EFFECT OF TREE GIRDLING, HARVEST TIME AND RIPENING TEMPERATURE ON 'HASS' AVOCADO FRUIT SKIN COLOUR DEVELOPMENT DURING RIPENING

BY

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MINI-DISSERTATION

Submitted in fulfilment of the

requirement for the degree of

Master of Science

in

Horticulture

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

2018

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DECLARATION

I, Hazel Sibuyi, declare that the mini-dissertation submitted to the University of Limpopo, for the degree of Master of Science in Horticulture has not previously been submitted by me for a degree at this or other University.

Ms H Sibuyi	**Date**

We certify that the above statement is correct.

Dr N Mathaba	**Date**
Supervisor	
Prof TP Mafeo	**Date**
Co-supervisor	

DEDICATION

I dedicate this mini-dissertation to my family, especially my daughter Khongelani Pretty Baloyi and my parents, Mr John Solomon Sibuyi and Mrs Sarah Vongani Sibuyi. They are my life's pillar of strength and through them I have witnessed strength and faith beyond measure through the toughest times. My mother will always be my role model.

I love you all, thank you for moulding me into the woman I am today.

ACKNOWLEDGEMENTS

The completion of this degree was successful with the help, support and love of many people. I would like to sincerely thank the following:

- My academic supervisors, Prof TP Mafeo and Dr N Mathaba for thier guidance throughout this study.
- I highly appreciate the support of the Laboratory assistant Mr J Mlimi and Mr J Ntandane.
- Senior student researchers (Mr S Mathe, Mr K Shikwambani and Mr T Sathege) for mentoring me through laboratory technique's and scientific writing.
- My colleagues Ms RG Baloyi and Ms RB Shibambu for their support, help and friendship.
- My parents for their endless love and support throughout all the academic years.
- My family and friend for being supportive, understanding and believing in my dreams.
- My best friend Mr LP Baloyi and my daughter K P Baloyi for their support, love and patient over number of years and for highly valuing my dreams.
- Above all, I praise and worship God the Almighty, my saviour for His love, grace, and for granting me the ability, strength and the wonderful opportunity to be able to achieve all that I have in my life.

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ABSTRACT

‘Hass’ avocado fruit changes skin colour from green to purple and then black during ripening. However, markets importing South African avocado fruit have been complaining about the ‘Hass’ skin colour not changing to purple/black during ripening. Thus, the study aimed to investigate the effect of tree girdling, harvest time and ripening temperature on ‘Hass’ avocado fruit skin colour development during ripening. The mature ‘Hass’ avocado fruit were harvested from girdled and non-girdled trees during early (April), mid- (May) and late (June) harvest times. Upon arrival, in the laboratory fruit were cold stored at 5.5°C for 28 days. After storage, fruit were ripened at 25, 21 and 16°C for 8, 6 and 4 days, respectively. After withdrawal from clod storage fruit were evaluated for skin colour development, ripening and physiological disorders (chilling injury). Fruit from girdled trees showed high maturity (low moisture content) when compared with fruit from non-girdled trees during early and mid-harvest. With respect to skin colour development, the results indicate that skin eye colour development of fruit from girdled and non-girdled trees minimally increased from emerald green (1) to olive green (3) across all harvest times, ripening temperature and ripening duration. However, late season fruit from non-girdled trees improved to purple (4) when ripened at 21°C when compared with fruit from girdled trees. In terms of objective colour, lightness, hue angle and chroma decreased for fruit from girdled and non-girdled trees, across all harvest times, ripening temperature and ripening duration. Lightness and hue angle of fruit from girdled trees were slightly reduced when compared with fruit from non-girdled trees, throughout all harvest times, ripening temperature and duration. Early and mid-season fruit harvested from girdled trees showed rapid decrease of chroma when compared with fruit from non-girdled trees, throughout ripening temperature and

duration. In terms of softening, fruit from girdled trees showed higher firmness loss and ripening percentage within 6 (16°C) and 4 (21 and 25°C) days when compared with fruit from non-girdled trees during early and mid-harvest, whereas, late harvest fruit from girdled trees reached higher ripening percentage and firmness loss within 4 days throughout ripening temperatures. With respect to cold damage, late harvested fruit from girdled trees showed higher external chilling injury when compared with non-girdled trees, throughout ripening temperature. In general, girdling treatment improved fruit maturity, ripening rate and firmness loss. However, the incidence of variable skin colouring of ‘Hass’ avocado fruit during ripening was also prevalent in early harvested fruit from girdled tree, irrespective of ripening temperature.

Keywords: girdling, harvest time, physiological disorder, ripening temperature, variable colouring

CHAPTER 1
GENERAL INTRODUCTION

1.1.Background

The South African avocado industry is export-oriented and it's fruit are largely exported to the European Union (79%), United Kingdom (18%) and Russian Federation (1.24%) (Food Trade SA, 2016). During 2013/14 season, approximately 51% of the total avocado fruit produced in South Africa were exported, while 49% were marketed locally (DAFF, 2015). In the 2014 season, the quantity of exported avocado fruit increased by 30% when compared with 2013 season (DAFF, 2015).

Avocado (*Persea americana* Mill.) are recognized as one of the top 20 commercial fruit produced in the world (Liu *et al.,* 2002), and considered as healthy food due to it's nutritional value (Bergh, 1992). Avocado fruit have a unique and vital combination of health features, such as B-complex vitamins, high ratio of unsaturated and saturatd fatty acids, a remarkable amount of potassium and iron, as well as a large amount of antioxidant substances, such as C7 sugars, α-tocopherol and β-carotene (Tesfay, 2009; Terasawa *et al.,* 2006). Furthermore, avocado fruit only undergo ripening process when detached from the tree (Hershkovitz *et al.,* 2009). However, ripening process in all avocado fruit types is accompaned by an increased in ethylene production and respiration rate followed by softening of mesocarp tissue (Kok *et al.,* 2010; Donetti, 2011) and skin colour change for 'Hass' cultivar (Cox *et al.,* 2004).

'Hass' avocado fruit skin colour changes from green to purple and then black during ripening (Cox *et al.,* 2004). Colour change is the characteristic of fruit physiological ripening stage. Therefore, skin colour change in 'Hass' avocado fruit after harvest has been used as a ripeness indicator by growers, exporters and consumers.

However, markets importing South African ‘Hass’ avocado fruit have been complaining about the skin colour not changing to purple then black during ripening (Mathaba *et al.,* 2015). Previously, several pre- and postharvest factors have been shown to be responsible for ‘Hass’ avocado fruit skin colour development during ripening. These factors included; harvesting season, harvest time, ripening temperature, ripening days after cold storage and orchard slope (Cox *et al.,* 2004; Donetti and Terry, 2012; Mathaba *et al.,* 2015; Nthai, 2016).

Harvest time plays a significant role in maturation, expected storage and shelf-life of an avocado fruit (Gamble *et al.,* 2010). Ripening temperature is a key factor associated with ripening physiological processes (Donetti, 2011). Moreover, skin sugars are known to contribute towards anthocyanin biosynthesis (Mita *et al.,* 1997) and anthocyanin are responsible for purple colour change in ‘Hass’ avocado fruit during ripening (Cox *et al.,* 2004). In other fruit crop such as grapes, fruit skin colour development has been improved by girdling (Casanova *et al.,* 2009). Girdling is the tree management practice used to improve carbohydrate accumulation in the fruit (Davie *et al.,* 1995). Furthermore, girdling was considered as an important practice responsible for improving fruit setting, yield as well as fruit quality (Nguyen and Yen, 2012).

1.2. Problem statement

‘Hass’ avocado fruit skin colour change as a ripeness indicator has been questioned by consumers, especially lucrative overseas markets (Mathaba *et al.,* 2015). Recently, harvest time and ripening temperature have been documented as major postharvest factors responsible for poor ‘Hass’ avocado fruit skin colour development during ripening (Cox *et al.,* 2004; Mathaba *et al*., 2015). Both harvest time and ripening temperature play a role in the concentration of fruit sugars (Liu *et*

al., 2002; Tesfay, 2009). Sugars have also been shown to contribute towards final skin colour change (Yamane and Shibayama, 2007). According to Mita *et al.* (1997), sugars have been known to trigger gene regulation, proteins and enzymes expression involved in anthoncyanin biosynthesis. Physiologically, girdling has been used in avocado, citrus, grape and peach fruit tree crops to manage carbohydrates partitioning and plant growth regulators (Goren *et al.,* 2003; Li *et al.,* 2003). To reduce the challenge of poor 'Hass' avocado fruit skin colour development, tree girdling needs to be considered. However, information about the interaction of tree girdling, harvest time and ripening temperature on colour development of 'Hass' avacado is not documented.

1.3. Rationale of the study

'Hass' avocado fruit skin colour uniformity is a key determinant of quality attribute used by both industry and consumers. However, 'Hass' avocado fruit poor skin colour development during ripening has been identified as a major challenge in some consignments from South Africa (Mathaba *et al*., 2015, Nthai, 2016). This has led to revenue loss from export markets due to poor 'Hass' avocado fruit skin colour development during ripening. Physiologically, tree girdling, harvest time and ripening temperature have been shown to affect the photosynthates content of the fruit (Nguyen and Yen, 2012), which also determines the peel colour development in fruit crops. Mohammad *et al.* (2012) reported that girdling stimulated 'Jambu madu' wax apple fruit skin colour development due to accumulation of sugars, especially sucrose. Girdled 'Hass' avocado tree reduced sap flow and improved photosynthates accumulation (Davie *et al.,* 1995). In 'Fuji' apple, girdling increased fruit size and sugar accumulation (Zhao *et al.,* 2013). Thus, the study proposes to determine combined effect of tree girdling, harvest time and ripening temperature on colour

development of ‘Hass’ avocado fruit. The information generated from this study will improve quality of exported ‘Hass’ avocado fruit from South Africa.

1.4. Aim

The aim of the study was to investigate the effect of pre- and post-harvest factors, and their interaction on skin colour development of ‘Hass’ avocado fruit during ripening.

1.5. Objective

To establish the combine effect of tree girdling, harvest time, ripening temperature and duration on skin colour development of ‘Hass’ avocado fruit during ripening.

1.6. Hypotheses

Tree girdling, harvest time and ripening temperatures would had an effect on skin colour development ‘Hass’ avocado fruit during ripening.

CHAPTER 2
LITERATURE REVIEW

2.1. Introduction

The South African avocado industry is export-oriented; and primarily, aimed at the European markets (DAFF, 2015). South African avocado production is dominated by 'Hass' (37%) and 'Fuerte' (37%) along with 'Ryan' (12%) and 'Pinkerton' (9%) cultivars (SAAGA, 2007). Normally, 'Fuerte' fruit usually dominate the early season market, followed by 'Pinkerton' fruit (April to June), while 'Hass' and 'Ryan' dominates the late season market (July to October) (Graham and Wolstenholme, 1991). However, the European markets have prefer a 'Hass' cultivar than the other cultivars (SAAGA, 2007). Avocado fruit 'Hass' is preferred by the European markets due to its rich and nutty taste and excellent shelf-life with few post-harvest problems when compared with other cultivars (DAFF, 2015). Furthermore, the European markets use 'Hass' avocado fruit skin colour change as ripeness guide. 'Hass' avocado fruit skin colour changes from green to purple then black during ripening (Cox *et al.,* 2004).

In the last 5 years, consumers have been complaining about poor 'Hass' avocado fruit skin colour change which compromises the credibility of the industry. Moreover, several pre- and post-harvest factors such as harvest time, orchard slope, ripening temperature and ripening days have been showed to be responsible for poor 'Hass' avocado fruit skin colour development (Cox *et al.,* 2004; Mathaba *et al.,* 2015). In other fruit crops such as 'Jambu madu' red wax apple and 'Flame seedless' grapes, skin colour change has been improved by girdling (Mohammad *et al.,* 2012; Soltekin *et al.,* 2016). Girdling is a pre-harvest practice used to improve carbohydrate accumulation in the fruit (Nguyen and Yen, 2012). This literature review would focus on work done and not yet done on the research problem.

2.2. Work done on the research problem

2.2.1. Importance of tree girdling

Girdling is a practice that involves the removal of ring bark around outer tree trunk circumference, thereby, reducing photosynthates and metabolites translocation from the leaves to the roots (Nguyen and Yen, 2012). In 'Hass' avocado trees, girdling reduced vegetative growth above girdled branch, therefore, increasing fruit set and yield (Köhne, 1992). In 'Murcott' citrus trees, girdling stimulated growth substance and improved carbohydrate accumulation in the girdled branch (Li *et al.,* 2003). Girdling also improved physico-chemical quality of 'Fuji' apple (Zhao *et al.,* 2013). According to Li *et al*. (2003), available carbohydrates in the girdled branch, leaves and bark are consumed by developing fruit. Therefore, the practice has been used to improve fruit tree productivity, fruit quality and size (Nguyen and Yen, 2012).

2.2.2. Effect of tree girdling on fruit quality

Fruit quality and size are essential attributes which influence marketability and yield component (Chaudhary *et al.,* 2014). In the South African avocado industry, acceptable fruit size ranges from 10 (346-362 g) to 30 (123-137 g) (Kassim *et al.,* 2013). In 'Hass' avocado, girdling has a tendency of overproduction, resulting in undersized fruit (<200 g) (Lahav *et al.,* 1972). Girdling increased production of small size 'Hass' avocado fruit due to higher crop load; thereby, decreasing the export quality (Köhne, 1992). However, girdling can also have a negative effect on fruit quality. Avocado fruit 'Hass' quality from girdled branches was down-graded due to rub-marks, sub-standard size and deformation when compared with fruit from control branches (Lahav *et al*., 1972). Furthermore, girdling has been confirmed to improve quality parameters, including skin colour on several fruit crops such as 'Jambu madu'

wax apple (Mohammad *et al.,* 2012) and ‘Aki Queen’ grapes (Yamane and Shibayama, 2007).

Skin colour is an essential parameter for fruit appearance and influences the consumer’s perception (Brouillard *et al.,* 1997). In ‘Hass’ avocado fruit, the purple colouration is due to increased anthocyanin content during ripening (Asthon *et al.,* 2006). Furthermore, carbohydrates (soluble sugars) induce and regulate genes, proteins and enzymes expression responsible for fruit anthocyanin biosynthesis (Mita *et al.,* 2006). Therefore, carbohydrates play a significant physiological role in fruit colour development, specifically, anthocyanin synthesis.

Girdling improved sugars and anthocyanins accumulation, subsequently, promoting skin colour development of ‘Jambu madu’ wax apple (Mohammad *et al.,* 2012), ‘Japanese’ persimmon (Kazutoshi *et al.,* 2009) and ‘Flame seedless’ grapes (Soltekin *et al.,* 2016). In ‘Aki Queen’ grapes, trees girdled 30 days after full bloom resulted in high anthocyanin accumulation which promoted berry colour development (Yamane and Shibayama, 2007). However, in ‘Kyoho’ grape, girdling lateral shoots 45 days after full bloom was not effective on berry colour development when compared with 30 days after full bloom (Yamamoto *et al.,* 1992).

2.2.3. Fruit maturity and indices

Fruit maturity is a growth developmental stage from flowering through maturation, finally, senescence (Kader, 1997). Maturity at harvest plays an essential role in determining storage-life and post-harvest quality of fruit (Lelièvre *et al.,* 1997; Kader, 1997). Gamble *et al*. (2010) indicated that ‘Hass’ avocado fruit should be harvested at a suitable physiological maturity in order to achieve an edible taste and firmness.

Maturity requirement standard of avocado fruit has led to the use of parameters such as moisture, oil and dry matter content (Kassim *et al.,* 2013).

Oil content was used to test maturity until fruit maturity could be measured using moisture content (Kader, 1997). According to Kruger *et al.* (1995), oil content is inversely proportional to moisture content, whereby, oil content increases with fruit maturity while moisture content decreases. Kruger and Claassens (1996) found that oil content was more variable than moisture content and oil increased faster following heavy rain and irrigation when compared with moisture content. In the South African avocado industry, moisture content is the preferred method to determine avocado fruit maturity (Blakey, 2011). In the avocado industry, the recommended moisture content ranges from 80 to 69% depending on cultivar (Kassim *et al.,* 2013).

2.2.4. Effect of fruit maturity on fruit quality

Avocado fruit maturity is determined according to the minimum standards for each specific cultivars and required storage-life (Hofman *et al.,* 2002). According to previous findings, avocado maturation stage can affect fruit quality parameters such as, susceptibility to physiological disorders and ripeness (Kader, 1997; Dixon *et al.,* 2003). Furthermore, fruit harvested too early and late in the season are more prone to physiological and pathological disorders than fruit harvested at proper maturity standard, respectively (Kader, 1997). Léchaudel and Joas (2006) reported that 'Cogshall' mango fruit harvested at an immature stage were associated with irregular ripening, high physiological disorders and off-flavour. Moreover, 'Hass' and 'Fuerte' avocado fruit harvested early were more susceptible to physiological disorders, shrivelling, and mechanical injury and showed poor ripening quality (Kader, 1997).

Tefera *et al.* (2007) reported that late harvested mango fruit might deteriorate quicker when compared with fruit harvested at proper maturity standard.

2.2.5. Effect of ripening temperature on avocado fruit quality

Ripening temperature is considered as the most important factor affecting fruit quality (Blakey *et al.,* 2012). In avocado fruit, ripening process is initiated by temperature, which needs to be increased and controlled after cold storage to reactivate enzyme activities (Bill *et al.,* 2014). The most suitable temperature for avocado fruit ripening is approximately 18-20°C (Hopkrik *et al.,* 1994). Meyer and Terry (2010) found that 'Hass' avocado fruit ripened at higher temperatures (30-40°C) did not ripen normally. Furthermore, Hopkrik *et al.* (1994) found that higher ripening temperatures (30-40°C) increased the risk of fungal decay, vascular browning and mixed ripening when compared with lower ripening temperature (15-18°C).

2.2.6. Ripening physiology

Fruit ripening involves a change in physiological and physical properties and once began; the process is irreversible (Adams-Phillips *et al.,* 2004). Ripening is the later stage of fruit maturity which involves changes in metabolic process such as respiration rate, skin colour development, flavours, aroma and texture (Alexander and Grierson, 2002). During fruit ripening, cell wall degradation is recognized after the action of enzymes such as pectin methylesterase (PE) and polygalacturonase (PG) and cellulase acting on pectin, hemicellulose, and cellulose (Cheng *et al.,* 2009; Zhou *et al.,* 2011). In general, avocado fruit ripen faster due to high carbon dioxide and ethylene production; and mostly, complete ripening within 4-8 days after harvest, depending on harvest time (Usenik *et al.,* 2008). Therefore, it is important to control ripening temperature in order to increase shelf-life after harvest (Zhou *et al.,* 2011).

In ‘Hass’ avocado fruit, skin colour is an important characteristic used by industry, producers and consumers as a ripening indicator (Arzate-Vazquez *et al.,* 2011). Furthermore, skin colour of fruits and vegetables is derived from the natural pigments which change as the plant reaches maturation and ripening stage (Valero and Serrano, 2010). Moreover, an avocado fruit comprise of considerable quantities of plant pigments such as carotenoids, chlorophylls and anthocyanins (Asthon *et al.,* 2006). Cox *et al.* (2004) and Lu *et al.* (2005) found that ripening in ‘Hass’ avocado fruit tends to accelerate degradation of skin carotenoids and chlorophyll, whereas, the green-yellow colour of the mesocarp is due to the presence of these pigments. However, ‘Hass’ avocado fruit purple colouration is due to increased anthocyanin (cyaniding 3-*O*-glucoside) content during ripening (Cox *et al*., 2004; Asthon *et al.,* 2006). Therefore, the presence of carotenoid, chlorophyll and anthocyanin pigments are essential in fruit tissue colouration during ripening (Brouillard *et al.,* 1997).

2.2.7. Chilling injury

Chilling injury is a permanent physiological damage to plant organ, cell and tissue and associated with prolonged exposure to temperature below critical threshold (Wang, 2001). Chilling injury symptoms develop rapidly when fruit are removed from chilling to non-chilling temperatures (Bower and Magwaza). In avocado fruit, chilling injury may manifests as internal and external damages with various symptoms that include cell membrane collapsing, pulp discolouration (brown or grey pulp), and small black pitted area to widespread sunken black lesions on the skin (Bower and Magwaza, 2004; Bower, 2005; Van Rooyen, 2009). Van Rooyen (2009) reported that mature green avocado fruit may fail to ripen normally after chilling. Woolf *et al.* (2005) found that the chilling damage will be less apparent after ripening due to skin blackening of ‘Hass’ avocado fruit.

2.3. Work not yet done on the research problem

The export market of South African avocado fruit has been complaining about variable colouring of early harvest ‘Hass’ cultivar (Mathaba *et al.,* 2015). Girdling technique has been widely used for many fruit crops to improve physiological partitioning of photosynthate (Li *et al.,* 2003), crop productivity (Goldschmidt, 1999), fruit size and alternative bearing (Agust *et al.,* 1992) and fruit quality (Nguyen and Yen, 2012). However, the effect of girdling on ‘Hass’ avocado fruit skin colour change during ripening has not been investigated, constitute the perceived gap in the research problem. Therefore, the study will focus on investigating the effect of harvest time, tree girdling, and ripening temperature on ‘Hass’ avocado fruit skin colour development during ripening.

CHAPTER 3
RESEARCH METHODOLOGY

3.1. Experimental sites

Matured ‘Hass’ avocado fruit were harvested at Nico Swart Farm (25° 4’0” S, 31°2’0” E) Kiepersol, Mpumalanga province. The orchard received the same management practices. The area receives an annual rainfall of < 667 mm, with a monthly minimum and maximum temperature of 13.4 and 26°C, respectively. After harvest, fruit were immediately transported to the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) post-harvest laboratory in Nelspruit (25°27’0” S, 30°58’ 0” E) for storage and analysis.

3.2. Experimental design, treatments and procedures

The experiment was arranged in a 2 × 3 × 3 × 3 factorial in completely randomized design (CRD) at the laboratory and replicated three times. The treatment factors were: 2 × girdling (girdled and non-girdled), 3 × harvest time (early May, mid- June and late July season), 3 × ripening temperature (16, 21 and 25°C) and ripening duration (8, 6 and 4 days).

The girdling process was performed early February 2016 using a girdling knife which simultaneously cut and remove the bark strip on the sub branches (Figure 3.1). The width of the girdle was between 1.5 to 2 mm. Fruit were harvested from the girdled and non-girdled tree branches at three different maturities; early May, middle June and late July. After each harvest time, fruit were sorted, graded and divided into 30 fruit per carton box, with each replicated three times. Thereafter, fruit were stored at 5.5°C for 28 days. After removal from cold storage, fruit were ripened at 16, 21 and 25°C for 8, 6 and 4 days respectively.

Figure 3.1 The girdled ‘Hass’ avocado tree branches where fruit were harvested

3.3. Determination of moisture content

Moisture content was determined at harvest from three fruit at each harvest time for girdled and non-girdled trees. Each fruit was weighed and then cut into halves with a fruit chopper (Figure 3.2 A and B). The seed together with the seed coat were removed. The skin was peeled with a potato peeler and flesh grated using a kitchen grater (Figure 3.2 C and D). A 10 g sample of the grated flesh from each fruit was weighed and oven dried (Model: 279, Ecotherm, Labotec, South Africa) at 30°C for 48 hours. Afterward, the samples were re-weighed using a weighing scale (Model: SBA 61, Scale Tec, Goettingen, Germany) to determine moisture content (Figure 3.2 E and F). The moisture content (%) was determined using the following equation:

$$Moisture\ content\ (\%) = \frac{(M_0 - M_1)}{M_0} \times 100$$

Where M_0 was the wet mass and M_1 represent dry mass

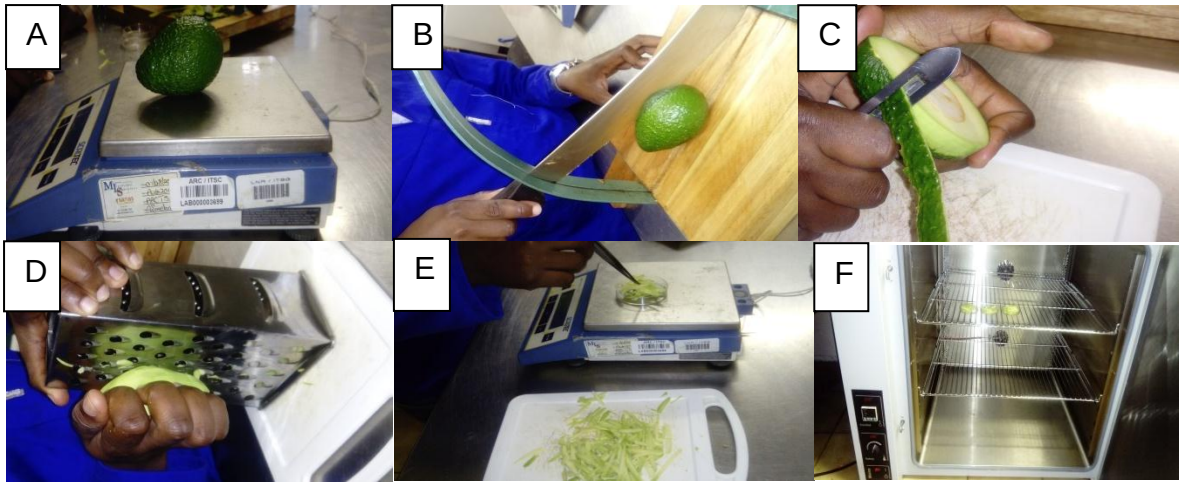

Figure 3.2 Measuring moisture content of 'Hass' avocado fruit – **A.** Weighing, **B.** Cutting, **C.** Peeling, **D.** Gratting, **E.** Weighing 10 g and **F.** Oven drying.

3.4. Determination of skin colour

'Hass' avocado fruit skin colour was determined both subjectively and objectively during ripening after removal from cold storage. Subjective skin colour was rated by using the following scale 1 = emerald green; 2 = forest green; 3 = olive green; 4 = purple; and 5 = black (Figure 3.3 A). Same fruit were then measured objectively using a chromameter (Model: DFM50, CR-400, Konica Minolta, Osaka, Japan) by averaging three measurements taken around the fruit equator (Figure 3.3 B). Colour was measured every other day until fruit were fully ripe. The values obtained were expressed in (L*, *a**, *b**,* C* and h^o) units. Where L* describe the lightness and darkness [L* = 0 (black) and *L** = 100 (white)], *a** specify the greenness or redness (where - *a** indicate greenness, whereas, + *a** indicate redness) and *b** indicates yellowness and blueness (where - *b** indicates blueness, whereas, + *b** indicates yellowness). Chroma (C*) value indicates the degree of saturation of colour whereas, hue angle (h^o) was the basic unit of colour (0^o = red; 90^o = yellow; 180^o = bluish-green and 270^o = blue). Hue angle (h^o) and Chroma (*C**) values were calculated based using the following formulas (McGuire, 1992):

$$Hue\ angle\ (h^o)\ =\ 180^o\ +\ [tan\ (a^*/b^*)] \quad 1$$

$$Chroma\ (C^*)\ = \sqrt{(a^*)^2 + (b^*)^2} \quad 2$$

Figure 3.3 Subjective 'Hass' avocado fruit skin colour ratings (**A**) and objective colour was determine by (**B**) using the chromameter.

3.5. Determination of firmness and ripening percentage

Fruit firmness was measured using a non-destructive automated Sinclair IQ^{TM} desktop firmness machine (Model: 51DFTB, Sinclair IQ^{TM} international, Norwich, United Kingdom), by taking four measurements along the fruit equator region (Figure 3.4). The reading ranges from 100 (Hard) to 0 (soft). Fruit was considered to be fully ripe at 25 Sinclair Units (SU). Ripening percentage was determined by dividing the number of fruit that were fully ripe (≥ 25 SU) by the total number of fruit evaluated and multiplied by 100.

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

Figure 3.4 Measuring 'Hass' avocado fruit firmness during ripening

3.6. Determination of chilling injury (CI)

'Hass' avocado fruit external quality was assessed after storage at 5.5° C and during ripening. External chilling damage was assessed two days after removal from cold storage, which appeared as grayish-brown discoloration of skin. Fruit were rated on a relative scale from 0 to 3 (1) 0 = no occurrence, (2) 0.5 = < 10%, (3) 1.0 = > 10 ≤ 20%, (4) 1.5 = >20 ≤ 50%, (5) 2.0 = >50 ≤ 75%, (6) 2.5 = >75 ≤ 90%, (7) 3.0 = ≥ 90% of the fruit surface damaged). Chilling injury percentage was calculated by using the formula by (White *et al.,* 2004).

$$CI\ (\%) = [\frac{(Number\ of\ fruit\ with\ CI\ symptoms)}{(Numberof\ evaluated\ fruit)}] \times 100$$

3.7. Data analysis

Analysis of variance (ANOVA) was generated using Genstat software version 16 (VSN International, Hemel Hempstead, UK) for window. Mean separation was done using Duncan Multiple Range Tests at P < 0.05.

CHAPTER 4
RESULT AND DISCUSSION

4.1. Results

4.1.1. Moisture content

An interaction of girdling treatment and harvest time had no significant effect (P > 0.05) on fruit moisture content at harvest (Appendix 1). However, moisture content of fruit from girdled and non-girdled trees significantly decreased with delayed harvest time (Figure 4.1). At early season, fruit harvested from girdled trees showed non-significantly lower moisture content when compared with fruit from non-girdled trees. Furthermore, same fruit moisture content trend was observed during mid-harvest. Contrary, late season fruit harvested from girdled trees showed higher moisture content when compared with non-girdled fruit.

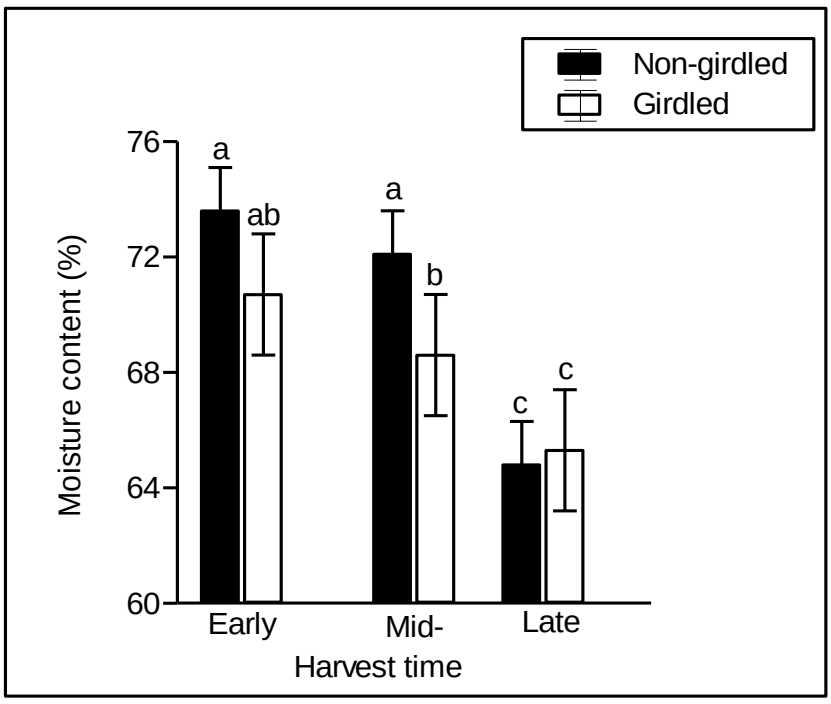

Figure 4.1 Effect of tree girdling and harvest time on ‘Hass’ avocado fruit moisture content.

4.1.2. Skin colouration

Eye colour

An interaction of girdling treatment, harvest time, ripening temperature and duration had no significant effect ($P > 0.05$) on fruit skin eye colour during ripening (Appendix 2). In general, skin eye colour development of fruit from girdled and non-girdled trees minimally increased from emerald green to olive green throughout all harvest time, ripening temperature and duration (Figure 4.2). During late harvest, fruit from non-girdled trees showed a non-significant increase in skin eye colour development when compared with early and mid-harvest fruit, regardless of ripening temperature and duration. With respect to ripening temperature, fruit from girdled and non-girdled trees also change skin eye colour during early and mid-season. However, fruit from non-girdled trees ripened at 21°C showed improved skin eye colour to purple when compared with fruit from girdled trees.

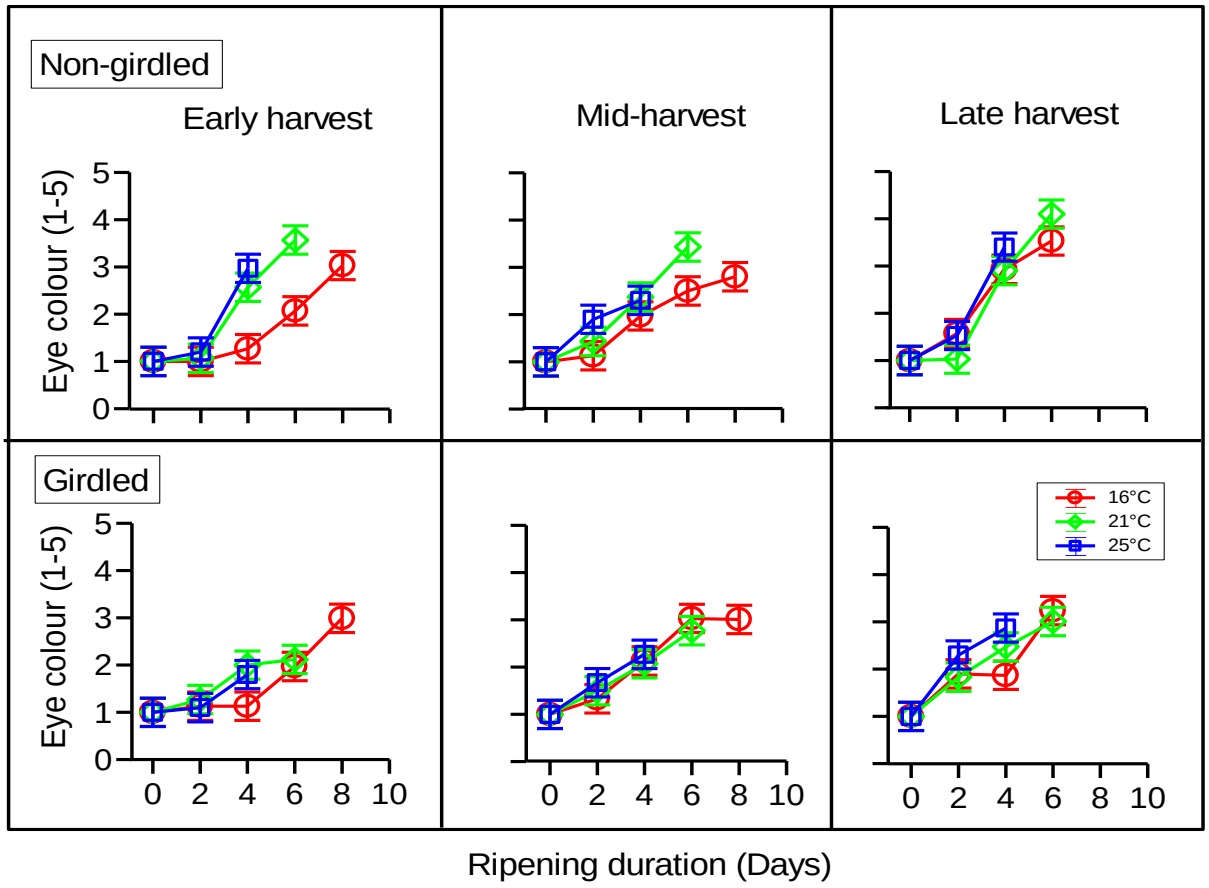

Figure 4.2 Effect of tree girdling, harvest time, ripening temperature and ripening duration on 'Hass' avocado fruit skin eye colour during ripening. The vertical bar represents the mean standard error.

Lightness (L^*)

Girdling treatment, harvest time, ripening temperature, ripening duration and their interaction had a significant effect ($P < 0.05$) on fruit skin lightness (L^*) during ripening (Appendix 3). Overall, fruit from girdled and non-girdled trees showed a decrease in lightness, throughout harvest time, ripening temperature and duration. Furthermore, skin lightness of fruit from girdled trees declined as ripening temperature increased, when compared with fruit from non-girdled trees, throughout all harvest time and ripening duration (Figure 4.3). At mid-season, fruit from non-

girdled trees showed lower lightness when compared with early and late harvest time, irrespective of ripening temperature and duration. Generally, higher skin L* was observed in fruit from girdled tree ripened at 16°C than at 21°C, with the lowest observed at 25°C, when compared with fruit from non-girdled trees, throughout harvest time and ripening duration. Contrary, late season fruit harvested from girdled trees and ripened at 16°C showed a sharp decreased in skin lightness from day 2 throughout ripening when compared with fruit from non-girdled trees.

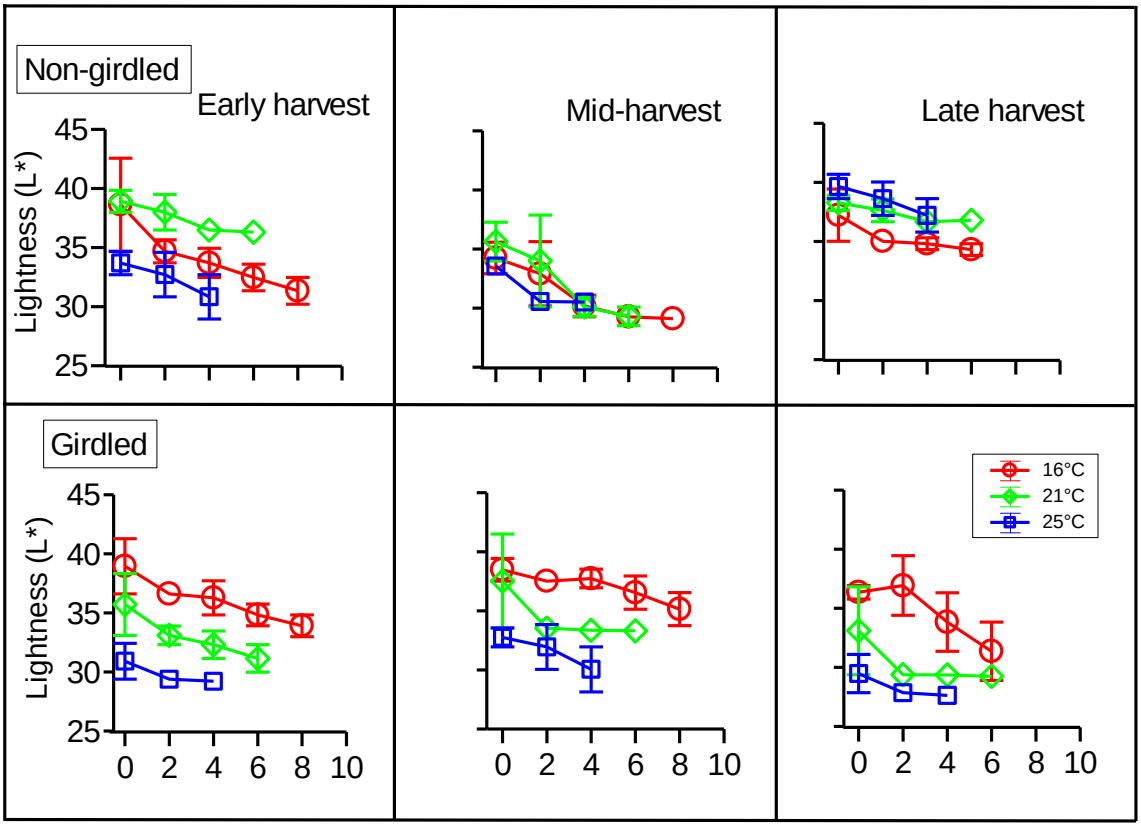

Figure 4.3 Effect of tree girdling, harvest time, ripening temperature and ripening duration on 'Hass' avocado fruit skin lightness (L*) during ripening. The vertical bar represents the mean standard error.

Hue angle ($h°$)

An interaction of girdling treatment, harvest time, ripening temperature and ripening duration had no significant effect (P > 0.05) on fruit skin hue angle ($h°$) during ripening (Appendix 4). In general, fruit from girdled and non-girdled trees showed a decrease in skin hue angle at all harvest time, regardless of ripening temperature and duration (Figure 4.4). However, fruit from non-girdled trees harvested during early and mid-season and ripened at 25°C showed lower hue angle when compared with fruit from girdled, throughout ripening duration. Furthermore, fruit from non-girdled trees during early and late harvest showed higher hue angle when compared with mid-harvest at all ripening temperature and duration. Moreover, fruit ripened at 25°C harvested from girdled and non-girdled trees showed lower skin hue angle when compared with fruit at 16 and 21°C, across all harvest time and ripening duration. At early and late season, fruit ripened at 16 and 21°C from non-girdled trees showed gradual decrease in hue angle when compared with mid-harvest, throughout ripening duration.

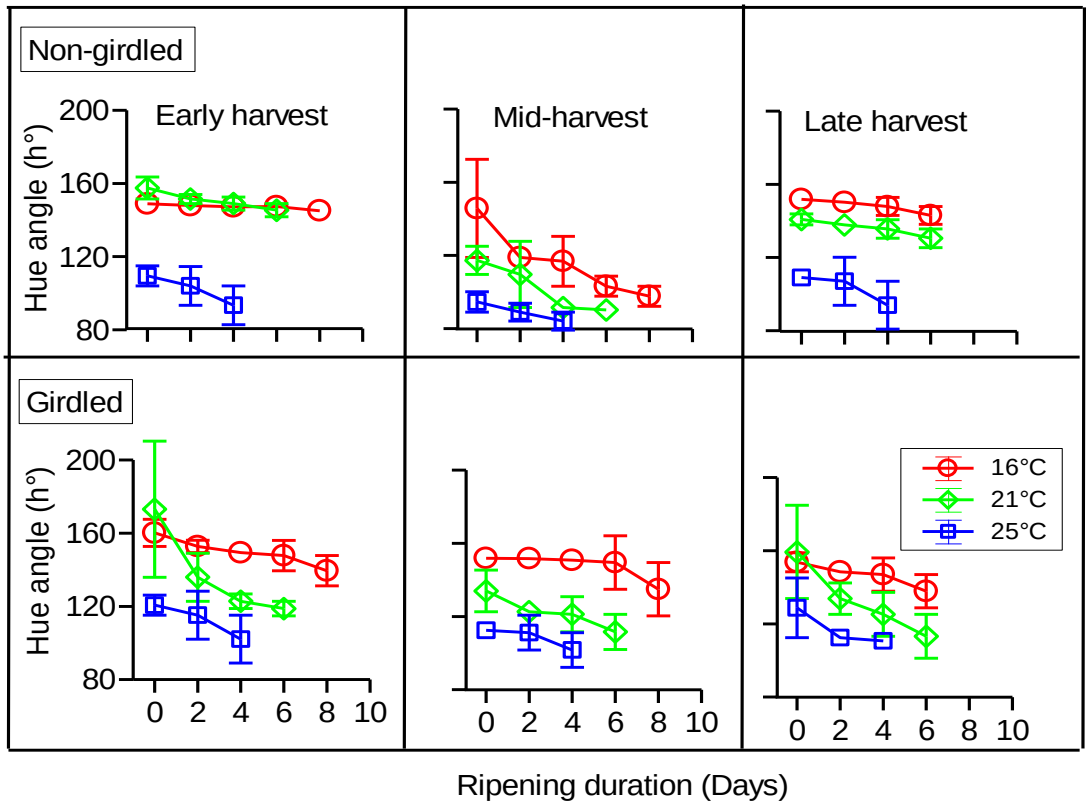

Figure 4.4 Effect of tree girdling, harvest time, ripening temperature and ripening duration on 'Hass' avocado fruit skin hue angle (*h*°) during ripening. The vertical bar represents the mean standard error.

Chroma (C^*)

An interaction of girdling treatment, harvest time, ripening temperature and ripening duration had a significant effect ($P < 0.05$) on fruit skin chroma (C^*) during ripening (Appendix 5). Overall, fruit harvested from girdled and non-girdled trees showed a decrease in skin chroma across all harvest time, ripening temperature and duration (Figure 4.5). Nevertheless, fruit from non-girdled trees showed a non-significant decrease in skin chroma during late harvest when compared with early and mid-harvest, regardless of ripening temperature and duration. Early and mid-harvest fruit from non-girdled trees ripened at 16°C showed higher skin chroma followed by 21°C, with the lowest for fruit ripened at 25°C throughout ripening duration. Additionally,

similar chroma values were observed on late harvested fruit from non-girdled trees when compared with fruit from girdled trees; however, fruit ripened at 25°C showed a rapid decrease throughout ripening duration.

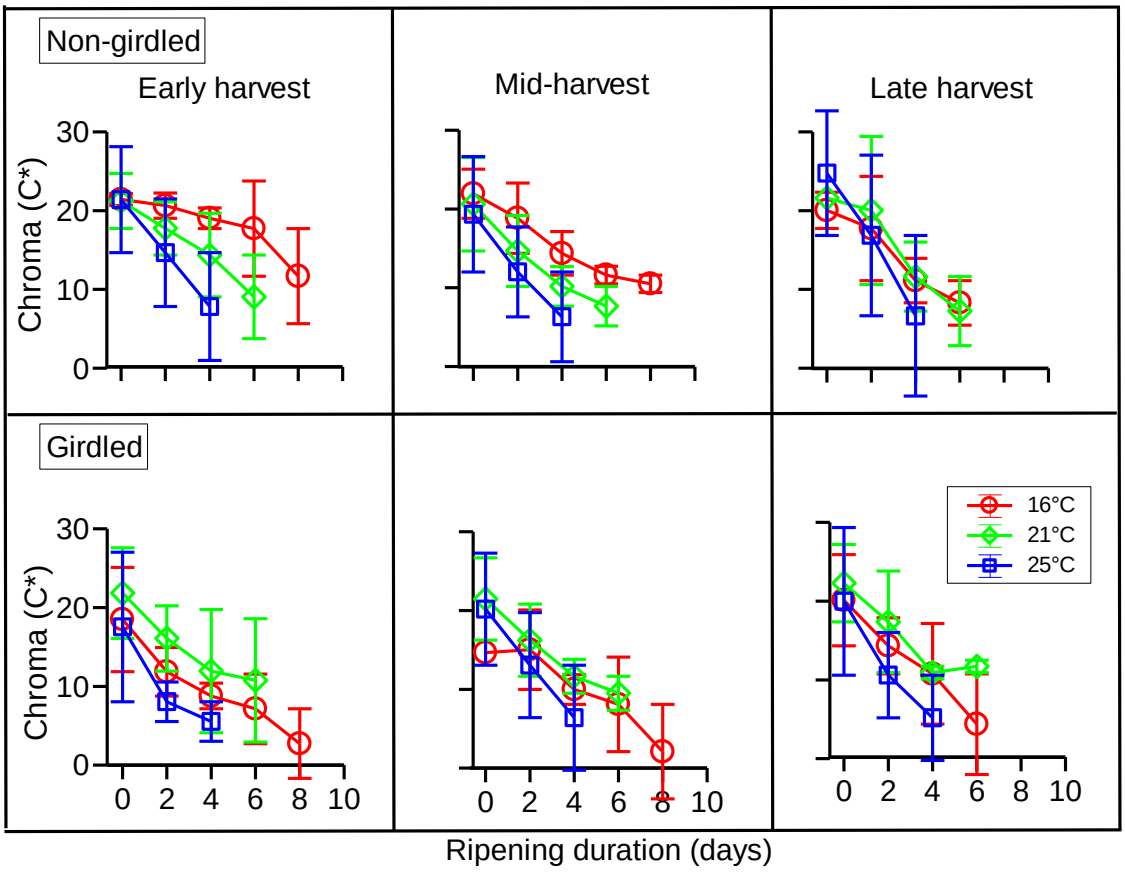

Figure 4.5 Effect of tree girdling, harvest time, ripening temperature and ripening duration on 'Hass' avocado fruit skin Chroma (C*) during ripening. The vertical bar represents the mean standard error.

4.1.3. Firmness

Girdling treatment, harvest time, ripening temperature and ripening duration and their interaction had no significant effect ($P > 0.05$) on fruit firmness loss during ripening (Appendix 6). The results showed that firmness of fruit from girdled and non-girdled trees declined significantly with delayed harvest time, across all ripening temperature and duration (Figure 4.6). However, fruit harvested from girdled trees showed higher

firmness loss when compared with fruit from non-girdled tree, throughout harvest time, ripening temperature and duration. Mid-season fruit harvested from non-girdled trees and ripened at 16°C were slightly firmer and had lower firmness loss than early and late harvested fruit, throughout ripening duration. Furthermore, fruit from non-girdled trees and ripened at 16°C showed lower firmness loss followed by 21°C; and subsequently, fruit at 25°C which showed higher firmness loss during mid-harvest, throughout ripening duration.

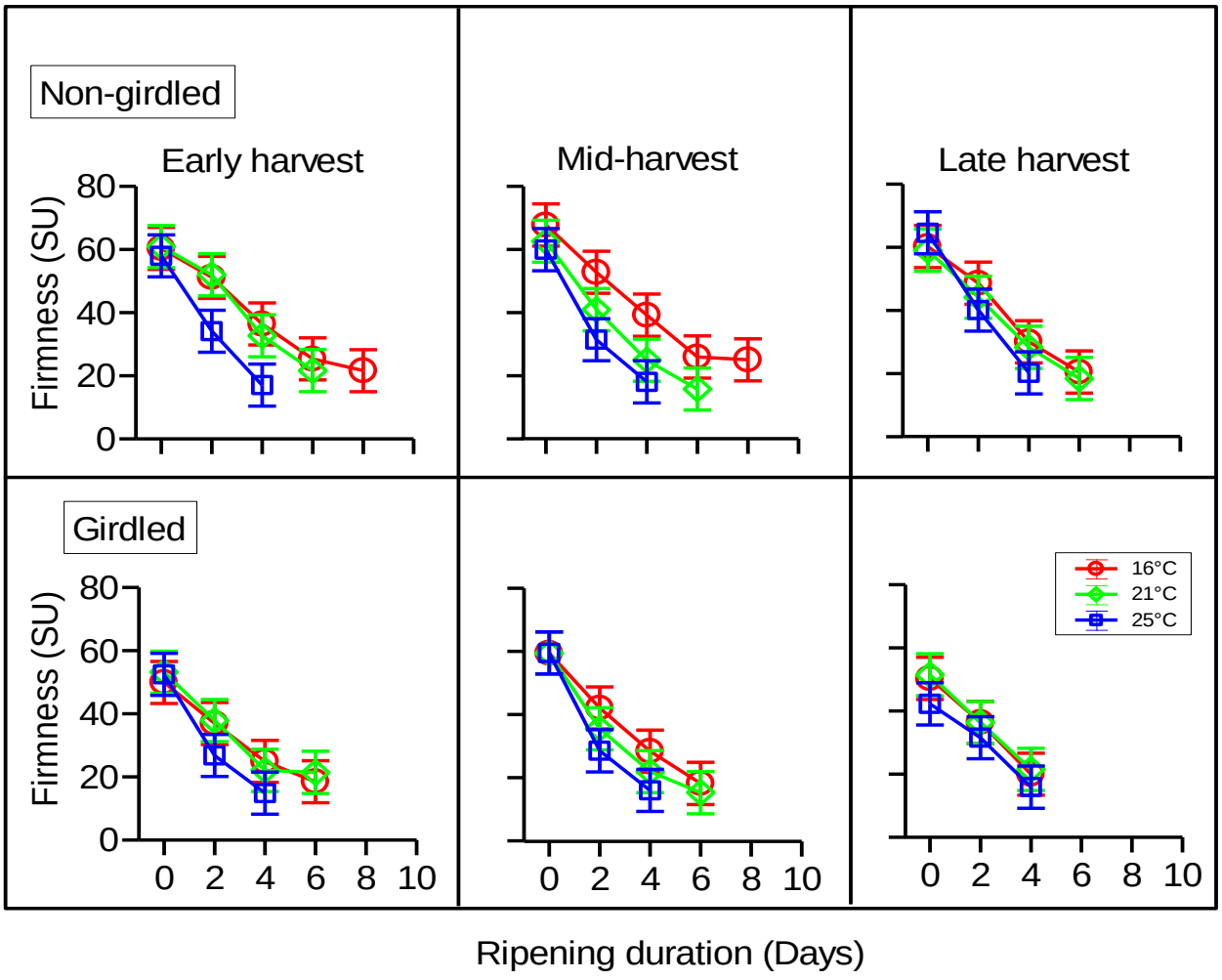

Figure 4.6 Effect of tree girdling, harvest time, ripening temperature and ripening duration on 'Hass' avocado fruit firmness during ripening. The vertical bar represents the mean standard error.

4.1.4. Ripening percentage

An interaction of girdling treatment, harvest time, ripening temperature and ripening duration had no significant effect ($P > 0.05$) on fruit ripening percentage during ripening (Appendix 7). With respect to girdling treatment and harvest time, there was no significant difference in ripening percentage, across all ripening temperature and duration (Figure 4.7). Furthermore, mid-season fruit harvested from non-girdled trees and ripened at 25°C showed a rapid increase in ripening percentage during day 2 when compared with early and late season fruit. Fruit from girdled trees and ripened at 25°C showed a rapid increase in ripening percentage when compared with 16 and 21°C during early harvest, throughout ripening duration. Early and mid-season fruit harvested from non-girdled trees reached higher ripening percentage within 8, 6 and 4 days at 16, 21 and 25°C, respectively. Fruit harvested from girdled trees during early and mid-season reached higher ripening percentage within 6 days (16 and 21°C) and 4 days (25°C). Higher ripening percentage was observed within 4 days on fruit from girdled tree during late harvest at all ripening temperature when compared with fruit from non-girdled trees, except for fruit ripened at 25°C.

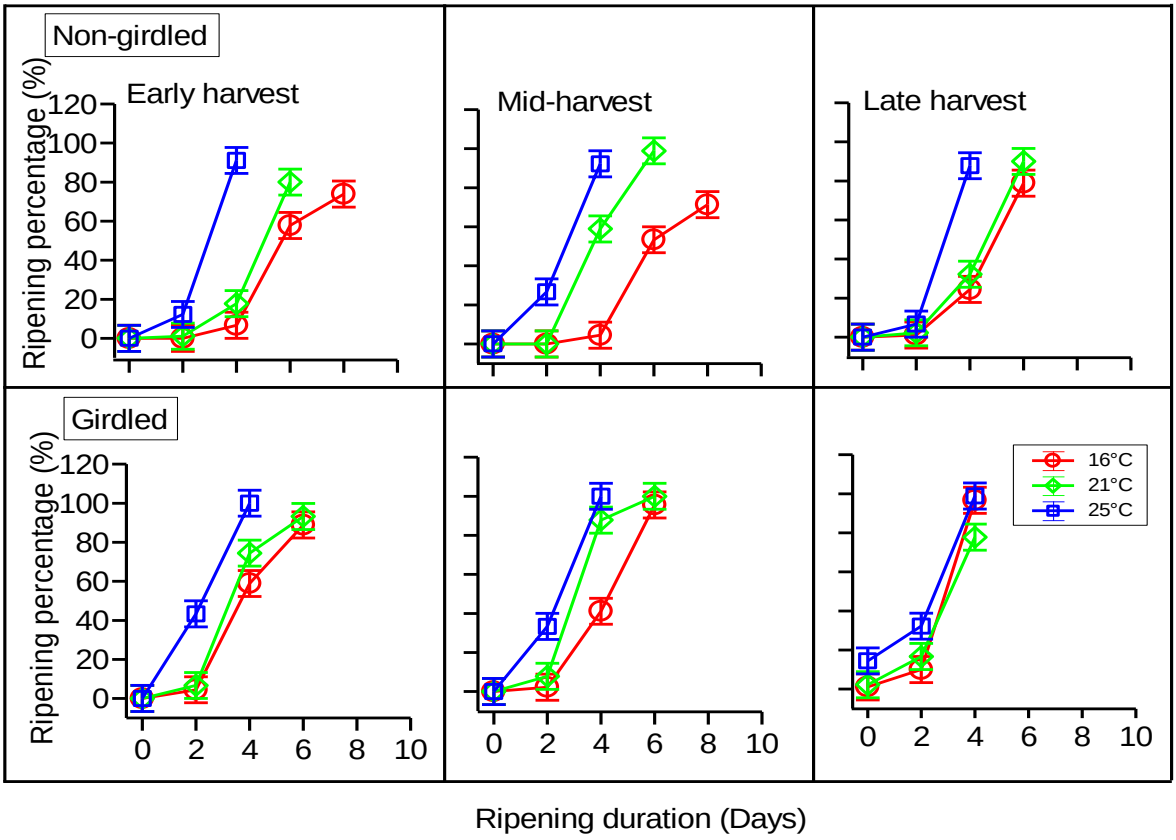

Figure 4.7 Effect of tree girdling, harvest time, ripening temperature and ripening duration on 'Hass' avocado fruit ripening percentage during ripening. The vertical bar represents the standard error of mean.

4.1.5. Chilling injury

An interaction of girdling treatment, harvest time and ripening temperature had no significant effect ($P > 0.05$) on 'Hass' avocado fruit external chilling injury incidence during ripening (Appendix 8). Fruit harvested from girdled trees during late season showed higher external chilling injury when compared with fruit harvested from non-girdled trees, throughout all ripening temperature (Figure 4.8). However, fruit from girdled trees and ripened at higher temperature (21 and 25°C) showed higher external chilling injury during early and mid-harvest when compared with late harvested fruit. Furthermore, early and mid-season fruit harvested from non-girdled trees and ripened at 25°C showed higher external chilling injury symptoms when

compared with fruit ripened at 16 and 21°C. Chilling injury incidence on late harvested fruit from girdled tree non-significantly decreased concomitant with increasing ripening temperature (16, 21 and 25°C).

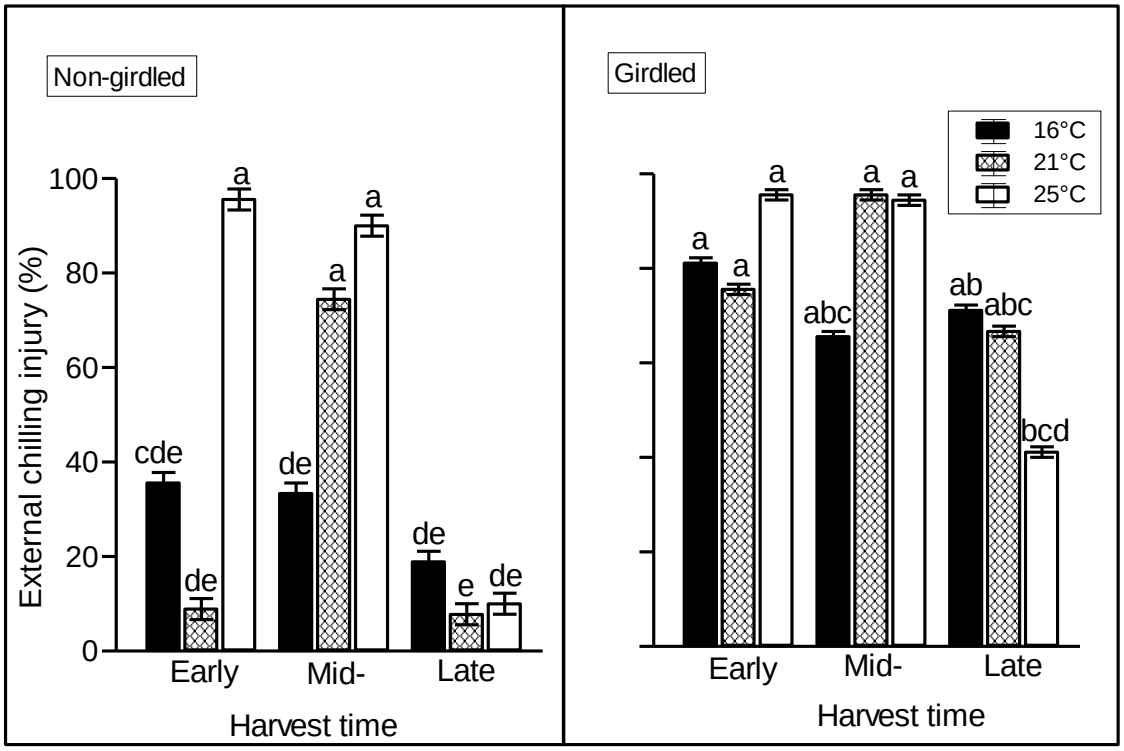

Figure 4.8 Effect of tree girdling, harvest time and ripening temperature on 'Hass' avocado fruit external chilling injury during ripening. Means with the same letter within the same panel were not significant different (P > 0.05) according to Duncan Multiple Range Test. The vertical bar represents the mean standard error.

4.2. Discussion

4.2.1. Moisture content

The South African avocado industry uses moisture content as the preferred maturity indicator, with an ideal recommended moisture content ranging between 69 to 80% depending on the cultivar (Mans *et al.,* 1995). In 'Hass' avocado fruit, 77% is the maximum recommended moisture content (Kassim *et al.,* 2013). In this study, moisture content of fruit harvested from girdled and non-girdled trees decreased with delayed harvest time (Figure 4.1). These findings were in agreement with Kruger *et al*. (1995), who found that moisture content of 'Hass', 'Fuerte', 'Edranol', 'Pinkerton' and 'Ryan' avocado fruit decreased as the harvest time progressed, subsequently, increased physiological fruit maturity. Whiley *et al*. (1988) indicated that 'Hass' avocado fruit attached to the tree and while the tree continues with its normal phonological cycles, concurrently, increase in maturity. This was associated with accumulation of carbohydrate with proceeding harvest date (Liu *et al.,* 1999b). According to Villa-Rodríguez *et al.* (2011), dry weight accumulation of avocado fruit was strongly related to oil content accumulation. Furthermore, it has been reported that moisture content has a divergent relationship with oil content, therefore, as oil content increase with maturity, moisture content decrease (Kruger *et al.,* 1995). In the present study, moisture content reduction in fruit from both girdled and non-girdled trees might be associated with dry weight accumulation which led to increased oil content as fruit maturity advances.

However, there was a notable difference in moisture content of fruit harvested from girdled and non-girdled trees at early and mid-harvest, with lower moisture content observed in fruit from girdled trees than non-girdled trees. In contrary, Lahav *et al*. (1972) found higher moisture content in 'Hass' avocado fruit from girdled branch than

non-girdled branch; the higher moisture content of fruit from girdled branch was associated with lower individual fruit weight. Lata *et al.* (2014) reported that girdling influenced maturity of 'Satluj purple' plum fruit due to high accumulation and availability of metabolites during development. Therefore, the lower moisture content of fruit from girdled trees in the present study might be associated with high carbohydrate accumulation during growth and development.

4.2.2. Skin colouration

Avocado growers, exporters and consumers consider 'Hass' avocado fruit skin colour as an important ripening indicator (Cox *et al.,* 2004). In this study, 'Hass' avocado fruit skin colour was measured objectively (Lightness (L*), Chroma (C*), and Hue angle (h°)) and subjectively (eye colour rating) during ripening (Figure 3.2). According to Lata *et al.* (2014), 'Satluj Purple' plum fruit skin colour change was due to chlorophyll degradation and accumulation of pigments such as anthocyanin and carotenoids. In general, fruit harvested from girdled and non-girdled trees showed a decrease in skin lightness (Figure 4.3), chroma (Figure 4.5) and hue angle (Figure 4.4), across all harvest time, ripening temperature and duration. Previous studies have reported that lightness, chroma and hue angle of 'Hass' avocado fruit decreased with ripening (Cox *et al.,* 2004; Donetti, 2011; Mathaba *et al.,* 2015; Mathaba *et al.,* 2016).

'Hass' avocado fruit harvested from girdled and non-girdled trees showed lower skin lightness and hue angle during mid-harvest when compared with early and late season, across all ripening temperature and duration. In the previous study, early season 'Hass' avocado fruit from South Africa showed higher lightness and hue angle when compared with mid- and late season at both ripening temperature (Donetti, 2011). Early harvested 'Hass' avocado fruit from girdled and non-girdled

trees and ripened at 16°C showed higher skin hue angle even when fully ripe. Similarly, early season 'Hass' avocado fruit from South Africa had greener skin colour with higher hue angle, whereas, fruit from Spain had darker and low hue angle at the end of shelf-life (Donetti, 2011).

'Hass' avocado fruit purple colouration was associated with increased anthocyanin synthesis and decline in chlorophyll content (Cox *et al.,* 2004; Asthon *et al.,* 2006). In this study, skin eye colour development of 'Hass' avocado fruit from girdled and non-girdled trees minimally increased from emerald green (1) to olive green (3) at all harvest time, ripening temperature and duration. However, 'Hass' avocado fruit from non-girdled trees ripened at 21°C had improved skin eye colour to purple (4) when compared with fruit from girdled trees during late harvest (Figure 4.2). Mathaba *et al.* (2015) found that 'Hass' avocado fruit colouring only increased to 3 (olive green) for early harvested fruit, while mid-season improved to 4 (purple) and reach maximum of 4-5 (purple or black) with late season. Girdling increased 'Flame seedless' grape fruit colour development from green (1) to pink (3) while control changed from green (1) to green-yellow (2). Colour change of 'Flame seedless' grape fruit was associated with increased anthocyanin accumulation during fruit development (Soltekin *et al.,* 2016).

At late season, fruit harvested from non-girdled trees showed increase in skin eye colour development when compared with early and mid-harvest fruit, thoughout ripening temperature and duration. However, in the previous study of Donetti and Terry (2014), early harvested 'Hass' avocado fruit from South Africa showed poor eye colour development when compared with fruit harvested during mid- and late season. Moreover, 'Hass' avocado fruit from non-girdled tree during early and mid-harvest showed similar trend of skin colour when ripened at higher temperature (21

and 25°C). Similar results were obtained for skin colour change of ‘Hass’ avocado fruit during ripening at 20 and 25°C (Cox *et al.,* 2004). Previously, ringing and girdling increased sugar accumulation and anthocyanin synthesis, thereby, an improved skin colour development of grapes (Soltekin *et al.,* 2016), pear and nectarine (Agusti *et al.,* 1998) and apple (Mohammad *et al.,* 2012) during ripening. In this study, poor ‘Hass’ avocado fruit skin colour development on fruit harvested from girdled trees might be associated with poor anthocyanin synthesis and low chlorophyll degradation during ripening.

4.2.3. Firmness

Avocado fruit firmness is an important determinant of assessing ripeness degree (Kassim *et al.,* 2013). In this study, ‘Hass’ avocado fruit harvested from girdled and non-girdled trees showed a decrease in firmness across all harvest time, ripening temperature and duration (Figure 4.6). However, higher firmness loss was observed in ‘Hass’ avocado fruit from girdled trees when compared with non-girdled trees, throughout all harvest time, ripening temperature and duration. The previous studies have indicated that girdling treatment resulted in a decreased firmness of ‘Rocha’ pear and ‘Satluj purple’ plums when compared with control, therefore, associated with the advanced fruit maturity (Soursa *et al.,* 2008; Lata *et al.,* 2014). The higher firmness loss of ‘Hass’ avocado fruit from girdled trees was associated with reduced moisture content and advanced maturity.

Mid-season ‘Hass’ avocado fruit from non-girdled trees and ripened at 16°C showed lower firmness loss followed by 21°C; and subsequently, 25°C when compared with early and late season fruit, throughout ripening duration. Previously, Donetti and Terry (2014), indicated that late harvested ‘ Hass’ avocado fruit were characterised by higher maturity; and therefore, resulted in higher firmness loss, regardless of

origin. Higher ripening temperature influenced a rapid decline in firmness and faster softening (Donetti, 2011). According to Donetti (2011), the response of 'Hass' avocado fruit maturity to firmness loss was dependable on different ripening temperature. Therefore, higher ripening temperature and advanced maturity contributed to higher firmness loss of 'Hass' avocado fruit harvested from girdled trees in this study.

4.2.4. Ripening percentage

Ripening process occurs as a result of a decrease in avocado fruit cell wall and membrane integrity, and an increase in respiration rate and ethylene production within 3-4 days of shelf-life depending on temperature (Van Rooyen and Bower, 2006; Kok *et al.,* 2010; Donetti, 2011). According to Mohammad *et al.* (2012), girdling influences a change in hormonal content of 'Jambu madu' wax apple fruit, consequently, enhanced ethylene production, thereby, promoting ripening. In this study, there was an increase in ripening percentage, irrespective of girdling treatment, harvest time, ripening temperature and duration. However, within 4 days, 'Hass' avocado fruit from girdled trees and ripened at 25°C reached 100% while fruit from non-girdled trees reached 90%, across all harvest time (Figure 4.7). Lata *et al.* (2014) found that girdling in combination with fruit thinning treatment resulted in higher firmness loss and resulted in an increased ripening rate of 'Satluj purple' plums when compared with control. The higher ripening percentage observed in 'Hass' avocado fruit from girdled trees might be due to high maturity and higher firmness loss, subsequently, increased climacteric respiration and ethylene production.

According to Hofman *et al.* (1997), advanced 'Hass' avocado fruit maturity result in higher ripening rate when compared with less mature fruit. In this study, early and

late season fruit harvested from girdled trees and ripened at 21°C showed lower ripening percentage when compared with fruit harvested during mid-season. In the previous work of Donetti (2011), mid-season fruit, ripened at 18 and 23°C showed higher ripening rate when compared with early and late season fruit due to higher production of ethylene. However, in this study, early and mid-season 'Hass' avocado fruit harvested from non-girdled trees reached higher ripening percentage within 8, 6 and 4 days at 16, 21 and 25°C, respectively. These results were similar to the findings of Mathaba *et al.* (2015), who found that 'Hass' avocado fruit ripened after 4, 6 and 8 days at 25, 21 and 16°C, respectively. According to Blakey and Bower (2009), ripening rate of 'Hass' avocado fruit increased higher with delayed harvest time and higher ripening temperature. In this study, the delay in ripening of 'Hass' avocado fruit held at 16°C could be associated with reduced firmness loss when compared with fruit at higher temperature.

4.2.5. Chilling injury

Temperature below critical threshold induces a change in cell membrane from liquid-crystalline to solid gel phase which lead to membrane permeability and solute leakage (Lysons, 1973; Siboza, 2013). Furthermore, chilling temperature also induces reactive oxygen species (ROS) accumulation which leads to chilling injury symptoms manifestation after removal from cold storage to higher temperature (Sharma *et al.,* 2012). According to Bolouri-Moghaddam *et al.* (2010), ROS is a reactive oxygen species which is always produced in the presence of oxygen and particularly under stress. In this study, the incidence of chilling injury was more severe in fruit from girdled trees when compared with fruit from non-girdled trees when ripened at 16 and 25°C, irrespective of harvest time. Purvis and Grierson (1982) indicated that an accumulation of reducing sugars in the 'Marsh' grapefruit

peel contribute to reduction of chilling injury incidence. Early and mid-season ‘Hass’ avocado fruit harvested from girdled trees and ripened at higher temperature showed higher chilling injury when compared with late season. These results were similar with previous findings of Bower and Magwaza (2004) who suggested that late harvested ‘Pinkerton’ avocado fruit showed reduced chilling susceptibility due to lower moisture content. In contrast, Dixon *et al.* (2003) reported that ‘Hass’ avocado fruit harvested early and late season had higher chilling injury when stored at 5°C for 28 days. Early and mid-season ‘Hass’ avocado fruit harvested from non-girdled trees and ripened at 25°C showed higher chilling injury symptoms when compared with at 16 and 21°C. Previously, Hopkirk *et al,* (1994), reported that ‘Hass’ avocado fruit ripened at higher temperature exhibited higher incidence and severity of chilling injury. According to Woolf *et al.* (2005), chilling injury degree of ‘Hass’ avocado fruit was closely associated with higher respiration rate and ethylene production, consequently, to advanced maturity and firmness loss. However, higher chilling injury incidence in early harvested ‘Hass’ avocado fruit from girdled and non-girdled trees could be due to high moisture content.

CHAPTER 5
SUMMARY AND CONCLUSION

5.1. Introduction

'Hass' avocado fruit skin colour changes from green to purple then black as ripening progress. However, there have been complains about 'Hass' avocado fruit skin colour not changing as expected. However, pre-harvest practise such as girdling has a potential to improve colour development of other fruit crops such as apples and grapes. Girdling is a management practice used to improve carbohydrate accumulation in the fruit. This technique has never been applied to 'Hass' avocado fruit crop to improve skin colour development, especially on early harvest. Harvest time has potential to influence variable colouring, expected shelf-life and fruit quality. Furthermore, ripening temperature has an influence on the ripening behaviour which indirectly influences colour development of the fruit. This study was carried out to investigated an interactive effect of tree girdling, harvest time ripening temperature and ripening duration on 'Hass' avocado fruit skin colour development during ripening.

5.2. Summary

Girdling treatment was assumed to improve an accumulation of photosynthates (sugars) on the fruit, thus, increased maturing, subsequently, higher firmness loss and ripening percentage of 'Hass' avocado fruit. However, the incidence of variable colouring in 'Hass' avocado fruit during ripening was predominant mainly from girdled tree harvested early in the season, according to eye colour rating, irrespective of ripening temperature and duration. Furthermore, chroma is a variable used to quantify colour and decreased rapidly in fruit from both girdled and non-girdled tree when ripened at 25°C, across all harvest time. Hue angle and lightness

of the fruit skin decreased gradually on ‘Hass’ avocado fruit from girdled tress during early and mid-harvest time, irrespective of ripening temperature and duration. Moreover, girdling treatment resulted in higher chilling injury incidence which had a greater influence on ‘Hass’ avocado fruit skin colour development during ripening. During early harvest, the variable colouring of ‘Hass’ avocado fruit from girdled trees was higher when compared with mid- and late harvested fruit.

5.3. Conclusion

In this study, the treatment combination of girdling tree, harvest time and ripening temperature had no effect on reducing variable colouring of ‘Hass’ avocado fruit. However, girdling improved fruit maturity, ripening rate and firmness loss in ‘Hass’ avocado fruit. Furthermore, the incidence of variable colouring of ‘Hass’ avocado fruit during ripening is also prevalent in early harvested fruit from girdled tree, irrespective of ripening temperature. Thus, the major causes of variable colouring of ‘Hass’ avocado fruit could be external chilling damage, harvest time (early harvest fruit) and to a lesser extent lower ripening temperature. Although, girdling treatment did not reduce the variable colouring of ‘Hass’ avocado fruit during ripening, it did improve the fruit maturity, ripening rate and internal quality. ‘Hass’ avocado fruit poor skin colour development during ripening requires further investigation in terms of the combination of tree girdling and early harvest time.

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Appendix: 1. Effect of tree girdling and harvest time on ‘Hass’ avocado fruit moisture content.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replications	9	84.82	9.42	0.88	
Harvest time (H)	2	544.93	272.47	25.40	<.001
Tree girdling (G)	1	58.02	58.02	5.41	0.025
G × H	2	46.53	23.27	2.17	0.126
Error	45	482.68	10.73		
Total	59	1216.98			

Appendix 2: Effect of tree girdling, harvest time, ripening temperature and ripening duration on ‘Hass’ avocado fruit skin eye colour.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replications	2		0.00542	0.00271	0.03	
Harvest time (H)	2		14.36387	7.18193	73.03	<.001
Tree girdling (G)	1		2.58784	2.58784	26.31	<.001
Ripening Temperature (T)	2		4.13312	2.06656	21.01	<.001
Ripening duration (D)	4		197.50267	49.37567	502.08	<.001
G × H	2		1.20205	0.60102	6.11	0.003
H × T	4		1.66638	0.41659	4.24	0.003
G × T	2		0.96654	0.48327	4.91	0.009
H × D	7	(1)	7.72075	1.10296	11.22	<.001
G × D	3	(1)	5.95146	1.98382	20.17	<.001
T × D	5	(3)	3.86993	0.77399	7.87	<.001
G × H × T	4		1.37026	0.34256	3.48	0.010
G × H × D	5	(3)	2.76708	0.55342	5.63	<.001
H × T × D	10	(6)	2.18893	0.21889	2.23	0.020
G × T × D	5	(3)	2.38575	0.47715	4.85	<.001
G × H × T × D	8	(8)	0.83310	0.10414	1.06	0.396
Error	127	(51)	12.48950	0.09834		
Total	193	(76)	150.29943			

Appendix 3: Effect of tree girdling, harvest time, ripening temperature and ripening duration on 'Hass' avocado fruit skin lightness (L^*).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replications	2		5.0396	2.5198	4.06	
Harvest time (H)	2		25.6001	12.8000	20.60	<.001
Tree girdling (G)	1		47.3459	47.3459	76.20	<.001
Ripening Temperature (T)	2		372.7572	186.3786	299.97	<.001
Ripening duration (D)	4		1802.0610	450.5153	725.09	<.001
G × H	2		10.3236	5.1618	8.31	<.001
H × T	4		21.6115	5.4029	8.70	<.001
G × T	2		99.0402	49.5201	79.70	<.001
H × D	7	(1)	96.7750	13.8250	22.25	<.001
G × D	3	(1)	18.3482	6.1161	9.84	<.001
T × D	5	(3)	39.5959	7.9192	12.75	<.001
G × H × T	4		47.9119	11.9780	19.28	<.001
G × H × D	5	(3)	22.8259	4.5652	7.35	<.001
H × T × D	10	(6)	12.0035	1.2004	1.93	0.046
G × T × D	5	(3)	17.0344	3.4069	5.48	<.001
G × H × T × D	8	(8)	11.5739	1.4467	2.33	0.023
Error	128	(50)	79.5293	0.6213		
Total	194	(75)	1636.8337			

Appendix 4: Effect of tree girdling, harvest time, ripening temperature and ripening duration on hue angle ($h°$) of 'Hass' avocado fruit skin.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replications	2		27.6	13.8	0.10	
Harvest time (H)	2		10148.2	5074.1	35.39	<.001
Tree girdling (G)	1		6797.1	6797.1	47.41	<.001
Ripening Temperature (T)	2		2040.7	1020.3	7.12	0.001
Ripening duration (D)	4		77143.0	19285.8	134.52	<.001
G × H	2		6971.3	3485.6	24.31	<.001
H × T	4		1814.1	453.5	3.16	0.016
G × T	2		73.1	36.5	0.25	0.775
H × D	7	(1)	6658.7	951.2	6.63	<.001
G × D	3	(1)	6037.0	2012.3	14.04	<.001
T × D	5	(3)	4863.8	972.8	6.78	<.001
G × H × T	4		1103.5	275.9	1.92	0.110
G × H × D	5	(3)	3345.3	669.1	4.67	<.001
H × T × D	10	(6)	4129.2	412.9	2.88	0.003
G × T × D	5	(3)	750.8	150.2	1.05	0.393
G × H × T × D	8	(8)	669.2	83.7	0.58	0.790
Error	128	(50)	18351.1	143.4		
Total	194	(75)	109234.1			

Appendix 5: Effect of tree girdling, harvest time, ripening temperature and ripening duration on 'Hass' avocado fruit skin chroma (C^*).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replications	2		2.364	1.182	0.45	
Harvest time (H)	2		579.979	289.989	110.26	<.001
Tree girdling (G)	1		6.648	6.648	2.53	0.114
Ripening Temperature (T)	2		2195.372	1097.686	417.37	<.001
Ripening duration (D)	4		11943.093	2985.773	1135.27	<.001
G × H	2		1121.353	560.677	213.18	<.001
H × T	4		2474.649	618.662	235.23	<.001
G × T	2		389.088	194.544	73.97	<.001
H × D	7	(1)	2561.428	365.918	139.13	<.001
G × D	3	(1)	1336.973	445.658	169.45	<.001
T × D	5	(3)	1433.650	286.730	109.02	<.001
G × H × T	4		1946.596	486.649	185.04	<.001
G × H × D	5	(3)	1989.096	397.819	151.26	<.001
H × T × D	10	(6)	3539.574	353.957	134.58	<.001
G × T × D	5	(3)	2679.066	535.813	203.73	<.001
G × H × T × D	8	(8)	3189.286	398.661	151.58	<.001
Error	128	(50)	336.641	2.630		
Total	194	(75)	29975.834			

Appendix 6: Effect of tree girdling, harvest time, ripening temperature and ripening duration on ‘Hass’ avocado fruit firmness.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replications	2		72.47	36.23	2.28	
Harvest time (H)	2		164.83	82.41	5.19	0.007
Tree girdling (G)	1		3053.16	3053.16	192.26	<.001
Ripening Temperature (T)	2		3753.12	1876.56	118.17	<.001
Ripening duration (D)	4		71217.36	17804.34	1121.14	<.001
G × H	2		185.15	92.58	5.83	0.004
H × T	4		745.15	186.29	11.73	<.001
G × T	2		358.74	179.37	11.29	<.001
H × D	6	(2)	491.29	81.88	5.16	<.001
G × D	3	(1)	352.66	117.55	7.40	<.001
T × D	5	(3)	738.61	147.72	9.30	<.001
G × H × T	4		268.33	67.08	4.22	0.003
G × H × D	5	(3)	151.70	30.34	1.91	0.097
H × T × D	10	(6)	179.54	17.95	1.13	0.345
G × T × D	5	(3)	117.45	23.49	1.48	0.201
G × H × T × D	8	(8)	114.56	14.32	0.90	0.517
Error	124	(54)	1969.20	15.88		
Total	189	(80)	49863.79			

Appendix 7: Effect of tree girdling, harvest time, ripening temperature and ripening duration on ripening percentage of 'Hass' avocado fruit.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replications	2		145.40	72.70	0.84	
Harvest time (H)	2		3122.21	1561.11	17.95	<.001
Tree girdling (G)	1		17414.72	17414.72	200.19	<.001
Ripening Temperature (T)	2		34023.57	17011.78	195.56	<.001
Ripening duration (D)	4		444176.75	111044.19	1276.50	<.001
G × H	2		933.59	466.79	5.37	0.006
H × T	4		5218.72	1304.68	15.00	<.001
G × T	2		2616.90	1308.45	15.04	<.001
H × D	6	(2)	2196.77	366.13	4.21	<.001
G × D	3	(1)	8061.66	2687.22	30.89	<.001
T × D	5	(3)	14009.54	2801.91	32.21	<.001
G × H × T	4		146.94	36.74	0.42	0.792
G × H × D	5	(3)	987.14	197.43	2.27	0.052
H × T × D	10	(6)	4832.63	483.26	5.56	<.001
G × T × D	5	(3)	8336.46	1667.29	19.17	<.001
G × H × T × D	8	(8)	1029.22	128.65	1.48	0.171
Error	124	(54)	10786.94	86.99		
Total	189	(80)	287517.06			

Appendix 8: Effect of tree girdling, harvest time and ripening temperature on external chilling injury of ‘Hass’ avocado fruit.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replications	2	659.6	329.8	1.07	
Harvest time (H)	2	15247.7	7623.8	24.77	<.001
Tree girdling (G)	1	16246.9	16246.9	52.78	<.001
Ripening Temperature (T)	2	4128.9	2064.4	6.71	0.004
G × H	2	1832.0	916.0	2.98	0.064
H × T	4	12292.3	3073.1	9.98	<.001
G ×T	2	3590.4	1795.2	5.83	0.007
G × H × T	4	1109.5	277.4	0.90	0.474
Error	34	10466.6	307.8		
Total	53	65573.9			