in oil content. This relationship is best illustrated by cultivars with a high oil content at maximum maturity such as ‘Hass’. Which is why ‘Gem’, ‘Ryan’ and ‘Fuerte’ cultivars were used in this experiment to find out any relation of oil content accumulation at maturity and beyond harvest maturity performance compared to ‘Hass’.

‘Hass’ avocado cultivar is known to have a greater tendency to hang on the tree after commercial harvest is achieved compared to other commercially grown cultivars. Since avocado fruits are known to accumulate oil with development to as much as 15 to 30% of total fresh mass, it is expected that 'on tree storage' of mature fruit will exert a continued demand for assimilate supply, with pronounced effects on tree phenology and subsequent productivity, which can result in alternate bearing (Kaiser and Wolstenholme, 1991). In this experiment at prolonged tree attachment period, oil content accumulation showed a continuous increase per cultivar, 18.01% for ‘‘Hass’’, 12.74 for ‘Fuerte’, 13.41% for ‘Gem’ and 16.98% for ‘Ryan’ (Figs 2-3). The increase in oil content can be due to different factors which includes: unrestricted cell division of avocado fruit which coincides with fruit enlargement or growth and oil accumulation because of correlation performance. Also, it can be due to the unusual biological nature of the avocado fruit since oil accumulation is unlimited if the fruit is attached on the tree. ‘Fuerte’ was harvested much earlier than other cultivars due to being an early maturing cultivar and lack ability to hang on the tree for much longer as fruit drop becomes a limiting factor. Environmental, and cultural practices can also have a contribution towards the increase in ‘Hass’ avocado oil content accumulation such as temperatures less than 31^{o}C in summer and greater than 4^{o}C in winter and relative humidity of above 32% during the growth period (Human, 1976).

Dry matter

Avocado mesocarp fruit tissue dry matter has a continuous increase in accumulation from initial sampling until prolonged fruit hanging period for all four cultivars. At initial sampling time, dry matter (DM) was 17.32% for ‘Hass’, 15.49% for ‘Fuerte’, 18.11% ‘Gem’ and 16.88% for ‘Ryan’ (Figs 4-5), measured at an early fruit growth stage. These results have a correlation with study’s findings of dry matter percentage at 8% oil content maturity stage, but the cultivars were neither approaching maturity or matured, which indicates that at 8% oil avocado fruit is not ideal for harvesting (Lee et al., 1983; Ranney, 1991). However, the dry matter accumulation pattern has similarities to oil content within all four cultivars therefore dry matter and oil content maturity indices correlates, and coincide with the avocado fruit growth

sigmoidal curve. As the cultivar approaches maturity, or at maturity, dry matter accumulation increased to 39.25% for 'Hass', 25.32% for 'Fuerte', 24.34% for 'Gem' and 20.66% for 'Ryan' (Figs 4-5), with 'Hass' being the greatest accumulator of mesocarp dry matter that increased to 30% and above, which confirms findings of (Johnston, 2006). The variation in dry matter between avocado cultivars could be dependent on various factors such as cultivar, region, and orchard and several pre- and postharvest factors (Requejo-Jackman et al. 2005). Variation in dry matter can occur for fruit from different orchard locations, and for fruit from different trees within orchards (Mans et al., 1995). Fruit aspect on the tree such as tree canopy, shaded parts and light interception on the fruit, fruit spacing and canopy density have been known to affect avocado fruit dry matter accumulation (Johnston, 2006). Dry matter is reduced in fruit from shaded parts of the tree, although the magnitude of this effect is variable and likely to depend on factors that govern the amount of light interception on the fruit such as tree size, spacing and canopy density (Hofman and Jobin-Decor 1999; Woolf et al. 1999). The presence of defects such as sunburn and ring neck can also increase dry matter content, as can postharvest conditions that affect the rates of water loss and respiration (Hofman and Jobin-Décor, 1999). Environmental conditions such as drought are also known to increase the dry matter content of South African fruit (Mans et al, 1995). The extended duration and timing of fruit set also influences dry matter accumulation in 'Pinkerton' in South Africa, with early set fruit being smaller and having higher dry matter than later set fruit (Sippel et al., 1994). Continuous increase in fruit dry matter during the late hanging period, is mostly related to fruit diameter increase and unrestricted cell division of avocado fruit attached to the tree as a concomitant reason or the favourable environmental conditions experienced by the orchard in 2016-2017 which could be high rainfall, optimum subtropical temperatures and no frost occurrence during the year. The high energy storage sources availability from the previous year can result in high dry matter expansion rate of ''Hass'' avocado fruit (Whiley et al., 1996), if cropping is primarily dependent on adequate carbohydrates reserves (Kaiser and Wolstenholme, 1994). Variation in dry matter of fruits of the same cultivar can be a result of the tree position in the orchard or the fruit position in the tree canopy.

Size

Avocado fruit size measurement showed significant differences between the four cultivars (Table.1), although the fruit were at different developmental stages at initial sampling, with 41.58 mm for 'Hass', 43.02 mm for 'Fuerte', 60.09 mm for 'Gem' and 63.35mm for 'Ryan' (Figs 6-7). These results confirm the correlation between oil content and dry matter

accumulation pattern in relation to fruit diameter. During winter period fruit diameter measurements showed nearly constant increase in size in all cultivars, meaning cell enlargement was at limited or reduced rate, because of unfavorable conditions such as minimum temperatures up to 7^{o}C, experienced at Everdon Estate orchards. Barmore (1976) stated that variation in fruit size and differences in growth rate between early and late maturing cultivars have been instrumental explained by anatomical studies. Much of this variation can be attributed to the flowering characteristics of the avocado tree (Barmore, 1976). Individual avocado trees of any cultivar may bloom over a period of several weeks (Platt, 1974). Hatton and Reeder (1972) have shown that on a tree, avocado fruits originating from known bloom dates are progressively smaller in size from the earliest to the latest bloom date. This is often attributed to differences in production practices, yield, water relations and climatic conditions (Barmore, 1976). Fruit size development is known to be related to fruit maturity, meaning the bigger the fruit the more it is ready for harvesting. Avocado fruit size has a significant difference between different fruits on the same and different trees. Fruit size showed a continuous increase as the cultivars approached maturity or at commercial harvest maturity, with 74.21 mm for 'Hass', 68.46 mm 'Fuerte', 76.68 mm for 'Gem' and 78.15 mm for 'Ryan' (Figs 6-7), showing significant recovery from winter period. Avocado fruit size development are dominantly dependent on the cultivar genetics or race, as Guatemalan avocado race is known to produce large size fruit (e.g. 'Hass'). However, in this study all cultivars were of large size ('Hass', 'Ryan', 'Fuerte' and 'Gem').

Avocado fruit size development during the late-hung of 'Hass' 'Ryan' and 'Gem' cultivars increased continuously up to (65 mm 'Hass', 74 mm 'Gem' and 76.5 mm 'Ryan') average diameter, then followed by fruit drop of few selected fruits. Increase in avocado fruit size development is due to the potential of continued cell division in fruits attached on the tree, and environmental conditions that are conducive to avocado fruit growth such as optimum rainfall received during the growing season, and cultural practices. Fruit size development is known to be related to fruit maturity, meaning the bigger the fruit the more it is ready for harvesting maturity. Avocado fruit size had significant difference between different fruits on the same and different trees, and picking times per cultivar ($p < .001$), (Table 2). Variation in fruit size of same cultivar can be due to cultural practices, yield water relation, and climactic conditions. The late-hung fruit size tend to have 70 mm or more diameter as the maximum size avocado fruit can achieve after the commercial acceptable maturity stage has been reached, which was followed by fruit drop.

5. CONCLUSION

To conclude, avocado commercially used maturity indicators such as oil content, dry matter and size are correlated towards fruit growth and development from pre- to postharvest periods, where during the very extended fruit attachment have a consistent continuous increase in accumulation, until the fruit drop period. Therefore, prolonging fruit attachment of late maturing avocado cultivars is convenient for commercial growers, due to reduced postharvest physiological disorders and ideal requirements for consumers.

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TABLE 2: P-values of analysis of variance for fruit growth and development between four avocado cultivars ‘‘Hass’’, ‘Fuerte’, ‘Gem’ and ‘Ryan’ at pre-harvest period, during 2016-2017 growing season.

		P-value		
Cultivar	Tree Load	DM(mg/kg)	Oil content (%)	Size (mm)
‘Hass’	High	< .001	< .001	0.812
	Low	< .001	< .001	< .001
‘Fuerte’	High	< .001	< .001	< .001
	Low	< .001	< .001	< .001
‘Gem’	High	0.001	< .001	< .001
	Low			< .001
‘Ryan’	High	< .001	< .001	< .001
	Low			< .001

TABLE 3: C.V. % -values of analysis of variance for fruit growth and development in four avocado cultivars ‘‘Hass’’, ‘Fuerte’, ‘Gem’ and ‘Ryan’ at pre-harvest period, during 2016-2017 growing season.

	C.V.%		
Cultivar	**Dry matter**	**Oil content**	**Fruit Size**
‘Hass’	8.9	12.4	274.5^{H}
			7.7^{L}
‘Fuerte’	4.2	8.5	8.8^{H}
			11.1^{L}
‘Gem’	7.8	4.7	9.3^{H}
			5.2^{L}
‘Ryan’	9.0	6.6	8.8^{H}
			8.7^{L}

H = High

L = Low

TABLE 4: LSD and S.E -values of analysis of variance for fruit growth and development in four avocado cultivars ‘‘Hass’’, ‘Fuerte’, ‘Gem’ and ‘Ryan’ at pre-harvest period, during 2016-2017 growing season.

	Oil %		**DM**		**SIZE**	
Cultivar	LSD	S.E	LSD	S.E	LDS	S.E
‘Hass’	2.084	0.724	2.877	0.999	75.29	27.09[H]
					2.390	0.858[L]
‘Fuerte’	1.762	0.671	1.174	0.402	2.140	0.769[H]
					3.259	1.625[L]
‘Gem’	0.7391	0.255	2.781	0.960	2.018	0.725[H]
					2.277	0.814[L]
‘Ryan’	1.250	0.610	2.731	0.943	2.463	0.886[H]
					3.591	1.286[L]

H = High

L = Low

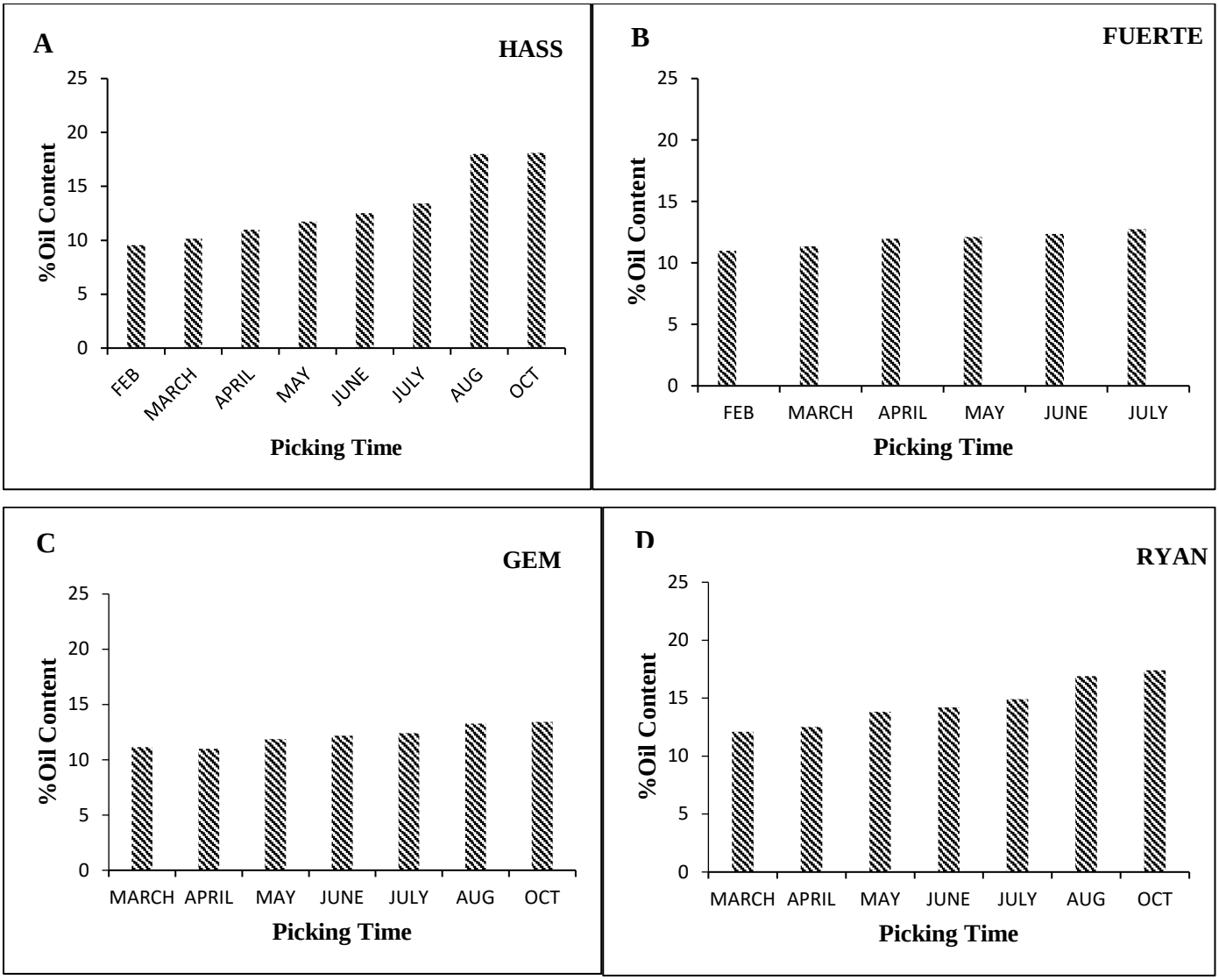

FIGURE 2: Alterations in oil content accumulation during fruit growth and development of 'Hass' (A), 'Fuerte' (B), 'Gem' (C), and 'Ryan' (D) avocado fruit for 2016-2017 growing season at Everdon Estate PMB.

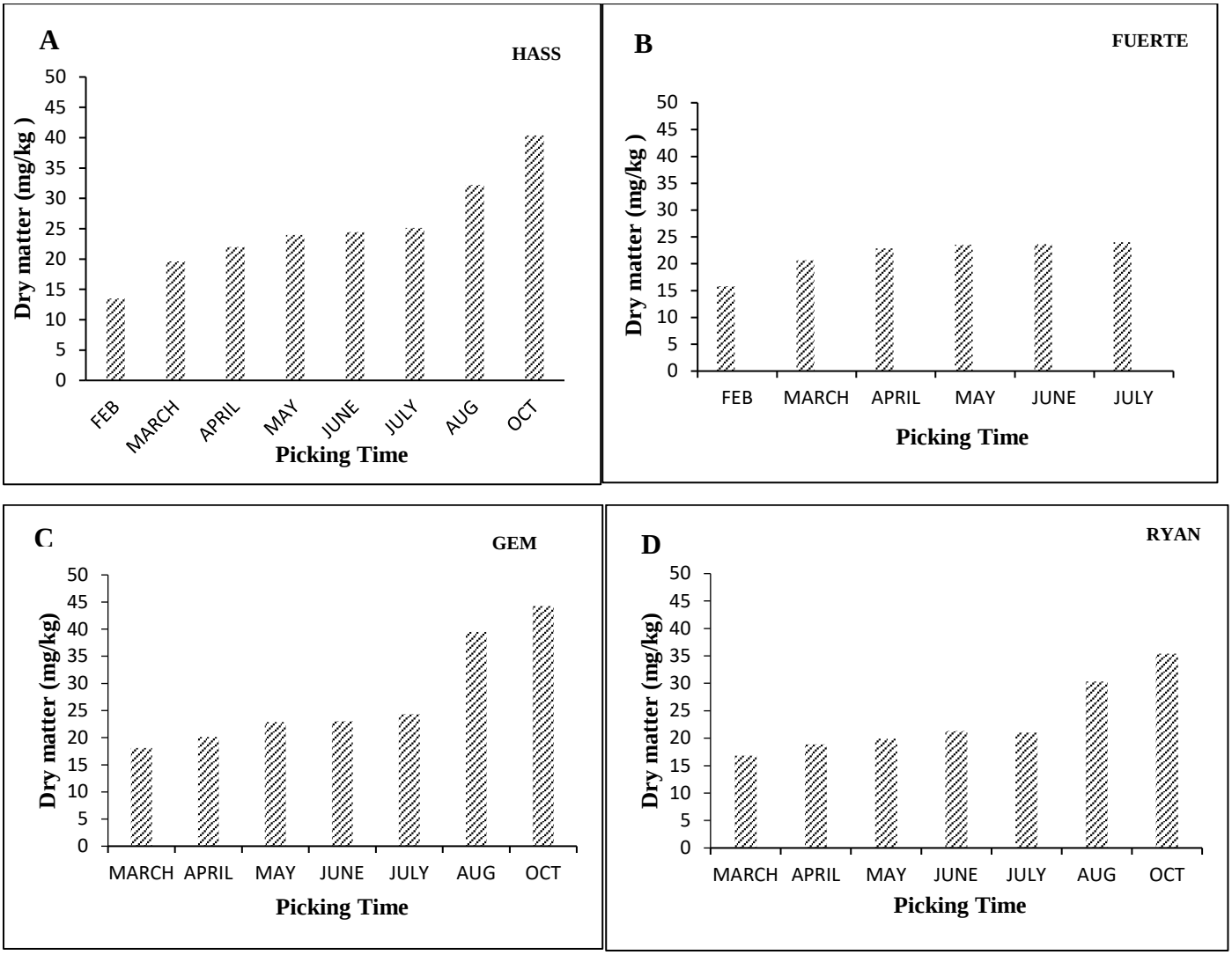

FIGURE 3: Alterations in dry matter accumulation during fruit growth and development of 'Hass' A, 'Fuerte' B 'Gem' C and 'Ryan' D avocado fruit for 2016-2017 growing season at Everdon Estate PMB.

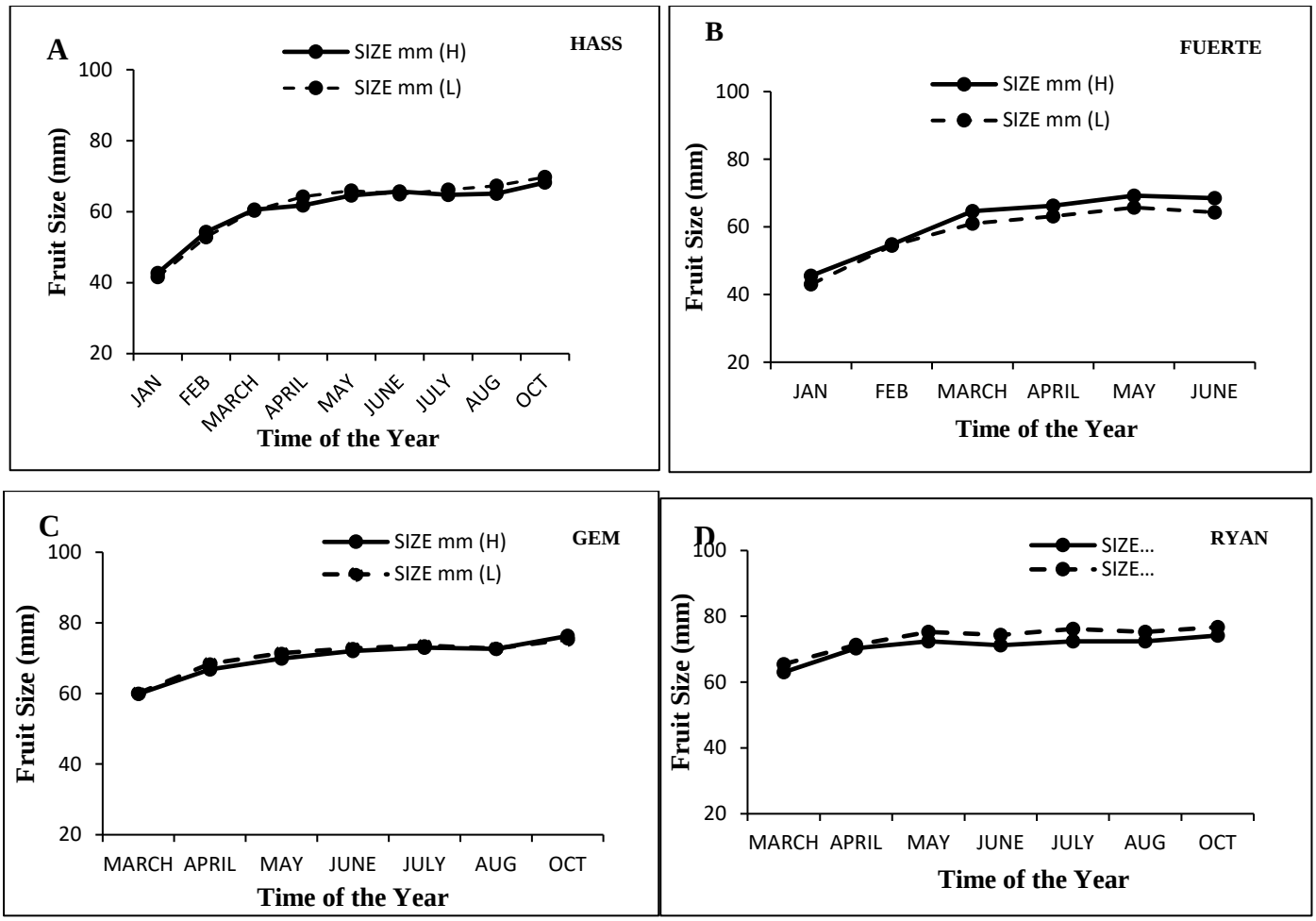

FIGURE 4: Alterations in fruit diameter during fruit growth and development of ‘‘Hass’’ A, ‘Fuerte’ B, ‘Gem’ C, and ‘Ryan’ D avocado fruit for 2016-2017 growing season at Everdon Estate PMB.

CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND OUTLOOK

NOSIPHO PRECIOUS MBELE

Discipline of Horticultural Science

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Sciences

University of KwaZulu-Natal

Pietermaritzburg

South Africa

INTRODUCTION

An intensive literature search was conducted regarding avocado fruit growth and development with effective factors mostly pre-harvest period, as a focal point. However, there is insufficient research under very extended harvesting period on avocado fruit still attached to the tree. An avocado fruit that is attractively desired from a consumer's perspective, with growers expecting quality and market productiveness is dependent on various internal and external contributors. In this research avocado carbohydrates (non-structural) as contributors during growth and development, were considered from pre-harvest till late harvesting period. As it has been stated and suggested that C7 sugars (D-Mannoheptulose and perseitol) have vast functions in avocado fruit, including the possibility to act as indicators of fruit sink strength (Bertling and Bower, 2005; 2006), provides protection of key enzymes essential for fruit growth and development against ROS, (Cowan, 2004; Bertling et al., 2007), and acting as "ripening inhibitors" as reported by Adato and Gazit, (1974) and Liu et al. (2002). Tesfay (2009) reported that C7 sugars in avocado form a part of a pool of antioxidant, which are of demand as defense mechanism when the fruit tissue is exposed to abiotic disorders. Calcium is one of the most in demand essential nutrient during fruit growth and development, as it is required in large amounts during early fruit set for cell division, and throughout development. Uchida (2000) reported that calcium provides plant disease resistance based on its ability to fortify cell walls. Avocado fruit growth and development during post-harvest extended attachment to the tree could be possible through unrestricted cell division, which requires calcium for cells protection and maintenance and sugars as carbohydrates providing energy. By measuring maturing indicators (oil content, and dry matter), in relation to more convenient harvest time towards the extended hanging period of the fruit, in correlation with fruit development pattern, helps to find a link towards double sigmoidal growth pattern.

Sugars accumulation during ontogeny stages

The ontogenic study of avocado fruit revealed that C7 sugars are in high levels during early fruit set, both D-Mannoheptulose and perseitol, but reach maximum peak accumulation in a few weeks after fruit set initiation. This confirms that small fruits are major sink of carbohydrates. Mesocarp and exocarp avocado tissues are a major sinks for D-Mannoheptulose and perseitol compared to the seed at fruit set developmental stage, as the avocado seed is mostly composed of starch. D-Mannoheptulose tends to be predominantly accumulated than perseitol in avocado fruit, as Tesfay et al. (2012) postulated that D-Mannoheptulose was found to be a primary photosynthetic product which acts as an energy source. However, Tesfay et al.

(2012) reported that while perseitol is the major C7 sugar in the seed, and could have been transported from the seed into the developing seedling, D-Mannoheptulose was not present in substantial amounts in the seed. It is likely, that this C7 sugar was released from its reduced form, perseitol, which therefore acted as the storage form of D-Mannoheptulose. As demonstrated by Liu et al. (2002), the high levels of C7 sugars at fruit set are associated with high rate of photosynthesis, as vegetative flush is most likely observed, during early fruit growth periods. This also explains the decline in D-Mannoheptulose in the mesocarp as the avocado fruit approaches/at maturity, because the rate of photosynthesis is at minimal and, the fruit tends to depend on the stored sugars as energy sources. Furthermore, the initiation of new growth commences with the formation of terminal buds and flowers which becomes the strong sinks of energy, resulting the fruit as a weak carbohydrate sink. The 'Hass' cultivar had the highest levels of D-Mannoheptulose and perseitol sugars (Figs 1) than other cultivars, followed by 'Ryan', 'Gem' and 'Fuerte' (Figs 2-4), which further explains the late maturity characteristic, and greater storage longevity of ''Hass''. 'Gem' and 'Ryan' avocado cultivars are also late maturing cultivars. Therefore, the fluctuating levels of D-Mannoheptulose accumulation throughout the experiment vary with the cultivar, and this needs to be further researched. Perseitol sugar alcohol is found predominantly in the avocado seed for all experimented cultivars, which in the fruit is at low levels mesocarp and exocarp. Perseitol at harvest maturity is found to be at in higher levels than D-Mannoheptulose in avocado fruit, which coincides with the findings by (Liu et al., 2002; Bertling and Bower, 2005; Tesfay et al., 2012) indicating that the presence of this sugar alcohol could be related to storability. C6 sugars (Sucrose and Glucose) seemed to be of less importance during fruit growth and development as low levels were detected in the fruit. Sucrose is more dominantly present in avocado seed than mesocarp and exocarp, and this finding confirms with (Liu et al., 1999; Bertling and Bower, 2005; Tesfay et al., 2012) results, which could possibility be providing energy for seed development. Stored cultivars ('Gem' and 'Ryan') under cold (8^{o}C) and room (21^{o}C) temperatures from fruits picked at maturity stage in mid-August, C7 sugar levels performance differs depending on the cultivar. The C7 sugar (perseitol and D-Mannoheptulose) levels at storage tended to decrease in concentrations, but varies in amounts with the cultivar. D-Mannoheptulose and perseitol decrease less under cold storage compared with room temperature storage. 'Ryan' fruit have high levels of D-Mannoheptulose and perseitol than 'Gem' (Table 1 and 2). And therefore 'Ryan' has high storability capacity and less susceptible to storage disorders, 'Ryan' fruit ripening process was a bit behind 'Gem' during storage, which confirms that C7 sugars inhibit respiration rate/ripening. Although at

maturity ‘Ryan’ trees shed a lot of leaves, this could explain the decline in D-Mannoheptulose in the mesocarp and exocarp fruit tissues, as it is a photosynthesis carbohydrate product. Therefore, C7 sugar concentration accumulation differed between the cultivars, but plays a significant roles during fruit growth and development at both pre- and postharvest stages.

Calcium performance during fruit growth and development

Calcium plays vital biochemical functions and supports many metabolic processes, in addition to activating several enzymatic systems, thus contributing to the proper development of plants (Mengel and Kirkby, 2000; Perez-Perez et al., 2008). The fundamental role of calcium is ensuring stability of the membrane and cell integrity (E*l* Habbasha and Ibrahim, 2015). calcium accumulation in avocado fruit at in high levels at early fruit set stages, where a peak is mostly achieved followed by a decline and constant loss towards maturity. As Wilkinson (1968) explained calcium uptake trend are influenced by cell division and enlargement, where rapid calcium uptake occurs during cell division stages of fruit growth, followed by slower uptake or even loss during cell expansion. However, cell division in avocado is unlimited throughout the growth and development phase, but maximum cell division occurs during early growth (Barmore, 1977), thereafter cell enlargement becomes predominant as cell division slows down (Bower, 1985) and the fruit approaches maturity. Bower (1985), explained the initial calcium uptake as the link between actively dividing cells and calcium accumulation, where actively dividing cells are strong calcium sinks (Bangerth, 1979). The rate of rapid calcium uptake in an avocado plant varies with the cultivar, ‘Hass’ as the highest accumulator of calcium (Figs 1) followed by ‘Ryan’ and then ‘Gem’ and ‘Fuerte’ (Figs 2-4). This coincides with the fact that ‘Hass’ has a high late hanging capability, with good shipping storage for both cold and ambient temperatures when compared with other cultivars. As Wills and Tirmazi (1982) illustrated, calcium is an inhibitor of ripening by reducing respiration rate and ethylene peak initiation. Therefore, an increase in transpiration rate and water uptake is favourable during the late growing period, towards an increase in calcium uptake by the fruit which will be beneficial in cell membrane protection and cell integrity throughout continuous cell division. Therefore, calcium availability plays a monumental role towards the unrestricted cell division of avocado fruit and late attachment ability with a continuous fruit diameter increase (Figs 1-4). As not many studies have been conducted regarding calcium concentration during an extended harvest period, when organic fertilizer is applied during maturity period of avocado fruit, surprisingly calcium absorption and concentration increases in avocado fruit, and it was observed for in all

cultivars (‘Hass’, ‘Ryan’ and ‘Gem’) (Figs 1-4). ‘Ryan’ fruit tend to have high rate of calcium uptake (Figs 4) compared to other cultivars, which explains the extended time it took to reach ripening when stored under 8^{o}C and 21^{o}C temperatures compared with ‘Gem’, and ‘Ryan’ which reached ripening after roughly 10 days under 21^{o}C and 30 days under 8^{o}C to a consumable state, when harvested in mid-August. The ability of calcium uptake increase at maturity could be of different reasons such as; increase in transpiration of the tree, increase in temperatures, sufficient irrigation, but further research needs to be conducted with regard to how? Therefore, avocado fruit is able to take up calcium during maturity stage or commercial harvesting time, which is important for fruit growth and cell protection. This justifies that avocado fruit growth does not only follow a single sigmoidal growth pattern but a double sigmoidal curve. Furthermore, the subjectivity of maturity identification is actual by preventing the ability of avocado fruit to reach natural readiness for consumption, preventing immature harvest, rubbery, watery and shrinking of avocado fruits which leads to market loss for the commercial growers. Although, avocado fruits with extended hanging period are not ideal for export but for local market, also depends with the cultivar, early maturing cultivars (‘Fuerte’) are not recommended but late maturing such as ‘Hass’, ‘Gem’ and ‘Ryan’ are recommended.

Fruit growth and development

Schroeder (1953) stated that, the avocado tree differs considerably from the typical deciduous fruit tree in that its period of bloom frequently extends over a very long time, sometimes lasting for six or more months. Avocado fruit growth is known to follow a single sigmoidal growth pattern, composed of rapid cell division in early fruit set, followed by a constant continuous reduced cell division and expansion as the fruit approaches maturity, with an unrestricted cell division as long as the fruit is attached to the tree. The development of cell size and cell number in the avocado fruit indicate that an increase in fruit growth results from both cell division and an increase in cell size during the early period of fruit development, but that cell division is the major factor concerned with increase in fruit size in the latter phase of fruit development (Schroeder, 1953). Previous studies have focused mainly on fruit growth from initiation of fruit set to commercial maturity or harvest, without further interest on the extended fruit hanging development. During the continuous hanging period of avocado fruit, increase in fruit size appears to occur in all parts simultaneously and continuously throughout the period while the fruit remains on the tree (Schroeder, 1953), where cell number increase is continuous throughout the life of the fruit. The increase in thickness of the fruit wall thus does not result entirely from cell expansion, but rather results from both cell division throughout the

fruit life and cell expansion (Schroeder, 1953). However, the rate of cell division and fruit size increase differs within the avocado fruit cultivars, which is attained through fruit diameter measurement. This could be due to various factors such as genetic composition of the cultivar, environmental conditions which are more suitable for that specific cultivar, and cultural practices. 'Hass' is one of the major late maturing avocado cultivars with a greater tendency to hang on the tree during extended harvesting period, with preserved good internal quality, and increase in oil percentage. 'Hass' under conducive growing environments could reach approximately 70 mm fruit size diameter and greater under prolonged tree attachment. During the extended fruit growth period, after the attainment of single sigmoidal curve, a notable moderate increase in fruit size was measured in all three late maturing cultivars (Figs 1, 3 and 4). This noticeable increase shows that avocado fruit does not strictly follow a single sigmoidal growth curve but rather a double sigmoidal curve when development is prolonged pass commercial harvest time. 'Gem' and 'Ryan' are cultivars of large size, which is explained by an increase in cell size and number continuously through extended harvest time. 'Fuerte' development past commercial harvest period was limited due to unintentional harvest of experimental trees by the orchard harvesters, which led to no further data collection. The avocado fruit does not soften until removed from the tree and may be held on the tree for periods up to six months or more following the attainment of acceptable horticultural maturity (Schroeder, 1953). Avocado fruit under extended harvesting tends to have continuous increase in oil content and dry matter. Fruit drop occurrence during very late extended harvest period is mostly experienced in avocado fruit, which could be for various causes (internal and external) such as; the decrease in D-Mannoheptulose as an energy source which also its decrease is associated with increase in respiration rate and ripening or the increase in ethylene production, radicle protrusion in the seed while still attached to the tree which becomes a strong sink for energy also an indication for new fruit growth season, ABA availability increase in the avocado fruit due to stress associated with environmental conditions, physiological disorders and pathogens.

CONCLUSIONS

C7 sugar (D-Mannoheptulose and perseitol) accumulation in avocado fruit fluctuates throughout the pre- and postharvest growth and development season, with D-Mannoheptulose as a predominant sugar in the mesocarp+exocarp while perseitol is dominant in the seed. C7 sugar accumulation pattern correlates with calcium levels in the avocado fruit, they are at high levels at early fruit set reaching a peak a couple weeks after fruit set, followed by a decline as the fruit approaches maturity, with continuous consistent low concentrations. Fertilizer

application during the late harvest period calcium uptake in avocado fruit tends to increase, while D-Mannoheptulose decreases with perseitol increasing. The high concentration of calcium in 'Hass' and 'Ryan' cultivars could explain the storage longevity of the fruits with good quality maintenance as it is postulated to inhibit ripening in avocado fruit. The FRAP assay was not able to extract sufficient antioxidant in 'Gem' and 'Ryan' cultivars in order to see any relation with sugar accumulation concentrations. During the prolonged avocado fruit attachment to the tree, D-Mannoheptulose could be the energy source for the fruit, with calcium providing protection for the cell membrane through continuous cell division which results in fruit size increase along with other exogenous and endogenous contributors of fruit growth and development. Avocado fruit growth pattern does not only follow a single sigmoidal pattern but a possible double sigmoidal pattern, due to the continuous increase in fruit size during extended attachment of the fruit past commercial harvest.

Future research and commercial implication

A vast number of pre-harvest fruit development studies have been conducted regarding all aspects till commercial harvest period, but studies that focuses on late hang avocado fruits are insufficient. The ability of avocado fruit to absorb calcium during late developmental stage, which changes the known calcium accumulation pattern needs to be further researched. Also, how does that calcium contribute towards fruit postharvest quality, and storage life? In depth focus on the relation between C7 sugar especial by D-Mannoheptulose and calcium during extended harvest period of avocado fruit and the t roles they should be researched.

Extending harvest period on late maturing avocado cultivars does not only increase oil content percentage but reduces immature harvesting which results in poor fruit quality at ripening which is rubbery, watery, with browning of the mesocarp, and small string like on the mesocarp which leads to negative impact to the market. Postharvest quality of the fruit develops during growth and maturation and is maintained, not improved, by postharvest conditions. Extending harvest period results in prolonged availability of desired fruits on the market which is good for the producer and the consumer. However, introducing 'Ryan' and 'Gem' in export market is recommended since they can absorb calcium at a late maturing stage, which contributes in ripening reduction and extending shelf life. Only late maturing cultivars are more relevant in late harvesting for both local and international markets, while early maturing cultivars such as 'Fuerte' are only of benefit under local market as they have poor storage quality.

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Table 1: C7 sugar concentrations in (mg/g DM), from mesocarp+exocarp fruit tissues harvested at early maturity stage in August 2017 at Everdon Estate, stored under cold (8^{o}C) and room (21^{o}C) temperatures

GEM COLD (mg/g DM)		ROOM (mg/g DM)	
D-Mannoheptulose	Perseitol	D-Mannoheptulose	Perseitol
(R ^{1}) 1.156706	2.636154	nd	nd
(R ^{2}) 1.009821	2.580462	nd	nd
(R ^{3}) 0.546902	1.247223	nd	nd
(R ^{4}) 0.652842	2.355125	nd	nd
(R ^{5}) 1.120582	2.002355	nd	nd

Nd = Not detected

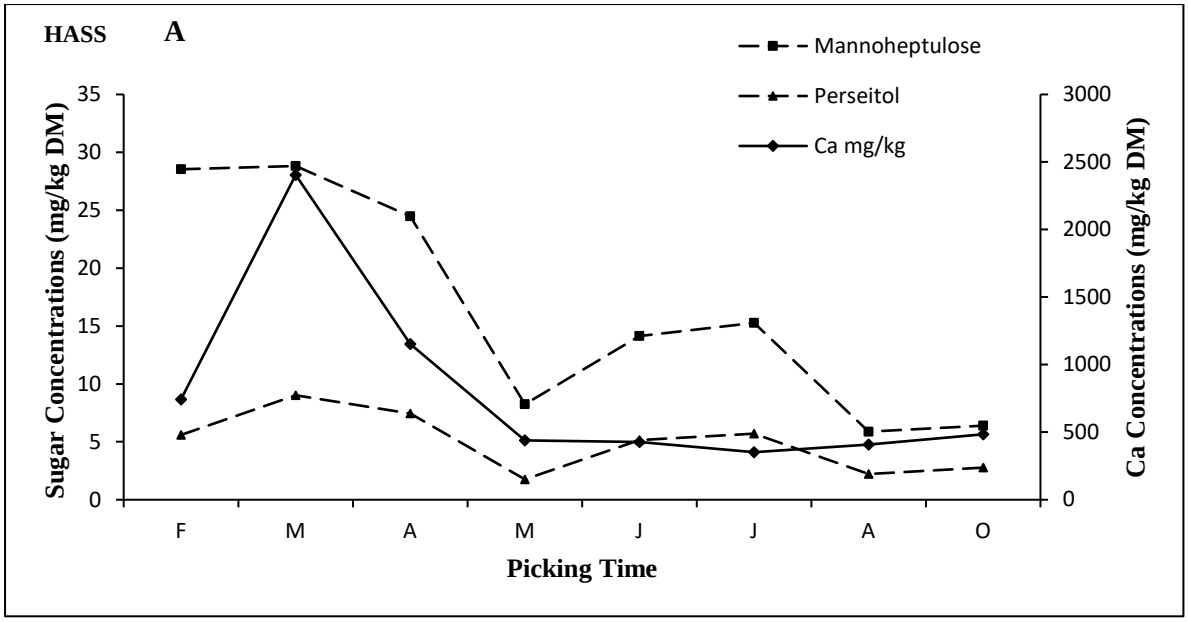
R = Replicate

Table 2: Table 2: C7 sugars concentrations in (mg/g DM), from mesocarp+exocarp fruit tissues harvested at early maturity stage in August 2017 at Everdon Estate, stored under cold (8^{o}C) and room (21^{o}C) temperatures.

RYAN COLD (mg/g DM)		ROOM (mg/g DM)	
D-Mannoheptulose	Perseitol	D-Mannoheptulose	Perseitol
(R ^{1}) 5.550457	2.847825	2.78413	0.982215
(R ^{2}) 7.220184	2.966733	1.203908	nd
(R ^{3}) 5.173541	3.397379	nd	nd
(R ^{4}) 8.271515	2.281240	1.921311	2.178752
(R ^{5}) 7.695215	2.655515	nd	nd

Nd = Not detected

R = Replicate



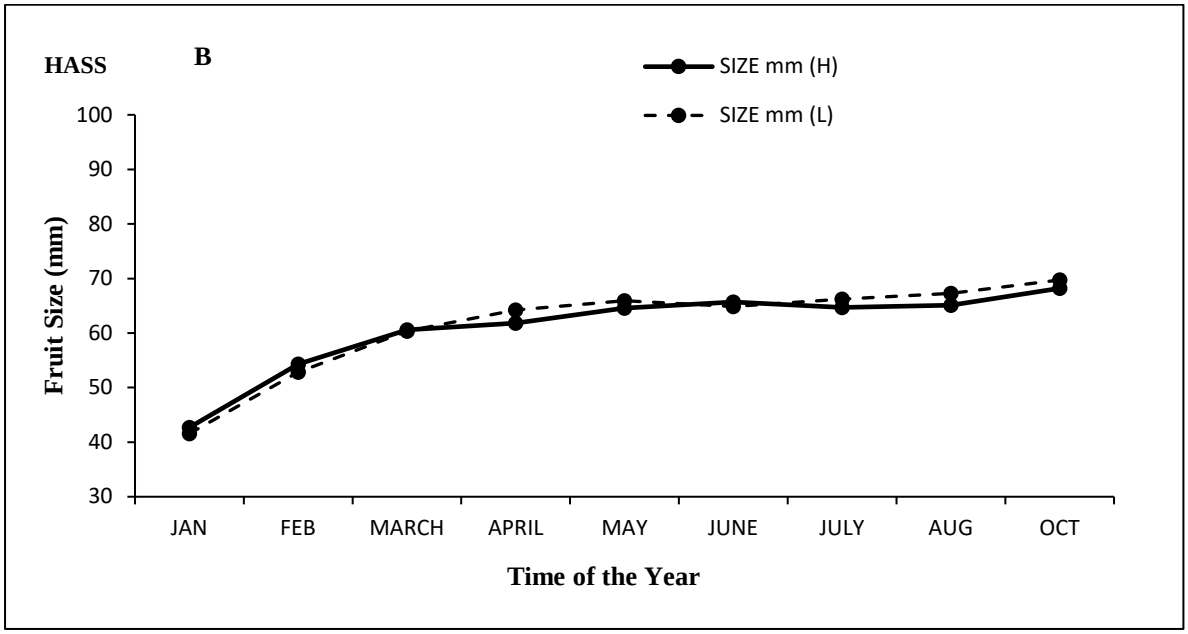
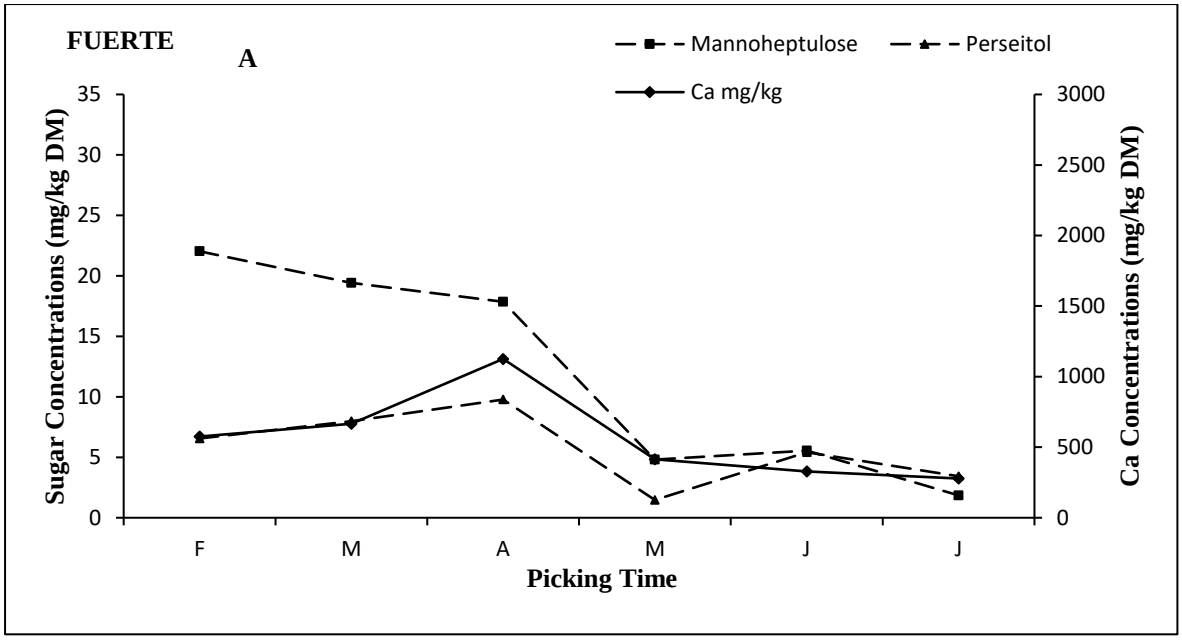


FIGURE 1: Sugars, calcium concentrations (A) and fruit size (B) comparison from early to prolonged avocado fruit mesocarp+exocarp tissue for ‘‘Hass’’, cultivar, grown at Everdon Estate during 2016/2017 growth season.



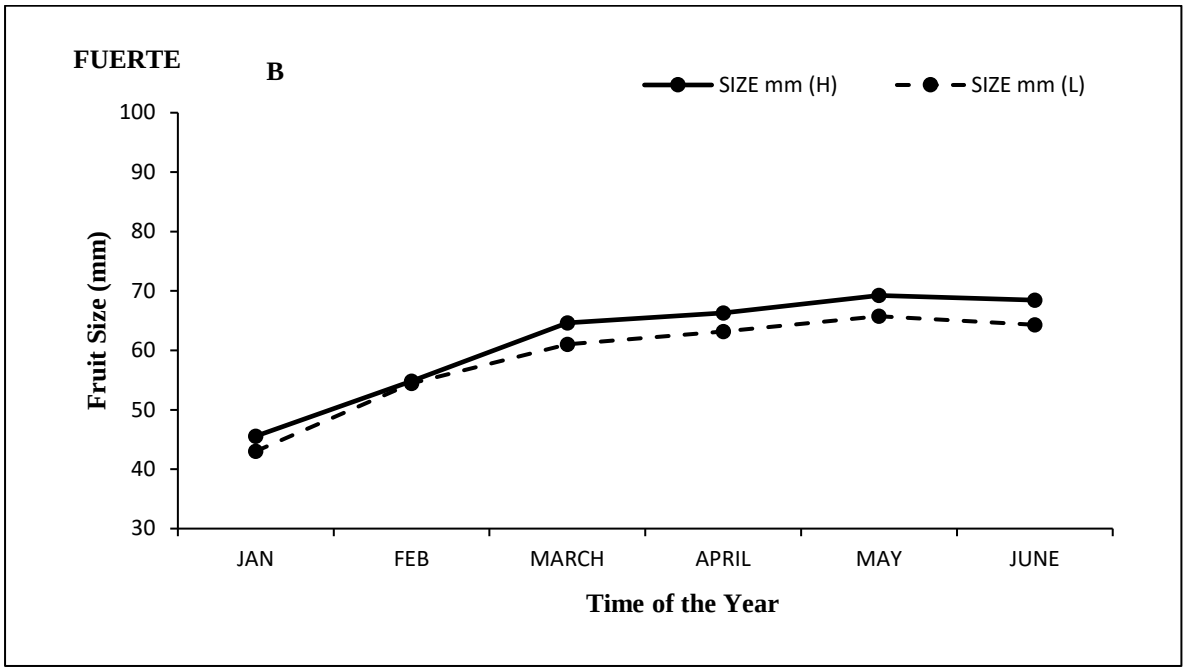
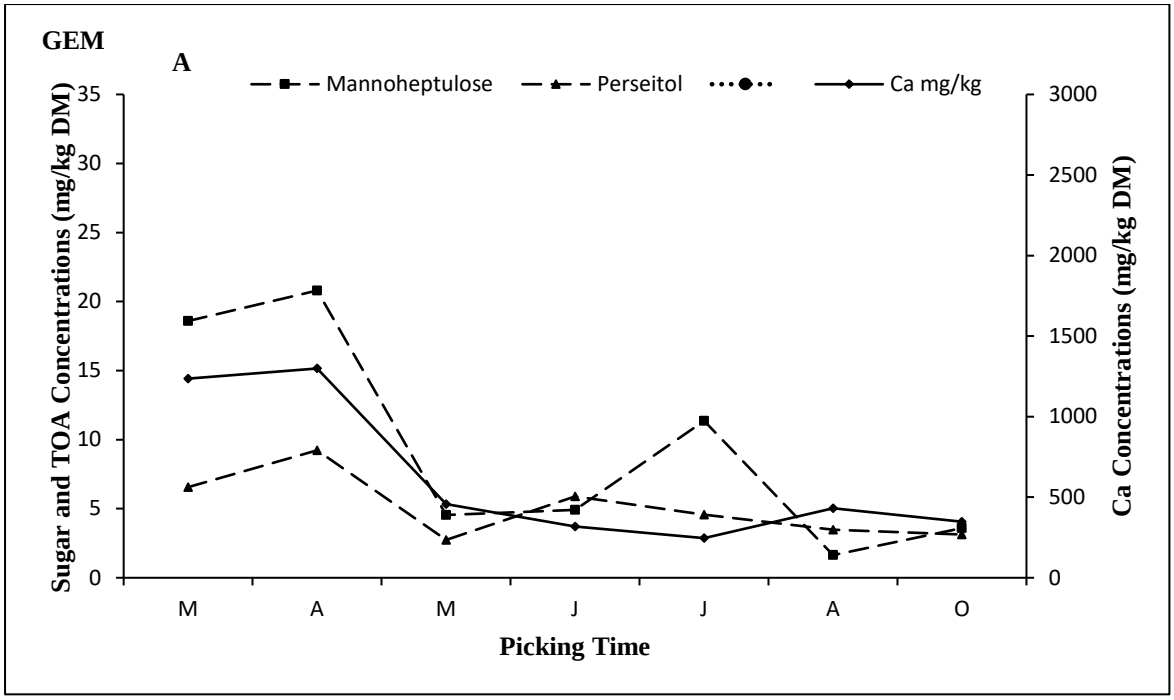


FIGURE 2: Sugars, calcium concentrations (A) and fruit size (B) comparison from early to prolonged avocado fruit mesocarp+exocarp tissue for ‘Fuerte’ cultivar, grown at Everdon Estate during 2016/2017 growth season.



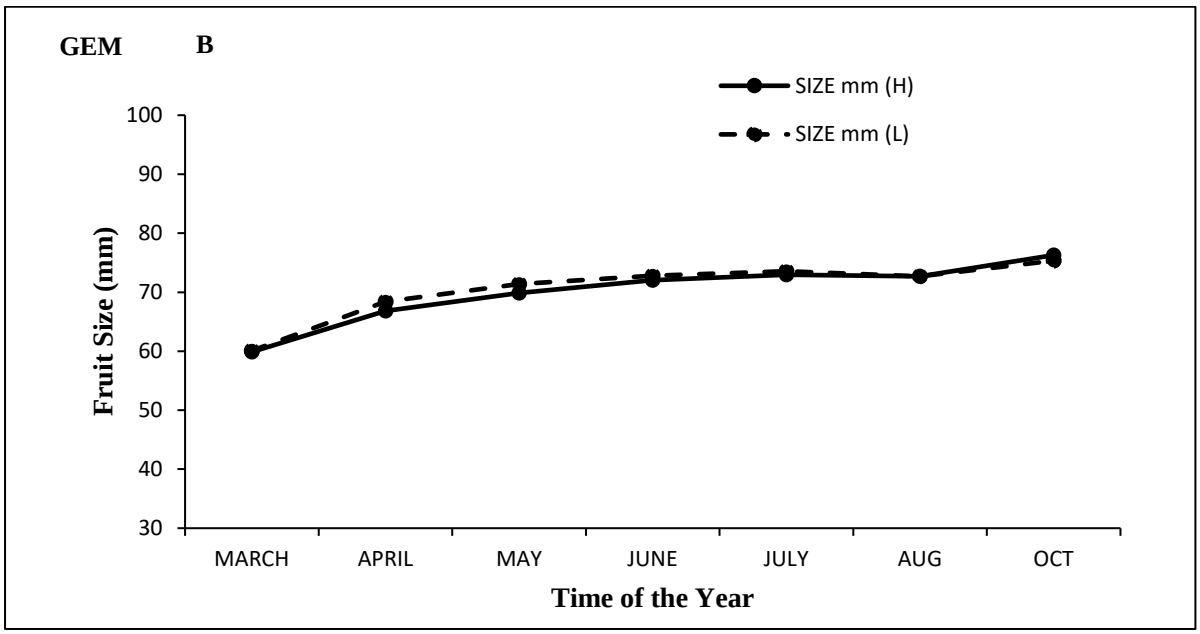
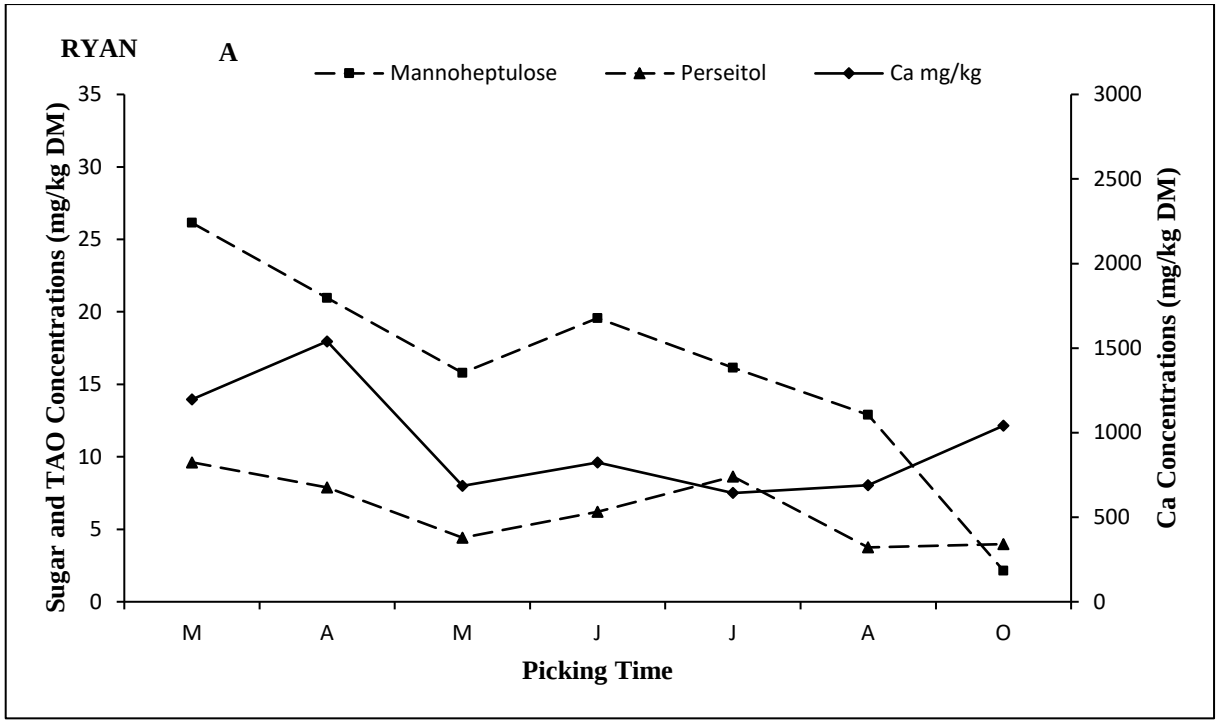


FIGURE 3: Sugars, calcium concentrations (A) and fruit size (B) comparison from early to prolonged avocado fruit mesocarp+exocarp tissue for 'Gem' cultivar, grown at Everdon Estate during 2016/2017 growth season.



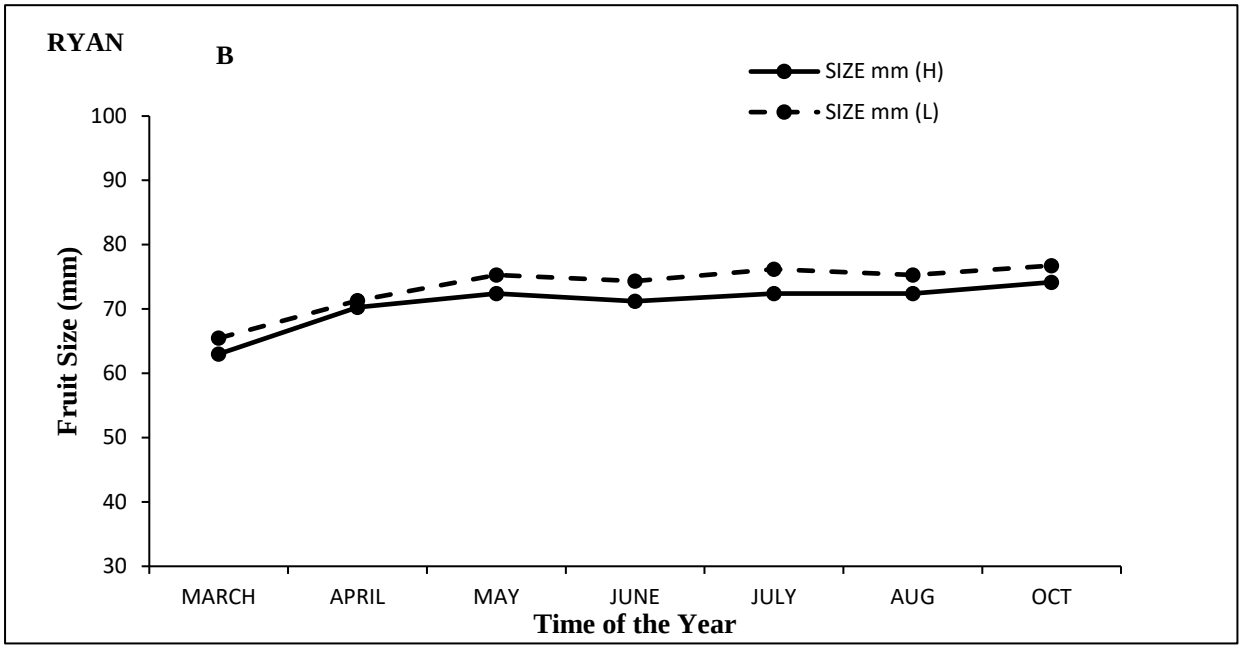


FIGURE 4: Sugars, calcium concentrations (A) and fruit size (B) comparison from early to prolonged avocado fruit mesocarp+exocarp tissue for 'Ryan' cultivar, grown at Everdon Estate during 2016/2017 growth season.