EFFECT OF PARTIAL ROOT-ZONE DRYING, STORAGE TEMPERATURE AND DAYS TO RIPENING ON POST-HARVEST QUALITY OF 'HASS' AVOCADO FRUIT

BY

KHANGWELO JESSICA MUKOVHANAMA

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SUPERVISOR   : PROF TP MAFEO (UL)
CO-SUPERVISORS   : DR N MATHABA (PPECB)
                              MR NJR ROETS (ARC-TSC)

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DECLARATION

I, Mukovhanama Khangwelo Jessica ███████ declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Horticulture has not been previously submitted by me or anybody for a degree at this or any other university. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

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Ms Jessica K Mukovhanama   Date

DEDICATION

This study is dedicated to my amazing parents, Mr and Mrs Mukovhanama and my late pastor (Lehasa Elvis).

ACKNOWLEDGEMENTS

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ABSTRACT

The South African Avocado Industry is continuously expanding with 'Hass' cultivar occupying the largest land and therefore, the most exported cultivar. Expansion of the industry implies an increased demand for irrigation water. However, South Africa is a water scarce country characterised by low and erratic rainfall, where fruit production relies heavily on irrigation. The agricultural sector is under an obligation to reduce water use due to the increasing demand for water by other economic sectors. Therefore, it must find efficient water use techniques to save water and ensure water availability for other economic sectors. Partial root-zone drying (PRD) is an irrigation technique which may lead to 50% reduction in water use, half of the root system is kept wet while the other half is left dry to a predetermined level of soil water depletion, the dry and the moist sides are alternated at regular intervals. During storage and transportation of fresh produce, low temperature is a post-harvest tool used to maintain quality, especially when fruit are destined for long distant export markets. This study investigated the effect of PRD and low temperature storage on post-harvest quality parameters of 'Hass' avocado fruit. The experiment was laid as a factorial arranged in randomised complete block design (RCBD). The treatment factors for chilling injury, electrolyte leakage and vascular browning were 2 x irrigation regimes (Full Irrigation (FI) vs. PRD) and 2 x storage temperatures (2.0 and 5.5°C). However, treatment factors for fruit weight loss, respiration rate, fruit firmness, ripening percentage and fruit skin colour were 2 x irrigation regimes (Full Irrigation (FI) vs. PRD), 2 x storage temperatures (2.0 and 5.5°C) and 4 ripening days (0, 2, 4 and 6 days). Mature 'Hass' avocado fruit were harvested from PRD and fully irrigated trees and thereafter fruit of each treatment were stored at 2.0 and 5.5°C for 28 days. Each treatment consisted of 6 replicates, with an exception of electrolyte leakage, whereby the experiment was replicated 4 times. After 28 days' storage at 2.0 and 5.5°C, fruit were ripened at 21°C. The effect of PRD and low temperature storage was determined by evaluating the following physico-chemical fruit parameters during ripening: external chilling injury, electrolyte leakage, fruit weight loss, respiration rate, firmness, fruit skin colour and vascular browning. During ripening, 'Hass' avocado fruit stored at 2.0°C showed significantly higher incidences of external chilling injury symptoms compared with

5.5°C, irrespective of irrigation treatment. Furthermore, an interaction between irrigation treatment and low storage temperature had a significant (P<0.05) effect on cell membrane electrolyte leakage. All evaluated fruit showed similar weight loss, irrespective of irrigation and storage treatment. Irrigation, storage temperature and ripening days did not have effect on respiration during ripening. Fruit reached the respiratory climacteric peak on the same day (day 2). Furthermore, there was no significant interaction effect (P>0.05) on fruit firmness. However, PRD treated fruit showed slightly low firmness when compared with control fruit. 'Hass' avocado fruit harvested from PRD and fully irrigated trees and stored at 5.5°C ripened quicker compared to fruit stored at 2.0°C. There was no significant interaction effect (P>0.05) on fruit skin lightness (L), chroma (C), hue angle (h°) and fruit eye colour due to irrigation and cold storage treatment. In general, fruit showed skin colour change from emerald green to approximately 75% coloured. Furthermore, irrigation and storage temperature did not have effect on vascular browning, however, there incidence was high on fruit stored at 2.0°C when compared with 5.5°C. The results of this study indicated that 'Hass' avocado fruit stored at 2.0°C was negatively affected by low temperature storage and this cold storage temperature is not recommended. PRD reduced water use during irrigation, however, its effect on post-harvest quality of 'Hass' avocado fruit subjected to 5.5°C must be further investigated before recommended for export markets.

Keywords: Chilling injury; PRD; Electrolyte leakage; Firmness; Physico-chemical parameters; Respiration rate; Vascular browning; Weight loss

CHAPTER 1
INTRODUCTION

1.1 Background

In South Africa, avocado (*Persea americana* Mill.) is a subtropical fruit of major economic importance (DAFF, 2015). Annually, South African Avocado Industry (SAAI) produces approximately 110 000 tons of fruit, of which approximately 62% is exported mainly to the European market (Potelwa and Ntombela, 2015). Some fruit are also exported to new markets, such as the United States of America and China. Due to an increase in the demand for South African avocados, the industry is growing at a rapid rate. Currently, the industry consists of more than 14 000 ha of commercial orchards, with 500 ha of new orchards planted annually to increase fruit supply to already existing markets (Donkin, 2012; DAFF, 2015). There are four main commercially grown cultivars, namely 'Hass', 'Fuerte', 'Pinkerton' and 'Ryan'. Hass consists of approximately 45% of commercial plantings (FFED, 2015). 'Hass' is the avocado cultivar with the highest consumer demand and contains a high number of fat soluble vitamin A and B and other beneficial antioxidants (Schaffer *et al.,* 2013). With an increase in the demand for water by other economic sectors, such as domestic, mining and industry, the agricultural sector is under pressure to reduce water use and use water more efficiently.

South Africa is a water scarce country, with an average rainfall of approximately 500 mm per annum. In addition, rainfall is erratic and mostly seasonal. The agricultural sector therefore relies heavily on irrigation to supplement rainfall during periods when rain is insufficient or absent. Currently, the agricultural sector uses approximately 62% of the available water for irrigation (National Water Resource Strategy, 2004). In order to reduce water usage in agriculture, new and more efficient irrigation techniques should be investigated and implemented. Globally, in arid areas with water scarcity, different irrigation techniques, such as Regulated Deficit Irrigation (RDI) and partial root-zone drying (PRD), have been tested (dos Santos *et al.,* 2003; Durovic *et al.,* 2012). RDI supplies the entire root-zone with less water than required by crop evapotranspiration of

the specific crop in question (Zegbe *et al.,* 2006). In contrast, PRD is achieved by maintaining half of the root system wet while the other half is left dry to a predetermined level of soil water depletion (Sephaskhah and Ahmadi, 2010). Previous studies demonstrated that trees irrigated with the same amount of water, PRD was more effective than RDI in maintaining high yields, good fruit quality and improving water use efficiency (WUE) (Stoll *et al.,* 2000).

The PRD technique has been studied and successfully applied in various crops. In 'Petoride' tomato (Zegbe *et al.,* 2006), and 'Golden Delicious' apple by (Zegbe and Serna-Perez, 2011), approximately 55 and 43% PRD increased irrigation efficiency, respectively. Furthermore, in 'Chemlali' olive (Ghrab *et al.,* 2013), maize (*Zea mays* L.) (Li *et al.,* 2007), 'Liseta' potato (Jovanovic *et al.,* 2010) and 'Monastrell' grape vines (De la Hera *et al.,* 2007) PRD resulted in increased growth of these crops. Similarly, PDR increased fruit yield and fruit quality of 'Ancho St. Luis' hot pepper (Dorji *et al.,* 2005), 'Rizamat' table grapes (Du *et al.,* 2008), 'Cedrico' tomato (Sun *et al.,* 2014), 'Golden Delicious' apples (Zegbe and Serna-perez, 2011), 'Chemlali' olive (Ghrab *et al.,* 2013) and 'Unica' potato (Yactayo *et al.,* 2013). 'Monastrell' grape berries produced under PRD had higher total anthocyanins and polyphenols content (De la Hera *et al.,* 2007). In addition, 'Carbernet Sauvignon' grape berries harvested from PRD treated vines had higher soluble sugar and sugar/acid ratio when compared with non-treatment (Du Toit *et al.,* 2003).

Low temperature is a treatment applied to fruit in order to maintain storage life during transit to distant markets and long-term storage. Depending on cultivar, storage temperature and duration, avocado fruit can withstand low temperature for up to 30 days (PPECB, 2008). As such, low storage temperature has been shown to maintain storage life of 'Hass' avocado fruit (Lallu *et al.,* 2004), 'Hass' and 'Fuerte' (Ahmed *et al.,* 2007), 'Sai Nam' mandarin orange (Shein *et al.,* 2008) 'Encore' tomato (Mutari and Debbie, 2011) and 'Royal Gala', 'Mondial Gala' 'Golden Delicious' and 'Red Delicious' apple (Jan *et al.,* 2012). Contrarily, 'Money Maker No. 2' eggplant stored at 0°C for 15 days developed chilling injury, while those stored at 10°C were not damaged (Concellon *et al.,* 2007). Similarly, low storage of 'Tanaka' lime at 9 and 10°C typically develop undesirable

yellow colour after 3 or 4 weeks and completely yellow after 8 weeks (Thamarath, 2009). In addition, previous studies have revealed that combined PRD application and low storage temperature had no significant effect on post-harvest quality of various crops. Durovic *et al.* (2012) reported that PRD did not have effect on post-harvest quality parameters such as total soluble solids (TSS), dry matter content, firmness and fruit weight loss after 24 weeks' storage at 0°C in 'Granny Smith' apple. However, there is no information on the combined effect of PRD, cold storage and days to ripening on post-harvest quality of 'Hass' avocado fruit.

1.2 Problem statement

There is an increased pressure on agricultural sector to reduce water use due to limited water supply. The, increase in production of avocado possesses a threat to limited resources such as water for agriculture. Thus, techniques that have a potential of increasing crop yield and quality as well as water use efficiency (WUE) such as PRD have been applied in agriculture (Sepaskhah and Ahmadi, 2010). However, there is dearth of information on the use of this technique in 'Hass' avocado production. Moreover, less is documented on post-harvest quality 'Hass' avocado fruit grown under PRD. Therefore, the aim of the study was to investigate whether PRD technique has influence on post-harvest quality of 'Hass' avocado fruit. The study also investigated whether PRD grown 'Hass' avocado fruit could withstand lower temperature in order to maintain storage life when held at high temperature during ripening.

1.3 Rationale of the study

The use of water saving irrigation techniques can ensure water availability for other economic sectors, including industrial users. Atkinson *et al.* (2011) reported that PRD is a technique that reduces irrigation water volume to less than the potential crop evapotranspiration, controlling stress level to improve fruit quality and yield. Therefore, this technique will play an important role in the reduction of water use for the entire avocado industry. Furthermore, its combination with low storage temperature will produce

avocado fruit that qualifies for lucrative export market allowing growth of South African avocado fruit sales into new markets while reducing water use.

1.4 Aim and Objective of the study

1.4.1 Aim

To determine effect of partial root-zone drying (PRD), low storage temperature and ripening duration on post-harvest quality parameters of 'Hass' avocado fruit.

1.4.2 Objective

To investigate whether 'Hass' avocado fruit grown using PRD technique and stored at low temperature would have better quality.

1.5 Hypothesis

The PRD technique and low temperature storage will have effect on post-harvest quality of 'Hass' avocado fruit.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Avocado fruit has a smooth and creamy texture, it contains high amount of monounsaturated fats and it is higher in fat than other fruits. Avocado fruit is also rich in fiber content and vitamins and minerals such as B-vitamin, vitamin K, potassium, copper, vitamin A and C (Schaffer *et al.,* 2013). Their consumption has been associated with the reduction of cardiovascular diseases. Furthermore, monounsaturated fats basal metabolic rate which helps in weight management program.

Avocado is a drought sensitive crop with yields decreasing considerably even after short periods of water shortage (Donkin, 2012). In addition, sensitivity to water stress is cultivar dependent, with some cultivars, such as 'Hass' being more sensitive to water stress than other cultivars, such as 'Fuerte' (Chartzoulakis *et al.,* 2002). In areas with limited water supply, irrigation efficiency plays an important role in optimal production of field crops and high-quality produce. The South African agricultural sector is the largest consumer of water where water is mainly used for irrigation purposes (Dennis and Dennis, 2012). As South Africa is a water scares country, there is legislative pressure on the agricultural sector to reduce water usage and use water more efficiently (Sepaskhah and Ahmadi, 2010). This also applies to the Avocado Industry, which is a rapidly expanding industry.

Currently, South African Avocado Industry (SAAI) grows at a rate of approximately 500 ha per annum. It is therefore of utmost importance to the SAAI to investigate means of saving water and use water more efficiently. Partial root-zone drying (PRD) can be considered as a viable option, as PRD was shown in a number of crops to improve water use efficiency (Stoll *et al.,* 2000). However, limited work on the effect of PRD on avocado has been done under South African conditions. South Africa is the only avocado producing country where avocado is not produced under a Mediterranean climate. Due to this unique situation, research results from other avocado producing countries cannot be directly applied under South African conditions. It is therefore critical that water savings techniques be tested under local conditions for the SAAI. The aim of this literature review

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was to highlight research gaps for future consideration on the effect of partial root zone drying on plant physiology, postharvest physiology and fruit quality of various crops and to propose its application on avocado.

2.2 Plant responses to water stress

Soil water availability is a key factor determining plant productivity of various crops. Low soil water availability for prolonged periods will restrict tree canopy development and reduce vegetative growth. This in turn will negatively impact flowering, fruit set, yield and fruit quality (Davies and Zhang, 1991). On the other hand, in some fruit tree species, such as citrus, water stress may support reproductive development rather than vegetative growth by improving flowering (Gu *et al.,* 2004). However, benefits of water stress, such as in citrus, may only apply for a specific phenological stage, with water stress at any other phenological stage being detrimental.

Plants respond to water stress by regulatory increase in hormone signals, such as abscisic acid (ABA) (Liu *et al.*, 2006). During water stress, ABA is produced in the plant roots and translocated to the guard cells in the leaves and through the xylem vessels (McCarthy *et al.*, 2002 and Liu *et al.*, 2006). The ABA is also synthesized in the guard cells (Davies *et al.,* 2002). Therefore, the concentrations of ABA increase inside the guard cells during water stress (Liu *et al.*, 2006). High levels of ABA in the leaves cause stomatal closure by adjusting guard cell osmotic potential. This involves the active transport of K^+ and Cl^- ions cross the guard cell plasma membrane, to neighbouring cells with subsequent water movement to the neighbouring cells. Movement of water out of the guard cells cause loss of turgor in the guard cells, which cause stomatal closure (Li *et al.,* 2007; Jiang and Zhang, 2002). The ABA can therefore be regarded as a stress signaling plant hormone, causing stomatal closure as a strategy to conserve water and reduce water stress.

Inappropriate irrigation scheduling or under-irrigation of avocado trees usually result in low yields and poor fruit quality (Lahav *et al.,* 2013). Chartzoulakis *et al.* (2002) subjected 'Fuerte' and 'Hass' avocado trees to water stress and found that 'Hass' was more sensitive to water stress. It was found that the relative water content of 'Hass' was lower

than that of 'Fuerte' causing the differences in sensitivity to water stress. Prolonged drought periods will limit plant growth (Chartzoulakis *et al.*, 2002), reducing fruit growth, fruit set and yield in avocado (Thorp *et al.,* 2010; Lechaudel and Joas, 2007). Water stress will further delay avocado fruit maturity and lead to poor fruit quality (Bezuidenhout and Bezuidenhout, 2014). It is, however, possible to subject avocado trees to different irrigation techniques in order to increase water use efficiency (WUE). One such irrigation technique that may potentially improve water use efficiency is partial root zone drying (PRD) and will be discussed in the following sections.

2.3 Partial root-zone drying (PRD)

Partial root-zone drying (PRD) is a modified irrigation technique, whereby, irrigation is applied to a volume of water less than the potential crop evapotranspiration (Atkinson *et al.,* 2011). Partial root-zone drying (PRD) involves spatial separation of dry and wet roots, which can easily be maintained during the entire season. In this instance, one half of the root-zone is irrigated, while the other half is left dry (Sephaskhah and Ahmadi, 2010). The dry and wet sides are alternated on a regular basis. Partial root-zone drying (PRD) was shown to reduce vegetative growth and control stress levels without compromising yield and fruit quality (Davies *et al.,* 2002; Kang and Zhang, 2004), as was found in 'Rizamat' table grapes (Du *et al.,* 2008), 'Cedrico' tomato (Sun *et al.,* 2014) and 'Chemlali' olive (Ghrab *et al.,* 2013). For effective PRD, the volumetric soil water content should be kept between field capacity on irrigated side and permanent wilting point on dry side of the root-zone (Zegbe and Serna-Perez, 2011). In this way, PRD can lead to water savings to an amount of 50%. During PRD application, roots on the dry side dehydrate. In response to the dehydration, the roots synthesize ABA, which is then transported to the leaves and shoots via the xylem (Figure 2.1). The plant subsequently responds to this signal by reducing stomatal conductance, transpiration and vegetative growth. Meanwhile, roots on the irrigated side of the root-zone absorb enough water to maintain high shoot water potential thereby preventing adverse effects on production and fruit quality (Saeed *et al.,* 2008; Ahmadi *et al.,* 2010). Therefore, PRD reduces water loss through transpiration, resulting in improved water use efficiency.
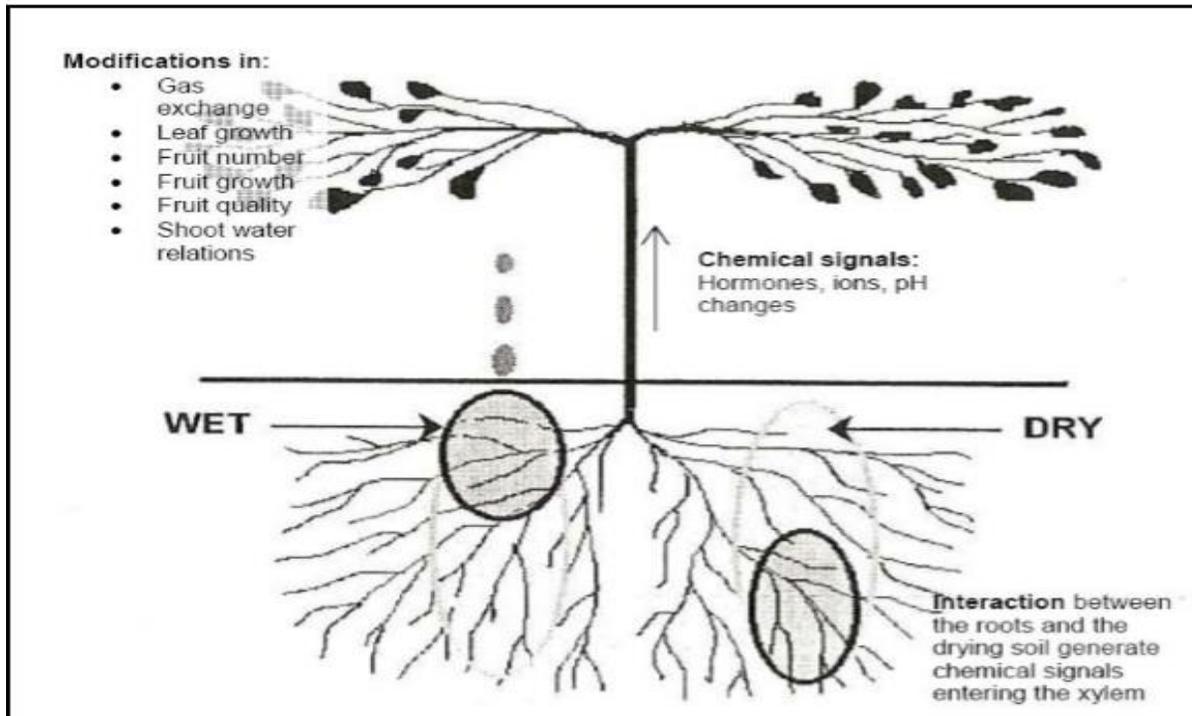
Figure 2.1 Schematic diagram of partial root zone drying presenting how roots interact with drying soil to produce chemical signals that affect plant responses to water stress (Dzikiti, 2007)

2.4 Effect of partial root zone drying (PRD) on water management and plant performance

2.4.1 Effect of PRD on water use efficiency (WUE)

Partial root-zone drying was shown to improve irrigation water use efficiency (WUE) of various perennial crops without negatively affecting yield and fruit quality (Stoll *et al.,* 2000). Irrigation was reduced by 45-50% and 47% in 'Fiji' and 'Golden Delicious' apples respectively (Leib *et al.*, 2006; Zegbe and Serna-Perez, 2011). Furthermore, PRD increased WUE by 30 to 50% on 'Petopride' tomato (Zegbe *et al.*, 2006), 'Flova' potato (Liu *et al.,* 2006), 'Monastrell' grapes (Du *et al.,* 2008), 'Fuji' apples (Leib *et al.,* 2006) and 'Unica' potato (Yactayo *et al.,* 2013). An increase in water use efficiency during PRD is associated with reduced transpiration, with a subsequent reduction in the water or irrigation requirement of the plant (Wang *et al.,* 2015). In addition, PRD constrain vegetative growth, which allows improved light penetration into the canopy (dos Santos

et al., 2003; Sun *et al.,* 2014). Reduced vegetative growth also allows for remobilisation of more nutrients and photo-assimilates from vegetative tissues to the fruit leading to improved fruit yield and quality (Gu *et. al.*, 2004).

2.4.2 Effect of PRD on fruit tree yield

Partial root-zone drying (PRD) caused no significant reduction in fruit number, yield per tree and total yield in 'Rizamat' table grapes (Du *et al.,* 2008). In another study by De la Hera *et al.* (2007), PRD increased yield of 'Monastrell' grapes by 43% due to increased cluster weight. Even in 'Grand Golden' papaya, PRD significantly increased fruit weight and yield per tree (De Lima *et al.,* 2015). However, PRD treated 'Carbenert Sauvignon' grapevines showed a sig1nificant reduction in yield when compared with full irrigation (Du Toit *et al.,* 2003). Moreover, Neuhaus *et al.* (2009) investigated the effect of PRD on 'Hass' avocado fruit from the middle of fruit growth to maturity. In this study, PRD reduced fruit number proportionately to the amount of irrigation applied. It is therefore evident that PRD may increase crop load or fruit weight to increase yields or reduce yields by causing higher fruitlet abscission. In addition, increased WUE when using PRD is not always associated with the increase in yield, since it might possess no effect on yield when less water is used.

2.5 Effect of PRD on post-harvest fruit quality parameters

Water stress may adversely affect important post-harvest quality parameters, such as fruit weight, firmness, fruit skin colour, respiration rate and physiological disorders. Moreover, it can modify cold storage behaviour of fruit by influencing disorders, thereby; making fruit unfit for export (Hershkovitz *et al.*, 2009). The effect of water stress induced by PRD on mentioned quality parameters is be reviewed.

2.5.1 Fruit weight loss

Water loss of horticultural commodities, including avocado, is a major challenge because it has a major effect on the quality and storage potential of fruit (Wills *et al.,* 2008). Reduced rates of fruit water loss at post-harvest delay ethylene production and reduce fruit sensitivity to chilling injury, thus; maintaining quality and shelf life (Blakey, 2011).

Delayed ethylene production due to reduced water loss will further prevent early or premature fruit ripening and therefore increase shelf life (Lallu *et al.,* 2004; Kok, 2011). Pre-harvest water stress reduced postharvest water loss of 'Hass' avocado fruit during cold storage at 5.5°C for 30 days (Bower and Cutting, 1987). Decreased post-harvest water loss also occurred for 'Granny Smith' apples subjected to pre-harvest PRD application and cold storage at 0°C for 24 weeks (Durovic *et al.,* 2012). Similar results were obtained for 'Pacific Rose' apples subjected to pre-harvest PRD application and being stored at 0±0.5°C for 10 weeks (Van Hooijdonk *et al.,* 2007).

For 'Golden Delicious' apples, PRD had no effect on postharvest water loss when fruit were stored for 18 days at 13-18°C (Zegbe and Serna-Perez, 2011). In addition, an experiment conducted by Zegbe *et al.* (2007) indicated that fruit water loss of 'Pacific Rose' apples were similar for PRD fruit and full irrigated fruit (FI), cold stored at 15°C for 18 days. Therefore, PRD application has the potential to influence post-harvest water loss of fruit. Reduced postharvest water loss may be as a result of changes in the structure of the fruit cuticle. Thickening of the cuticle may occur during water stress to reduce water loss while still attached to the tree (Mpelasoka *et al.,* 2000). Cracked cuticles, open calyx and skin lenticels are major ways by which fruit lose water during cold storage and ripening (Zegbe and Serna-Perez, 2011). However, Mpelasoka *et al.* (2000) specified that PRD technique does not modify epidermis to hasten or delay fruit water loss at post-harvest.

2.5.2 Respiration rate and ethylene production

Water stress has been reported to increase respiration rate due to sugar accumulation in 'Virosa' tomato produced under greenhouse condition using deficit irrigation (Pulpol *et al.,* 1996). Respiration rate of water stressed banana fruit held at 17°C showed a clear 9 days' period of low carbon dioxide production before reaching climacteric peak climacteric (Burdon *et al.,* 1994). In avocado fruit, the ethylene peak occurs just before and during the respiratory climacteric rise (Martinez-Romero *et al.,* 2007). An increase in respiration rate and ethylene production is followed by an increase in the activities of enzymes that

causes hydrolysis and breakdown of cell wall components to cause fruit softening or ripening (Martinez-Romero *et al.,* 2007). Usually water stress causes earlier ethylene production, leading to earlier fruit ripening (Gindaba, 2014; Ripoll *et al.,* 2014).

An increase in respiration rate and ethylene production is followed by an increase in the activities of enzymes that causes hydrolysis and breakdown of cell wall components to cause fruit softening or ripening (Martinez-Romero *et al.,* 2007). Usually water stress causes earlier ethylene production, leading to earlier fruit ripening (Gindaba, 2014; Ripoll *et al.,* 2014). For 'Hass' avocado, water stress hastened ethylene production leading to an earlier climacteric peak. This caused earlier fruit ripening (Blakey, 2011). Therefore, the ethylene production pathway is highly reliant on water for biochemical reactions (Lechaudel and Joas, 2007; Blakey, 2011). The effect of PRD on respiration rate and ethylene production in fruit crops has not been widely documented, and there is therefore limited information on this topic.

2.5.3 Fruit firmness and ripening

Fruit firmness is an important ripening indicator of many horticultural fruit and vegetables (Mutari and Debbie, 2011). Ripening can be defined as a process that lead to a change in fruit colour, texture, firmness, flavour, aroma, sugars and other nutritive components making a fruit attractive for consumption (Dixon, *et al.,* 2005). However, ripening time of most horticultural fruit subjected to water stress is normally shortened after storage, followed by high incidence of physiological disorders (Kassim, 2013). Once fruits are harvested, softening occur due to the degradation of cell wall integrity, which occur concurrently with increased activity of ripening enzymes. Irrespective of irrigation treatment, firmness of 'Chok Anan' mango fruit decreased rapidly during ripening at 27°C (± 2°C) reaching values of 250-350 N/100 g, under PRD, regulated deficit irrigation (RDI) and full irrigation (Spreer *et al.*, 2007). Durovic *et al.* (2012) reported that firmness of 'Granny Smith' apple decreased about 35% after 3-day shelf-life during 24 weeks of storage at 0°C when using PRD, deficit irrigation (DI) and full irrigation. In this instance, fruit from the PRD treatment were the firmest. In addition, firmness loss of 'Cripps Pink'

apple fruit harvested from PRD treated trees were lower than fruit of fully irrigated trees. This was most likely as a result of reduction in cellular hydration and increased flesh compactness (Wan Zaliha and Singh, 2009).

'Hass' avocado fruit harvested from water stressed trees and stored at 5.5°C for 30 days followed by ripening at 22°C, ripened after 2 days compared to fruit from fully irrigated trees that ripened after 4 to 5 days (Bower, 1984). Cellulose is the major component of cell walls of fruit, with cellulase playing a major role in breaking down cellulose to cause fruit softening. Another major component of cell walls is pectin, which is broken down by polygalacturonase (PG) during fruit softening. Usually an increase in these two ripening enzymes occur simultaneously or directly after the ethylene climacteric peak (Kok, 2011). Cellulase hydrolyses the cellulose in the cell walls and disrupt the cell wall matrix. Polygalacturonase (PG), then breaks down the pectin in the cell walls to cause final fruit softening (Jeong *et al.,* 2002; Dixon *et al.,* 2005). It was shown that fruit from water stressed plants exhibit increased PG activity, thus, leading to quicker fruit softening (Lechaudel and Joas, 2007; Gindaba, 2014). For avocado, it was shown that pre-harvest water stress increased ABA concentrations in fruit. Increased ABA concentrations initiate ripening by inducing ethylene synthesis, which then increased fruit respiration rates and hastened fruit softening (Blakey *et al.*, 2009).

2.5.4 Fruit skin colour

Fruit skin colour is an important post-harvest quality parameter, which consumers use to the decision of purchasing fresh produce (Magwaza and Tesfay, 2015). Water stress, however, can affect fruit skin colour (Pulupol *et al.,* 1996). Thorp *et al.* (1997), conducted an experiment to determine the response of 'Hass' avocado fruit to water stress. Fruit were stored for three weeks at 6°C, followed by ripening at 20°C. Fruit subjected to water stress displayed uneven skin colour during ripening at 20°C. Application of PRD to 'Petoride' tomato improved the red colouring (lower $h°$ angle) of fruit when compared to fruit harvested from fully irrigated plants (Zegbe-Domınguez *et al.,* 2003). Application of PRD on 'Chok Anan' mango had no significant effect on fruit colour. Both the exocarp

and mesocarp of the mango fruit turned yellow similarly when expressed by $h°$ values (Spreer *et al.,* 2007). Application of PRD on 'Monastrell' grapes had no effect on total phenolic compounds and anthocyanins of the fruit (De la Hera *et al.,* 2007) and therefore no effect on fruit colour. Furthermore, dos Santos *et al.* (2003) reported that anthocyanin and phenols were significantly high on PRD treated 'Catelao' grape berries when compared with deficit irrigation and commercially irrigated fruit. Improved fruit skin colour in the 'Catelao' red grape variety might be presumably due to increased contents of anthocyanins and phenols (dos Santos *et al.,* 2003).

Higher concentrations of anthocyanins and phenols in fruit from PRD treatments might be due to increased exposure of fruit to sunlight (Dry *et al.,* 2000; Du Toit *et al.,* 2003). It has been previously reported that PRD retards vegetative growth, reducing canopy density and shading thereby causing higher exposure of fruit to the sun (Davies *et al.,* 2002; Kang and Zang, 2004; Du *et al.,* 2008). Furthermore, higher concentration of total anthocyanins in PRD fruit, might be due to high starch accumulation during early fruit development, which is later converted into sugars (Pulupol *et al.,* 1996; Sun *et al.,* 2014). Sun *et al.* (2014) further stipulated that fruit are preferential sinks of carbohydrates under water deficits conditions such as PRD. In such instance, more sugars may be translocated to fruit. Sugars, especially sucrose, play an important role in anthocyanin synthesis (Gu *et al.,* 2004; He *at al.,* 2010). Anthocyanin is a secondary metabolite and is also responsible for 'Hass' avocado fruit skin colour development (Cox *et al.,* 2004). Therefore, PRD has the potential to increase anthocyanin concentration of 'Hass' avocado fruit, thus, improving skin colour.

2.5.5 Physiological disorders

Most physiological disorders occur when fruit are exposed to low temperature that is above the freezing point of the produce (Wang and Wang, 2009). Pre-harvest water stress increases physiological disorders such as mesocarp discoloration, vascular browning and body rot (Blakey, 2011). Calcium is an important component in strengthening cell membranes and it has low mobility, therefore, it requires an adequate amount of water for translocation (Blakey, 2011). Low water availability result in low

calcium (Ca^{2+}) partitioning to fruit during early fruit development. Therefore, low calcium contents in fruit will lead to weakened cell membranes causing fruit to be more susceptible to various disorders and infections. In addition, pre-harvest water stress increased the activity of polyphenol oxidase (PPO) in ripe avocado fruit cold stored for extended periods. Polyphenol oxidase (PPO) catalyses the oxidation of o-quinone in the fruit mesocarp to a brown melanin pigment, causing browning of the mesocarp. This disorder is known as diffuse mesocarp discoloration (Hershkovits *et al.,* 2009). Increased PPO activity reported is associated with increased concentrations of ethylene (Pesis *et al.,* 2002), which was shown earlier is caused by water stress. Furthermore, high amounts of abscisic acid (ABA) produced during water stress may reduce fruit tissue tolerance to chilling injury, which may also lead to increased incidences of physiological disorders in fruit (Gindaba, 2014).

2.6. Effect of low storage temperature on post-harvest physico-chemical fruit quality

Maintaining superior quality during export of avocado fruit is a major challenge for high profitable markets. Temperature is a critical component that shortens a produce storage-life, as it regulates many biological processes (Kassim *et al.,* 2013). Low temperature storage is the most effective method to slow deteriorative metabolic and pathological processes in harvested fruit (Kok, 2011). It is therefore also used to maintain quality of various0 avocado fruit by maintaining them in the most desirable state for the longest possible time to be suitable for long distance shipping during export (Mutari and Debbie, 2011). Even though low temperatures have a positive effect on maintaining storage life and quality of most fruit and vegetable, it may result in various physiological disorders. The following post-harvest quality parameters are affected by cold storage:

2.6.1 Chilling injury

Storage temperatures below 13°C has been reported to cause chilling injury in a number of avocado cultivars (Crisosto *et al.*, 2003). Chilling injury is the permanent and irreversible damage to plant cells as results of exposing fruit to low temperatures that is below the freezing point of the produce (Hershkovitz *et al.*, 2009). Chilling injury can manifest in many forms including pitting, pulp browning near the seed, failure to soften when transferred to higher temperatures, off-flavours, vascular straining and mesocarp browning (Pesis *et al.*, 2002; Kassim *et al.,* 2013). However, avocado fruit from different cultivars respond differently to low temperatures, with each cultivar having an optimum storage temperature used to maintain quality. Selection of storage temperatures is also dependent on the storage duration and season (Van Rooyen and Bezuidenhout, 2010). South African 'Hass' avocado must be cold sterilised at 2°C for 28 days as a phytosanitary measure when fruit are destined for export market (Van Rooyen, 2009; Van Rooyen and Bezuidenhout, 2010). In order to maintain quality, prevent and minimize chilling injury of South African avocado during export, low storage temperature between 5 and 6°C (varying with cultivar and stage of fruit maturity) is recommended for up to 4 weeks (Woolf *et al.,* 2003; Perez *et al.,* 2004). When 'Hass' avocado fruit was stored at 1°C, they developed severe chilling injury during ripening at ambient temperature (±24°C) when compared with fruit stored at 5.5°C for 28 days (Van Rooyen and Bezuidenhout, 2010). Therefore, the incidence of chilling injury increases considerably when fruit are stored at lower temperatures than the recommended temperature regimes.

Chilling injury leads to loss in plasma membrane integrity of the fruit cells causing increased electrolyte leakage (Gonzalez-Aguilar *et al.,* 2000). In this instance chilling injury occurs as results of cold damage disrupting the order of the membrane lipids, thereby, increasing its permeability (Dorria *et al.,* 2010). Cellular structure is protected from reactive and damaging compounds (reactive oxygen species (ROS) by anti-oxidants formed in the cells. However, under oxidative stress condition there is an imbalance between anti-oxidants and ROS, resulting in high formation of damaging compounds (ROS). These compounds (ROS) causes membrane damage resulting in various post-harvest browning disorders (Mittler, 2002). Thus, electrolyte leakage is an indicator of

membrane permeability. In addition, Crisosto *et al.* (2003) showed that chilling injury symptoms becomes more visible when fruit were held at ambient temperature after withdrawal from cold storage. Chilling injury of 'Jinyou-1' cucumber fruit was highly correlated with increased in skin tissue electrolyte leakage when stored at 2°C (Mao *et al.*, 2007). Similar results were reported on the avocado cultivars 'Árad' and 'Éttinger' stored at 5°C. Fruit showed severe chilling injury symptoms (mainly mesocarp discoloration) as a result of higher electrolyte leakage during ripening at 20°C (Hershkovitz *et al.*, 2009).

2.6.2 Fruit weight loss

In fruits, low temperature storage results in reduced rates of water loss at post-harvest (Hershkovitz *et al.,* 2009). Fruit weight loss increases rapidly after withdrawal from cold storage when held at higher temperatures (Burdon *et al.*, 2005). This was supported by Mutari and Debbie (2011), whereby, weight loss of 'Encore' tomato fruit stored at 20°C was higher when compared with fruit stored at 12°C. Similarly, it was shown that weight loss was significantly higher for 'Sai Nam Phueng' and 'See Thong' tangerine orange stored at 22°C compared to storage at 5°C (Roongruangsri *et al.,* 2013). Even for 'Hass' and 'Fuerte' avocado fruit, weight loss was significantly higher during ripening at 20°C, when compared to cold storage at 5 and 9°C (Ahmed *et al.,* 2007). Fruit weight loss further increased as the cold storage period increased as was found for preconditioned 'Hass' avocado fruit stored at 3°C and evaluated after 1, 23 and 46 days of cold storage (Mandieta *et al.*, 2016). Increased water loss is mainly caused by two factors: 1) Postharvest water loss caused by increased membrane permeability (Bower and Magwaza, 2004) and 2) Water loss as a result of increased fruit respiration rate (Burdon *et al.*, 2005; Mutari and Debbie, 2011).

2.6.3 Respiration rate

Respiration is a natural process that take place in every living organism, whereby, glucose is oxidized to carbon dioxide and water for energy production (Mutari and Debbie, 2011). 'Hass' avocado fruit display a characteristic respiratory pattern that follow an increase in

respiration rate prior to the onset of ripening (Perez *et al.,* 2004). It rises to a maximum called the climacteric peak, prior to ripening, which is then followed by a subsequent decline in respiration rate (Forero, 2007). 'Hass' avocado fruit held at 20°C to ripen showed increased respiration rates on the second ripening day and thereafter, start to decrease after the fourth day until the fruit were fully ripe (Perez *et al.,* 2004). Furthermore, Donetti and Terry (2012) reported that 'Hass' avocado fruit stored at 5 to 6°C for 24 to 27 days reached the respiratory climacteric peak on the second and the third day, respectively during ripening when held at 18 or 23°C. Respiration rates increased with an increase in storage temperature causing fruit to ripen quicker at higher temperatures (Kassim *et al.,* 2013). Previous results were reported by Mutari and Debbie (2011) on 'Encore' tomato, showing that respiration rate was significantly higher when fruit were stored at 20°C when compared with fruit stored at 12°C fruit.

2.6.4 Fruit firmness

Decrease in avocado firmness indicate softening or ripening, while unripe fruit are usually hard (Arzate-Vazquez *et al.,* 2011). According to Blakey *et al.* (2014), fruit exposed to high temperature ripens faster due to increased metabolic rates. In accordance, 'Fuerte and 'Hass' avocado fruit stored at 8°C softened quicker than fruit stored at 4 and 6°C after 3 weeks (Pesis *et al.,* 2002). Moreover, 'Cochoro' tomato fruit stored at 4°C were significantly firmer when compared with fruit stored at 20 and 30 after 21 days (Tadesse *et al.,* 2015). Cell wall components such as cellulose, hemicellulose and pectin are responsible for strengthening cell walls causing firm fruit (Blakey, 2011). Ripening enzymes, namely, cellulase, endo-polygalacturonase, pectin methyl esterase, β-1,4-xylosidase, β-1,4-xylanase and β-galactosidase works concurrently on cell wall components to bring about fruit softening and ripening (Blakey *et al.,* 2009). These ripening enzymes catalyze the solubilisation and depolymerisation of pectin and cellulose in the cell walls to cause fruit softening (Magwaza and Tesfay, 2015). Low temperature storage suppresses the activity of ripening enzymes thereby preventing or delaying fruit softening during cold storage (Blakey *et al.,* 2009). However, activity of ripening enzymes increases rapidly after removal from cold leading to normal ripening of fruit (Kok, 2011).

2.6.5 Fruit skin colour

During ripening, 'Hass' avocado fruit skin colour changes from green to purple black due to chlorophyll degradation and accumulation of anthocyanins, especially cyaniding-3-O-glucoside (Cox *et al.*, 2004; He *et al.,* 2010). Decrease in chlorophyll content in the fruit skin is associated with increasing maturity and is used as a measure for visual assessment of fruit quality (Mutari and Debbie, 2011). Storage temperature, maturity and storage time are important factors that can affect fruit colour development (Bezuidenhout and Bezuidenhout, 2014). Cold shock treatment (CST) was shown to delay 'Hass' avocado fruit colour development at post-harvest (Pesis *et al.,* 2002). Chen *et el.* (2017) also reported that hue angle of non-treated ''Hass' avocado fruit decreased from initial value of 42.17 to 27.3 and 27.51 indicating purplish black colour, while cold shock treated 'Hass' fruit exhibited final value of 29.34 and 29.86. Cold shock treatment therefore delayed colour change from green to purple/black in this study. Furthermore, 'Hass' avocado fruit stored at 5.5°C developed poor skin colour after ripening at ambient temperature (±24°C) early in the harvesting season when compared to fruit stored at 1°C (Van Rooyen, 2009). Thorp *et al.* (1997) reported that ripe 'Hass' avocado showed improved skin colour after storage at 6°C for three weeks, however, the colour was not uniform. Therefore, cold-storage temperature has a major effect on fruit colour development.

2.7 Conclusion

Reduction in irrigation water usage by the agricultural sector can ensure that more water is available for the increasing demand of other economic sectors, including domestic and industrial users. Partial root-zone drying (PRD) is an effective irrigation technique that can be employed to improve water use efficiency. Application of PRD improve WUE by reducing irrigation water usage to an amount of 50% while maintaining high yields and acceptable fruit quality. Maintaining good fruit quality for export, however, needs additional technologies to prevent ripening and spoilage during transit. Cold storage is an effective technology that allows preservation of fruit during long-distance shipping to

export markets. In addition, as a phytosanitary requirement, low temperature storage (2°C) is mandatory, but involved a number of risks, such as chilling injury and poor internal fruit quality. Even though the effect of PRD and post-harvest cold storage has been investigated for a number of fruit crops. There is dearth of information on the combined effect of PRD and low temperature storage on post-harvest quality of avocado fruit. The study was therefore initiated to investigate the combination effect of PRD, low temperature storage and ripening time on post-harvest quality of 'Hass' avocado fruit.

CHAPTER 3
MATERIALS AND METHODS

3.1 Experimental sites and procedures

The experiment was conducted in a commercial orchard at Avo Valley (25°29'39" S; 30°55'54" E), near Nelspruit, Mpumalanga province, South Africa. Fourteen year-old 'Hass' avocado trees, grafted on 'Duke 7' rootstocks and planted at a spacing of 4 x 4 m (625 trees per hectare) were used. The orchard soil is classified as Hutton soil with the topsoil as having sand, silt and clay content of 68, 5 and 27%, respectively. The sub-soil having sand, silt and clay content of 66, 3 and 31%, respectively. Apart from irrigation, all trees received standard cultural practices used for local commercial production throughout the experimental duration. For PRD experiment, only one portion of the root-zone was irrigated with the remaining portion not irrigated. The irrigated and non-irrigated sides were alternated at three-week intervals initially using micro sprinklers with a 20 L/h delivery rate. The dry half was covered with plastic sheets to prevent rainwater (Figure 3.1 A). For the control, the root-zone was fully irrigated as commercial requirement (40 L/h) (Figure 3.1 B).

3.2 Treatments and experimental designs

Avocado fruit 'Hass' were harvested at commercial maturity, randomly from inside and outside canopies of the two irrigation methods (PRD and full irrigation). Thereafter, harvested fruit were transported to the Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC) post-harvest laboratory in Nelspruit (25°28'0" S; 30°58'0" E) for post-harvest storage and analysis. The experiment was laid as a factorial arranged in randomised complete block design (RCBD). The treatment factors for chilling injury, electrolyte leakage and vascular browning were 2 x irrigation regimes (Full Irrigation (FI) vs. PRD) and 2 x storage temperatures (2.0 and 5.5°C). However, treatment factors for fruit weight loss, respiration rate, fruit firmness, ripening percentage and fruit skin colour were 2 x irrigation regimes (Full Irrigation (FI) vs. PRD), 2 x storage temperatures (2.0 and 5.5°C) and 4 ripening days (0, 2, 4 and 6 days). Mature 'Hass' avocado fruit were

harvested from PRD and fully irrigated trees and thereafter fruit of each treatment were stored at 2.0 and 5.5°C for 28 days. Each treatment consisted of 6 replicates, with an exception of electrolyte leakage, whereby the experiment was replicated 4 times. After 28 days storage at 2.0 and 5.5°C, fruit were ripened at 21°C. The effect of PRD and low temperature storage was determined by evaluating the following physico-chemical fruit parameters during ripening: external chilling injury, electrolyte leakage, fruit weight loss, respiration rate, firmness, fruit skin colour and vascular browning.

Post-harvest treatments

In post-harvest laboratory, fruit from each irrigation treatment were divided in two batches. Thereafter, drenched into 50 L of water containing 10.5 mL prochloraz solution (0.021% v/v). Fruit were sorted, graded and then packed into avocado creates, each with 15 fruit replicated six times per treatment. Each replicate per treatment was subjected to low storage temperature of 5.5 and 2°C for 28 days. After withdrawal from cold storage, 'Hass' avocado fruit were ripened at 21°C for 4 days. Ripening was monitored by measuring mass, colour, firmness and respiration rate until fruit were fully ripe. Five randomly selected fruit per replication on each treatment were used for evaluations.

Figure 3.1 (**A**) Partial root-zone drying (PRD) covered with plastic sheets versus (**B**) control irrigation of 'Hass' avocado fruit.

3.3 Data collection
The following physico-chemical quality parameters were measured: skin colour, moisture content, respiration rate, weight loss, firmness, ripening percentage, electrolyte leakage and physiological disorders.

3.3.1 Physiological disorders
External chilling injury was assessed immediately after removing fruit from cold storage using the following formula:

$$\text{CI (\%)} = \frac{Number\ of\ fruit\ with\ CI\ symptoms}{Total\ number\ of\ fruit\ evaluated} \times 100$$

Thereafter, when fruit were fully ripe, physiological disorder associated with water stress and low temperatures were assessed. Fruit were cut open into half when fully ripe with a blade knife and evaluated for vascular browning: The incidence of vascular browning were assessed based on 3 hedonic scale where 0 = 0%; 1 = 10%; 2 = 25% and 3 = 50%.

3.3.2 Electrolyte leakage (EL)

Electrolyte leakage (EL) was determined immediately after withdrawing fruit from cold storage after 28 days. Electrolyte leakage is quantified from electrical conductivity measurement, reflecting on the biochemical changes occurring during and after storage. Montoya *et al.* (1994) method with minor modification was used to determine electrolyte leakage. Three avocado fruit from each treatment were used to determine electrolyte leakage. Sample disks were removed from the fruit using a 10 mm in diameter cork borer. Initial electrolyte leakage (EL_1) was obtained after shaking the disks in 10 mL ultra-pure water for 3h using electrical conductivity (EC) meter (Hi991301N, E1- Hamma Instruments, Israel). Thereafter, samples were placed in a shaking hot water bath controlled at 100°C for 1 hour, allowed to cool at room temperature and the second electrolyte leakage (EL_2) was measured. Electrolyte leakage percentage was then calculated from the following equation:

Total electrolyte leakage (%) = (EL_1/ EL_2) ×100
Where:
EL_1 = Initial electrolyte leakage reading
EL_2 = Final electrolyte leakage reading

3.3.3 Fruit weight loss

Individually selected fruit were weighed after cold storage at 2-day intervals until they were fully ripe. Fruit weight was measured using an electronic weighing scale (SBA 61, Scaltec instruments, Heiligenstadt-Germany). Fruit weight loss was obtained by calculating the difference between initial weight on the first day after cold storage and fruit weight on the ripening day. The data was expressed as percentage weight loss using the following formula:

Weight loss (%) = $\frac{Initial\ fruit\ weight - Fruit\ weight\ at\ ripening}{Initial\ fruit\ weight} \times 100$

Figure 3.2 Measuring fruit weight of 'Hass' avocado fruit during ripening

3.3.4 Ripening percentage

Fruit were evaluated for ripening using firmness (SU) readings, when they were kept at the ripening room set at 21°C. Fruit were considered ripe upon reaching an average reading of ≤ 25 SU which is the eating soft stage. Fruit were further kept in the experiment for further physiological disorders analyses. Ripening percentage was then calculated using the following formula:

Ripening (%)= $\frac{Number\ of\ ripe\ fruit}{Number\ of\ fruit\ evaluated} \times 100$

3.3.5 Determining fruit firmness

Fruit firmness was measured from five fruit after during entire ripening using a non-destructive automated Sinclair IQTM desktop machine (Model: 51DFTB, International LTD, Jerrold, Bowthorpa, Norwich, NR5, 9. D, England). Each fruit was placed on top of the rotating disc of the Sinclair desktop machine taking four measurements along the equatorial part of the fruit. Firmness readings were recorded in Sinclair unit (SU) to a computer with data acquisition and analysis program. When the reading on Sinclair desktop machine reaches ≤ 25 SU, fruit were considered ripe.

Figure 3.3 Measuring fruit firmness of 'Hass' avocado fruit during ripening

3.3.6 Determination of respiration rate

Each of the five randomly selected 'Hass' avocado fruit were weighed and then incubated in a 3 L glass jar for 1 hour. Carbon dioxide concentration was then measured using Map-Pak gas analyser (AGC Map-Pak Analyser Range, German) after the set time. The headspace CO_2 concentration was converted to respiration rate taking into consideration fruit mass, fruit volume, free space in the container and ambient CO_2 concentration expressed in µmol CO_2 kg^{-1} hr^{-1}.

Figure 3.4 Measuring respiration rate (CO_2) of 'Hass' avocado fruit during ripening

3.4.7 Fruit skin colour

Fruit colour measurements were taken after withdrawing fruit from cold storage and during ripening using subjective and objective method. Subjectively colour was determined using avocado eye colour rating, using the following scale: 1, emerald; 2, forest green; 3, approximately 25% coloured; 4, approximately 75% coloured; 5, purple; 6, black. Colour variability among the fruit was determined as an average value. Colour was also determined objectively using a Minolta Chroma Meter (CR- 400, Konica Minolta, and model: DFM50, Osaka, Japan). Three measurements were taken at an equatorial region of the fruit to determine lightness or darkness (L*), redness or greenness (a*) and yellowness or blueness (b*) of avocado fruit.

The parameters associated to colour measurement were:

L*= lightness or brightness

a* = redness or greenness

b* = yellowness or blueness

In this manner, L*, a* and b* describe a three dimensional space whereby L* (Lightness) is the vertical exis with the values varying from 100 for perfect white and 0 for black. Values of a* and b* indicate green-red and blue-yellow. Chroma describe the length of colour vector in the cartesian plane formed by a* and b* values. Where hue angle determines the position of such vector. From these colour parameters chroma (C) and hue angle were determined as follows (McGuire,1992):

$$C = \sqrt{a^2 + b^2} \qquad hue\ angle = \tan^{-1}\left(\frac{a}{b}\right)$$

Figure 3.5 Measuring fruit colour parameters of 'Hass' avocado fruit during ripening

3.4 Data analysis

Data was subjected to the analysis of variance (ANOVA) using GenStat 16th edition statistical software (VSN International, Hemel Hempstead, UK). Treatment means were separated using Duncan's Multiple Range Test at 5% level of probability.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 External chilling injury

An interaction between irrigation regime and storage temperature had no significant effect (P>0.05) on external chilling injury of fruit during ripening (Appendix 1). However, irrigation and cold storage temperature had a significant effect (P = 0.013 and < 0.001, respectively) on external chilling injury (Appendix 1). Pre-harvest application of PRD resulted in a significant increase in the incidence of external chilling injury during post-harvest cold storage, especially for fruit stored at 2°C. For fruit stored at 2°C, fruit from the PRD had almost a 30% higher incidence of chilling injury, compared to fruit from the fully irrigated (Figure 4.1). For the 5.5°C, the incidence of external chilling injury for fruit from the PRD was approximately 25% higher than for the fully irrigated treatment (Figure 4.1). As expected, the lower (2°C) storage temperature increased the incidence of external chilling injury, regardless of irrigation treatment. For the fully irrigated fruit stored at 2°C, had approximately a 40% higher incidence of chilling injury compared to fruit stored at 5.5°C. For the PRD, external chilling injury was approximately 50% higher for fruit stored at 2°C, compared to the PRD fruit stored at 5.5° (Figure 4.1).
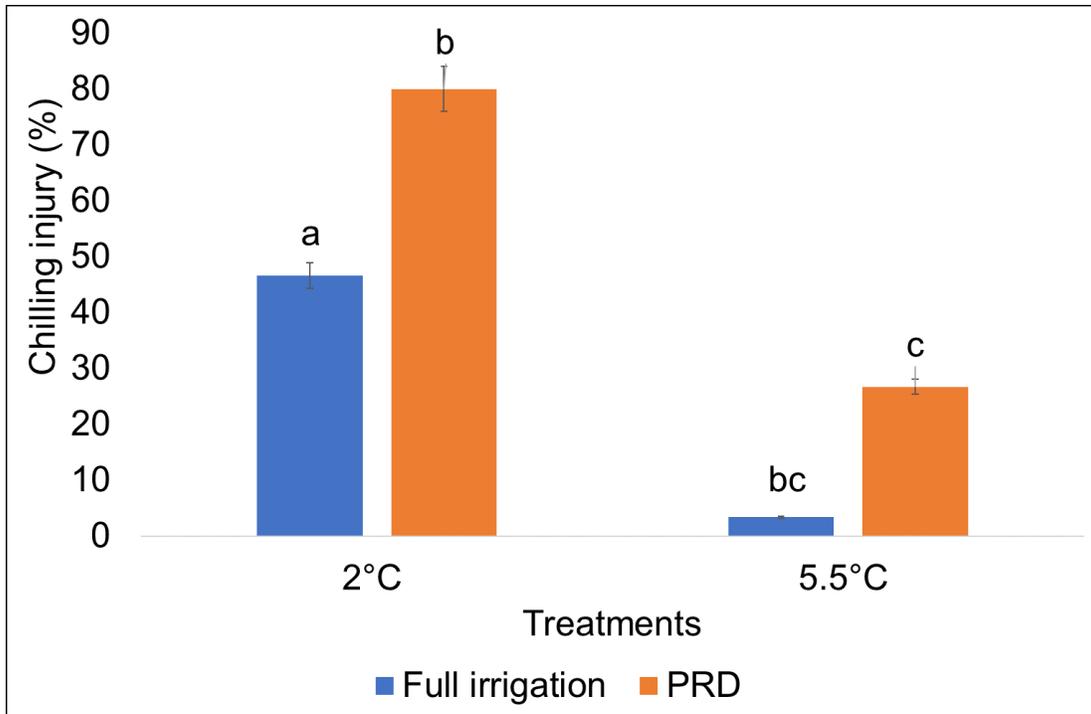
Figure: 4.1 Effect of partial root-zone drying (PRD) and low storage temperature on external chilling injury of 'Hass' avocado fruit cold stored for 28 days at 2.0 and 5.5°C. (Means with the same letter do not differ significantly at P ≤ 0.05)

4.1.2 Electrolyte leakage (EL)

An interaction between irrigation regime and cold storage temperature had a highly significant effect (P<0.001) on electrolyte leakage of (Appendix 2). However, irrigation treatments (PRD and full irrigation) had no significant effect (P = 0.21) on e on electrolyte leakage of studied fruit. Fruit from PRD treatment exhibited slightly higher electrolyte leakage when compared with fruit from fully irrigated treatment. For PRD and full irrigation fruit stored at 2°C exhibited almost similar electrolyte leakage values (Figure 4.2). Storage temperature had no significant effect on electrolyte leakage. For the 2.0°C storage regime, there was no significant difference between the PRD and fully irrigated treatment on electrolyte leakage (Figure 4.2). For fruit stored at 5.5°C, fruit from PRD treatment had significantly high higher electrolyte leakage than fruit from fully irrigated treatment. Electrolyte leakage was approximately 3% higher for the PRD treatment, compared to fully irrigated treatment.
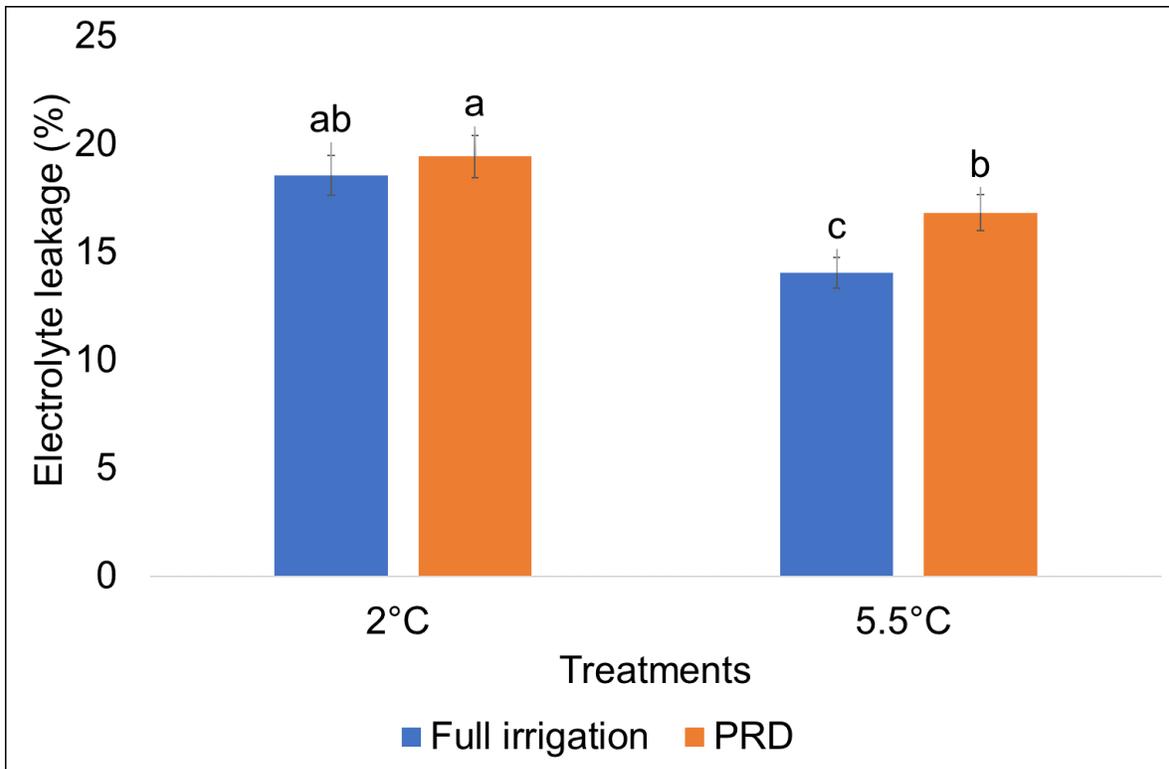
Figure 4.2 Effect of partial root-zone drying (PRD) and low storage temperature on electrolyte leakage of 'Hass' avocado fruit cold stored for 28 days at 2.0 and 5.5°C. (Means with the same letter do not differ significantly at P ≤ 0.05)

4.1.3 Fruit weight loss

There was no significant interaction effect (P>0.05) between irrigation type, storage temperature and ripening time on weight loss percentage of 'Hass' avocado fruit during ripening (Appendix 3). Fruit from both the PRD and fully irrigated treatments, stored at 2°C exhibited similar weight loss for day 2, 4 and 6 during ripening (Figure 4.3 A). Weight loss, however, increased significantly from day 0 to 2 and again from day 2 to day 4 and again from day 4 to day 6 (Figure 4.3 A). Fruit lost approximately 0.75% weight per day during ripening. For fruit stored at 5.5°C, PRD and fully irrigated treatments displayed similar weight loss on day 2 during ripening (Figure 4.3 B). On day 4, fruit from PRD treatment lost significantly more weight (3.18%) than fruit from the fully irrigated (2.9%) treatment during ripening (Figure 4.3 B). Fruit harvested from PRD and fully irrigated trees

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thereafter, stored at 5.5°C were ripe and already terminated from the experiment on the 6th ripening day. Weight loss for fruit stored at 5.5°C was also approximately 0.75% per day. In general, 'Hass' avocado fruit harvested from PRD and fully irrigated treatment exhibited similar weight loss percentage during ripening after withdrawal from both 2.0 and 5.5°C storage temperature.
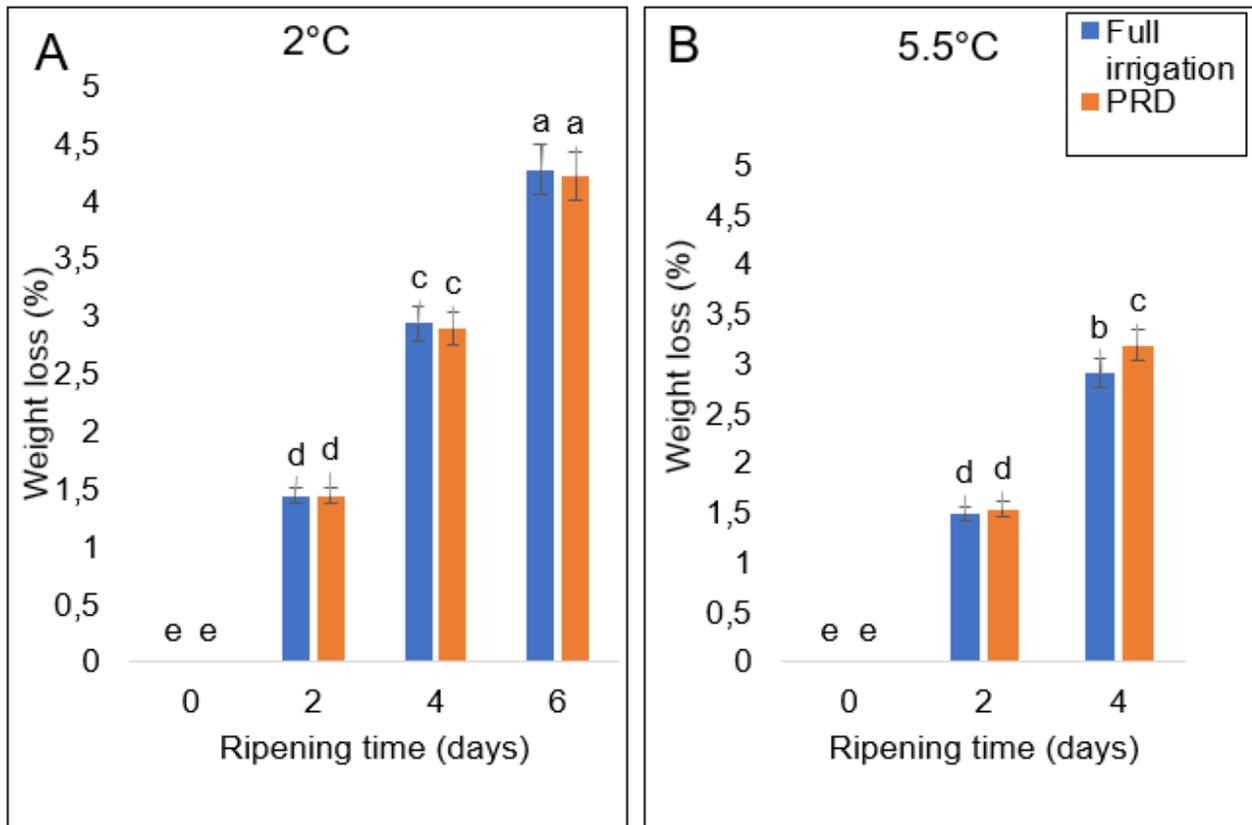

Figure 4.3 Effect of partial root-zone drying (PRD) and low storage temperature on weight loss of 'Hass' avocado fruit cold stored for 28 days at 2.0 (A) and 5.5°C (B). (Means with the same letter do not differ significantly at P ≤ 0.05)

4.1.4 Respiration rate

There was no significant interaction effect (P>0.05) on 'Hass' avocado fruit respiration rate due to irrigation type, storage temperature and ripening time during ripening (Appendix 4). Fruit from both the PRD and fully irrigated treatments reached a climacteric peak on the second day of ripening, regardless of cold storage temperature regime

(Figure 4.4). In general, fruit harvested from PRD treated trees and stored at 2°C had the highest respiration rate of 3467 µmol CO_2 Kg^{-1} hr^{-1} on the 2nd ripening day. However, fruit harvested from PRD treated trees and stored at 5.5°C exhibited lowest respiration rate of 98, 2844 and 1885 µmol CO_2 Kg^{-1} hr^{-1} after 0, 2 and 4 ripening days, respectively. Contrary, fruit harvested from control treated trees and stored at 5.5°C had high respiration rate of 1029, 3086 and 2329 µmol CO_2 Kg^{-1} hr^{-1} after 0, 2 and 4 ripening days, respectively. Furthermore, fruit harvested from control treated trees and stored at 2.0°C also exhibited high respiration rate of 1527, 1685 and 1638 µmol CO_2 Kg^{-1} hr^{-1} on 0, 4 and 6 ripening days, respectively. Overall, fruit harvested from PRD treated trees and stored at 5.5°C exhibited the lowest respiration rate when compared with control fruit.
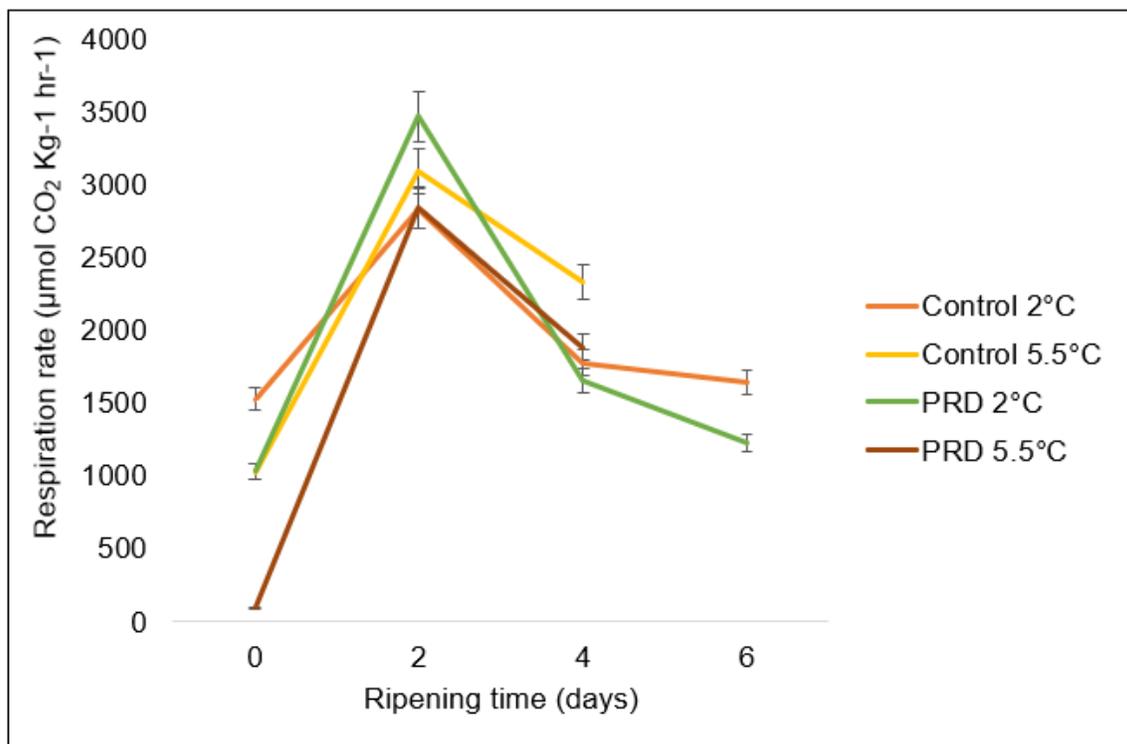

Figure 4.4 Effect of partial root-zone drying (PRD) and low storage temperature on respiration rate of 'Hass' avocado fruit cold stored for 28 days at 2.0 and 5.5°C

4.1.5 Fruit firmness

An interaction between irrigation type, storage temperature and ripening time was not significant (P>0.05) on fruit firmness during ripening (Appendix 5). However, firmness values of fruit obtained from PRD and fully irrigated treatment thereafter, stored at 5.5°C were similar on the first ripening day. In the present study, fruit harvested from fully irrigated treatment and stored at 2.0°C showed the highest firmness values from the 2nd ripening day until they ripen. In addition, firmness values of fruit harvested from fully irrigated trees, thereafter, stored at 5.5°C was high (43.67) and (23.7) after 2 and 4 ripening days, respectively (Figure 4.5). However, fruit harvested from PRD treated trees and stored at 5.5°C exhibited low fruit firmness values of 40.6 and 20.63 after 2 and 4 ripening days, respectively. Fruit from PRD and fully irrigated trees thereafter, stored at 5.5°C were ripe on day 4 and removed from the experiment. Moreover, fruit harvested from PRD treated trees and stored at 2.0°C had firmness values of 25.53 and 14.67 after 4 and 6 ripening days, respectively. In general, fruit harvested from PRD treated trees exhibited slightly low firmness values when stored at both 2.0 and 5.5°C during the entire ripening time.
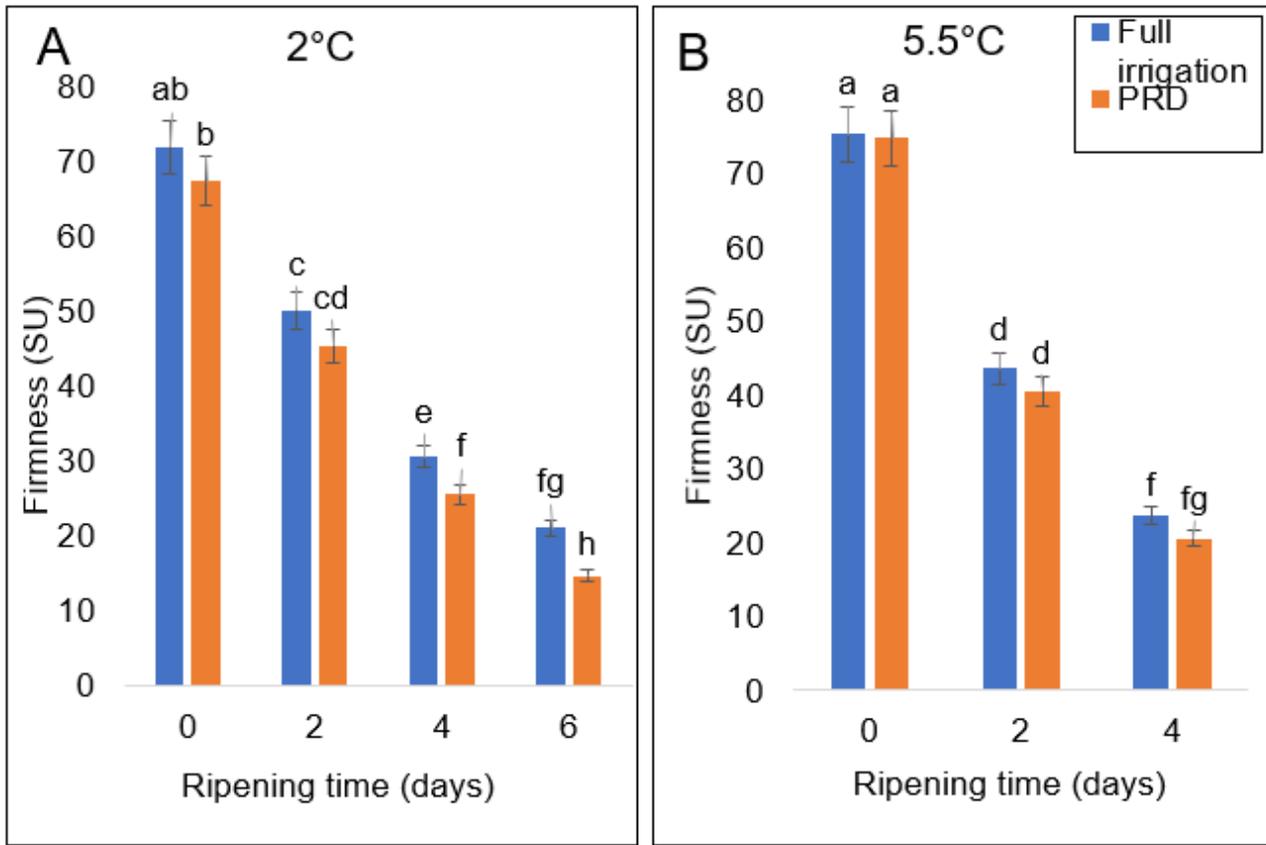
Figure 4.5 Effect of partial root-zone drying (PRD) and low storage temperature on firmness of 'Hass' avocado fruit cold stored for 28 days at 2.0 (A) and 5.5°C (B). (Means with the same letter do not differ significantly at P ≤ 0.05)

4.1.6 Ripening percentage

The interaction between irrigation, storage temperature and ripening time had no significant effect (P>0.05) on ripening percentage of studied fruit (Appendix 6). Avocado fruit harvested from the PRD and fully irrigated treatments, stored at 5.5°C had only 4 days shelf-life when compared with fruit stored at 2.0°C that had 6 days shelf-life (Figure 4.6). None of the fruit ripened (0%) at day 0 and 2, irrespective of irrigation regime and cold storage temperature. On day 4, a significantly higher number of fruit were ripe for the 5.5°C cold storage regime when compared to the 2°C cold storage regime. In addition, significantly more fruit for the PRD treatment were ripe on day 4 when compared to the fully irrigated treatment. Even on the sixth day during ripening, significantly more fruit

(~20% more) were ripe for the PRD treatment compared to the fully irrigated treatment. Fruit from both PRD and fully irrigated treatment, thereafter, stored at 2.0°C were considered ripe when the average firmness was ≤ 25 on the 4th day of ripening. Whilst, fruit stored at 5.5°C from both PRD and fully irrigated treatment were considered ripe and terminated from the experiment on the 4th ripening day, when average firmness was ≤ 25 SU.
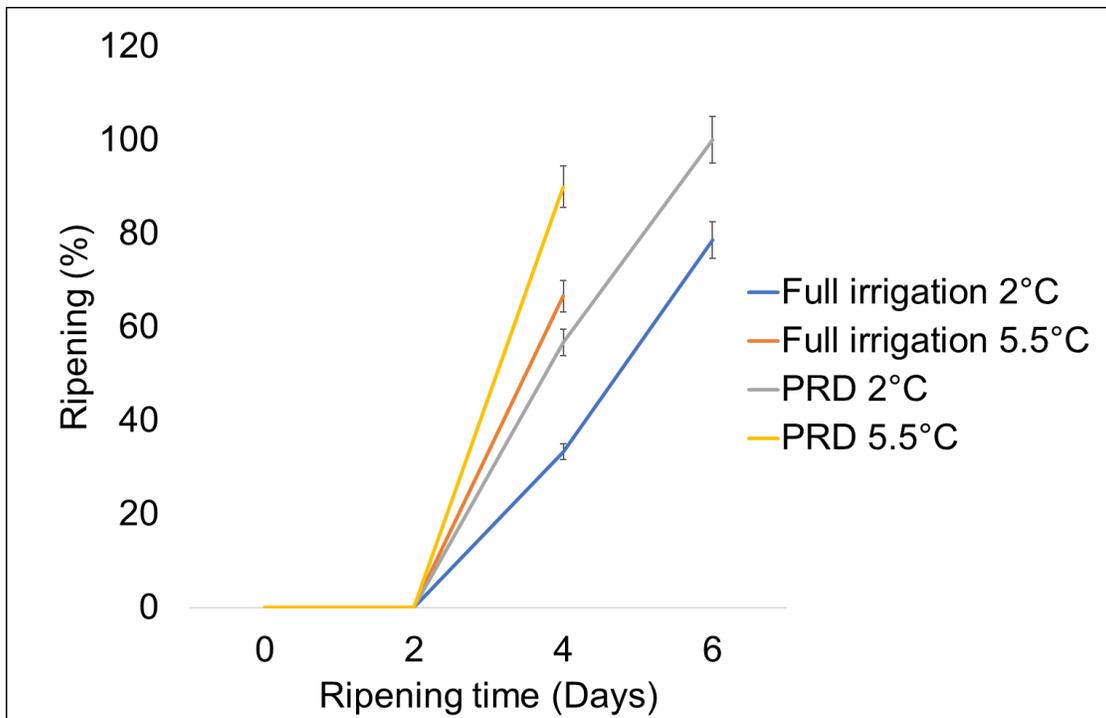
Figure 4.6 Effect of partial root-zone drying (PRD) and low storage temperature on ripening percentage of 'Hass' avocado fruit cold stored for 28 days at 2.0 and 5.5°C

4.1.7 Fruit skin colour

Lightness

An interaction between irrigation type, storage temperature and ripening time had no significant effect (P>0.05) on fruit skin lightness (L) during ripening (Appendix 7). However, fruit harvested from PRD treated trees and stored at 2.0°C showed the lowest skin lightness value of 33.30, 33.51 and 26.71 after 0, 2, and 6 ripening days, respectively (Table 4.1). Contrarily, fruit harvested from control treated trees and stored at 5.5°C

exhibited highest skin lightness values of 36,72 and 29.19 after 0 and 4 ripening days, respectively. Interestingly, fruit harvested from PRD and control trees exhibited similar skin lightness after storage at 5.5°C during 2 ripening days. Furthermore, fruit harvested from PRD and control trees and cold stored at 2.0 and 5.5°C exhibited the same skin lightness on the 4th ripening day. Moreover, skin lightness values of fruit harvested from PRD and control fruit also showed similar values on 4th and 6th ripening day after withdrawal from 2.0°C storage temperature. Fruit skin lightness values vary from 100 for pure white and 0 for black. Lightness values significantly decreased as fruit ripened from green to purple/black, indicating a decrease in peel brightness.

Chroma

An interaction between irrigation type, storage temperature and ripening time had no significant effect ($P > 0.05$) on skin chroma (C) during ripening (Appendix 8). In general, chroma (C) values of fruit decreased rapidly during ripening, irrespective of irrigation treatment and storage temperature regime (Table 4.1). From day 0 to day 2 the decrease in chroma values was between 21.1 and 35.6%. From day 2 to day 4 the decrease in chroma values was between 54.2 and 59.0%, while it was between 30.0 and 37.5% on day 6. Fruit harvested from PRD treated trees and stored at 2.0 and 5.5°C showed the highest skin chroma values of 23.20 and 24.25, respectively directly after removal from cold storage. On the second, fourth and sixth day of the ripening period, fruit chroma values did not differ significantly between the different irrigation and cold storage regimes. Chroma values determine the length of colour vector formed by a* and b* and hue angle determine such vectors giving the actual fruit colour.

Hue angle (*h°*)

An interaction between irrigation type, storage temperature and ripening time (days) had no significant effect ($P > 0.05$) on fruit hue angle (*h°*) during ripening (Appendix 9). In general, there was a colour change in fruit skin colour (lower *h°* angle) of the studied fruit irrespective of storage and irrigation treatment. On day 0 of the ripening period, hue angle values were not significantly different between the different treatments and cold storage temperature regimes (Table 4.1). On day 2 of the ripening period, the hue angles of the

PRD treatment stored at 5.5°C was significantly lower than other treatments. This indicated that the colour change from green to purple was quicker for the PRD treatment stored at 5.5°C compared to the other treatments. Hue angle values decreases as the fruit changes colour from green to purple/black. The hue angle values for the different treatments and cold storage regimes decreased with between 11.4 and 28.0% from day 0 to day 2. From day 2 to day 4, the hue angle values decreased with between 13.0 and 28.4% for the different treatment and cold storage regimes. On day 4 of the ripening period, the hue angle values did not differ significantly between the different irrigation and cold storage regimes. Hue angle values further decreased for the 2°C treatments from day 4 to day 6 of the ripening period. However, on day 6, there were no significant differences between the hue angles of fruit from the fully irrigated and PRD treatments.

Eye colour

An interaction between irrigation type, storage temperature and ripening time had no significant effect ($P>0.05$) on skin eye colour during ripening (Appendix 10). Fruit harvest from PRD and control trees exhibited the same skin eye colour (1: emerald green) after withdrawal from 2.0 and 5.5°C storage temperature on day 0 during ripening (Table 4.1). Furthermore, fruit harvested from control treated trees and cold stored at 2.0 and 5.5°C showed similar skin eye colour on the 4th ripening day. Studied fruit harvested from PRD treated tress and stored at 5.5°C showed the highest skin eye colour values of 2.23 and 3.63 after 2 and 4 ripening days, respectively. However, fruit harvested from control treated trees and stored at 2.0°C exhibited low skin eye colour values of 1.8 and 3.4 on the 2nd and 4th ripening days. Furthermore, fruit harvested from PRD treated fruit stored at 2.0°C had low skin eye colour values of 1.57, 3.3 and 4.43 after 2, 4 and 6 ripening days, respectively. The highest skin eye colour was observed on control treated fruit stored at 2°C on the 6th ripening day. In general, fruit harvested from PRD and control treated trees, thereafter, stored at 5.5°C had had high eye colour values when compared with 2.0°C fruit. However, PRD fruit stored at 5.5°C exhibited slightly high eye colour than control throughout ripening.

Table 4.1. Skin colour of 'Hass' avocado fruit using partial root-zone drying (PRD) and control irrigation during ripening after withdrawal from low storage temperature of 2 and 5.5°C.

Irrigation method	Storage temperature (°C)	Ripening time (days)	Fruit skin colour parameters			
			Lightness (L*)	Chroma (C)	Hue angle (*h°*)	Eye colour
Full irrigation	2.0	0.0	35.75 ab	21.71 b	146.50 a	1.00 e
	5.5		36.72 a	22.20 ab	147.03 a	1.00 e
PRD	2.0		33.51 cd	23.20 ab	147.22 a	1.00 e
	5.5		35.55 ab	24.25 a	147.19 a	1.00 e
Full irrigation	2.0	2.0	35.15 abc	17.12 c	120.14 b	1.80 cd
	5.5		34.69 bcd	16.60 c	119.91 b	1.90 cd
PRD	2.0		33.30 d	14.95 c	130.50 b	1.57 d
	5.5		34.28 bcd	15.97 c	106.07 c	2.23 c
Full irrigation	2.0	4.0	28.85 ef	7.67 d	93.41 cd	3.40 b
	5.5		29.19 e	7.61 d	95.80 cd	3.43 b
PRD	2.0		28.88 ef	6.35 de	96.40 c	3.30 b
	5.5		28.51 ef	6.54 de	92.30 cd	3.63 b
Full irrigation	2.0	6.0	27.23 efg	4.79 e	75.43 e	4.90 a
	5.5		*	*	*	*
PRD	2.0		26.71 g	4.51 e	82.10 de	4.433 a
	5.5		*	*	*	*

* Denote missing values. Values which do not sharing common letters differ significantly at P < 0.05

4.1.8 Vascular browning

Irrigation type and storage temperature had no significant effect (P>0.05) on vascular browning incidence during ripening (Appendix 11). Avocado fruit harvested from control treated trees, thereafter stored at 5.5°C did not show any vascular browning symptoms after storage and during ripening at ambient temperature (Figure 4.7). Furthermore, fruit harvested from PRD treated trees also exhibited the lowest vascular browning symptoms of 6.67% after storage at 5.5°C. However, fruit harvested from PRD treated trees exhibited the highest vascular browning symptoms (16.67%) after storage at 2.0°C. Moreover, mesocarp vascular browning symptoms of 'Hass' avocado fruit harvested from control treated trees was 10% after storage at 2.0°C. In general, fruit obtained from PRD

and control treated trees, thereafter stored at 2°C showed more visible symptoms of vascular browning when compared with fruit stored at 5.5°C storage temperature.
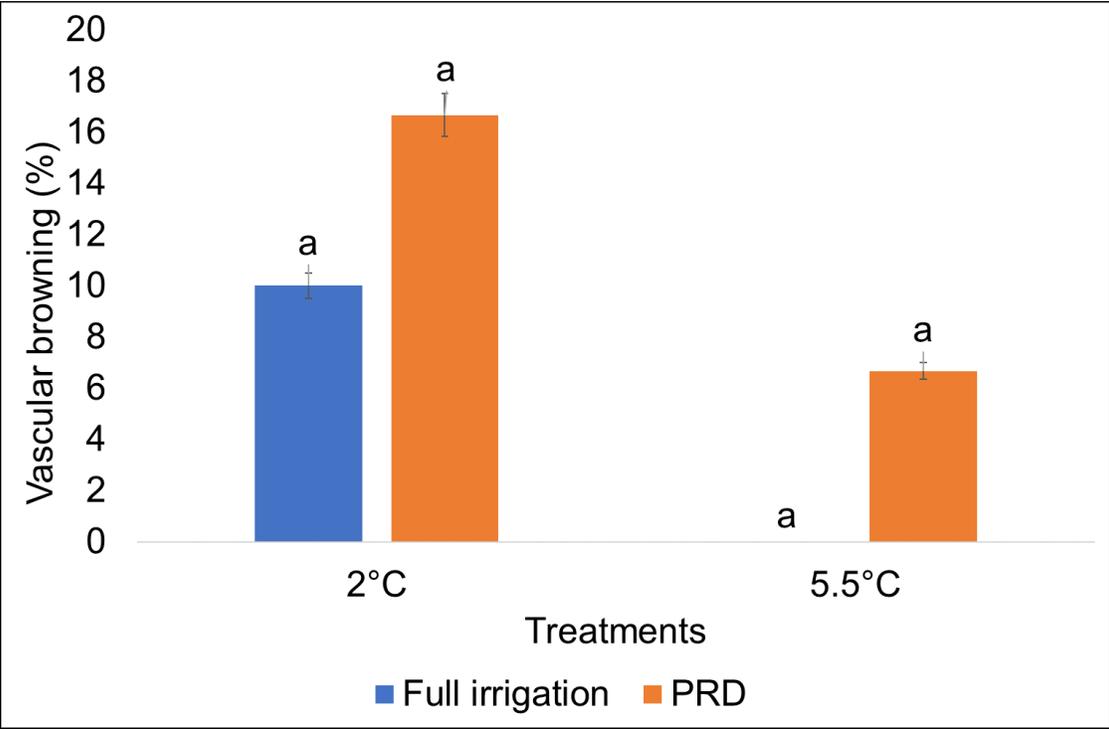
Figure 4.7 Effect of partial root-zone drying (PRD) and low storage temperature on vascular browning of 'Hass' avocado fruit cold stored for 28 days at 2.0 and 5.5°C. (Means with the same letter do not differ significantly at P ≤ 0.05)

4.2 DISCUSSION

4.2.1 External chilling injury

In this study, visible chilling injury incidence was recorded in fruit harvested from PRD treated trees when compared with the control fruit, especially for 2.0°C storage temperature (Figure 4.1). In addition, the incidences of chilling injury were significantly higher for fruit stored at 5.5°C compared to fruit stored at 2°C, irrespective of treatment (Figure 4.1). These results are similar to an earlier finding where 'Hass' avocado fruit stored at 1°C had higher incidences of chilling injury than fruit stored at 5.5°C (Van Rooyen and Bezuidenhout, 2010). Chilling injury of the studied fruit manifested as pitting and black lesions on the fruit surface after withdrawal from low temperature storage during ripening. These findings agreed with Woolf *et al.* (2003) who found that pitting, scalding and blackening after low storage temperature have been the most common external chilling symptoms in 'Hass' avocado fruit during ripening. Chilling injury symptoms started to be visible after the 2nd day during ripening when fruit were ripened at 21°C, due to the weakening of cell wall. The main function of anti-oxidants is to scavenge reactive oxygen species (ROS) and maintain the balance between anti-oxidants and ROS to allow normal metabolism to continue (Mittler, 2002). However, during low temperature storage, such as at 2°C, the balance between anti-oxidants and reactive oxygen species (ROS) is easily disrupted, therefore, forming damaging compounds, mainly ROS (Tesfay *et al.,* 2010). These compounds (ROS) cause membrane damage manifesting as visible symptoms of chilling injury (Mittler, 2002). Similarly, Crisosto *et al.* (2003) specified that chilling injury increases when fruit were kept at high temperatures after withdrawal from low temperature storage.

Calcium plays an important role in strengthening and maintaining cell wall structures in the fruit by its interaction with pectin acids in the cell wall (Blakey, 2011). Therefore, fruit with adequate calcium levels are generally firmer and less prone to various physiological disorders and chilling injury. Lower water availability (as for PRD), will result in less uptake of plant minerals, including calcium, from the soil. Low calcium uptake, especially during early stages of fruit development, would give rise to an increase in the incidences of post-

harvest physiological disorders (Neuhaus *et al.,* 2009). In this study, it could be hypothesised that the significant increase in chilling injury of fruit harvested from PRD treated trees could possibly be attributed to low calcium content.

4.2.2 Electrolyte leakage (EL)

Membrane electrolyte leakage was significantly higher for fruit stored at 2.0°C when compared with 5.5°C after withdrawal from low temperature storage (Figure 4.2). Similarly, Montoya *et al.* (1994) reported an increase in leakage which highly correlated with chilling injury for 'Hass' avocado fruit after 46 days of storage at 6°C. For fruit stored at 5.5°C, the PRD treatment displayed significantly higher electrolyte leakage when compared to the fully irrigated treatment. This was not the case for fruit stored at 2°C, most likely because the damaging effect caused by the lower storage temperature, overwrite the effect of irrigation regime. An increase in electrolyte leakage of fruit is associated with loss of plasma membrane integrity of cells during cold storage (Gonzalez-Aguilar *et al.,* 2000). This loss in membrane integrity is most likely the cause of higher incidences of chilling injury in fruit stored at 2°C.

There appear therefore to be a strong relationship between chilling injury and electrolyte leakage. Such high correlation between chilling injury and electrolyte leakage was found in 'Jinyou-1' cucumber stored at 2°C (Mao *et al.,* 2007). Similarly, in a study carried out on two avocado cultivars, 'Arad' and 'Ettinger', showed that increased chilling injury coincided with increased electrolyte leakage (Hershkovitz *et al.,* 2009). External chilling injury is caused by low temperatures. This causes disruption of the cell membrane lipids, which subsequently increases the permeability of the membranes. Higher permeability of the membranes causes increased electrolyte leakage (Dorria *et al.,* 2010). Lower cold storage temperatures and extended periods of cold storage can be expected to cause more damage to cells, resulting in higher electrolyte leakage compared to short cold storage periods and higher storage temperatures (Van Rooyen and Bower, 2006). This explains why the lower storage temperature had higher electrolyte leakage. Higher electrolyte leakage for the PRD treatment at 5.5°C is most likely caused by weaker cell

membranes caused by water stress during PRD application (see earlier discussion on the role of calcium in membrane strength).

4.2.3 Fruit weight loss

Similar results were recorded for 'Golden Delicious' apples, whereby, PRD had no significant effect on postharvest water loss and weight loss of fruit stored at 13-18°C for 18 days (Zegbe *et al.*, 2007). In the case of apples, it was found that PRD had no effect on the ultra-structure of the epidermis of fruit that could affect water loss explaining the reason that no significant effect on water loss was recorded (Mpelasoka *et al.*, 2000). It would therefore be important to investigate if PRD can have any significant effect on the ultra-structure of the avocado fruit epidermis. Furthermore, increased weight loss might also be influenced by tree water status during harvest (Lallu *et al.*, 2004). During harvest of the fruit for the current trial, tree water status, measured as midday stem xylem water potential, was similar for the fully irrigated and PRD treatments (data not shown). 'Hass' avocado fruit withdrawn from 2.0 and 5.5°C storage exhibited similar weight loss percentage during ripening, irrespective of irrigation treatment (Figure 4.3). Similarly, Ahmed *et al.* (2007) reported that increase in weight loss of 'Hass' avocado fruit during ripening at 20°C was similar after withdrawal from 5 and 9°C. Furthermore, fruit weight loss in the present study might be the result of water loss associated with ripening time and irrigation type and storage. According to Burdon *et al.* (2005) and Kok (2011), ripening time of avocado fruit can influence on water loss during ripening.

4.2.4 Respiration rate

Fruit respiration rate started at a low rate, then increased to a maximum (climacteric peak) where after it declined again. Irrespective of irrigation type and storage temperature a clear climacteric peak was visible for all fruit (Figure 4.4). The observed pattern is typical of climacteric fruit, such as avocado, where a rise in respiration rate occurs just prior to ripening (Kassim *et al.,* 2013). Fruit harvested from PRD and fully irrigated trees, thereafter; stored at both 2 and 5.5°C showed different magnitudes of the climacteric peak

during ripening. For fruit stored at 5.5°C, respiration rates of fruit from the PRD treatment was lower than for fruit from the fully irrigated treatment. For fruit stored at 2°C the fully irrigated treatment displayed lower respiration rates (Figure 4.4). It is not certain why the two different cold storage regimes yielded opposite results. However, similar to the results for fruit stored at 2°C, it was found that for 'Fuji' apple, respiration rates were two times higher for fruit from PRD trees compared to fruit from fully irrigated trees (Leib *et al.*, 2006).

4.2.5 Fruit firmness

In general, firmness values of fruit after withdrawal from low storage temperature decreased during the entire ripening period, irrespective of irrigation type and storage temperature (Figure 4.5). Similarly, an experiment on 'Chok Anan' mango showed that fruit firmness decreased rapidly during ripening at 27°C (± 2°C) reaching values of 250 to 350 N/100 g, under control irrigation, PRD and regulated deficit irrigation (RDI) (Spreer *et al.*, 2007). Likewise, Durovic et al. (2012) reported that firmness of 'Granny Smith' apple decreased about 35% after 3-day shelf-life during 24 weeks of storage at 0°C. The firmest fruit were recorded in PRD treatment when compared with deficit irrigation (DI) and fully irrigated fruit.

In the present study, fruit harvested from PRD treated trees thereafter, stored at 2.0 and 5.5°C had slightly lower firmness values when compared with fully irrigated treatment (Figure 4.5). Similarly, Leib *et al.* (2006) reported that PRD treated 'Fuji' apple had slightly lower firmness when compared with fruit from fully irrigated treatment after ripening at 20°C for 14 days. Contrarily, firmness loss of 'Cripps Pink' apple harvested from PRD treated trees have been reported to be lower than fully irrigated fruit, assumable due to reduction in cellular hydration and increased flesh compactness (Wan Zaliha and Singh, 2009). Evaluated fruit harvested from PRD and full irrigation lost firmness more rapidly after withdrawal from 5.5°C when compared with 2.0°C fruit during ripening (Figure 4.3). Changes in the cell wall structure are the most important physiological event that occurs during fruit softening or ripening (Blackey *et al.,* 2014). In general, rapid firmness loss in

fruit from the PRD and fully irrigated treatment, thereafter, stored at 5.5°C, might be due to water loss and high temperature which hastened metabolic rate when compared with 2°C fruit. Hastened metabolic rate will lead to earlier solubilisation and depolymerisation of polysaccharides in the cell with subsequent quick softening of fruit (Magwaza and Tesfay, 2015).

4.2.6 Ripening percentage

Fruit harvested from PRD treated trees ripened quicker when compared with fruit from the fully irrigated treatment, irrespective of storage temperature (Figure 4.6). However, fruit harvested from PRD and control, thereafter, stored at 5.5°C had a short shelf-life (4 days) when compared with fruit stored at 2.0°C (6 days shelf-life). Dixon *et al.* (2004), showed that, 'Hass' avocado fruit stored at 5°C for 4 weeks and ripened at 19.5°C ripened quicker and showed lower incidences of stem-end rot and brown patches when compared with fruit stored at 2.0°C during ripening at 19.5°C. It was shown that with increased temperatures, increased ripening enzyme activity, respiration rate and ethylene synthesis occurred (Perez *et al.,* 2004; Ferero, 2007), which explains why fruit ripened quicker at 5.5°C, compared to 2°C. Water stress caused by the dry side of the PRD treatment probably increased synthesis of ABA (Jiang and Zhang, 2002). Increased ABA concentrations in avocado fruit caused by pre-harvest water stress has been shown in previous studies to increase ethylene synthesis causing subsequent earlier ripening (Bower and Cutting. 1987; Blakey *et al.*, 2009; Kassim *et al.*, 2013). This would explain why fruit from the PRD treatment ripened earlier than fruit from the fully irrigated treatment.

4.2.7 Fruit skin colour

Irrigation treatment and cold storage temperature had no significant effect on the colour change f of the fruit. According to the eye colour data none of the fruit coloured fully to the purple/black colour but only coloured to approximately 75% coloured (eye colour 4). However, there was a change in fruit skin colour (decreased $h°$ angle) fruit irrespective of storage and irrigation treatment. The results were more pronounced on fruit harvested from the PRD and fully irrigated treatment stored at 5.5°C expressed by hue angle values of 92.3 and 95.8 when fully ripe, respectively (Table 4.1).

For 'Petopride' tomato fruit from PRD treated plants, fruit had more intense red colour (lower $h°$) when compared with fruit from fully irrigated plants (Zegbe-Domınguez *et al.*, 2003; Zegbe *et al.,* 2006). Moreover, 'Chok Anan' mango fruit harvested from PRD, deficit irrigation and fully irrigated plants turned yellow expressed by $h°$ values around 80-90 and 90-95 for exocarp and mesocarp, respectively (Spreer *et al.,* 2007). In addition, Pulpol *et al.* (1996) also more intense red colour for water stressed 'Virosa' tomato cultivated under greenhouse conditions. Improvement in skin colour of 'Hass' avocado fruit might be due to high conversion of starch to sugars under high temperature (5.5°C) when compared with 2.0°C. Anthocyanins are mainly responsible for the purple/black skin colour of 'Hass' avocado fruit. Cyanidin-3-O-glucode is the main anthocyanin responsible for 'Hass' fruit skin colour (Cox *et al.,* 2004). Sugars plays an important role in anthocyanin synthesis (He *et al.,* 2010) and any factor affecting sugar synthesis or translocation will influence final fruit skin colour. It is speculated that pre-harvest factors could cause 'Hass' fruit skin not colour fully to purple/black at post-harvest.

4.2.8 Vascular browning

Irrigation method and low storage temperature did not have effect of vascular browning (Figure 4.7). A study carried out by Zegbe *et al.* (2006) in 'Petopride' tomato fruit produced under PRD, had higher incidences of blossom-end rot and body rot compared with fully irrigated tomato plants. This was mainly observed in fruit with low calcium levels. However, incidence of vascular browning was higher in fruit stored at 5.5°C when compared with 1°C fruit after 28 days storage (Blakey *et al.,* 2014). Pre-harvest water

stress increased fruit browning at post-harvest, due to low calcium partitioning to fruit at post-harvest. It was found in tomato subjected to PRD treatment that fruit had lower calcium levels and higher incidences of physiological disorders (Zegbe *et al.,* 2006). As calcium is important in strengthening membrane structures, it is expected that low calcium levels will result in weaker cell membranes making fruit more susceptible for physiological disorders and infection by post-harvest pathogens. However, in the current treatment and storage temperature had no effect on the incidence of vascular browning. If calcium plays a role in the occurrence of vascular browning, it may be postulated that PRD did not affect calcium levels of fruit and therefore not affected the incidences of vascular browning. This is supported by a study carried out by Neuhaus *et al.* (2009) who found that application of PRD did not affect fruit calcium levels of 'Hass' avocado fruit.

CHAPTER 5

SUMMARY, RECOMMENDATIONS AND CONCLUSION

5.1. Summary

This showed that the application of PRD and low temperature storage (2°C) affected ripening and some of the measured physico-chemical properties. Fruit harvested from PRD treatment had significantly higher incidences of chilling injury when compared with fruit harvested from the fully irrigated treatment. In addition, chilling injury was also higher for the 2°C cold storage temperature regime compared with 5.5°C storage temperature fruit. Moreover, fruit harvested from PRD treatment thereafter stored at 2°C also led to a significant increase in membrane electrolyte leakage. Treatment factors had no effect on ripening percentage, while 2°C cold storage temperature regime delayed fruit ripening with two days. PRD treatment and low temperature storage had no effect on fruit weight loss and firmness during ripening.

In addition, fruit showed a skin colour change, irrespective of storage temperature and irrigation type during ripening. An interaction between irrigation, low storage temperature and ripening time had no significant effect on respiration rate. All treatments showed respiratory climacteric on the second day after removal from cold storage, however, respiration rate was significantly higher for PRD fruit stored at 2.0°C. The incidence of vascular browning was not affected by PRD treatment and low temperature storage of 2°C.

5.2 Recommendations

It was shown in previous studies that the hormone abscisic acid had a significant effect on ripening physiology of water stressed fruit. It is therefore, suggested that further studies should be conducted to determine changes in ABA concentration of fruit when

subjected to pre-harvest PRD application and determine its effect on ripening and fruit quality.

As mentioned, the PRD treatment increased chilling injury and electrolyte leakage. It is therefore postulated that application of PRD causes weakening of cell walls of fruit to cause increased susceptibility to chilling injury and increased electrolyte leakage. In general, calcium is known to play a vital role in strengthening cell walls and make fruit less susceptible to physiological disorders and fungal infections that cause fruit rots. It is therefore suggested that studies must be conducted to determine the effect of PRD on fruit nutrient levels.

In addition, PRD has been found to increase fruit sugar content in other crops. Sugars play an important role in anthocyanin synthesis. Anthocyanins are responsible for 'Hass' avocado fruit skin colour development during ripening. Therefore, it may be useful to determine the effect of PRD on sugar and anthocyanin metabolism in order to explain any effect of PRD on skin colour.

In the present study only, external chilling injury and vascular browning were evaluated, while fungal rots and other physiological disorders were not investigated. It is important that fungal rots and other physiological disorders be included in future research as they play important role in internal fruit quality when exporting fruit.

Subjecting avocado fruit to low storage temperature, such as in this study is therefore, not recommended for fruit producers.

5.3 Conclusion

The present study indicated that irrigation type and low storage temperature had an effect on some physico-chemical quality parameters of fruit assessed during ripening. Fruit stored at 2.0°C had high incidences of chilling injury, which make fruit more susceptible to fungal infections and negatively affect fruit appearance. Partial root-zone drying (PRD) reduced water use during irrigation, however, its effect on post-harvest quality of 'Hass'

avocado fruit subjected to 5.5°C must be further investigated and address the mentioned gaps in knowledge before recommendations on PRD can be made.

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APPENDICES

Appendix 1: Analysis of variance (ANOVA) of chilling injury during ripening.

Source of variation	DF	SS	MS	F	P
Replication	5	1883.3	376.7	0.62	
Irrigation	1	4816.7	4816.7	7.93	0.013
Temperature	1	14016.7	14016.7	23.06	<.001
Irrigation*temperature	1	150.0	150.0	0.25	0.627
Error	15	9116.7	607.8		
Total	23	29983.3			

Appendix 2: Analysis of variance (ANOVA) of electrolyte leakage during ripening.

Source of variation	DF	SS	MS	F	P
Replication	3	4.977	1.659	0.96	
Irrigation	1	13.359	13.359	7.72	0.021
Temperature	1	3.572	3.572	2.07	0.185
Irrigation*temperature	1	50.339	50.339	29.10	<.001
Error	9	15.567	1.730		
Total	15	87.814			

Appendix 3: Analysis of variance (ANOVA) of fruit weight loss percentage during ripening.

Source of variation	DF MV*	SS	MS	F	P
Replication	5	0.28042	0.05608	1.65	
Irrigation	1	0.03166	0.03166	0.93	0.338
Temperature	1	0.08388	0.08388	2.47	0.121
Ripening days	3	245.66948	81.88983	2413.95	<.001
Irrigation*temperature	1	0.08613	0.08613	2.54	0.116
Irrigation* ripening days	3	0.06035	0.02012	0.59	0.622
Temperature*ripening days	2 (1)	0.04728	0.02364	0.70	0.502
Irrigation*temperature* ripening days	2 (1)	0.09058	0.04529	1.34	0.270
Error	64 (11)	2.17111	0.03392		
Total	82 (13)	185.56570			

*Denote missing values

Appendix 4: Analysis of variance (ANOVA) of respiration rate during ripening.

Source of variation	DF MV*	SS	MS	F	P
Replication	5	672290	134458	1.30	
Irrigation	1	3110107	3110107	29.97	<.001
Temperature	1	552029	552029	5.32	0.024
Ripening days	3	61300359	20433453	196.90	<.001
Irrigation*temperature	1	1944440	1944440	18.74	<.001
Irrigation* ripening days	3	3328793	1109598	10.69	<.001
Temperature*ripening days	2 (1)	3976377	1988189	19.16	<.001
Irrigation*temperature* ripening days	2 (1)	202314	101157	0.97	0.383
Error	65 (10)	6745585	103778		
Total	83 (12)	75337988			

*Denote missing values

Appendix 5: Analysis of variance (ANOVA) of fruit firmness during ripening.

Source of variation	DF MV*	SS	MS	F	P
Replication	5	233.00	46.60	2.37	
Irrigation	1	392.90	392.90	19.96	<.001
Temperature	1	36.07	36.07	1.83	0.181
Ripening days	3	43951.91	14650.64	744.25	<.001
Irrigation*temperature	1	103.73	103.73	5.27	0.025
Irrigation*ripening days	3	46.99	15.66	0.80	0.501
Temperature*ripening days	2 (1)	431.93	215.96	10.97	<.001
Irrigation*temperature*					
ripening days	2 (1)	14.67 7.34	0.37	0.690	
Error	65 (10)	1279.53	19.69		
Total	83 (12)	39419.62			

*Denote missing values

Appendix 6: Analysis of variance (ANOVA) of ripening percentage.

Source of variation	DF MV*	SS	MS	F	P
Replication	5	2852.9	70.6	3.01	
Irrigation	1	3375.1	3375.1	17.83	<.001
Temperature	1	3347.5	3347.5	17.68	<.001
Ripening*days	3	150791.8	50263.9	265.52	<.001
Irrigation*Temperature	1	25.2	25.2	0.13	0.716
Irrigation*Ripening days	3	3540.9	1180.3	6.23	<.001
Irrigation*Ripening days	2 (1)	5390.0	2695.0	14.24	<.001
Irrigation*Temperature*					
Ripening*days	2 (1)	45.0	22.5	0.12	0.888
Error	65 (10)	12305.8	189.3		
Total	83 (12)	130781.0			

*Denote missing values

Appendix 7: Analysis of variance (ANOVA) of fruit skin lightness during ripening.

Source of variation	DF	MV*	SS	MS	F	P
Replication	5		1.262	0.252	0.14	
Irrigation	1		17.131	17.131	9.57	0.003
Temperature	1		8.952	8.952	5.00	0.029
Ripening days	3		1143.238	381.079	212.90	<.001
Irrigation*temperature	1		2.217	2.217	1.24	0.270
Irrigation*ripening days	3		9.698	3.23	1.81	0.155
Temperature*ripening days	2	(1)	9.622	4.811	2.69	0.076
Irrigation*temperature* ripening days	2	(1)	3.892	1.946	1.09	0.343
Error	65	(10)	116.349	1.790		
Total	83	(12)	1112.16			

*Denote missing values

Appendix 8: Analysis of variance (ANOVA) of fruit skin chroma during ripening.

Source of variation	DF	MV*	SS	MS	F	P
Replication	5		13.307	2.661	0.65	
Irrigation	1		0.773	0.773	0.19	0.665
Temperature	1		4.075	4.075	1.00	0.322
Ripening days	3		4969.398	1656.466	404.82	<.001
Irrigation*temperature	1		3.700	3.700	0.90	0.345
Irrigation*ripening days	3		38.414	12.805	3.13	0.032
Temperature*ripening days	2	(1)	1.811	0.905	0.22	0.802
Irrigation*temperature* ripening days	2	(1)	1.367	0.683	0.17	0.847
Residual	65	(10)	265.968	4.092		
Total	83	(12)	4519.891			

*Denote missing values

Appendix 9: Analysis of variance (ANOVA) of fruit skin hue angle during ripening.

Source of variation	DF *		SS	MS	F	P
Replication	5		575.6	115.1	0.94	
Irrigation	1		0.2	0.2	0.00	0.967
Temperature	1		357.8	357.8	2.91	0.093
Ripening days	3		66621.6	22207.2	180.54	<.001
Irrigation*temperature	1		687.3	687.3	5.59	0.021
Treatment*ripening days	3		30.9	10.3	0.08	0.969
Temperature*ripening days	2	(1)	612.7	306.3	2.49	0.091
Irrigation*temperature* ripening days	2	(1)	442.4	221.2	1.80	0.174
Error	65	(10)	7995.5	123.0		
Total	83	(12)	61556.9			

*Denote missing values

Appendix 10: Analysis of variance (ANOVA) of fruit eye colour during ripening.

Source of variation	DF *		SS	MS	F	P
Replication	5		0.6403	0.1281	0.77	
Irrigation	1		0.0727	0.0727	0.44	0.510
Temperature	1		0.7327	0.7327	4.43	0.039
Ripening days	3		197.6963	65.8988	398.58	<.001
Irrigation*Temperature	1		0.5044	0.5044	3.05	0.085
Irrigation*ripening days	3		0.5721	0.1907	1.15	0.334
Temperature*ripening days	2	(1)	0.4556	0.2278	1.38	0.259
Irrigation*temperature* ripening days	2	(1)	0.2411	0.1206	0.73	0.486
Error	65	(10)	10.7467	0.1653		
Total	83	(12)	154.6114			

*Denote missing values

Appendix 11: Analysis of variance (ANOVA) of vascular browning during ripening.

Source of variation	DF	SS	MS	F	P
Replication	5	2733.3	546.7	1.60	
Treatment	1	266.7	266.7	0.78	0.391
Temperature	1	600.0	600.0	1.75	0.205
Treatment*temperature	1	0.0	0.0	0.00	1.000
Error	15	5133.3	342.2		
Total	23	8733.3			