
School of Agriculture and Environment

**Pre-harvest factors affecting fruit quality in sweet oranges with an
emphasis on albedo breakdown**

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**This thesis is presented for the Degree of
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of
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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature _____

Date _____

Dedication

*I dedicate this thesis to my mother, Xuan Dao Vo, and my father, Duc Vinh Pham,
for their love, incredible patience and endless support.*

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Abstract

Albedo breakdown known as creasing, a physiological disorder, due to abnormal separation of cells leading to the formation of irregular fractures in the white tissue (albedo) causing the creases of sweet orange rind. It causes serious economic losses to the sweet orange growers in Australia and in other orange producing areas of the world.

Fruit quality, particularly albedo breakdown has been influenced by various factors such as plant water relations, genetic factors, plant nutritional status and plant growth regulators. My research investigated the development of the incidence and the severity of albedo breakdown during fruit maturation and ripening, the effects of severity of albedo breakdown on fruit quality among locations and cultivars of 'Navel' sweet orange. I also elucidated the influence of deficit regulated irrigation, exogenous application of surfactants added in calcium solution, exogenous application of boron and the role of ethylene in the incidence of albedo breakdown, textural properties of the rind and fruit quality of 'Navel' sweet oranges.

The incidence and the severity of albedo breakdown increased rapidly after commercial harvest. The incidence and severity of albedo breakdown in 'Washington Navel' orange differed from location to location, with the lowest at Harvey as compared to three other locations. Regardless of locations and cultivars, the severity of albedo breakdown did not affect juice content, soluble solids concentration, titratable acidity, ascorbic acid, citric acid and malic acid except for decreasing succinic acid and increasing tartaric acid. Locations and cultivars significantly influenced these fruit quality parameters.

The application of deficit irrigation (50% and 75% water supply of control trees) improved fruit quality in terms of increased soluble solids concentrations and acidity levels without affecting percentage of juice, pH of juice, ascorbic acid and individual organic acids in 'Navelina' sweet orange. The enhancement of the uptake of Ca in leaf, rind, and pulp of the fruit and the reduction in the incidence of albedo breakdown were obtained with the application of different surfactants added into aqueous solutions of 2% $\text{Ca}(\text{NO}_3)_2$ starting from 81 days after full bloom (DAFB) at 10-day intervals. Among four tested surfactants, 'Tween 20' (0.05%) was the most

effective in enhancing Ca uptake, reducing albedo breakdown and improving textural properties of rind and fruit firmness while maintaining the other important fruit quality attributes in 'Washington Navel' sweet orange.

The foliar application of boron enhanced the concentration of boron and calcium in the leaf, rind and pulp. The single spray application of boron in early summer at $600 \text{ mg}\cdot\text{L}^{-1}$ was the most effective in increasing boron concentration in the leaf, rind and pulp of fruit, reducing the incidence of albedo breakdown and improving textural properties of rind and fruit firmness without affecting any the other fruit quality attributes in 'Washington Navel' sweet orange.

Rind of fruit with albedo breakdown produced the higher ethylene production than the normal fruit. The exogenous application of ethylene inhibitors including AVG ($200 \text{ mg}\cdot\text{L}^{-1}$) and CoSO_4 ($300 \text{ mg}\cdot\text{L}^{-1}$) reduced the incidence of albedo breakdown and improved the rind textural properties in 'Washington Navel' sweet orange. Ethylene seems to be involved in the incidence of albedo breakdown.

In conclusion, the severity of albedo breakdown did not affect the major attributes of fruit quality in 'Navel' sweet oranges. The applications of deficit irrigation, exogenous 2% $\text{Ca}(\text{NO}_3)_2$ containing 'Tween 20' as a surfactant and the exogenous spray application of boron ($600 \text{ mg}\cdot\text{L}^{-1}$) influenced the incidence and severity of albedo breakdown without affecting other fruit quality parameters. Ethylene seems to be associated with the incidence of albedo breakdown in 'Washington Navel' sweet orange.

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List of symbols and abbreviations

%	Percent
°C	Degree Celsius
AB	Albedo breakdown
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of variance
AVG	Aminoethoxyvinylglycine
C ₂ H ₄	Ethylene
Ca	Calcium
CaCl ₂	Calcium dichloride
cm	Centimetre
Co	Company
Cv	Cultivar
DAFB	Days after full bloom
DAS	Days after spray
DI	Deficit irrigation
DPPH	1,1-diphenyl-2-picryl-hydrazyl
EDTA	Ethylene diamino tetra acetic acid
FAO	Food and Agriculture Organisation
Fig.	Figure
g	Gram
GA ₃	Gibberellic acid
gal	gallon
GC	Gas chromatography
h	Hour
ha	Hectare
HCl	Hydrochloric acid
HLB	Hydrophile-lipophile balance
HPLC	High performance liquid chromatography
IAA	Indole-3-acetic acid
ICP-AES	Radial Inductively Coupled Plasma Atomic Emission Spectrometry
K	Potassium

kg	Kilogram
kPa	kilo pascal
L	Litre
lat.	Latitude
LSD	Least significant different
Ltd.	Limited
m	Metre
M	Molar
MeOH	Methanol
Mg	Magnesium
mg	Milligram (s)
min.	Minute
mL	Millilitre
mm	Millimetre (s)
mM	Millimole
Mn	Mangan
mQ	milliQ
N	Newton
N	Nitrogen
NaOH	Sodium hydroxide
NH ₄	Ammonium
nL	Nanolitre
NO ₃	Nitrate
ns	not significant
NSW	New South Wales
O ₂	Oxygen
<i>P</i>	Probability
Pa	Pascal
pH	Symbol denoting hydrogen ion in a solution
PRD	Partial rootzone drying
psi	Pounds per square inch
<i>r</i>	correlation coefficient
RH	Relative humidity
rpm	Rotation per minute

RT	Retention time
s	Second (s)
SEM	Standard errors of means
SAM	S-adenosylmethionine
sp.	Species
SSC	Soluble solids concentration
TA	Titrateable acidity
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
U.K.	United Kingdom
USA	United State of America
UV	Ultra-violet
v/v	Volume to volume
WHO	World Health Organisation
µg	Microgram (s)
µL	Microliter (s)
µM	Micromole (s)

CHAPTER 1

General introduction

Oranges (*Citrus sinensis* [L.] Osb.) are one of the major fruit crops in the world with an estimated production of 63,906,064 tonnes in 2007 (FAOSTAT, 2009). In Australia, oranges are the second most important fruit crop after grapes with an estimated production of 585,000 tonnes which accounted for approximately 49% of the Australian total fruit production in 2007 (FAOSTAT, 2009). 'Navel' oranges contribute to 46% of Australian orange production and the supply for fresh fruit market is available in winter from June to August. The citrus exports had the largest total volume (24%) as compared to the other horticultural products exports from Australia (Horticulture Australia Limited, 2008). Oranges are grown in New South Wales, Victoria, South Australia, Queensland and Western Australia, but New South Wales has the largest share in orange production (Australian Bureau of Statistics, 2009). Citrus industry of Western Australia contributed 15,000 tones to the Australian citrus production. Oranges are grown from Gingin in the North to Bunbury in the South of Perth where there is a temperate Mediterranean climate, suitable soils and availability of good quality irrigation water (Foord et al., 2004; Hancock, 2008). There was an increase of 25% in production from 2000 to 2004 in Western Australia (Foord et al., 2004).

Sweet oranges probably originated from central China and North- East India including 'Navel' oranges, common oranges, pigmented oranges and acidless or sugar oranges. Among them, 'Navel' oranges are mainly produced for fresh market. They are known as the large, seedless and earlier maturing type as compared to the other types of oranges. Late season 'Navel' oranges are designated for export leading to the expansion of 'Navel' areas (Horticulture Australia Limited, 2004). However, the recurring incidence of albedo breakdown is a major problem affecting the fruit quality in 'Navel' oranges particularly the appearance of fruit.

Albedo breakdown also known as creasing is a physiological disorder due to cracks in the internal white tissue (albedo) causing puffiness of orange peel (Treeby and Storey, 2002). The development of crease is connected to the degradation of pectin that is an important component in cell walls of fruit tissue. The loosening of the connections between cells is the result of this degradation.

Albedo breakdown causes considerable losses to the growers due to the dramatic reduction in price by the down-grading of orange value for fresh market in Australia (Pellizo, 1997; Sneath, 1987; Storey and Treeby, 1994) and other citrus producing areas in the world (Ali et al., 2000; Bower, 2004; Gambetta et al., 2000; Jones et al., 1967; Li et al., 2009; Monselise et al., 1976). A very high proportion of fruit (from 50 % to 90%) could be influenced by albedo breakdown in some localities in South Africa and Australia, respectively (Goldie, 1998). It is estimated that with each 1% of reduction in albedo breakdown, producers' income will increase by 1 to 2 million dollars in Australia and Israel (Gilfillan et al., 1981; Goldie, 1998; Monselise et al., 1976; Pellizo, 1997; Sneath, 1987; Treeby and Storey, 1994).

The relationship between albedo breakdown and fruit quality has been established by those who reported that higher specific gravity, a thinner peel, a higher percentage of juice, a lower acid content, and especially a lower ascorbic acid content were associated with albedo breakdown in comparison to normal fruit (Jones and Embleton, 1967; Jones et al., 1967; Sneath, 1987). Higher total soluble solids and acid ratio in fruit with albedo breakdown indicated that fruit with albedo breakdown matured earlier than normal fruit at the same time on the same tree (Jones and Embleton, 1967). Similarly, Treeby and Storey (1994) reported that albedo breakdown adversely affected fruit quality. In contrast, Goldie (1998) reported that internal fruit quality parameters were not affected by albedo breakdown. The research work reported on the effects of albedo breakdown on fruit quality is sporadic and inconclusive thus warrants further investigation.

Various factors affecting incidence of albedo breakdown in sweet oranges have been reported such as plant water relations (Agustí et al., 2004; Gonzalez-altozano and Castel, 1999; Sneath, 1987; Treeby et al., 2007), genetic factors (Agusti et al., 2003; Bevington et al., 1993; Moulds et al., 1995; Treeby et al., 1995), plant nutritional status (Ali et al., 2000; Bower, 2004; Jones et al., 1967; Storey et al., 2002; Treeby and Storey, 2002) and plant growth regulators (Dick, 1995; Embleton et al., 1973; Jona et al., 1989; Treeby and Storey, 1994; Tugell et al., 1993).

It has been reported that albedo breakdown may be due to water stress during the late dry summer and autumn periods (Sneath, 1987). However, Treeby (1996) claimed that it is not associated with water stress. Albedo breakdown incidence seems to be a result of the rapid increase in fruit size after the first eight weeks of fruit

development (Tugell et al., 1993). Medium fruit size (62 – 79 mm in diameter) were to be more prone to the albedo breakdown than the smaller fruit (less than 62 mm in diameter) and the bigger fruit (more than 80 mm in diameter) (Storey et al., 2003). Deficit irrigation has been reported as being a very good method for controlling fruit growth in ‘Navel’ oranges (Hutton et al., 2007). It has been reported that a reduction in the incidence of albedo breakdown occurred with the application of regulated deficit irrigation and partial rootzone drying (water applied at 50% of control trees) over the whole growing seasons in ‘Bellamy’ navel oranges (Treeby et al., 2007). The application of deficit irrigation in the second year at the flowering and fruit set phases reduced incidence of albedo breakdown in mandarins (Gonzalez-altozano and Castel, 1999). However, no research work has been reported on the effects of deficit irrigation on albedo breakdown and fruit quality in sweet orange grown under Mediterranean climate of Western Australia.

A limited success in the reduction of albedo breakdown in sweet oranges has been reported with application of potassium nitrate, potassium sulphate as well as phosphorous (Bevington et al., 1993; Jones et al., 1967). Nutritional factors which result in thicker rind reduced the incidence of albedo breakdown in sweet orange (Ali et al., 2000; Bevington et al., 1993; Embleton et al., 1973; Jones et al., 1967; Monselise et al., 1976). Some authors reported that lower levels of Ca in oranges are associated with albedo breakdown (Storey et al., 2002; Treeby and Storey, 2002). Treeby and Storey (2002) showed that the application of five foliar sprays of either 0.11% or 0.33% calcium starting in December – January period or the January - February period at an early stage of ‘Navel’ orange fruit growth resulted in decreased albedo breakdown as calcium sprays increased the Ca levels in the rind and albedo of the fruit. However, increased calcium concentration in fruit does not always eventuate with the foliar applications of calcium solution as calcium is xylem mobile (Schonherr, 2001; Treeby and Storey, 2002) and cuticles are the first barriers to prevent the penetration of calcium into fruit (Schonherr, 2001). Therefore, the attempt to enhance the uptake of calcium into fruit with different surfactants has been reported in apples (Roy et al., 1996) and mango (Singh et al., 2000). Whilst, no information is available on the effects of different surfactants on improving the uptake of calcium with the foliar spray application of calcium and relating this to the incidence of albedo breakdown in sweet oranges.

Boron has been reported to be involved in increasing the soluble forms of calcium and stimulating its movement into the apple fruit (Shear, 1975; Zude et al., 1997). The binding calcium to the cell walls was associated with the presence of boron. Smith and Reuther (1950) reported that the absorption of calcium was reduced with the high boron concentration in the leaves and fruit of citrus. The foliar application of boron have been reported to increase fruit size, soften the rind of fruit, decrease the rind thickness and increase the percentage of juice and the ascorbic acid content in sweet orange (Tariq et al., 2007). However, Smith (1955), Boaretto et al. (1997) and Maurer and Truman (2000) reported that yield, fruit size, rind thickness and fruit quality parameters such as juice content, soluble solids concentration and titratable acidity were not affected with the foliar application of boron in 'Navel' and 'Valencia' oranges. No research work has been reported on the effects of foliar application of boron on the incidence of albedo breakdown in oranges.

The role of ethylene, a plant hormone, is well known in basic plant processes such as fruit maturity, ripening, and senescence (Ladaniya, 2007; Rath and Prentice, 2004). Ethylene has been reported to regulate fruit colour, flavour, chemical composition and texture in citrus fruits (Ladaniya, 2007; Oetiker and Yang, 1995). Monselise et al. (1976) reported that fruit with albedo breakdown produced higher concentrations of ethylene in the internal atmosphere of the fruit ($0.09 \text{ mL}\cdot\text{kg}^{-1}$) than the normal fruit ($0.04 \text{ mL}\cdot\text{kg}^{-1}$) in 'Valencia Late' orange. Exogenous application of ethylene has been reported to increase respiration rate, stimulate ripening, and enhance colour in citrus fruits (Agusti et al., 2002; Al-Mughrabi et al., 1989; Burg, 2004; Ladaniya, 2007; Monselise et al., 1976; Porat et al., 1999). An increase in peel puffing in Satsuma mandarin was observed with the exogenous application of ethephon ($250 \text{ mg}\cdot\text{L}^{-1}$) seven days before harvest (Burg, 2004; Ladaniya, 2007). The research work reported on the role of ethylene in causing albedo breakdown in sweet oranges is not known.

To date the research work conducted on the effects of severity of albedo breakdown on fruit quality, management of this disorder with deficit irrigation and exogenous application of Ca containing different surfactants and B are sporadic and inconclusive. Therefore in the current investigations, the effects of severity of albedo breakdown on fruit quality of 'Navel' sweet oranges were investigated. I also investigated the effects of regulated deficit irrigation, exogenous application of Ca

containing different surfactants and B on the incidence of albedo breakdown, textural properties of the rind and fruit quality of ‘Navel’ sweet oranges.

The main objectives of my research were to:

1. Determine the influence of severity of albedo breakdown on fruit quality in ‘Navel’ oranges.
2. Investigate the effects of deficit irrigation on plant water relations, yield, incidence of albedo breakdown and fruit quality in ‘Navelina’ orange.
3. Elucidate the effects of different surfactants on uptake of calcium, fruit quality, incidence of albedo breakdown and the textural properties of rind and fruit in ‘Washington Navel’ orange.
4. Explore the influence of foliar application of boron on the incidence of albedo breakdown and the textural properties of rind and fruit in ‘Washington Navel’ orange.
5. Investigate the role of ethylene in albedo breakdown, textural properties of rind and fruit in ‘Washington Navel’ orange.

CHAPTER 2

General literature review

2.1. Introduction

Sweet oranges (*Citrus sinensis* [L.] Osb.) are probably native to central China and North East India. Sweet oranges are the most widely grown group and contribute to the largest production of all commercial citrus species in the world. Orange was first introduced from Brazil into Australia by colonists of the First Fleet and planted in New South Wales in 1788 (Davies and Albrigo, 1994; Spiegel-Roy and Goldschmidt, 1996). Based on the morphological characteristics, chemical constituents, and for convenience, sweet oranges are divided into four distinct groups including the common or round oranges, the ‘Navel’ oranges, the pigmented (red) oranges and the acidless (sugar) oranges. The round oranges are the most important commercial group of sweet oranges and are mainly used for processing. The second group of sweet oranges in the planted area and in the production are the ‘Navel’ oranges which are primarily planted for the fresh fruit market (Davies and Albrigo, 1994; Godden, 1988; Spiegel-Roy and Goldschmidt, 1996).

Orange production increased slightly in Asia, Europe and Oceania while it decreased in America and remained relatively stable in Africa from 2005 to 2007 (Table 2.1).

Table 2.1. Orange production (tonnes) by different continents during 2005 – 2007 (FAOSTAT, 2009)

Continents	Year		
	2005	2006	2007
America	34,724,403	34,321,385	34,045,552
Asia	16,231,571	17,005,493	17,419,153
Europe	5,827,226	6,044,901	6,199,986
Africa	5,585,416	5,666,007	5,647,433
Oceania	507,351	580,365	593,940
World	62,875,967	63,618,151	63,906,064

Major orange producing countries and their total orange production (%) in 2007 are shown in Fig. 2.1. Brazil was the largest orange producer which accounted for 28%

of world orange production, followed by the United State of America that contributed about 12% to world orange production (FAOSTAT, 2009).

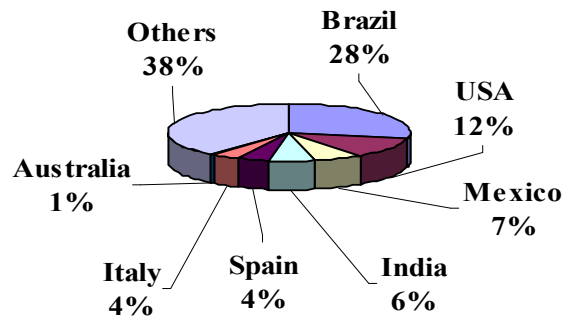


Figure 2.1. Orange production in different countries of the world in 2007 (FAOSTAT, 2009)

In 2006, approximately 2.76 million tonnes of fresh oranges were exported from different countries (Table 2.2).

Table 2.2. Fresh oranges exports by principal countries in the world in 2006 (FAOSTAT, 2009)

Countries	Quantity (tonnes)	Value (1000 \$)
Spain	1,311,605	958,301
South Africa	1,006,917	317,233
United State of America	546,503	370,693
Egypt	282,698	65,272
Morocco	262,612	117,771
Greece	227,298	107,659
Turkey	219,401	89,651
Argentina	177,703	59,774
Netherlands	176,912	119,600
Australia	127,536	100,306
Italy	100,633	68,742
World	5,317,682	2,762,121

Spain contributed to the largest exporting quantities of oranges in the world with the production of 1,311,605 tonnes. South Africa and United State of America were also

the major exporters for fresh fruit export markets (FAOSTAT, 2009) although the United States is known for orange production mainly for processing due to their climatic and industrial capacity to produce high-quality, processed, frozen concentrate orange juice (Davies and Albrigo, 1994). In 2006, Australia ranked the eleventh among the other orange exporting countries with 127,536 tonnes which accounted 23.33% of total world orange exports (Table 2.2).

The import of fresh oranges was approximately 5.5 million tonnes by different countries in 2006 (Table 2.3). Russian Federation led the world in importing fresh oranges. Western European countries were significant orange importing countries including The Netherlands, France, Germany and United Kingdom (Table 2.3). Canada and China also imported major amounts of fresh oranges.

Table 2.3. Major orange importing countries in the world in 2006 (FAOSTAT, 2009)

Countries	Quantity (tonnes)	Value (1000\$)
Russian Federation	509,842	281,359
Netherlands	438,794	271,067
Germany	438,101	302,265
France	407,991	316,597
United Kingdom	346,711	214,823
Saudi Arabia	323,842	111,922
Canada	209,189	137,875
China, Hong Kong SAR	174,117	144,301
Belgium	148,425	128,435
World	5,504,652	3,315,632

SAR = Special Administrative Region

In Australia, oranges are the second important fruit crop after grapes with its production estimated at 585,000 tonnes from harvested area of 28,500 ha in 2006. The Australian orange production contributed to only 1% of the total world orange production (Fig. 2.1).

Oranges are grown in New South Wales, South Australia, Queensland, Victoria and Western Australia but the most Australian orange production comes from New South

Wale (224,800 tonnes) in 2006 – 2007, followed by South Australia (167,400 tonnes) (Australian Bureau of Statistics, 2009).

Although citrus industry of Western Australia contributes to only 5% of the total Australian citrus production, oranges in Western Australia have a good colour and taste leading to top grade in Australia. In Western Australia, oranges are grown from Gingin in the North to Bunbury in the South of Perth where soils and climate are suitable and a good quality of irrigation water is available (Foord et al., 2004).

2.2. Fruit growth

Citrus fruit consists of two morphological regions: the pericarp (peel or rind) and the endocarp (pulp). The external layer of pericarp is called flavedo and the white internal layer is known as the albedo (Godden, 1988; Iglesias et al., 2007; Ladaniya, 2007; Spiegel-Roy and Goldschmidt, 1996). According to the characteristics of the fruit growth, the development of the citrus fruit can be divided into three stages (Bain, 1958; Godden, 1988; Iglesias et al., 2007; Ladaniya, 2007; Spiegel-Roy and Goldschmidt, 1996). The length of the stages can be slightly changed depending on the citrus fruit variety and location. Generally, the length of stage I is from about 4 to 9 weeks. This stage is the cell division period lasting from full bloom to cell division completed in all tissues except the outermost cell layers. The pericarp grows very quickly and the albedo can reach 90% of the fruit volume at this fruit development stage (Iglesias et al., 2007; Ladaniya, 2007; Spiegel-Roy and Goldschmidt, 1996). In ‘Valencia’ oranges, this period can be longer depending on the date of the blossom (Bain, 1958; Godden, 1988). In Australia, this stage normally lasts from October (full bloom) to about mid December in ‘Navel’ oranges (Hutton et al., 2007). Stage II, a critical growth period, is the cell enlargement with the rapid morphological and physiological changes of fruits. At this stage the endocarp growth becomes very active and the volume percentage of the albedo is decreased (Godden, 1988; Iglesias et al., 2007; Ladaniya, 2007; Spiegel-Roy and Goldschmidt, 1996). In Australia, this stage lasts about 29 weeks and starts from mid December to mid July in ‘Valencia’ oranges (Bain, 1958) and to mid May in ‘Navel’ oranges (Hutton et al., 2007). Stage III is the fruit maturation period in which morphological, anatomical, and physiological changes are decreased. The flavedo colour also changes from yellow to orange in this stage (Bain, 1958; Iglesias et al., 2007; Ladaniya, 2007; Spiegel-Roy and Goldschmidt, 1996). This stage starts after the growth period (Stage II)

completion and finishes at the end of the harvest. In ‘Navel’ oranges in Western Australia, the stage III starts from mid May to November (Hutton et al., 2007).

2.2.1. Fruit weight, volume and size

The typical changes in weight, volume and size of the orange fruit during fruit growth were reported as indicated in Fig. 2.2 (Bain, 1958). During stage I the fruit size increased mainly because of the growth of the pericarp. The data in the Fig. 2.2 shown clearly that the stage II was the most important stage of the citrus fruit growth.

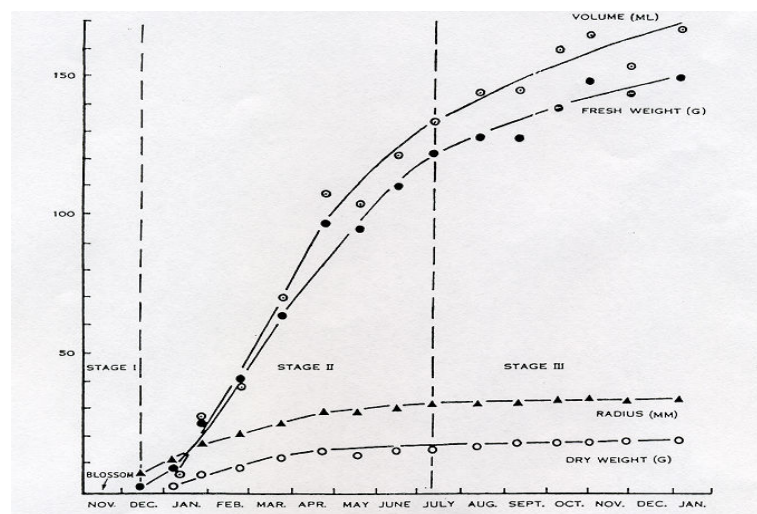


Figure 2.2. Growth in fruit volume and fresh weight during fruit development in 1954 (Bain, 1958).

The maximum increase in fresh fruit weight and fruit size occurred during stage II due to the rapid growth of the pulp segments (Bain, 1958; Godden, 1988; Spiegel-Roy and Goldschmidt, 1996). During stage III the growth rate of volume, fresh weight and radius of orange fruit decreased significantly. It has been reported that some factors affected the fruit size and fresh weight. Firstly, water stress can cause a reduction in fruit size, as the application of deficit irrigation decreased the fruit weight in mandarins (Gonzalez-altozano and Castel, 1999; Verreyne et al., 2001), grapefruits (Ritenour et al., 2003), oranges (Hutton et al., 2007; Treeby et al., 2007), lemons (Domingo et al., 1996) and pears and apples (Behboudian and Mills, 1997). Secondly, nutrition is an important factor, which affects the fruit size and weight. Koo and Reese (1977) applied N and K treatment to ‘Temple’ orange tree for six

years and found that the K-treatment had a positive effect on fruit size and weight, while the N-treatment had the significantly negative effect on these fruit parameters.

2.2.2. Rind weight and thickness

In Valencia oranges, rind reached to the maximum in thickness at the end of the cell division stage. The rind became thinner during the cell enlargement and rind thickness slightly increased during the stage III with the expanding pulp (Fig. 2.3) (Bain, 1958; Spiegel-Roy and Goldschmidt, 1996).

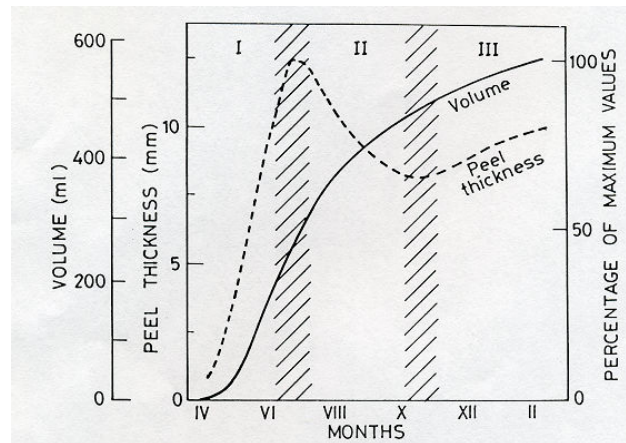


Figure 2.3. Growth of rind thickness and fruit volume in the developing fruit of Valencia oranges (Spiegel-Roy and Goldschmidt, 1996)

It has been reported that the rootstock is the main factor affecting the fruit rind thickness (Hutton et al., 2007; Treeby et al., 2007). The association of nutritional factors with rind thickness is also well known. Fruit with high phosphorous levels had thinner rind while rind was thicker in fruit with high levels of nitrogen and potassium (Bevington et al., 1993; Dick, 1995; Jones et al., 1967; McIntosh, 1998; Moulds et al., 1995; Sneath, 1987). Tariq et al. (2007) reported that rind was softer and thinner with the boron foliar application in sweet orange. It is well known that water deficit generally results in decreased fruit size (Davies and Albrigo, 1994; Kriedemann and Barrs, 1981) which may lead to an increase of rind thickness (Kriedemann and Barrs, 1981). Similarly, an application of water stress during fruit growth resulted in thicker fruit rind at maturation in oranges (Domingo et al., 1996; Ritenour et al., 2003; Treeby et al., 2007). Physiological disorders may be other factors affecting rind thickness. Jones and Embleton (1967), Jones et al. (1967) and

Sneath (1987) reported that rind was thinner in fruit with albedo breakdown than rind in normal fruit.

2.2.3. Juice content

Juice content reached at maximum during the enlarging cell phase and then reduced till fruit maturation (Fig. 2.4) (Bain, 1958; Spiegel-Roy and Goldschmidt, 1996).

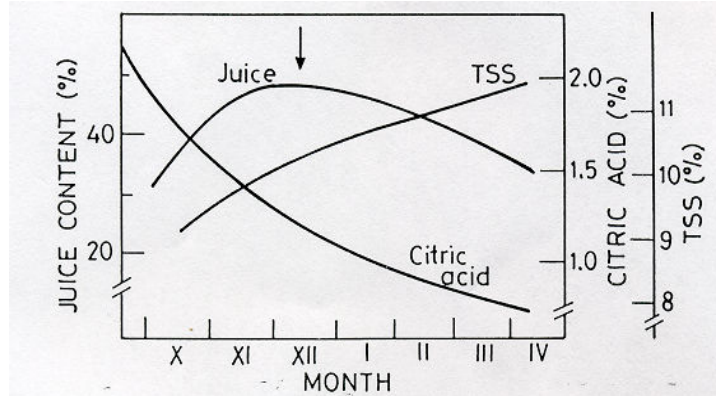


Figure 2.4. Changes of juice content, soluble solids concentration and citric acid during fruit development (Bain, 1958).

The effect of fertilizers on the juice content of the orange fruit was studied by different research groups, but unfortunately the reported results did not show agreement. The most intensive study on the ‘Temple’ orange had shown that K and P treatments did not have any significant effect on juice content whilst, the N treatment reduced this fruit parameter. These results are contrary to those reported on ‘Round’ oranges (Koo and Reese, 1977). A relationship between irrigation and juice content has been reported. Lower juice content may be associated with water stress anytime during fruit growth and development in citrus (Ritenour et al., 2003). In contrast, Verreyne et al. (2001) and Velez et al. (2007) indicated that juice content was not affected with the application of deficit irrigation in citrus. Physiological disorders may contribute to the changes of juice content, as juice content was significantly higher in fruit with albedo breakdown than that in normal fruit (Jones and Embleton, 1967; Jones et al., 1967; Sneath, 1987).

2.2.4. Phytohormones

It is well known that phytohormones play important roles in regulating the growth, development, maturity, ripening and senescence of citrus fruit during fruit growth

and development. Levels of auxins, gibberellins and cytokinins were high in young fruitlets while the levels of abscisic acid (ABA) and other growth inhibitors were higher during fruit maturation and senescence (Bain, 1958; Spiegel-Roy and Goldschmidt, 1996).

The involvement of gibberellic acid in set and development of citrus fruit has been reported (Iglesias et al., 2007). The role of gibberellic acid in promoting cell division and cell enlargement indicated the association of gibberellic acid with the initial growth of fruit. A number of previous studies reported that the foliar application of gibberellic acid ($20 \text{ mg}\cdot\text{L}^{-1}$) during an early stage of fruit growth (30 to 40 mm in diameter) significantly reduced albedo breakdown in sweet oranges (Dick, 1995; Gambetta et al., 2000; Gilfillan et al., 1981; Jona et al., 1989; Monselise et al., 1976; Moulds et al., 1995; Treeby and Storey, 1994; Tugwell et al., 1996). This suggested that gibberellic acid is also a factor influencing the compactness of albedo tissues, reducing cuticle permeability and delaying senescence (Agusti et al., 2002; Embleton et al., 1973; Iglesias et al., 2007; Ladaniya, 2007; Zaragoza et al., 1996).

Cytokinins are involved in cell division as high levels of cytokinins were observed in developing ovaries at anthesis in citrus (Iglesias et al., 2007).

It has been reported that auxins play a role in activation of cell enlargement as the levels of auxins were high during the beginning of phase II of citrus fruit growth (Iglesias et al., 2007; Ladaniya, 2007). The significant increase in final fruit size with the application of auxins including 2,4-DP and 3,5,6-TPA at the cell enlargement stage of fruit development indicated that auxins contribute to the controlling fruit size during the rapid growth phase of citrus fruit development (Agusti et al., 2002).

The relationship of ethylene and fruit growth and development has been reported in the literature. The enhancement of colour in citrus fruit with the application of exogenous ethylene or ethephon indicated an important role of ethylene in fruit maturity, ripening, and senescence (Agusti et al., 2002; Al-Mughrabi et al., 1989; Burg, 2004; Ladaniya, 2007; Monselise et al., 1976; Porat et al., 1999). The increase in peel puffing in Satsuma mandarin with the application of ethephon ($250 \text{ mg}\cdot\text{L}^{-1}$) seven days before harvest suggested that ethylene is also a factor responding to wounding or aging (Burg, 2004; Ladaniya, 2007).

2.3. Fruit quality

2.3.1. Soluble solids concentration

Soluble solids in orange fruit are mainly made of carbohydrates and organic acids which are recognised as the stable compounds and can reach 85% of the total soluble solids. The other chemical compounds (about 15 percent of the total soluble solids) contributing to the soluble solids concentration of orange juice are inorganic compounds, amino acids, ascorbic acid, and small amount of pectins, essential oils, esters, glucosides, and other organic compounds which are relatively unstable (Sinclair, 1961). Soluble solids concentration in the orange fruit is varied from 10% to 20% of the fruit fresh weight (Davies and Albrigo, 1994). Soluble solids concentration is affected by fruit size. Sinclair (1961) reported that an increase of soluble solids concentration was obtained with a decrease in size of fruit. Irrigation management has been reported to be related to soluble solids concentration. The significantly higher soluble solids concentration was observed in fruit under deficit irrigation in mandarins (Gonzalez-altozano and Castel, 1999), oranges (Hutton et al., 2007; Treeby et al., 2007) and 'Marisol' Clementine (Verreynne et al., 2001). The possible effect of fertility on the soluble solids concentration in orange fruit has been studied. It was found that P fertilization decreased the soluble solids concentration slightly, while the N and K fertilization did not show any effect on this parameter in 'Temple' orange (Koo and Reese, 1977). Moss and Higgins (1975) found that the Ca concentration in the leaf contributed to the brix/acid ratio in 'Late Valencia' oranges in New South Wales. Location may be another factor influencing soluble solids concentration. Titratable acidity in 'Autumn Gold' varied from 0.74% to 0.92% depending on locations in California (Kahn et al., 2007). Genetic factor is well known to be associated with fruit quality. Pretel et al. (2004) found that soluble solids concentration was significantly different among orange varieties. Percentage of total soluble solids acid ratio was significantly higher in fruit with albedo breakdown than in normal fruit (Jones et al., 1967).

2.3.2. Titratable acidity

Acidity is the main factor which affects the orange fruit taste. Total acidity in the fresh orange juice includes the major amount of citric acid, followed by malic acid, oxalic acids and lesser amounts of other related acids (Davies and Albrigo, 1994; Iglesias et al., 2007). It was proposed that the citric acid formation during fruit

development occurred with the tricarboxylic acid cycle simultaneously. The total acid content of the orange juice could reach 5 volume percent (Spiegel-Roy and Goldschmidt, 1996). Soluble solids concentration increased and the acidity reduced at the end of stage II and toward to maturation in 'Valencia' oranges (Bain, 1958; Davies and Albrigo, 1994). It was found that the titratable acidity in orange fruit was affected by soil fertility. N and K fertilization increased the acid content in 'Temple' orange juice, while P fertilization showed the reverse effect (Koo and Reese, 1977). Irrigation is another factor that affects the titratable acidity in orange juice. Treeby et al. (2007) found that deficit irrigation increased the titratable acidity in orange juice at maturity.

2.3.3. Organic acids

Organic acids are principle source of acidic taste in fruit. The major acid attributed to the organic acids is citric acid in fruit juice, followed by malic acid. Succinic, oxalic and tartaric acids are in low concentrations in citrus juice (Clements, 1964a; Davies and Albrigo, 1994; Iglesias et al., 2007; Karadeniz, 2004; Matsumoto and Shiraishi, 1981; Pretel et al., 2004; Shaw and Wilson, 1938). It has been reported that seven organic acids including citric, malic, quinic, tartaric, succinic, oxalic and ascorbic acids were found in the pulp of citrus fruit in acidless and acidic varieties. Oxalic, quinic and citric acids changed during fruit development whilst malic, tartaric, succinic and ascorbic acids remained stable. Quinic acid was the major organic acid and accounted from 46% to 64% of the total organic acids during the first 50 days of fruit development. Citric acid constituted 45% of the total organic acids in the pulp of mature fruit (Albertini et al., 2006). Clements (1964b) found that there was a tendency of reduction in citric and malic acids in juice in early season to prior to maturity during fruit development in 'Valencia' sweet oranges. Malic acid was the second major organic acid and accounted approximately 20% of the total organic acids in juice of 'Valencia' and 'Navel' oranges. Concentration of citric acid was low ($0.01 - 0.02 \text{ meq}\cdot\text{g}^{-1}$) in both albedo and flavedo of fruit while the higher concentration of malic acid was observed in albedo and flavedo ($0.01 - 0.03 \text{ meq}\cdot\text{g}^{-1}$ and $0.03 - 0.07 \text{ meq}\cdot\text{g}^{-1}$, respectively) in 'Valencia' and 'Navel' oranges.

2.3.4. Concentrations of total sugars

Three well known sugars including glucose, fructose and sucrose were found in most mature orange fruit. In orange fruit, the amount of sucrose was much higher than

fructose and glucose. The sugar content in orange fruit increased more rapidly at ripening stage than rapid growth period. It was reported that the sugar concentration in orange juice ($87.8 - 110.6 \text{ mg}\cdot\text{mL juice}^{-1}$) was higher than in the orange rind ($67.4 - 83.4 \text{ mg}\cdot\text{g fresh weight}^{-1}$) (Ladaniya, 2007).

2.3.5. Ascorbic acid

Ascorbic acid or vitamin C has been well known as an important part of the human nutrition. Citrus fruit is known as a major dietary source of ascorbic acid, although the precise biosynthetic pathway of this compound has not been identified. The ascorbic acid concentration in citrus juice increased at early fruit development stage and decreased at rapid growth period and ripening stage (Ladaniya, 2007). The concentration of ascorbic acid has been reported to be attributed to some factors. Ascorbic acid was attributed to genetic factor. Pretel et al. (2004) worked on 'Navelina' and other sixteen traditional orange varieties and found that the concentration of ascorbic acid was highest ($77.99 \text{ mg}\cdot 100\text{g}^{-1}$) in 'Capuchina' variety and lowest ($29.47 \text{ mg}\cdot 100\text{g}^{-1}$) in 'Blanca' variety. The ascorbic acid content in the orange peel ($1.3 - 2.2 \text{ mg}\cdot\text{g fresh weight}^{-1}$) was much higher than in the orange juice ($0.4 - 0.6 \text{ mg}\cdot\text{mL juice}^{-1}$) (Eaks, 1969; Sinclair, 1984). It has been suggested that irrigation management was associated with the ascorbic acid concentration in citrus. Ascorbic acid was higher in lemon under deficit irrigation (Domingo et al., 1996). Significantly lower ascorbic acid was found in fruit with albedo breakdown than in normal fruit (Jones et al., 1967). However, the ascorbic acid content in citrus fruit was almost unchanged after harvest and during storage (Spiegel-Roy and Goldschmidt, 1996).

2.3.6. Total antioxidants

Antioxidants have been known as the anti carcinogenic agents. Antioxidant content and activity in the fresh orange juice become a major topic for some research groups (Huang et al., 2007; Rapisarda et al., 2008; Rapisarda et al., 1999). Unfortunately, very limited data are reported in the literature to date. The antioxidant content in the fresh orange juice is changed significantly depending on cultivars, environmental conditions of growing and fruit maturity (Rapisarda et al., 1999). The five main antioxidant groups including phenols, anthocyanins, flavanones, hydroxycinnamic and ascorbic acid in different orange juices were monitored and the experimental results showed that the antioxidant content varied in a very wide range in the orange

juice. The maximum total phenols ($1147.2 \mu\text{g}\cdot\text{mL}^{-1}$), anthocyanins ($278,42 \mu\text{g}\cdot\text{mL}^{-1}$) and flavanones ($444.52 \mu\text{g}\cdot\text{mL}^{-1}$) were found in Moto IV orange juice and the minimum concentrations of these antioxidant groups (total phenols ($361.4 \mu\text{g}\cdot\text{mL}^{-1}$), anthocyanins (not detectable) and flavanones ($202.3 \mu\text{g}\cdot\text{mL}^{-1}$) were found in 'Washington Navel' orange juice. The maximum total hydroxycinnamic acid ($140.2 \mu\text{g}\cdot\text{mL}^{-1}$) was found in the 'Moro III' orange juice. Like the other antioxidants, ascorbic acid concentration in orange juices changed significantly. The maximum concentration of this compound ($781.4 \mu\text{g}\cdot\text{mL}^{-1}$) was found in the 'Tarocco II' orange juice, while the minimum ($417 \mu\text{g}\cdot\text{mL}^{-1}$) was found in the 'Washington Navel' orange juice again.

The effect of storage time at 6°C on the antioxidant profile of five orange varieties ('T. Messina', 'T. Meli', 'Moro', 'Ovale' and 'Valencia') was studied. The research results suggested that in 'T. Messina', 'T. Meli' and 'Moro' oranges, the anthocyanins, flavanones and hydroxycinnamic acids concentration increased with storage time, but ascorbic acid had reverse effect slightly. In 'Ovale' and 'Valencia' oranges, the effect of storage time on the antioxidant profile had different trend, the flavanone concentration decreased with the storage time, while the ascorbic acid increased slightly (Rapisarda et al., 2008).

2.4. Albedo breakdown

2.4.1. Physiology of albedo breakdown

Albedo breakdown, also known as creasing, is a physiological disorder with cracks in the internal white tissue (albedo) causing puffiness of orange peel (Bevington et al., 1993; Jones et al., 1967; Monselise et al., 1976; Sneath, 1987; Treeby and Storey, 1994; Treeby and Storey, 2002; Tugell et al., 1993). Albedo breakdown was recognised and reported in the literature as an old problem to the citrus industry (Jones and Embleton, 1967). After that, this problem has been studied by many scientists to identify the factors or combinations of the factors, which cause this serious problem of orange fruit production. The albedo breakdown is extremely difficult to study because there is no visual symptom observed before its appearance (Jones et al., 1967). Jones et al. (1967) suggested that albedo breakdown is a result of water stress. Monselise et al. (1976) reported that albedo breakdown can be recognised as a natural aging process of the orange fruit as it is associated with an earlier senescence of albedo due to the higher pectolytic activity (PE) and the content

of water-soluble pectin in affected fruit. As a consequence, the development of creases is connected to the degradation of pectin leading to the loosening of the connection between cells (Monselise et al., 1976). Later on, Li et al. (2009) found that albedo breakdown is a result of increased loss of pectin in the cellular walls of rind tissue of sweet oranges. These results are consistent with the findings of those who suggested the association of the activity of a pectin gelling enzyme and albedo breakdown (Bower, 2000). The results of Jones et al. (1967), Monselise et al. (1976) and Bower (2000) reported a contrary to the findings of Storey and Treeby (1994) who proposed that albedo breakdown is a result of the formation of fractures in albedo tissue during the post colour-break period. Albedo breakdown is greatly caused by changes in cell wall cohesion of adjoining cells at the middle lamella leading to the separation of white albedo cells (Moulds et al., 1995; Treeby and Storey, 1994). A number of previous studies reported that mineral nutrition including P, B, Ca, Mn, S, K and Mo in the trees and fruit was involved in albedo breakdown (Bower, 2004; Gambetta et al., 2000; Jones et al., 1967; Moulds et al., 1995; Treeby and Storey, 1994). Tugell et al. (1993) suggested that rapid increases in fruit size after first eight weeks of fruit development can cause albedo breakdown due to forming cracks in albedo tissue underneath the rind. Although albedo breakdown commonly observed visibly after colour break, it is well known that albedo breakdown develops at an early stage of fruit growth (in the first two months after petal-fall) (Bower, 2000; Monselise et al., 1976; Treeby and Storey, 1994).

2.4.2. Economic loss

Albedo breakdown causes a serious economic losses to the Australian citrus industry (Pellizo, 1997; Sneath, 1987; Treeby and Storey, 1994) and other citrus producing areas in the world including California (Ali et al., 2000; Jones et al., 1967), Israel (Monselise et al., 1976), Uruguay (Gambetta et al., 2000), South Africa (Bower, 2004) and China (Li et al., 2009). It can affect up to 50% to 90% of fruit in some localities in South Africa and Australia, respectively, and the potential returns to Australian and Israeli citrus producers is estimated at 1 to 2 million dollars for each percentage reduction of albedo breakdown (Gilfillan et al., 1981; Goldie, 1998; Monselise et al., 1976; Pellizo, 1997). More than 60% of fruit were discarded because of albedo breakdown which was the most dominant single cause (followed by hail and mechanical bruises) in Israel (Monselise et al., 1976). It is a huge cost for

the citrus industry as it causes fruit being rejected at the packing shed after the most money investment in fruit has been done including irrigation, fertilizer application, pesticides, harvest and grading (Pellizo, 1997). Approximately 15% to 30% or more of 'Navel' orange fruit were eliminated from packing due to albedo breakdown in Australia and Uruguay, respectively, in some years (Gambetta et al., 2000; Tugell et al., 1993).

2.4.3. Factor affecting incidence of albedo breakdown

The factors affecting albedo breakdown of citrus fruit can be divided into three groups.

2.4.3.1. Natural conditions of the orchard

2.4.3.1.1 Climate

2.4.3.1.1.1 Temperature

Albedo breakdown incidence is not consistent from year to year, so that temperature during the season becomes one of the most interesting climate factors of albedo breakdown. Ali et al. (2000) reported that the temperature at an early stage of the orange fruit development is involved in the albedo breakdown incidence as the average maximum and minimum temperatures in February prior to flowering have been correlated with creasing problem at harvest. Treeby's research results agreed with the above conclusion as he found that the summer temperature has a strong influence on albedo breakdown incidence in orange fruit (Treeby et al., 1995). It has been reported that the radial temperature gradient across the fruit is related to the albedo breakdown incidence as initial development of creasing occurs on the shaded side of the fruit toward the trunk (Jones et al., 1967). Albedo breakdown increased with the increasing range of temperature between maximum and minimum during the season (Shear, 1975; Sneath, 1987). In the Uruguay conditions, however, the albedo breakdown has not been influenced by the mean temperature (Gambetta et al., 2000).

2.4.3.1.1.2 Light

The incidence of albedo breakdown was more serious on the shaded side of the fruit than the exposed side. This means that lack of sun light may cause the albedo breakdown in sweet orange (Bevington et al., 1993; Treeby, 1996).

2.4.3.1.1.3 Relative humidity

Another important climatic factor, which affects the albedo breakdown, is the humidity. The albedo breakdown has been reported to be related to the sudden changes in relative humidity at fruit colour break (Gonzalez-altozano and Castel, 1999). Agusti et al. (2001) reported that the sudden changes in relative humidity at fruit colour break seem to be responsible for the development of albedo breakdown. Moisture stress is greatly associated with increased incidence of albedo breakdown (Dick, 1995; McIntosh, 1998). These results are opposite to the findings of Gambetta et al. (2000) who concluded that neither the relative humidity nor the rainfall may contribute to albedo breakdown incidence.

2.4.3.1.2 Water stress

Water stress is one of the most commonly discussed topics in the albedo breakdown literature. Albedo breakdown has been blamed on water stress during the late dry summer and autumn periods (Sneath, 1987). In contrast, Treeby (1996) found that the water stress does not seem to contribute to albedo breakdown.

2.4.3.1.3 Location

It has been reported that the albedo breakdown incidence varies from location to location in a given year. Unfortunately the specifications of the location (soil types, latitude, altitude) could not be found in the literature (Jones et al., 1967).

2.4.3.2. Tree factors

2.4.3.2.1 Rootstocks and scion cultivars

‘Navel’ orange is more likely susceptible to albedo breakdown than ‘Valencia’ and other sweet orange varieties (Sneath, 1987). Rootstock plays a major part in the incidence and severity of albedo breakdown in ‘Navel’ oranges (Agusti et al., 2003; Moulds et al., 1995; Treeby et al., 1995). The difference of the uptake of water or nutrient in rootstocks and scions might be a factor influencing the incidence of albedo breakdown (Treeby et al., 1995). Incidence of albedo breakdown was lower on ‘Bellamy Navel’ orange trees grafted on sweet orange and ‘Cleopatra’ mandarin rootstock than those on citranges and trifoliolate orange (Moulds et al., 1995; Treeby et al., 1995). Additionally, the highest proportion of fruit with albedo breakdown came from trees on rough lemon and Rangpur lime in ‘Bellamy Navel’ oranges (Moulds et al., 1995). Agusti et al. (2003) showed that the lower incidence of albedo breakdown was recorded on the tree grafted on sour orange than that on Carrizo citrange.

2.4.3.2.2 Crop loads and fruit size

It has been reported that albedo breakdown incidence are associated with fruit size (Jones et al., 1967; McIntosh, 1998; Moulds et al., 1995; Sneath, 1987; Treeby et al., 2000) and crop loads (Jones and Embleton, 1967; Sneath, 1987; Tugell et al., 1993). Albedo breakdown was greater in the smaller fruit (Jones et al., 1967). The higher incidence of albedo breakdown was observed in the trees which had higher yield (Jones and Embleton, 1967; Sneath, 1987). Consistent with this result, Jones et al. (1967) reported that albedo breakdown incidence was positively related to the number of fruit per tree as the percentage of smaller fruit is higher on the trees which produce higher yield (McIntosh, 1998). The results obtained on fruit size were opposite to the findings in Australia where the medium fruit (62-79 mm) were more likely to be susceptible to albedo breakdown than the smaller fruit (<62 mm) and the very large fruit (>79 mm) (McIntosh, 1998; Moulds et al., 1995; Treeby et al., 1995). Crop loads and the fruit number per tree do not contribute to incidence of albedo breakdown (Gambetta et al., 2000; Treeby et al., 2000).

2.4.3.2.3 Fruit position

Albedo breakdown was generally more severe on the fruit which were on the south half than those on the north half of the tree. The visual symptoms of albedo breakdown has also observed first on the fruit on the south half of the tree under southern California conditions (Jones et al., 1967). Creasing is more prevalent on the fruit, which are inside the canopy. It has been reported that albedo breakdown is often detected on the shaded side of a single fruit (Bevington et al., 1993; Jones et al., 1967; Treeby et al., 2000).

2.4.3.2.4 Tree age

It has been reported that the albedo breakdown incidence increased with the age of the tree (Moulds et al., 1995; Tugell et al., 1993). Treeby et al. (2000) suggested that tree age may contribute to incidence of albedo breakdown. Unfortunately, no related data has been reported.

2.4.3.2.5 Fruit age

Albedo breakdown is often worse when fruit remain longer on the tree in 'Navel' oranges (Dick, 1995; Jones et al., 1967; McIntosh, 1998; Moulds et al., 1995; Storey and Treeby, 2002).

2.4.3.2.6 *Nutrients*

2.4.3.2.6.1 Nitrogen, potassium, phosphorous and magnesium

Nutritional factors which affect the rind thickness are highly associated with albedo breakdown. A number of previous studies reported that fruit with high phosphorous levels were more likely to be susceptible to albedo breakdown as these fruit had thinner rind. High levels of nitrogen and potassium in fruit increased rind thickness resulting in the lower albedo breakdown incidence (Bevington et al., 1993; Dick, 1995; Jones et al., 1967; McIntosh, 1998; Moulds et al., 1995; Sneath, 1987). Ali et al. (2000) and Lovatt (2000) reported that both peel K and P concentrations at maximum rind thickness showed significantly positive correlation with rind thickness in October and significantly negative correlation with albedo breakdown incidence at harvest. The relationship of rind thickness in October and rind K concentration at maximum rind thickness explained 75% of the variation in albedo breakdown at harvest in 'Valencia' sweet oranges in California. Gambetta et al. (2000) found that concentrations of P and K were higher in the fruit with albedo breakdown than that in the normal fruit but there was no relationship between N concentration and albedo breakdown. However, albedo breakdown still develops on the fruit in which the levels of N, P and K are optimal. These results indicated that other factors are more important than N, P and K (Bevington et al., 1993; Jones et al., 1967). Mg did not contribute to albedo breakdown (Gambetta et al., 2000). In contrast, Mg concentration was lower in fruit with albedo breakdown than in the normal fruit, although there were no differences in Mg concentration in the rind of fruit with albedo breakdown and normal fruit (Storey and Treeby, 2002).

2.4.3.2.6.2 Calcium and Boron

Calcium and boron affect the albedo breakdown incidence as discussed below in this chapter.

2.4.3.3. *Cultural practises*

2.4.3.3.1 *Irrigation and water management*

Treeby et al. (2007) found that albedo breakdown incidence was significantly lower (48%) with the application of deficit irrigation (DI) and partial rootzone drying (PRD) over the whole growing seasons of 1999 and 2000 to 'Bellamy' Navel orange grafted on five rootstocks in comparison to control (60%) in New South Wales,

Australia. Similarly, Gonzalez-altozano and Castel (1999) concluded that reduced irrigation (by replacing 25% and 50% of potential evapotranspiration) at flowering and fruit set stage of fruit growth in 1995 and 1996 on 'Clementina de Nule' mandarin (*Citrus clementina* Hort. ex Tan.) resulted in the higher albedo breakdown incidence in the 25% treatment than in the 50% treatment in 1995. Both reduced irrigated treatments reduced the incidence of albedo breakdown (less than 1%) in 1996.

2.4.3.3.2 Nutrition

Jones et al. (1967) reported that N and K interaction and nitrogen rate influence albedo breakdown incidence. A significantly reduced incidence of albedo breakdown was a result of the soil application of increased N rate without application of K; however, albedo breakdown was not significantly affected with increased N rate or the high rate of K in 'Valencia' oranges during 4 year period from 1953 to 1956. The application of N in summer resulted in the lower incidence of albedo breakdown than the spring application. The soil application of only K reduced albedo breakdown (Jones and Embleton, 1967; Jones et al., 1967). Jones et al. (1967) also reported that two foliar sprays of KNO_3 solution at 40 lb·100 gal. water⁻¹ commencing on March and on May, 1964 significantly reduced albedo breakdown from 42.6% to 27.2% in 'Valencia' orange. Five foliar sprays of 2% $\text{Ca}(\text{NO}_3)_2$ starting at early stage of fruit development decreased albedo breakdown incidence in 'Washington Navel' oranges (Treeby and Storey, 2002).

2.4.3.3.3 Plant growth regulators

Many previous reports have indicated the involvement of gibberellins in albedo breakdown. The foliar application of GA_3 (20 mg·L⁻¹) at an early fruitlet stage (30 to 40 mm in diameter) significantly reduced incidence of albedo breakdown in 'Valencia' and 'Navel' sweet oranges in Israel (Jona et al., 1989; Monselise et al., 1976), South Africa (Gilfillan et al., 1981), Uruguay (Gambetta et al., 2000) and Australia (Dick, 1995; Moulds et al., 1995; Treeby and Storey, 1994; Tugwell et al., 1996). The foliar sprays of a combined solution of GA_3 (20 mg·L⁻¹) and a mixture of ammonium mono and di-phosphate (4%) and ammonium hydroxide (1%) in November was more effective in reducing albedo breakdown than spray GA_3 alone but it delayed colour development (Monselise et al., 1976). The spray application of GA_3 (20 mg·L⁻¹), acidified to pH 4, during cell expansion resulted in the significantly

lower incidence and severity of albedo breakdown than those during cell division (Bevington et al., 1993; Dick, 1995; Moulds et al., 1995; Treeby and Storey, 1994; Tugell et al., 1993). In Uruguay, spray application of GA₃ (20 mg·L⁻¹) alone were equally effective with the foliar sprays of the combination of GA₃ and potassium nitrate (2%) or mono-amonium phosphate (2%) commencing at fruit of 40 mm to 55 mm in diameter (Gambetta et al., 2000).

2.5. Regulated deficit irrigation

2.5.1. Concept of regulated deficit irrigation

Deficit irrigation term has been used since 1970 for a practical irrigation method, at which the plant water status is kept below the maximum water potential for any part of the plant during their development. Deficit irrigation can be used to control the growth of the tree over the critical period. Moreover, deficit irrigation can be applied to control the growth of citrus fruit at any development stage. Due to the quick response of this method, deficit irrigation is widely used for citrus (Domingo et al., 1996; Gonzalez-altozano and Castel, 1999; Hutton et al., 2007; Pérez-Pérez et al., 2009; Treeby et al., 2007), apple, peach, pear (Behboudian and Mills, 1997; Kriedemann and Goodwin, 1988) and olive (Rouina et al., 2007). Deficit irrigation effectiveness depends on the time of application during the year. Generally, deficit irrigation is most effective at the active growth phase of the organs of the tree. The application of deficit irrigation in early season or during flowering will inhibit the fertilization while deficit irrigation applied at late season will reduce fruit size and yield. The application of deficit irrigation after harvest will increase the flower density in the next season and reduce the shoot and radial trunk growth. For long time application of deficit irrigation, it is difficult to establish the relationship between deficit irrigation and fruit growth as different effects between glasshouse and field experiment were observed (Behboudian and Mills, 1997). The other advantage of deficit irrigation is the improvement of water use efficiency. This is very important for the region, where water supply is limited (Kriedemann and Goodwin, 1988).

2.5.2. Regulated deficit irrigation and soil, plant water status

2.5.2.1. Soil water content

Soil water content is one of the most important parameters for the irrigation planning as it affects the root development and water up-take to the tree directly (Hutton et al., 2007). As consequence, the photosynthetic and stomatal conductance was significantly affected by the soil water availability (Rouina et al., 2007). The relative water content values were used in the praxis of irrigation planning as there is no optimum volumetric soil water content for different types of tree and soil types. Rouina et al. (2007) reported that under the same irrigation conditions for different types of soil such as: sandy soil and sandy loam clay soil, the leaf water potential and the tree growth were differently affected. The application of deficit irrigation has strong effect on the plant water status on the 'Fino' lemon trees under low water retention capacity soil (Domingo et al., 1996).

2.5.2.2. Leaf water potential

Leaf water potential is sensitive with the deficit irrigation, especially at the late season application. It has been reported that stopping of irrigation in phase III of the fruit growth significantly reduced the leaf water potential (Pérez-Pérez et al., 2009). Domingo et al. (1996) reported that the leaf water potential decreased during deficit irrigation periods for both applied methods (reducing water all year except rapid fruit growth period and reducing at the rapid fruit growth period) on the Fino lemon tree. Similarly, Rouina et al. (2007) found that the leaf water potential for irrigated tree was -1.95 MPa while for the deficit irrigation trees were in the range of -3.2 MPa and -4.71 MPa in olives.

2.5.2.3. Transpiration and stomatal conductance

Plant water relation is mainly controlled by transpiration. Through transpiration process water escapes from the plant in the form of vapour through the stomata on the leaf. A high percentage of water in the plant will be lost and will affect the plant water status due to the large surface of the leaves (Kriedemann and Barrs, 1981).

Transpiration process is affected by many factors. One of the major factors is the CO₂ absorption. Water vapour diffuse from the evaporating surface of the leaf, but through this surface the CO₂ is absorbed in the plant. This process will add a

significant resistance to the water diffusion rate (Kramer and Boyer, 1995; Kriedemann and Barrs, 1981).

The rate of transpiration depends firstly on the plant condition itself and secondly on the leaf surrounding environment. The major factors of the plant conditions affecting the transpiration rate are the supply of water to the evaporating surfaces, the energy for water vaporisation and the resistances in the vapour path way. Temperature, absolute humidity and wind velocity around the plant are the main environmental factors because they affect vapour pressure different between the leaf surfaces and the outside air. As a consequence, it affects the driving for the water diffusion through the leaf directly (Kramer and Boyer, 1995).

Stomata, located on the leaf, are the most important organ of the plant for the transpiration process. Stomata can open and close to control the plant water status. Normally, they open during day time and close in the night (Kriedemann and Barrs, 1981). Under water deficit conditions, stomata will close to stop the transpiration process. Through this action the plant can reduce the water loss. The response of the stomata to the water deficit is different depending on varieties. ‘Valencia’ orange trees can sustain severe water stress with minimal or no detrimental effects (Hutton et al., 2007).

2.5.2.4. *Photosynthetic rate*

It is well-known that citrus plants have lower photosynthetic rates than other fruit trees, such as apple and peach. This may be due to the high stomatal resistance to CO₂ diffusion which restricts its access to carboxylation sites (Papadakis et al., 2004).

Deficit irrigation strongly affects the photosynthetic of the tree as the shoot growth will be reduced by low xylem water potential. If the activity of water potential reduces further, the stomata will close and transpiration process will stop (Kriedemann and Goodwin, 1988).

2.5.3. Impact of regulated deficit irrigation on the vegetative and reproduction growth of plants

2.5.3.1. *Vegetative growth*

Deficit irrigation affects the plant growth throughout the year but the organs of the plant will be affected differently depending on the time of its application. Generally, the application at the more active stage of the organ does the stronger effects on them (Behboudian and Mills, 1997). Similarly, Kriedemann and Goodwin (1988) reported that deficit irrigation can be used over the critical growth period of the targeted organs to get the strongest impacts. The response of the plant under deficit irrigation depends on the genetic factors. It has been reported that the plants react differently under deficit irrigation (Hutton et al., 2007). Treeby et al. (1995) reported that the ‘Bellamy’ Navel orange trees grafted on the Trifoliate orange rootstock had the strongest effect as compared to the other four rootstock tested. Furthermore, it has been suggested that ‘Valencia’ orange trees can handle water stress better than the other sweet orange tree varieties (Hutton et al., 2007).

2.5.3.2. *Shoot growth*

Behboudian and Mills (1997) reported that deficit irrigation significantly reduces the shoot growth of the plant because shoot is one of the most active parts of the plant. The shoot growth is reduced when the xylem water potential in the plant is lower than -0.6MPa (Hutton et al., 2007).

2.5.3.3. *Root growth*

It has been reported that the deep-rooted plants have less effect of deficit irrigation than the other plant types as the roots can access water from the wet lower soil layers (Kriedemann and Barrs, 1981). It is well known that orange tree has relatively shallow root system. The root system is reduced further by deficit irrigation, the very strong effect of the deficit irrigation on the orange tree can be expected (Hutton et al., 2007). The root to shoot ratio increased under deficit irrigation leading to the plant more tolerant to cope with the deficit of soil moisture (Behboudian and Mills, 1997).

2.5.3.4. *Trunk growth*

A part of a tree in the slowest response to the deficit irrigation is trunk (Kriedemann and Barrs, 1981). It has been reported that the trunk growth has been reduced under deficit irrigation and has very little impact from the time of application (Behboudian and Mills, 1997).

2.5.3.5. *Leaf growth*

Perez-Perez et al. (2009) reported that under deficit irrigation, not only the number of the leaves was reduced through the reduction of the shoot but also the size of the leaves was significantly reduced. As the final result, the total leaf area of the tree was strongly reduced.

2.5.3.6. *Fruit yield and quality*

The relationship between yield and deficit irrigation has been reported in the literature. Kriedemann and Barrs (1981) concluded that an increase of water stress in the tree canopy may be associated with a high crop loads in citrus. Gonzalez-altozano and Castel (1999) reported that the application of deficit irrigation (25% and 50% of water control treatment) at flowering and fruit set reduced yield 62% and 28%, respectively, while the application of these irrigation treatments at initial fruit enlargement reduce yield from 25% to 11%, respectively, due to fewer fruit number per tree in 'Clementina de Nule' mandarin in 1995. Yield was decreased 17% due to smaller fruit size with these irrigation treatments in 1996. Treeby et al. (2007) found that reduced water volumes by applied deficit irrigation and partial rootzone drying decreased crop loads in 'Bellamy Navel' oranges. In contrast, Ortuno et al. (2008) reported that deficit irrigation applied based on the maximum daily trunk shrinkage did not affect total yield and total number of fruit per tree in adult 'Fino' lemon tree. Similarly, Perez-Perez et al. (2009) concluded that deficit irrigation applied by cut-off irrigation in phase III of fruit growth did not affect fruit yield in 'Lane Late' sweet oranges.

It is well known that deficit irrigation greatly influences the fruit quality parameters in citrus. Ritermour et al. (2003) reported that juice content was lowered with the water stress anytime during fruit growth and development in citrus. Gonzalez-altozano and Castel (1999) and Verreyne et al. (2001) reported that the application of deficit irrigation increased the total soluble solids, acids in the juice and the ratio

of total soluble solids and titratable acidity in mandarins. Significant increase in soluble solids concentration due to deficit irrigation was observed in oranges (Treeby et al., 2007; Hutton et al., 2007). Domingo et al. (1996) found that deficit irrigation improved concentration of ascorbic acid in lemon fruit. Verreyne et al. (2001) and Velez et al. (2007) indicated that peel and juice content was not affected with water deficit in citrus.

It has been reported that the rapid increase in fruit size after the first eight weeks of fruit development can cause the albedo breakdown incidence (Tugell et al., 1993) and deficit irrigation is a very good method to control the growth of the orange fruit (Hutton et al., 2007). Therefore, deficit irrigation should be a good tool for controlling albedo breakdown in sweet oranges. Treeby et al. (2007) reported that deficit irrigation reduced the incidences of moderate and severe albedo breakdown at the end of the season in 'Bellamy Navel' oranges. Gonzalez-altozano and Castel (1999) applied deficit irrigation at the flowering and fruit set phases, which are the critical periods of citrus fruit development and reduced the incidence of albedo breakdown.

2.6. Nutrients

2.6.1. Calcium

2.6.1.1. Physiology and functions of calcium in plants

Calcium is one of the essential macro-nutrients for plant growth and development. It builds the structure and permeability of cell membrane and stimulates cell division and elongation. Ca forms cross-links within the pectin polysaccharide matrix resulting in the strong structural rigidity of the cell wall (Easterwood, 2002). Calcium is a major part of cell wall as cell wall, especially in the middle lamella, stores 60% of calcium and the rest of calcium is in the cell membrane (Poovaiah, 1988; Huang et al., 2008). Therefore, the calcium deficiency in the cell walls during ripening results in solubilization of pectin and acceleration of senescence (Zaragoza et al., 1996). Calcium is released from the pectins in the middle lamella by the interaction of the pectinesterase and polygalacturonase leading to loosening and separating the cell wall. In addition, the leak in the membrane occurred, when calcium outside the cytosol decreased (Poovaiah, 1988).

2.6.1.2. The involvement of Ca in albedo breakdown

It has been reported that the lower Ca concentration in rind and pulp has been associated with albedo breakdown in ‘Bellamy Navel’ sweet oranges in Australia (Treeby et al., 1995; Storey et al., 2002; McIntosh, 1998). Contrarily, Lovatt (2000) reported that albedo breakdown was significantly attributed to higher Ca concentration in rind in ‘Valencia’ sweet oranges in California. Inconsistent with these results, Ca does not seem to influence albedo breakdown in ‘Washington Navel’ oranges in Uruguay (Gambetta et al., 2000) or Ca seems to be less important in contributing to albedo breakdown than Mo or S in ‘Washington Navel’ sweet oranges in South Africa (Bower, 2004). Interestingly, albedo breakdown seems to be the results of non-uniform distribution of Ca within the albedo tissue as Ca concentration in the leaves of the tree on which fruit were affected by albedo breakdown were adequate (Treeby, 1996; McIntosh, 1998). Storey and Treeby (2002) reported that the ratios of K/Ca and Mg/Ca in albedo and pulp tissues were positively correlated with the albedo breakdown incidence in ‘Bellamy Navel’ sweet oranges in New South Wales. It has also been suggested that the ratios of K/Ca and Mg/Ca in the fruit were better indices than the Ca, K and Mg concentration alone.

2.6.1.3. Absorption of calcium

Foliar applications of calcium solution have not always significantly increased the calcium concentration in fruit as calcium is xylem mobile (Schonherr, 2001; Treeby and Storey, 2002) and cuticles are the first barriers to prevent the penetration of calcium into fruit (Schonherr, 2001). Saure (2005), Harker and Ferguson (1991) and Schlegel and Schonherr (2002) reported that Ca needs to be applied directly to the fruit surface to improve the penetration of Ca into apple fruit because the position of spray droplets affects the penetration of calcium chloride and trichomes, stomata and lenticels were also involved in penetration of all inorganic salts including CaCl_2 through the apple fruit cuticles (Schlegel and Schonherr, 2002; Manganaris et al., 2005). These authors also claimed that the calcium penetration is highly correlated to a large number of trichomes which densely covered fruitlet surface in ‘Golden Delicious’ and ‘Cox Orange Pippin’ apple cultivars during this period. Therefore, spraying foliar calcium salt solution before June drop resulted in the highest rates of calcium penetration into apple fruits. Various factors have been reported to contribute to the increased calcium in the fruit such as the proportion of the fruit

surface, the velocity of penetration and the concentration of salt solution. High rate of calcium penetration during droplet drying on stomatous surfaces may be associated with rapid penetration into guard cells and stomatal infiltration in apple and pear leaves (Schlegel and Schonherr, 2002; Schonherr, 2001). In addition, calcium can be penetrated through cracks and discontinuous surface of fruits. The higher penetration rate of calcium chloride is associated with higher humidity in pear leaf cuticles as increased swelling of cuticles was highly correlated with increasing humidity (Schmitz-Eiberger et al., 2002; Schonherr, 2001 and Schonherr, 2000).

2.6.1.4. Improvement of calcium uptake

2.6.1.4.1 Chemicals used to enhance calcium uptake

Calcium uptake can be improved with the application of the soluble form such as calcium nitrate or calcium chloride (Easterwood, 2002). It has also been reported that surfactant is an important factor involving the enhancement of the calcium uptake. Surfactant is known as a surface-active agent to improve physico-chemical characteristics of a spray solution and consequently to increase the efficiency of foliage-applied agrochemicals. Surfactants, which have hydrophilic and lipophilic groups, create the bridges between the aqueous solution and lipophilic waxes. The value of hydrophilic-lipophilic balance (HLB) mainly affected the improvement of mineral nutrient absorption by leaves. Ethoxylated alcohols, alkylphenols, sorbitant and alkylamines are the most frequently applied surfactants in agriculture. In theory, the surfactants which have the higher HLB value are more effective in the penetration of nutrients through the cuticular membrane. Wojcik (2004) reported that in practice absorption of leaf-applied nutrients is very effective when the optimal HLB values of the used non-ionic surfactants are ranged within 15-17. No research work has been reported on the effects of different surfactants in improving uptake of calcium in citrus fruits and warrants to be investigated.

2.6.1.4.2 The pathway of surfactants to improve the calcium uptake

It has been reported that surfactants can enhance the uptake of calcium into plant tissues through five pathways. Firstly, surfactants enhanced the uptake of Ca ions due to improving a distribution of Ca ions as surfactants resulted in the lower contact angles of spray solution on the leaf surface (Schmitz-Eiberger et al., 2002; Harker and Ferguson, 1991; Schlegel and Schonherr, 2002). Greene and Bukovac (1974)

found that the stomatal penetration of NAA and silver nitrate into pear leaves was more effective with surfactants due to a decreased surface tension between the surface of solution drop and leaf surface. Similarly, Schonherr (2000) and Schonherr (2001) used stomatous isolated pear leaf cuticular membranes and found that the rate constants of Ca^{2+} penetration was highly increased by surfactants as retention and wetting of leaves can be improved due to a sufficiently low reduction of the surface tension between the liquid and leaf. These results are in agreement with those who found that surfactant should be added into the foliar spray solution of iron in citrus because the decreased surface tension of spray solution resulted in a good wetting of the waxy citrus leaves which are hard to wet. Therefore, stomatal penetration was more effective (Neumann and Prinz, 1974). Secondly, surfactants also induce the penetration of solutes through the stomata, cuticular membranes and the cell wall, eliminate or decrease the air layer between the liquid and leaf surfaces (Wojcik, 2004). Thirdly, an increase in binding capacity of the cuticle to Ca^{2+} was as a result of surfactants in improving the calcium uptake due to a reduction in the drying of droplets (Wojcik, 2004). Stock et al. (1992) reported that the foliar uptake of organic compound was improved with surfactant 'Tween 20' which had humectant properties to remain moist throughout the uptake in field beans and peas. Roy et al. (1996) and Saftner et al. (1997) demonstrated that pre-treatment of apple fruit with 'Tween 20', 'Tween 80' and 'Tergitol 15-S-9' enhanced Ca uptake as long alkyl chains in these surfactants are the moiety agents. Roy et al. (1996) pointed out that 'Triton X-100' resulted in a higher Ca uptake compared to 'Tween 20' or 'Tergitol 15-S-9' as 'Triton X-100' had an alkylbenzen moiety which was more effective in absorbing Ca due to having better wetting agents. Harker and Ferguson (1991) reported that the rate of Ca^{2+} transport was increased with the addition of 'Armoblen T25' and 'Tween 20' due to the increased binding capacity of the cuticle to Ca^{2+} . Therefore, Ca^{2+} was removed from the solution. The Ca^{2+} contents in apple fruit were also increased with the application of 'Armoblen NPX' or 'Tween 20'. Fourthly, the interaction between the added surfactants and the cuticles by diffusing into the cuticle along hydrophilic-lipophilic interfaces was also important pathways to induce the foliar uptake of organic compound. This process caused the dilation of hydrophilic pores leading to the decreased resistance of the cuticle by increasing in permeability of the cuticle to polar solutes. Finally, surfactants also removed sites of adsorption by damaging and extracting cuticle wax. In consequence, the mobility of

solutes was increased (Stock et al., 1992; Harker and Ferguson, 1991). Harker and Ferguson (1991) claimed that the adsorption of Ca ions was increased with added 'Armoblen T25' but decreased with applied 'Tween 20' in apple fruit.

2.6.2. Boron

2.6.2.1. Physiology and functions of boron in plants

Boron is an essential micronutrient for plant growth and fruit quality (Dong et al., 1997; Maurer and Truman, 2000; Papadakis et al., 2003). Boron plays important roles in the citrus tree (Haas, 1929; Matoh, 1997; Haas, 1945; Zekri and Obreza, 2003). Firstly, boron involves in sugar translocation and carbohydrate metabolism to keep the tree growth processes active and normal. Secondly, boron plays an important role in plant cell wall formation. As consequence, boron is required at the site of active cell division. Thirdly, boron plays a key role in flowering, pollen-tube growth, fruit fertilisation processes, N-metabolism and hormone activity. Boron also interacts with the other nutrients to control the tree growth, for example, transports potassium to guard cells for the proper control of internal water balance and maintains calcium in a soluble form to insure its proper utilization (Zekri and Obreza, 2003). The relationships between B and Ca are well established. Boron assists in binding calcium to the cell walls. Shear (1975) and Zude et al. (1997) reported that boron increased the soluble forms of calcium and promoted calcium movement into the apple fruit. High boron concentration in the citrus trees depresses calcium absorption (Smith and Reuther, 1950).

2.6.2.2. Boron mobility in the citrus trees

Most of the total boron content (50.9-92.2%) of the citrus plant is retained in the leaves and boron distribution within the citrus tree follows the following order: Basal leaf > top leaf > bark > root > stems > wood (Papadakis et al., 2003). Boron is found in the tree in two forms: boric acid (water soluble) and B-rhamnogalacturonan II complex (water insoluble) (Matoh, 1997). Boron is not redistributed from the old to the young organs in citrus plant (Boaretto et al., 2007). In orange fruit, the rind contains more boron than the pulp. The boron concentration in the orange fruit is related to the ratio between fruit and leaf number (Haas, 1945).

It has been reported that boron absorption and distribution in the citrus tree depend on the citrus genotype and boron mobility mechanisms within the tree remain unclear

(Papadakis et al., 2003). It is well known that xylem mobility of boron is the main pathway within the tree and phloem mobility of boron is limited in the citrus tree due to the B-status of the tree (Boaretto et al., 2006; Boaretto et al., 2007). This conclusion is derived from two important research results. Firstly, for boron xylem translocation, the formation of Polyol-B-Polyol complexes in the photosynthetic tissue is necessary. Polyols (sorbitol, manitol, dulcitol) are found in many green trees but not in the citrus trees (Boaretto et al., 2006). Secondly, Papadakis et al. (2003) watered six boron concentrations with two-day intervals during 3-month period into two-year-old seedlings of sour orange and ‘Swingle’ citrumelo and found that the movement of boron is not in phloem as boron concentration in the leaf was much higher than that in other parts of the tree and boron concentration in the young leaves is much lower than in the old leaves for both genotypes. In contrast, Storey and Treeby (2000) analysed fruit nutrients during a period of 34 weeks with fortnight intervals on the ‘Bellamy Navel’ orange grafted on trifoliolate orange rootstock and firstly found that manganese mobility is in the xylem. Therefore, boron should be translocated in phloem as boron and manganese move into fruits by different pathways. Secondly, the lower concentration of boron in citrus fruit than those in the leaf reflected the result of a significant movement of boron within citrus plant via phloem. Thirdly, boron can form complexes with fructose and myo-inositol, which involve in the boron transport in the phloem. Fructose and myo-inositol are present in citrus trees.

Three other boron mobility mechanisms are proposed in the literature without any clear or direct evidences. Papadakis et al. (2003) proposed two different boron mobility mechanisms in the citrus trees: firstly, boron mobility can occur in xylem through transpiration processes and secondly, boron transport can follow the plasmalemma permeability through the cell wall mechanism. Furthermore, it has been reported that boron can form B-chelating compounds, which involve in the boron mobility in the rhizosphere (Papadakis et al., 2003).

2.6.2.3. Boron deficiency

Boron deficiency is more common than deficiency of any other micronutrients in citriculture and it affects all species of the citrus trees (Boaretto et al., 2008). The boron deficiency symptoms of the citrus trees appear when the boron concentration in the leaf is lower than $21 \text{ mg}\cdot\text{kg}^{-1}$ (Maure and Taylor., 1999; Hardy and Huett,

2005). Boron deficiency leads to the slight thickening of the leaves, chlorosis, curl downward and premature shedding of the leaves (Zekri and Obreza, 2003). The boron deficient trees have the splits in the bark of the branches, trunk and rootstock. Gum is often found in the vessels of the branches (Haas, 1945). Boron deficiency is easy to detect by citrus fruit observations. The fruits are hard and dry. The albedo is thicker than normal and has brownish discolorations (Zekri and Obreza, 2003). In oranges, the fruit are undersized, lumpy and misshapen (Foroughi et al., 1973). Gum deposit around the fruit axis and in the albedo of the rind is often detected (Haas, 1945). The grapefruit shows clearly brown discolorations in the albedo (Haas, 1945). Under the boron deficient condition, citrus trees have limited flowering and gum can be seen at the tip of the flower. High fruit premature abscission is observed due to boron deficiency.

2.6.2.4. Boron toxicity

High boron concentration in the soil can injure citrus trees seriously (Haas, 1929). Boron toxicity of citrus tree occurs when the boron concentration in the leaf is higher than $260 \text{ mg}\cdot\text{kg}^{-1}$ (Hardy and Huett, 2005). The boron toxicity symptoms appear on the leaves clearer than any other parts of the tree. Firstly, the leaves become mottled between the veins near the tip, then this part turn to yellow, followed by a slight tip burn. Finally, the leaves become chlorotic and drop prematurely (Haas, 1929; Haas, 1945). The boron toxicity is not equal for all citrus varieties, for example, leaves of 'Eureka' lemon seedlings have boron toxicity symptoms clearly at the boron concentration of $2 \text{ mg}\cdot\text{kg}^{-1}$, while the leaves of 'Valencia' orange seedlings are unaffected under similar conditions (Haas, 1929). High boron concentration damages the leaf tissues and reduces the calcium absorption. As a consequence, the growth processes of the tree are stopped.

2.6.2.5. Boron fertilizer applications

In commercial fertilizer alkalimetal Borates (borax, potassium borate ($\text{K}_2\text{B}_4\text{O}_7\cdot 4\text{H}_2\text{O}$) are the most common boron source for boron soil application as known as granubor. Solubor, which contains boric acid and sodium borates ($\text{Na}_2\text{B}_8\text{O}_{13}\cdot 4\text{H}_2\text{O}$, $\text{Na}_2\text{B}_4\text{O}_7\cdot 5\text{H}_2\text{O}$, $\text{Na}_2\text{B}_{10}\text{O}_{16}\cdot 10\text{H}_2\text{O}$) is formulated for boron foliar application with its high solubility. Manganese borate (MnB_4O_7) has been used as boron source as well. Although manganese borate is water insoluble compound, but it can cause boron toxicity or treat boron deficiency successfully (Haas, 1929). Boron is leached easily

by high rainfall and leads to a temporary low boron concentration in the soil due to the water solubility of borax.

The most common boron source in the commercial fertilizers is borax and boric acid. Boron fertilizer can be applied to the foliage or soil as citrus tree absorbs boron through roots and leaves (Boaretto et al., 2006). For foliar application, boric acid is the most common boron source due to its high water solubility ($5.7\text{g}\cdot 100\text{mL water}^{-1}$) (Zekri and Obreza, 2003). Boron is applied to the citrus plants usually as foliar spray (Tariq et al., 2007; Zekri and Obreza, 2003; Abd-Allah, 2006; Maurer and Taylor, 1999; Nguyen and Nguyen, 2006). This favourite method for boron application offers two important advantages. Firstly, foliar spray is more effective than soil application in correcting boron deficiency in citrus (Tariq et al., 2007). Secondly, foliar spray is safer than soil application as it provides better practical control in the field. Furthermore, boron soil application is not effective during dry springs and causes boron toxicity if applied during the summer rainy season (Zekri and Obreza, 2003). Nguyen and Nguyen (2006) reported that the foliar application of boron to citrus trees should be done before flowering to get the maximum yield improvement. Boron application to the citrus trees must be carried out frequently due to the low mobility and restricted redistribution from the old to the young tree parts (Hardy and Huett, 2005).

2.6.2.6. Effects of boron on plant yield and fruit quality

Boron deficiency in orange production is reported very often. This problem causes citrus fruit yield reduction around the world (Foroughi et al., 1973; Boaretto et al., 2006; Haas, 1945). Hard fruit, small fruit size and low yield are the major causes of boron deficiency resulting in economic loss. An inadequate level of boron in the fruit resulted in poor fruit quality in apple (Shorrocks and Nicholson, 1980). Rajput et al. (1976) found that foliar application of boron can improve growth, flowering, fruiting and fruit quality in mango. It has been reported that foliar boron application at the concentrations from $100 - 250 \text{ mg}\cdot\text{L}^{-1}$ on six-year-old 'Cam sanh' orange trees (*Citrus nobilis* var. *typical* Hassk) before flowering improved the yield (Nguyen and Nguyen, 2006). In combination with zinc, boron foliar spray increased the yield of orange trees up to 83.02%. This result clearly showed positive interactions among the micro-nutrients. Foliar application of boron on citrus trees can cure the "hard fruit" problem and eliminate the lumps formation in the albedo and increase the fruit

size, soften the rind and decrease the rind thickness. An increase of the percentage of juice and the ascorbic acid content was a result of boron foliar application (Tariq et al., 2007). In contrast, Smith and Reuther (1950), Boaretto et al. (1997) and Maurer and Truman (2000) reported that foliar application of boron did not improve yield, fruit size, rind thickness and fruit quality parameters such as juice content, soluble solids concentrations and titratable acidity in 'Navel' and 'Valencia' oranges. Apparently, no research work has been reported on the effects of B application on albedo breakdown in sweet oranges.

2.7. Ethylene

2.7.1. Role of ethylene

Ethylene is well known as a ripening hormone involved in the basic process of fruit maturity, ripening, and senescence. Ethylene occurs naturally in fruit and accelerates the fruit softening due to disintegrating cell membranes making them leakier (Rath and Prentice, 2004; Ladaniya, 2007). The role of ethylene in changing fruit colour, flavour, chemical composition and texture in citrus fruits has been reported (Ladaniya, 2007; Oetiker and Yang, 1995). Based on the ethylene production patterns during fruit maturation, citrus fruit are known as non-climacteric (Porat et al., 1999).

Ethylene is produced in the citrus tissues from a methionine amino acid by following three enzymatic reactions. The simplified scheme for ethylene biosynthesis pathway in plants can be described in Fig. 2.5.

Second reaction of this ethylene synthesis process is the slowest reaction and is affected by the gene expression of the citrus tissues. *S*-adenosyl-*l*-methionine (SAM) simultaneously forms polyamines, which negatively affects the ethylene production in several plant tissues and retards senescence process in excised leaves and protoplast (Ladaniya, 2007; Wang and Joseph, 2002; Even-Chen et al., 1982). 1-aminocyclopropane-1-carboxylic acid (ACC) is an intermediate compound in ethylene biosynthesis. The ACC is higher in wounding tissues than the fresh fruit tissues which contain a very low amount of ACC. The ethylene production is continuously maintained by protein as the inhibitors of protein synthesis reduce the ethylene biosynthesis in aged albedo tissue (Ladaniya, 2007).

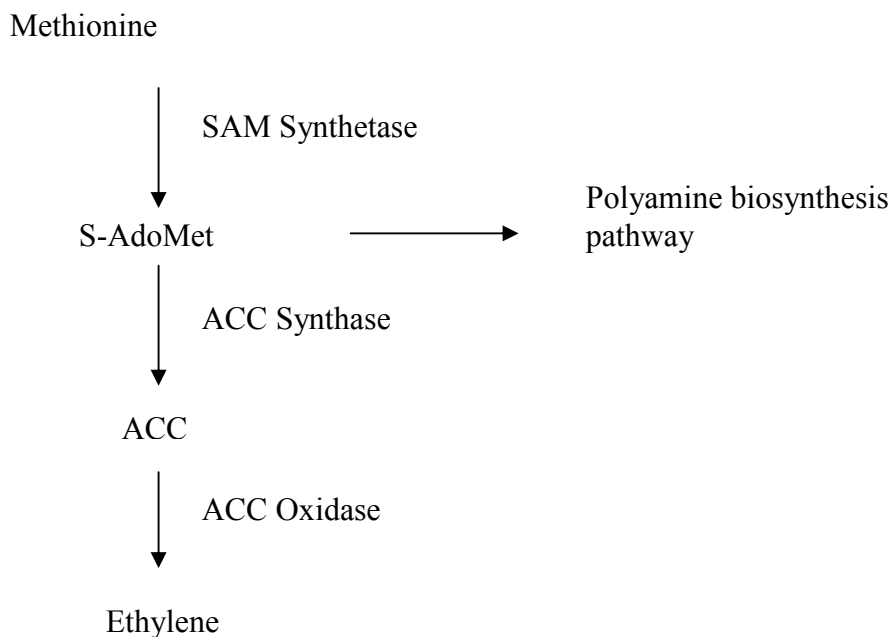


Figure 2.5. The simplified biosynthesis pathway of ethylene in plant

2.7.2. Endogenous ethylene

The rate of ethylene evolution is very low in citrus fruits ($<0.1 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). A large amount of ethylene was observed in the young, immature citrus fruits during the June drop (Hyodo, 1977; Ladaniya, 2007). Endogenous concentration of ethylene in citrus fruits has been reported to be very low ($2 \text{ nL}\cdot\text{h}^{-1}\cdot\text{fruit}^{-1}$) (Ladaniya, 2007). The smaller and green oranges and grapefruits produced the higher amount of ethylene than the bigger and mature ones during the fruit growth. The amount of ethylene in grapefruits, ‘Valencia’ and ‘Navel’ oranges decreased to 0.4, 0.3 and $0.2 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, respectively, when the fruit weight increased to 62, 50 and 70 g, respectively, in the harvest of August 16. All those fruit produced no detectable ethylene (less than $0.01 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) at harvest on September 4 when the fruit weight increased to 120, 64 and 87 g for grapefruits, ‘Valencia’ and ‘Navel’ oranges, respectively (Eaks, 1970). Rasmussen (1975) monthly measured the ethylene in internal atmosphere of fruit in four citrus cultivars during seven months from December to July and reported that amount of ethylene was higher in the fruit of two early maturing orange ‘Hamlin’ and ‘Pineapple’ (up to $95 \text{ nL}\cdot\text{L}^{-1}$) than the late maturing ‘Valencia’ and ‘Lam Summer’ (less than $25 \text{ nL}\cdot\text{L}^{-1}$). He also reported that cellulase activity and loosening were increased as ethylene and abscisic acid increased at the fruit maturity in four

citrus cultivars. Wounding due to fungal attacks, insect damage or freezing injury increased the amount of ethylene and changed the metabolism in harvested citrus fruit tissues (Ladaniya, 2007; Hyodo, 1997). The increase of endogenous concentration of ethylene in excised albedo tissue of citrus fruit indicated the response of ethylene to wounding and aging (Hyodo and Nishino, 1981). Hyodo (1977) worked on the isolated albedo tissues of 'Satsuma' mandarin fruit and reported that ethylene evolution increased dramatically and peaked at maximum after incubation of 30 hours and then decreased steady in sliced albedo tissues. Ethylene concentration was higher in late maturity fruit with albedo breakdown ($0.09 \text{ mL}\cdot\text{kg}^{-1}$) than the normal fruit ($0.04 \text{ mL}\cdot\text{kg}^{-1}$) on the same day in 'Valencia Late' orange (Monselise et al., 1976).

2.7.3. Exogenous application of ethylene

The citrus fruit can respond to exogenously applied ethylene by an increased respiration and promoting ripening although they produce a very low amount of ethylene (Porat et al., 1999, Ladaniya, 2007). Earlier research work indicated that exogenously applied ethylene or ethephon significantly improved colour in citrus fruits (Agusti et al. 2002; Ladaniya, 2007; Porat et al., 1999; Burg, 2004; Monselise et al., 1976; Al-Mughrabi et al., 1989). Porat et al. (1999) placed fruits in various concentrations of 1-methylcyclopropene (1-MCP) ($0 - 100 \text{ nL}\cdot\text{L}^{-1}$) and then exposed them to $10 \text{ }\mu\text{L}\cdot\text{L}^{-1}$ ethylene for 60 hours and found that both 1-MCP and ethylene did not affect the loss of fruit weight and fruit firmness on green or orange 'Shamouti' oranges. The association of ethylene and wounding or aging has been reported by Burg (2004) and Ladaniya (2007) who concluded that the application of ethephon ($250 \text{ mg}\cdot\text{L}^{-1}$) seven days before harvest increased peel puffing in 'Satsuma' mandarin. Ladaniya (2007) also stated that application of exogenous ethylene or ethephon increased amount of nootkatone which is an indicator of ripening or senescence in the rind of 'Star Ruby' grapefruit. In contrast, Al-Mughrabi et al. (1989) reported that the application of two foliar sprays of either $100 \text{ mg}\cdot\text{L}^{-1}$ or $200 \text{ mg}\cdot\text{L}^{-1}$ ethrel commencing before colour break of the fruit did not affect fruit physical characteristics such as fruit weight, rind thickness, rind weight and juice percentage in 'Balady' orange. Soluble solids concentration, acidity and ascorbic acid were also not affected with the foliar application of ethrel. Similarly, exposure of fruit to ethylene or ethephon did not increase pectolytic activity (PE) which is

involved in promoting senescence in sound mature 'Valencia Late' orange fruit (Monselise et al., 1976).

2.7.4. Regulation of ethylene

Ethylene biosynthesis is regulated by various factors of plant development and environment such as germination, fruit ripening, flower senescence, low temperature (Yang and Hoffman, 1984). The mechanical wounding and auxin and other regulators are also known to promote the ethylene production but the ethylene biosynthesis is blocked by ethylene biosynthesis inhibitors such as aminoethoxyvinylglycine (AVG) or Co^{2+} ion (Yang and Hoffman, 1984; Yu and Yang, 1979; Ladaniya, 2007). AVG is an effective inhibitor of ethylene production in plant tissues (Hyodo and Nishino, 1981; Ladaniya, 2007; Yang and Hoffman, 1984). It is also reported that AVG blocked the conversion of SAM to ACC resulting in the inhibition of the ethylene biosynthesis (Hyodo and Nishino, 1981; Even-Chen et al., 1982). However, a combination of AVG (0.2 mM) and ACC (0.5 mM) did not inhibit ethylene production in the aged albedo tissue of citrus fruit indicating that wounding and aging promote the formation of ACC and the conversion of ACC to ethylene in the citrus albedo tissue (Hyodo and Nishino, 1981). Contrarily, Yu and Yang, (1979) reported that AVG inhibits the ethylene synthesis due to inhibition of the IAA-induced ethylene production. Burg (2004) reported that treatment of fruit with AVG reduced peel puffing in 'Satsuma' mandarin.

It has been reported that cobalt is an ethylene action blocker which inhibits the conversion of ACC to ethylene (Lau and Yang, 1976; Yu and Yang, 1979; Yang and Hoffman, 1984). Lau and Yang (1976) treated apple tissues with CoCl_2 at 1 or 10 μM and found that application of Co^{2+} (0.1 mM) strongly inhibited the ethylene production. The application of Co^{+2} at concentration of 50 μM inhibited 75% of the ethylene formation while the ethylene synthesis was abolished in the presence of 1 mM Co^{+2} in mung bean hypocotyl (Yu and Yang, 1979). Even-Chen et al. (1982) worked on aged peel discs in 'Mature Shamouti' orange fruit and found that the combination of 2 mM Co^{2+} and 100 mM Na-phosphate inhibited 80% ethylene production due to inhibition of the conversion of ACC to ethylene. The exact role of ethylene in causation of albedo breakdown is not known and yet to be investigated.

CHAPTER 3

General materials and methods

3.1. Plant and fruit materials

Sweet orange trees growing in commercial orchards at different locations in Western Australia were used for the experiments as described below.

1. A commercial Niela orchard located in Bindoon (Latitude 31° 23' S, longitude 116° 06' E): the 'Navelina' sweet orange trees [*Citrus sinensis* (L.) Osbeck] were 12 years old and grafted on 'Swingle citrumelo' (*Poncirus trifoliata* [L.] Raf.) x (*Citrus paradisi* Macf.) rootstock and were used for deficit irrigation experiment. They were spaced 6.00 m x 2.00 m with a north-south row direction. The soil is gravely pale sandy loam with poor water holding capacity.
2. A commercial Westralian Fruits orchard located in Gingin (Latitude 31° 21' S, longitude 155° 55' E): twenty- two years old 'Washington Navel' orange trees [*Citrus sinensis* (L.) Osbeck] grafted on [*Poncirus trifoliata* (L.) Raf.] rootstock were used for the experiments of the development of albedo breakdown during fruit maturation, surfactants added into Ca spray solution and boron spray. The trees were spaced 7.50 m between rows and 2.70 m within rows and rows directed north-south. Trees were irrigated by micro sprinkler system installed under trees in each row. The soil is a sandy loam.
3. A commercial orchard located in Gingin, Western Australia: three sweet orange varieties from 'Navel' orange group [*Citrus sinensis* (L.) Osbeck] including 'Leng Navel', 'Autumn Gold' and 'Washington Navel', were used for the experiments of the effects of the severity of albedo breakdown on fruit quality among locations and the role of ethylene in albedo breakdown. The soil is a sandy loam. All selected sweet orange trees were 22 years old grafted on 'Troyer citrange' hybrid rootstock [*Citrus sinensis* (L.) x *Poncirus trifoliata* (L.) Raf.]. Trees were planted in a north-south row direction (6.50 m between rows and 1.50 m within rows).

4. Rosy Reds, the commercial orange orchard located in Chittering (Latitude 31° 28' 60S; longitude 116° 5' 60E): twelve-year-old 'Washington Navel' sweet orange trees were grafted on 'Troyer citrange' hybrid rootstock [*Citrus sinensis* (L.) x *Poncirus trifoliata* (L.) Raf.] and used for the experiment on the effects of the severity of albedo breakdown on fruit quality among cultivars. The trees were spaced 6.00 m x 2.00 m and planted in north-south row direction. The soil texture is red loam.
5. Harvey Citrus located at Harvey (Latitude 33° 05' S; longitude 115° 54' E): thirty-year-old 'Washington Navel' sweet orange trees were grafted on 'Troyer citrange' hybrid rootstock [*Citrus sinensis* (L.) x *Poncirus trifoliata* (L.) Raf.] and used for the experiment on the effects of the severity of albedo breakdown on fruit quality among cultivars. Trees were spaced 6.00 m x 2.50 m and rows directed north-south. The orchard soil is clay loam.
6. A commercial Fawcett Orchards at Serpentine (Latitude 33° 22' S; longitude 115° 59' E): thirtynine-year-old 'Washington Navel' sweet orange trees were grafted on 'Troyer citrange' hybrid rootstock [*Citrus sinensis* (L.) x *Poncirus trifoliata* (L.) Raf.] and used for the experiment on the effects of the severity of albedo breakdown on fruit quality among cultivars. Trees were spaced 7.60 m x 3.80 m and planted in north-south row direction. The soil is clay loam.

For fruit quality experiment, similar cultural practices including irrigation, fertilization, weed control and pest management were applied to all the experimental trees at Gingin, Chittering, Serpentine and Harvey.

3.2. Determination of soil volumetric water content

Soil volumetric water content (θ) was determined at depths of 300 mm and 600 mm within the main rootzone where 80% of orange roots was distributed (Kriedemann and Barrs, 1981; Mikhail and El-Zeftawi, 1979) using a MPM 160 moisture probe (ICT International Pty Ltd, Armidale, New South Wales, Australia). Two measurements were done at 50 cm away from the tree trunk on both sides of each orange tree (Mikhail and El-Zeftawi, 1979) when the orchard was irrigated after six hours (four measurements per treatment unit) (Kriedemann and Barrs, 1981). Soil volumetric water content was expressed as percentage.

3.3. Measurement of midday stem water potential (ψ_{md})

Midday stem water potential (ψ_{md}) was determined from 11.00 to 13.00 using a pressure chamber (Model 3000, Soil Moisture Equip. Corp, Santa Barbara, CA, USA) and expressed as MPa. Measurements were monitored on two fully exposed mature leaves per tree from the middle third of the tree (four leaves per treatment unit). Leaves were covered with aluminium foil at least two hours before taking reading and carefully excised at the petiole using a surgical blade (Paramount Surgimed Ltd., Okhla Pahse-II, New Delhi, India) and then placed in chamber within 20 seconds of collection. High purity nitrogen gas (concentration 1.4 sm³, U.N. No. 1066, BOC Gases Australia Ltd., Lismore, NSW, Australia) was used to apply pressure into the chamber until leaf sap appeared at the cut cross-sectional area of vascular tissue.

3.4. Measurement of stomatal conductance

Stomatal conductance was determined from 11.00 to 13.00 using a leaf porometer AP4 [(Model Sc-1 (Steady State Diffusion Porometer), Decagon Devices Inc., Pullman, WA, USA)]. Measurements were taken on two fully exposed mature leaves and one mature leaf at the shade side of canopy (three leaves per tree).

3.5. Estimation of endogenous ethylene

Endogenous ethylene concentration of the orange rind was determined following the procedure described below.

3.5.1. Extraction of endogenous ethylene

Endogenous ethylene from fruit rind was extracted following a partial vacuum method as discussed by Saltveit (1982). The system to extract internal ethylene consisted of a plastic desiccator filled to 75% capacity with the saturated ammonium sulphate aqueous solution. The outlet located on the desiccator head, was connected to a vacuum pump. This system consisted of a pressure indicator and a small pump (model 2107 CD18-194A, Dynapumps, Perth, Australia). A small glass container with the internal volume of 200 mL collection flask was used to collect ethylene. The collection container was filled with the saturated ammonium sulphate solution and placed in the desiccator with the small opening faced upward. The small opening of funnel shape container was sealed with a rubber septum (Altech Associates, Inc. Illinois, USA). Orange rind was carefully removed from normal fruit and fruit with

albedo breakdown. Two fruit of each group (normal and albedo breakdown fruit) were used for ethylene determination. The rind samples were put into a small net bag. The peel net bag was first dipped into ‘Tween ‘20 solution (0.01%) for 20 seconds at room temperature and then placed in the collection container. The air-bubbles-free collection container sat in the desiccator fairly stable. The desiccator was vacuumed and the pressure was kept constant at 85 p.s.i for six minutes. At the end of this process, one mL gas was collected from the collection container through the rubber septum using a 5 mL gas tight syringe (SGE, International Pvt. Ltd., Victoria, Australia) and immediately injected into a gas chromatograph (Agilent Technologies, 6890 N Network GC system, Palo Alto, CA, USA).

3.5.2. Specifications of Gas Chromatograph

A gas chromatograph (Agilent Technologies, 6890 N Network GC system, Palo Alto, CA, USA) was used for determining concentrations of endogenous ethylene.

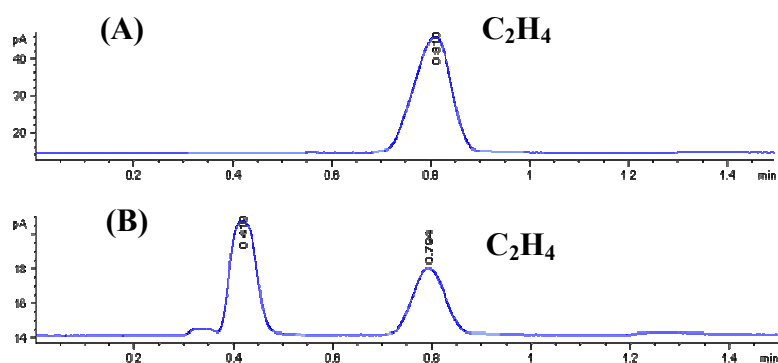


Figure 3.1 A typical chromatogram of $8.0 \mu\text{L}\cdot\text{L}^{-1}$ ethylene standard (A) and ethylene in orange rind (B).

It was fitted with a 2 m-long stainless steel Supelco Porapapak-Q column (outer diameter 1/8 inches, mesh size 80/100) and a flame ionization detector. The carrier gas was nitrogen. The GC-Column, injector and detector temperature were kept constant at 110°C , 100°C and 250°C , respectively.

Endogenous ethylene peak was identified by comparing the retention time with standard ethylene. The ethylene production was calculated by using ethylene peak area and calibration curve and expressed as $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{hour}^{-1}$.

3.6. Nutrient analysis

3.6.1. Sample preparation

Fully developed six - month old spring flush leaves (25 /tree) from non-fruiting shoot and five fruit per tree were randomly collected for nutrient analysis. The leaves and fruit from each tree were collected from unshaded position at about 1.50 m height at the north, east, south and west points of the tree. All leaves and fruit collected were free of damage from insects or diseases.

3.6.2. Measurement of mineral concentrations

Following washing, the leaves, rind and pulp of fruit were dried in an oven at $60^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 72 hours and milled and sieved through a 1 mm screen.

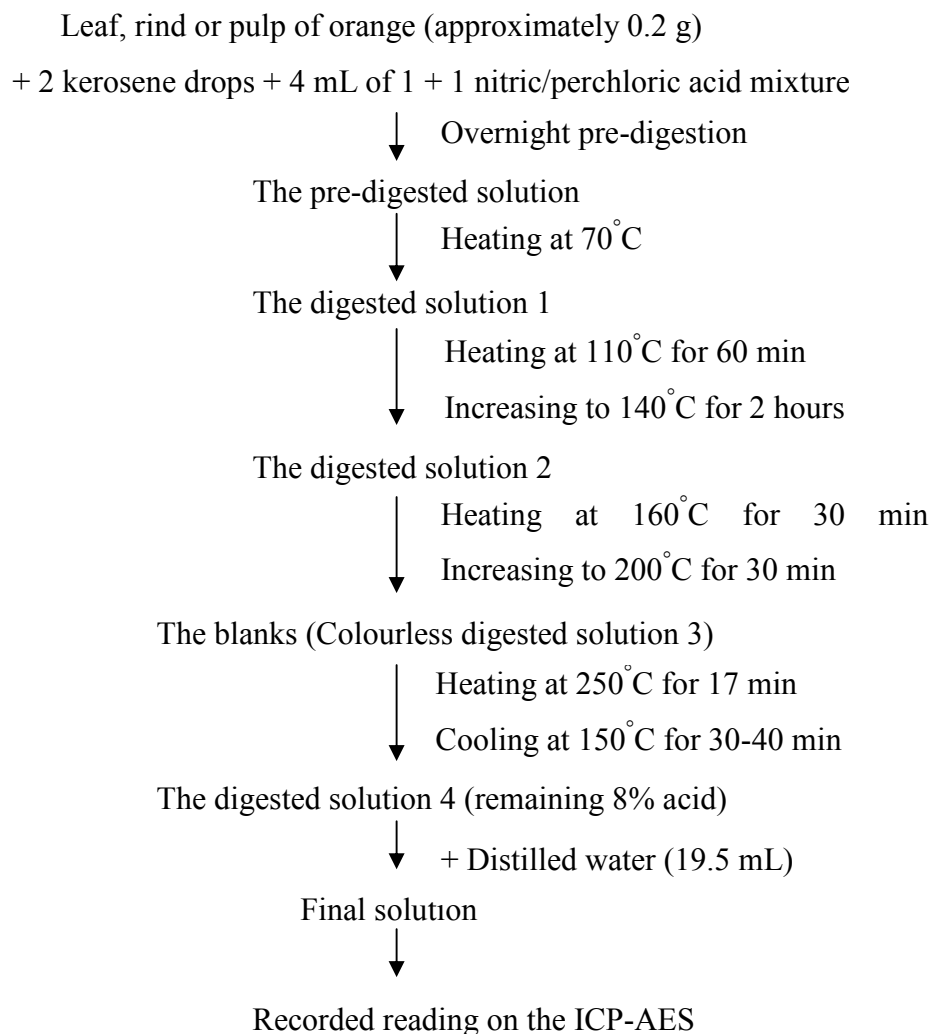


Figure 3.2 Flow chart of mineral analysis from leaf, rind and pulp of oranges (McQuaker et al., 1979).

Powered samples were digested by heating for 5 hours with a mixture of nitric acid (70% w/w) and perchloric acid (70% w/w) (ratio 1:1 in volume). After cooling period, the digest solutions were diluted with distilled water to form the final solutions. Mineral concentrations were analysed by using Radial Inductively Coupled Plasma Optical Emission Spectrometry (VISTA – PRO, CCD Simultaneous ICP-OES, VARIAN, Australia) which operated in simultaneous mode (McQuaker et al., 1979).

3.7. Determination of albedo breakdown incidence and severity

The incidence of albedo breakdown was examined on the fruit from whole tree. Following the recommendation of Treeby and Storey (2002), based on the appearance of fruit surface, three categories of albedo breakdown were assigned: none (no albedo breakdown), moderate (0% to 50 % of fruit surface affected) and severe (more than 50% of fruit surface affected). The incidence of albedo breakdown was expressed as percentage of fruit affected with albedo breakdown. The following formula was used to calculate the albedo breakdown (AB) severity based on using a rating scales described above by multiplying the number of fruit scored with the same value of the hedonic scale with the corresponding scale number. Finally, the resultant number was divided by the total number of fruit.

$$\text{AB severity (\%)} = \frac{\left[\sum (\text{Rating number} \times \text{number of fruit in rating category}) \right]}{\text{Total of fruit assessed} \times \text{highest rating value}} \times 100$$

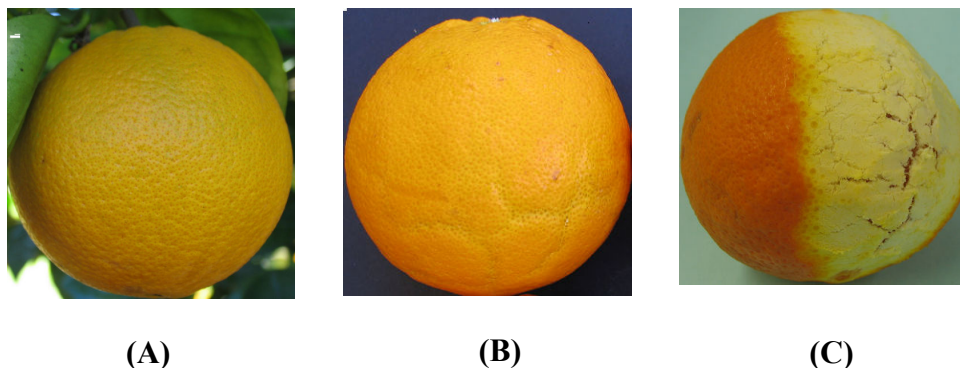


Figure 3.3. A normal fruit (A), typical appearance of fruit with albedo breakdown (B) and cracks in the albedo (C)

3.8. Texture profile analysis

Textural properties of rind such as hardness, adhesiveness, cohesiveness, springiness, fracture, tensile strength force and fruit firmness from normal fruit and fruit with albedo breakdown were determined using a texture analyser as described in details below (TA Plus, AMETEK Lloyd instruments Ltd., Hampshire, UK). A personal computer with Nexygen® software was interfaced to a texture analyser. A 5/16 Magness-Taylor probe, with a 500 N load cell was used for the measurement of textural parameters.

3.8.1. Rind puncture test

Rind sample from two fruit groups (normal and fruit with albedo breakdown) were cut in the size of 2.5 cm wide x 0.6 cm thick using a slicer (Zyliss Easy slice 2" folding Mandolin slicer, Swiss) to give uniform sections for determining rind puncture test.

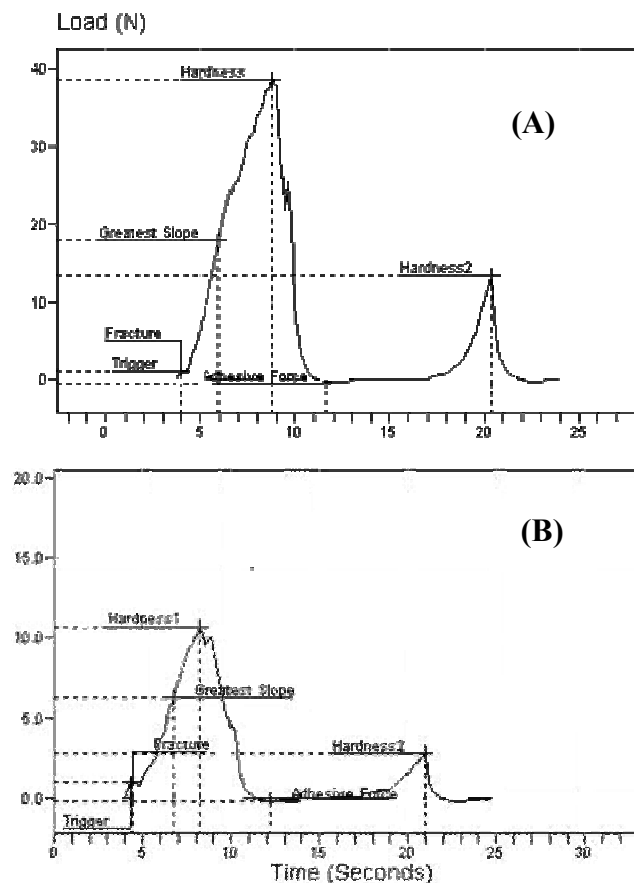


Figure 3.4. A typical curves for puncture test of normal fruit rind (A) and fruit rind with albedo breakdown (B) from a textural analyser (TA Plus, AMETEK Lloyd instruments Ltd., Hampshire, UK)

Two rinds samples were dissected 90 degrees apart per fruit. Ten fruit of each mentioned fruit group were tested from each tree. The rind sample was placed onto the flat plate. A cylinder probe of 4.00 mm diameter attached to the load cell was used. The speed of probe was 50 mm/min. Hardness is the maximum force of the first penetration when the rind sample is contacted to the probe at 70% of rind sample thickness. Cohesiveness was measured as the ratio of the work area during the second compression and the work area during the first compression. Springiness (mm) was measured as the ratio of the detected height of the product on the second compression and the original compression distance. Fracture force (N) is the force at the first significant peak during the first compression of product.

3.8.2. Rind tensile strength test

The rind tensile test was determined to measure the behaviour of the orange peel up to the rind deflection of 10 mm.

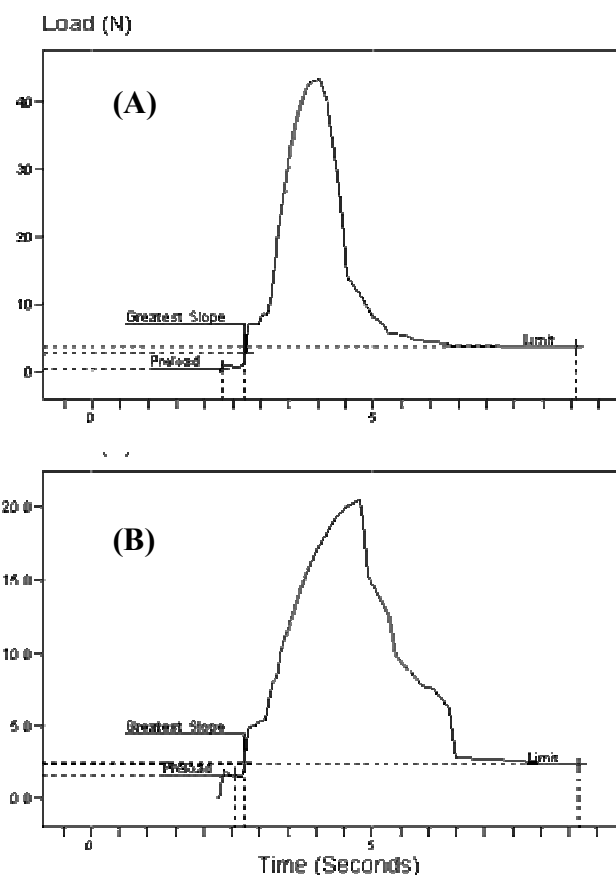


Figure 3.5. A typical curves for tensile strength test of normal fruit rind (A) and albedo breakdown fruit rind (B) from a textural analyser (TA Plus, AMETEK Lloyd instruments Ltd., Hampshire, UK)

A rind sample section was carefully removed from each fruit in the size of 2.5 cm wide x 5.0 cm length x 0.6cm thick using a slicer to give uniform sections. Ten fruit of each fruit group (normal and albedo breakdown fruits) were used for each test. A sample section of orange rind was held using two clamps. One clamp was fixed to the base of the machine while another one was attached to the moveable load cell. The rind sample was subjected to axial tensile loading until rind deflection of 10.0 mm at the crosshead speed of 100 mm/min and preload of 10 N. The rind tensile strength force was calculated at the maximum load and limit points where the rind deflection occurred.

3.8.3. Fruit compression test

The fruit with the height of about 8.5 cm were used for each compression test. Each fruit was placed between two flat plates with the stem axis perpendicular to the plate. The crosshead speed was 200 mm/min. This test was completed at strain of 25% of fruit height. Ten fruit of each fruit group (normal and albedo breakdown fruits) were sampled for each test.

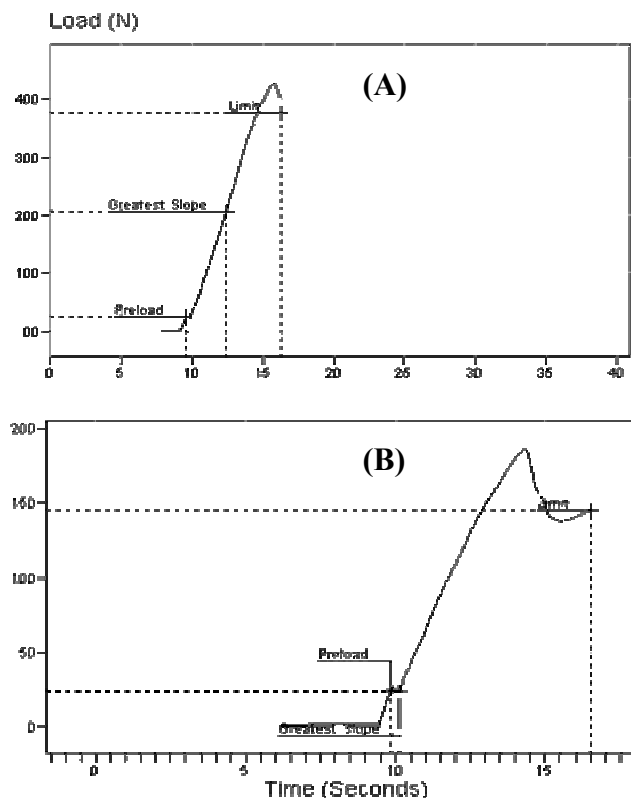


Figure 3.6. A typical curves for burst test of normal fruit rind (A) and albedo breakdown fruit rind (B) from a textural analyser (TA Plus, AMETEK Lloyd instruments Ltd., Hampshire, UK)

3.9. Determination of rind, flavedo and albedo thickness

Ten mature fruit collected at north, east, west and south points of each tree at about 1.5 m high were sampled to determine rind, flavedo and albedo thickness using an electronic digital calliper. The measurements were done at the equatorial region of each fruit.

3.10. Determination of rind and pulp dry matter contents

Ten mature fruit collected at north, east, west and south points of each tree at about 1.5 m high were sampled to determine dry matter contents. Fruit were first washed with tap water and then in distilled water twice to remove soil or dust. Rind was carefully removed from the pulp. Rind and pulp were separately cut into small pieces and dried in an oven at $60^{\circ}\text{C} \pm 2^{\circ}\text{C}$ to constant weight. Dry matter was expressed as $\text{g} \cdot 100 \text{ g}^{-1}$ fresh sample.

3.11. Determination of fruit quality parameters

Five mature fruit selected at north, east, west and south sides of each tree at about 1.5 m high were weighed and the juice was squeezed using a citrus juicer (Sunbeam citrus juicer, TE 2600, Sunbeam Co. Ltd., made in China to Sunbeam's specification). The freshly extracted juice was used for determining fruit quality parameters including percentage of juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and organic acids.

3.11.1. Juice content

Juice content was calculated using the formula below and expressed as percentage.

$$\% \text{ Juice} = \frac{\text{Juice weight} - \text{Jug weight}}{\text{Fruit weight}} \times 100$$

3.11.2. pH of juice

Juice pH was assessed using a digital pH meter (Cyberscan pH 510, Eutech Instrumnet Pte Ltd., Singapore). The electrode probe was dipped into 20 mL of freshly extracted juice. The reading was taken when the output stabilized.

3.11.3. Soluble solids concentration

Soluble solid concentrations (SSC) were recorded by measuring the refractive index using an infrared digital refractometer (Atago-Palette PR 101, Atago Co. Ltd, Itabashi-Ku, Tokyo, Japan) at 20°C and expressed as percentage.

3.11.4. Titrable acidity

Titrate acidity (TA) was determined by titration to phenolphthalein endpoint. 0.1 N NaOH was added into a mixed solution including 5 mL freshly extracted juice, 10 mL neutral water (pH = 7) and six drops of phenolphthalein until the solution just started to change colour to pink. This was the end point of titration. The following formula was used to calculate titrate acidity which was expressed as gram per 100 mL citric acid.

$$\text{Titrate acidity (g} \cdot 100 \text{ mL}^{-1}) = \frac{\text{Titre value} \times \text{molarity of NaOH} \times 64}{\text{Volume of sample}}$$

3.11.5. Ascorbic acid

Ascorbic acid concentration was determined following the combined method of Jagota and Dani (1982) and Malik and Singh (2005).

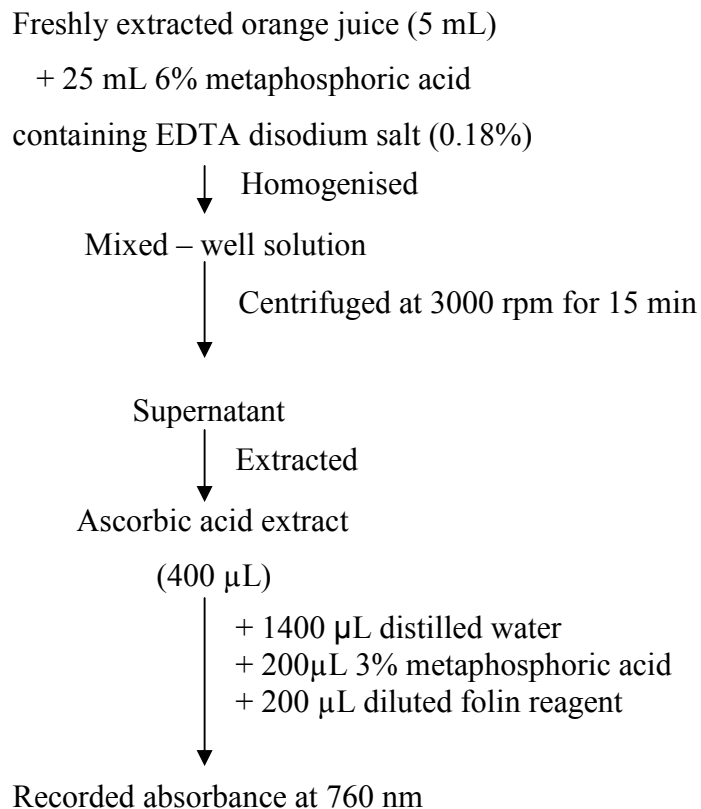


Figure 3.7. Flow chart of determining ascorbic acid from freshly orange juice.

Freshly extracted juice (5 mL) was mixed well in 25 mL of 6 % metaphosphoric acid containing 0.18% (w/v) of ethylenediamine tetraacetic acid disodium salt (EDTA). The solution was then centrifuged at 3000 rpm for 15 minutes using a centrifuge (Eppendorf Centrifuge 5810R, Hamburg, Gemany).The sample was prepared by mixing of 400 μ L supernatant, 200 μ L (3%) metaphosphoric acid, 1.4 mL distilled water and 200 μ L diluted folin reagent (5 mL deionised water : 1 mL folin reagent). After 10 minutes, the absorbance of sample was taken at 760 nm using a UV-vis spectrophotometer (Jenway 6405, Dunmow, Essex, U.K.). Ascorbic acid concentration was calculated using a standard curve of L-ascorbic acid and expressed as mg ascorbic acid per 100 mL fresh juice.

3.11.6. Individual organic acids

3.11.6.1. Chemicals

Citric, malic and tartaric acids purchased from Sigma-Aldrich, St. Louis, U.S.A and succinic acid purchased from Fluka, Buchs SG, Swizeland.

3.11.6.2. HPLC analysis

Organic acid concentrations were determined by using the high performance liquid chromatograph (HPLC Waters 1525 Binary HPLC Pump, Model code 5CH, Model; Waters 2414 Refractive Index Detector, Model code 487 and Waters 717 plus Auto sampler, Model code 71P). Freshly extracted juice (1 mL) was diluted with 19 mL mQ water. The diluted juice was centrifuged at 5000 rpm for 10 minutes using a centrifuge (Eppendorf Centrifuge 5810R, Hamburg, Gemany). The juice sample (20 μ L) was injected into HPLC system after being filtered through a 0.22- μ m nylon syringe filter (Altech Associates, Baulkham Hills, New South Wales, Australia). A Bio Rad Aminex HPX – 87 ion exclusion column (300 x 7.8 mm) preceded by a Cation-H Bio Rad Micro-Guard column (30 x 4.6 mm) was installed in a high performance liquid chromatograph system. The degassed mobile phase was 0.005 M H₂SO₄. The flow rate of the mobile phase was 0.6 mL/min. The temperature for the operation of the column and the column guard was 65°C. Absorbance of the column effluent at the wavelength of 210 nm was recorded for individual organic acids analysis purpose. Organic acids concentration was calculated using a standard curve of citric acid, malic acid, succinic acid and tartaric acid and expressed as g.L fresh juice⁻¹.

3.11.6.3. Standard preparation

Standard solutions of individual organic acids were prepared as following: both citric and malic acids ($1 \text{ g}\cdot\text{L}^{-1}$); succinic and tartaric acids ($0.1 \text{ g}\cdot\text{L}^{-1}$). The standard solutions volume of 4, 8, 12, 16 and 20 μL were injected into the HPLC system following the same procedure conditions as described in Section 3.11.6.2. The calibration curve for each organic acid was generated using Water Breeze Software (Version 3.30) by plotting the peak areas from the chromatogram against the injected weight of the related organic acid. The UV detector was set at 210 nm for the calibration works for the mentioned organic acids. The relationship between the peak area and amount is shown by ‘a’ (slope), ‘b’ (intercept) and r values in Table 3.2. ‘a’ and ‘b’ represent the coefficients of the regression equation $y = ax + b$, where x is amount of the organic acid, y is peak area and r is correlation coefficient of the equation. The ‘r’ values for all individual organic acids were very high showing almost perfect linearity ($r = 0.999$).

3.11.6.4. Elution orders and retention times

The elution order and retention times of the various organic acids identified in ‘Navel’ sweet orange juice sample are shown in Table 3.1. The characteristics of the calibration curves for organic acids are shown in Table 3.2. The chromatogram in Fig. 3.6 (A) and Fig. 3.6 (B) show the organic acids peak in the standard solution and ‘Navel’ sweet orange juice, respectively.

Table 3.1. Elution order and retention times of different organic acids used for identifying the organic acids concentration in ‘Navel’ sweet orange juice.

Elution order	Standard	Retention time (min)	Detection wavelength (nm)
1	Citric	15.88	210
2	Tartaric	17.09	210
3	Malic	18.85	210
4	Succinic	23.46	210

Table 3.2. Analytical characteristic of the calibration curves of the mentioned organic acids.

Standard	Slope (a)	Intercept (b)	r
Citric	2.13e+005	-1.46e+004	0.999
Tartaric	2.56e+005	-4.31e+003	0.999
Malic	1.63e+005	-4.71e+004	0.999
Succinic	1.10e+005	-2.14e+003	0.999

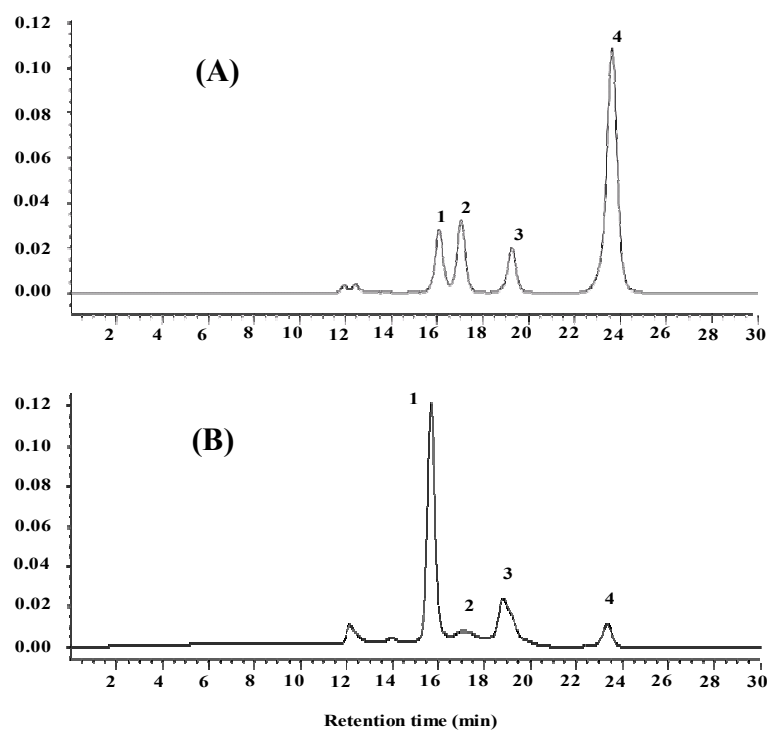


Figure 3.8. HPLC chromatograms of the standard solution (A) and ‘Navel’ sweet orange juice (B) at 210 nm. Peak 1: Citric acid; peak 2: Tartaric acid; peak 3: Malic acid; peak 4: Succinic.

3.12. Statistical analysis

The data from Experiments 1, 2 and 3 were subjected to one-way ANOVA and data from Experiments 4 and 5 were subjected to two-way ANOVA using Genstat 9 release 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The least significant difference (Fisher's protected LSD) was calculated at $P \leq 0.05$. To ensure the validity of statistical analysis, all the variables of ANOVA were checked.

CHAPTER 4

Development of albedo breakdown during fruit maturation, the relation between location and the incidence of albedo breakdown and the severity of albedo breakdown among locations and cultivars influencing fruit quality in ‘Navel’ sweet oranges [*Citrus sinensis* (L.) Osbeck.]

Abstract

During fruit maturation, the incidence of albedo breakdown was recorded four times (at 265 ± 5 , 286 ± 5 , 323 ± 5 and 332 ± 5 days after full bloom) in ‘Washington Navel’ sweet orange in 2007 and 2008. It was also recorded at four different locations in Western Australia (Gingin, Chittering, Serpentine and Harvey) and the effects of its severity on fruit quality were investigated in 2006. The effects of the severity of albedo breakdown on fruit quality in three different cultivars of ‘Navel’ oranges (‘Leng Navel’, ‘Autumn Gold’ and ‘Washington Navel’) were also studied in 2005. Sampled fruit were classified in three categories of albedo breakdown incidence in 2005 and four categories in 2006 based on the surface of fruit being affected. The incidence and severity increased slowly after colour break but they increased quickly after commercial harvest in 2007 and 2008. The significantly lowest incidence and the severity in ‘Washington Navel’ sweet orange were observed at Harvey in comparison to three different locations in 2006. Regardless of locations and cultivars, the severity of albedo breakdown did not affect the major internal fruit quality attributes such as juice content, titratable acidity, concentration of soluble solids, ascorbic acid, citric, and malic acids whilst it significantly decreased concentration of succinic acid in 2005 and 2006 and increased tartaric acid in 2005. Location and cultivar significantly affected fruit quality parameters in ‘Navel’ sweet oranges.

4.1. Introduction

Albedo breakdown is a physiological disorder of sweet orange fruit characterised by the cracking of white albedo tissues due to an abnormal separation of cells underneath the rind resulting in a weak rind. The initial development of albedo breakdown occurs at the early fruit growth period after the completion of cell division (Jona et al., 1989; Storey and Treeby, 1994). However, it is usually only

visible at the colour break stage during fruit development and tends to increase during fruit maturation (Bower, 2004; Dick, 1995; Jones et al., 1967; Moulds et al., 1995; McIntosh, 1998). Albedo breakdown has been responsible for dramatic economic losses to the Australian citrus industry with an estimation of \$20 to \$40 millions each year due to 20% or more of fruit being lost from export market for 'Navel' oranges (Pelizzo, 1997).

Climatic, cultural practices, genotype, and nutritional conditions have been reported to be associated with the incidence of albedo breakdown. The incidence and severity varies from year to year and from location to location and among cultivars (Sneath, 1987; Treeby et al., 1995). Treeby et al. (1995) reported that trees grafted on sweet orange had significantly lowest incidence of albedo breakdown than those grafted on Carrizo citrange or Troyer citrange. Mediterranean sweet oranges had higher incidence of albedo breakdown than other midseason varieties or 'Navel' or 'Valencia' oranges (Le Roux and Crous, 1938). Hearn (1988) reported that 'Sunstar' and 'Midsweet' cultivars grafted on 'Carrizo citrange' had no incidence of albedo breakdown while 'Pineapple' cultivar was susceptible with albedo breakdown in Florida.

Fruit produced from highly productive trees, small fruit and fruit with thin peel will increase susceptibility to albedo breakdown (Moulds et al., 1995; Jones et al., 1967; Tugell et al., 1993; Ali et al., 2000). Thus, nutritional factors which increase peel thickness are negatively correlated with albedo breakdown in sweet oranges. The higher levels of nitrogen and potassium in the tree reduce albedo breakdown as they increase the fruit rind thickness whilst, high levels of phosphorus in the fruit increase albedo breakdown due to thinner fruit rind (McIntosh; 1998, Tugell et al., 1993; Jones et al., 1967). In contrast, Treeby et al. (1995) found no relationship between crop load, small fruit and albedo breakdown in sweet orange. Tree physiological factors are associated with albedo breakdown as fruit held longer on the tree after commercial harvest stage results in higher incidence of albedo breakdown (Tugell et al., 1993; Dick; 1995; Moulds et al., 1995; McIntosh, 1998; Sneath, 1987; Jones et al., 1967). Old tree is positively correlated to the albedo breakdown (Tugell et al., 1993).

Fruit with albedo breakdown had significantly higher specific gravity, thinner peel, higher percentage of juice, lower acid content, including ascorbic acid, as compared

to normal fruit (Jones et al., 1967; Jones and Embleton, 1967; Sneath, 1987). It has also been reported that fruit with albedo breakdown was more mature as they had a significantly higher percentage of total soluble solids and acid ratio than normal fruit (Jones and Embleton, 1967). In contrast, Goldie (1998) reported that internal fruit quality parameters were not affected with albedo breakdown.

Information on the influence of severity of albedo breakdown on fruit quality or the effects of locations on albedo breakdown incidence and fruit quality is sporadic and inconclusive. These observations prompted to investigate the effects of fruit maturity on development of albedo breakdown and have also investigated whether fruit quality parameters are affected by severity of albedo breakdown among different locations and sweet orange cultivars for ‘Navel’ oranges.

4.2. Materials and methods

4.2.1. Experimental site, plant materials

4.2.1.1. Experiment 1: Development of albedo breakdown during fruit growth and maturation

The experiment was carried out in 2005 to 2008 at a commercial orchard located in Gingin, Western Australia (Latitude 31° 21' S, longitude 155° 55' E). Twenty-two years old orange trees grafted on ‘Troyer citrange’ hybrid rootstock [*Citrus sinensis* (L.) x *Poncirus trifoliata* (L.) Raf.] with a 7.5 m by 2.7 m spacing were used for the experiment. The row direction was north – south. The soil was a sandy loam. All the experimental trees received similar cultural practices.

Fruit diameter was measured on ten fruit tagged per tree during fruit development and maturation period from 97 ± 5 DAFB to commercial harvest with three weeks interval by using an electronic digital calliper. After commercial harvest (286 ± 5 DAFB), fruit diameter was still measured two times at 323 ± 5 and 332 ± 5 DAFB. The albedo breakdown incidence was determined four times at 265 ± 5 DAFB (when albedo breakdown can be visibly observed), 286 ± 5 DAFB (at commercial harvest), 323 ± 5 and 332 ± 5 DAFB (after commercial harvest). The incidence was determined by assessment of all fruit on the tree based on the appearance of fruit surface as described in Section 3.7. Percent fruit with albedo breakdown was calculated. The experimental design was a randomised block design with four replications. Two uniform trees were treated as an experimental unit.

4.2.1.2. Experiment 2: Incidence of albedo breakdown in ‘Washington Navel’ at different locations and the effects of its severity on fruit quality

The experiment was carried out in July 2006. ‘Washington Navel’ sweet orange trees grafted on Troyer citrange hybrid rootstock [*Citrus sinensis* (L.) x *Poncirus trifoliata* (L.) Raf.] were used at four commercial orchards located in four distinct locations including Gingin, Chittering, Serpentine and Harvey, Western Australia. In Gingin, the soil is sandy loam. Twenty-five years old orange trees were used for the experiment. Planting distance is 6.50 m x 1.50 m. In Chittering, the soil texture is red loam. Trees were 12-years old spaced at 6.0 m x 2.0 m. In Serpentine, the soil is clay loam. Thirty-nine years old trees with a 7.60 m by 3.80 m spacing were selected. In Harvey, the orchard soil is clay loam. Trees were thirty years old and spaced at 6.00 m x 2.50 m. The row direction was north – south in all the orchards at the locations. All the experimental trees at all the locations received similar cultural practices including irrigation, fertilizers, weed control and pest management.

One hundred fruit were harvested from 2 metre-squares from one side throughout another side of each tree from the middle canopy. Two hundred fruit were from each replication and albedo breakdown incidence was recorded. Following the method of Treeby and Storey (2002) with some modifications, based on the appearance of fruit surface, four categories of albedo breakdown were determined: nil (no albedo breakdown), slight (less than 25% of fruit surface affected), moderate (from more than 26% to less than 50% of fruit surface affected) and severe (more than 51% of fruit surface affected). The albedo breakdown incidence and severity was calculated as described in Section 3.7.

Ten fruit in similar size of each albedo breakdown category of two trees from each location were chosen to form a replication for determination of fruit quality parameters.

The experimental design was two-factor completely randomised block design with four replications. At each location, two uniform trees were treated as an experimental unit.

4.2.1.3. Effect of severity of albedo breakdown on fruit quality for different cultivars of 'Navel' oranges

The experiment conducted in July 2005. Three cultivars of 'Navel' sweet orange group [*Citrus sinensis* (L.) Osbeck] including 'Leng Navel', 'Autumn Gold' and 'Washington Navel', were selected at the commercial orchard located in Gingin, Western Australia. 'Leng Navel', 'Washington Navel' and 'Autumn Gold' are early, mid and late maturing cultivars, respectively (Lacey and Foord, 2006). The soil was a sandy loam. All selected sweet orange trees were twenty-two years old grafted on 'Troyer citrange' hybrid rootstock [*Citrus sinensis* (L.) x *Poncirus trifoliata* (L.) Raf.]. Tree spacing was 6.00 m between rows and 1.50 m within rows planted in north – south row direction. All the experimental trees received similar cultural practices during the growing season.

Four uniform trees of each cultivar mentioned above were selected for this experiment. Forty fruit from each tree of each cultivar were randomly collected and classified according to the albedo breakdown severity at the commercial harvest maturity. Based on the affected surface of fruit, the albedo breakdown grade was divided into three categories: nil (no albedo breakdown), moderate (1-50 % of fruit surface affected) and severe (>50% of fruit surface).

Single tree was treated as an experimental unit and included four replications for determination of fruit quality parameters. The experimental layout was two-factor completely randomised block design.

4.2.1.4. Determination of other fruit quality parameters

Five fruit of each albedo breakdown category of each tree from each cultivar were sampled for determination of other parameters of fruit quality. Juice was squeezed by using a citrus juicer (Sunbeam citrus juicer, TE 2600, Sunbeam Co. Ltd., China) for determination of juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and individual organic acids as mentioned in Section 3.11.

4.2.1.5. Ascorbic acid

Ascorbic acid concentration was determined following the combined method of Jagota and Dani (1982) and Malik and Singh (2005) as detailed in Section 3.11.5. Ascorbic acid concentration was expressed as mg ascorbic acid·100 mL fresh juice⁻¹.

4.2.1.6. Individual organic acids

Organic acid concentration was determined by using the high performance liquid chromatograph (HPLC) as described in Section 3.11.6.

4.2.2. Statistical analysis

The data from Experiment 1 were subjected to one-way ANOVA and those from Experiments 2 and 3 to two-way ANOVA using Genstat 9 release 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The least significant difference (Fisher's protected LSD) was calculated at $P \leq 0.05$. To ensure the validity of statistical analysis, all the assumptions of ANOVA were checked.

4.3. Results

4.3.1. Incidence of albedo breakdown during fruit development and maturation

The fruit diameter increased rapidly from 97 ± 5 DAFB to 244 ± 5 DAFB and then the growth rate became moderate until commercial harvest. Later on, the fruit diameter was unchanged until 332 ± 5 DAFB (Fig. 4.1A). The trends of fruit growth recorded as diameter in 2005-2006 and 2006-2007 were similar.

The first visible symptom of albedo breakdown appeared after colour break at 244 ± 5 DAFB when fruit have almost developed to full size in 2007 and 2008. Later on, the albedo breakdown incidence dramatically increased until 332 ± 5 DAFB during both years. The severity of albedo breakdown increased steadily after 244 ± 5 DAFB to commercial harvest (286 ± 5 DAFB). After this period, it increased markedly at later stages of harvest up to 332 ± 5 DAFB (Fig. 4.1B).

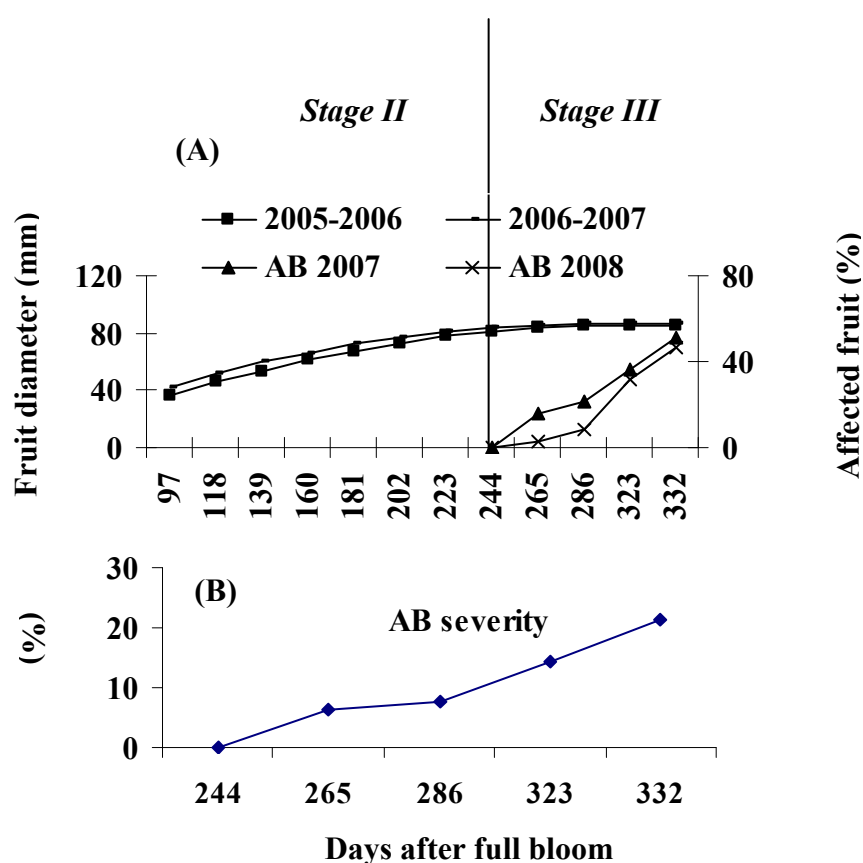


Figure 4.1. Changes in fruit diameter (mm) in 2005-2006 and 2006-2007, the incidence of albedo breakdown in 2007 and 2008 during fruit development in ‘Washington Navel’ (A) and severity of albedo breakdown (B) during fruit development and maturation in 2007. Commercial harvest was at 286 ± 5 DAFB.

4.3.2. Incidence of albedo breakdown in ‘Washington Navel’ at different locations

The albedo breakdown incidence and severity were significantly lowest in ‘Washington Navel’ orange at Harvey as compared to Gingin, Chittering and Serpentine in 2006. The incidence and severity of albedo breakdown in ‘Washington Navel’ orange did not differ significantly at Gingin, Chittering and Serpentine in 2006 (Fig. 4.2).

4.3.3. Effects of severity of albedo breakdown on fruit quality at different locations

Fruit weight was not significantly affected by the different levels of severity of albedo breakdown at all the locations (Table 4.1). The different levels of severity of albedo breakdown did not significantly affect juice content, its pH, soluble solids concentration (SSC), titratable acidity, ascorbic acid and individual organic

acids except decreasing succinic acid at Gingin, Chittering, Serpentine and Harvey in 2006 (Tables 4.1 and 4.2).

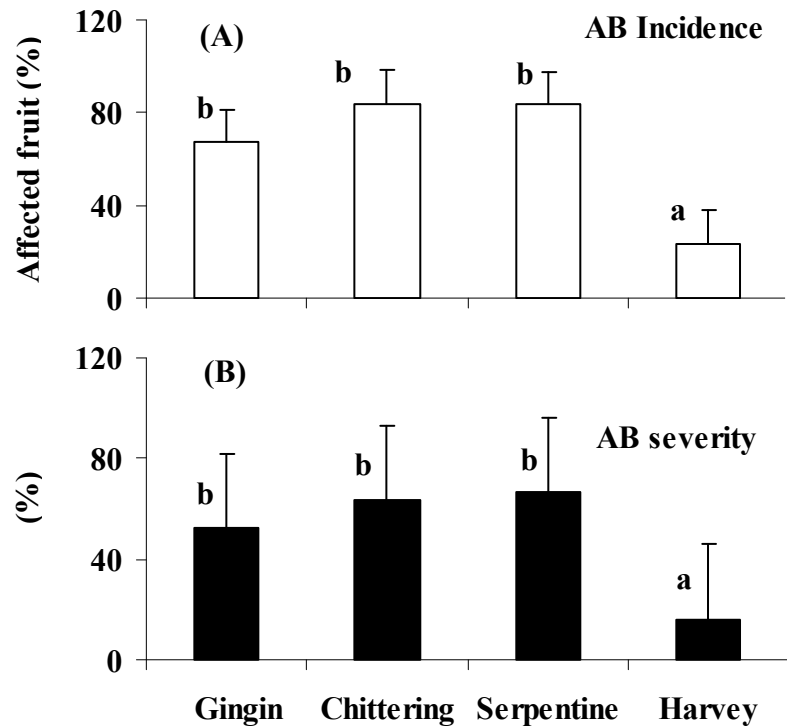


Figure 4.2. Incidence of albedo breakdown (%), (A) and albedo breakdown severity (%), (B) in ‘Washington Navel’ sweet orange at different locations in 2006. Different letters on means indicate significant differences at $P \leq 0.05$. $n = 4$ replications. Vertical bars represent standard error of differences of means.

Different locations significantly affected the juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and individual organic acids (Tables 4.1 and 4.2). The interactions between different levels of severity of albedo breakdown and location were found to be non-significant for fruit weight, juice content, pH, SSC, titratable acidity, ascorbic acid and all individual organic acids except succinic acid (Tables 4.1 and 4.2).

Table 4.1. Effect of different severities of albedo breakdown on fruit weight, juice content (%), pH, soluble solids concentration (SSC) (%) and acidity (mg·100 mL fresh juice⁻¹) in “Washington Navel” orange at different locations in 2006. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Location	Tree age	Severity of AB	Fruit weight (g)	Juice content	Juice pH	SSC	Acidity
Gingin	25	Nil	237.0	42.05	3.17	13.80	1.06
		Slight	231.4	42.89	3.14	13.55	1.01
		Moderate	221.5	42.14	3.11	13.55	1.04
		Severe	211.4	41.98	3.12	13.48	1.07
Mean			225.3A	42.27C	3.13C	13.59A	1.05C
Chittering	12	Nil	220.4	47.37	3.43	12.78	1.42
		Slight	217.1	48.35	3.41	13.30	1.46
		Moderate	211.5	47.42	3.40	13.23	1.49
		Severe	217.0	45.92	3.33	12.98	1.40
Mean			216.5A	47.26B	3.39A	13.07B	1.44A
Serpentine	39	Nil	166.3	39.77	3.16	12.38	1.34
		Slight	159.7	39.52	3.17	12.38	1.34
		Moderate	182.5	41.30	3.16	12.75	1.28
		Severe	174.0	41.82	3.13	12.50	1.33
Mean			170.6B	40.60C	3.15C	12.50C	1.25B
Harvey	30	Nil	224.7	47.29	3.24	12.25	1.22
		Slight	215.1	49.92	3.24	12.55	1.24
		Moderate	216.5	50.42	3.25	12.78	1.21
		Severe	205.5	51.39	3.21	13.05	1.23
Mean			215.4A	49.75A	3.23B	12.66C	1.26B
LSD ($P \leq 0.05$)	AB Severity		ns (4.41)	ns (0.64)	ns (0.02)	ns (0.11)	ns (0.03)
	Location		12.6	1.82	0.05	0.31	0.07
	SOAB x Location		ns (8.81)	ns (1.28)	ns (0.03)	ns (0.21)	ns (0.05)

AB = albedo breakdown. SOAB = Severity of albedo breakdown. Nil = no albedo breakdown. Slight = < 25% of fruit surface affected with AB. Moderate = 25% - <50% of fruit surface affected with AB. Severe = ≥ 50 of fruit surface affected with AB. n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Table 4.2. Effect of different severities of albedo breakdown on levels of ascorbic acid ($\text{mg}\cdot 100 \text{ mL fresh juice}^{-1}$) and individual organic acids including citric, malic, succinic and tartaric acids ($\text{g}\cdot\text{L fresh juice}^{-1}$) in ‘Washington Navel’ orange at different locations in 2006. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Location	Tree age	Severity of AB	Ascorbic acid	Organic acids			
				Citric	Malic	Succinic	Tartaric
Gingin	25	Nil	61.85	9.35	3.90	0.90	0.40
		Slight	58.11	9.21	3.88	0.88	0.40
		Moderate	58.52	9.57	3.89	0.87	0.41
		Severe	55.60	9.29	3.86	0.83	0.43
Mean			58.52C	9.35D	3.88C	0.87D	0.41D
Chittering	12	Nil	61.52	14.71	4.91	1.14b	0.59
		Slight	62.27	14.94	4.90	1.13b	0.62
		Moderate	63.44	15.25	5.17	1.27ab	0.66
		Severe	62.05	15.02	5.27	1.35a	0.68
Mean			62.32B	14.98A	5.06B	1.22C	0.64A
Serpentine	39	Nil	68.04	13.02	5.44	2.53a	0.45
		Slight	68.50	12.51	5.32	2.07b	0.48
		Moderate	69.77	13.60	5.21	1.95b	0.53
		Severe	66.68	13.21	5.19	1.54c	0.56
Mean			68.25A	13.08B	5.29A	2.02B	0.51C
Harvey	30	Nil	67.88	11.99	5.44	3.56a	0.50
		Slight	68.67	12.33	5.39	3.39ab	0.50
		Moderate	67.86	12.09	4.99	2.73c	0.59
		Severe	67.60	12.37	5.06	2.23d	0.62
Mean			68.00A	12.19C	5.22AB	2.98A	0.55B
	AB severity		ns	ns	Ns	0.21	ns
LSD			(0.70)	(0.19)	(0.06)		(0.01)
($P \leq 0.05$)	Location		1.98	0.57	0.17	0.21	0.05
	SOAB x Location		ns	ns	Ns	0.42	ns
			(1.39)	(0.39)	(0.35)		(0.04)

AB = albedo breakdown. SOAB = Severity of albedo breakdown. Nil = no albedo breakdown. Slight = < 25% of fruit surface affected with AB. Moderate = 25% - <50% of fruit surface affected with AB. Severe = ≥ 50 of fruit surface affected with AB. n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

4.3.4. Effect of the severity of albedo breakdown on fruit quality in different cultivars of ‘Navel’ orange

The different levels of severity of albedo breakdown did not significantly affect juice content, its pH, soluble solids concentration, titratable acidity, and ascorbic acid in ‘Leng Navel’, Golden Atum’ and ‘Washington Navel’ cultivars (Tables 4.3 and 4.4).

Amongst all individual organic acids detected in the fruit juice, different levels of severity of albedo breakdown have significantly affected concentrations of succinic acid and tartaric acid in all the three cultivars. The higher levels of severity of albedo breakdown have reduced the concentrations of succinic acid while it has increased tartaric concentration in the fruit juice (Table 4.4). The juice content, its pH, soluble solids concentration, ascorbic acid and succinic acid and tartaric acid differed significantly among different cultivars. The interactions between different levels of severity of albedo breakdown and cultivars were found to be non-significant for juice content, pH, SSC, titratable acidity, and all the individual organic acids whereas it was significant for ascorbic acid (Tables 4.3 and 4.4).

Table 4.3. Effect of different severities of albedo breakdown on percentage of juice, juice pH, soluble solids concentration (%) (SSC) and acidity (mg·100 mL fresh juice⁻¹) in different cultivars of ‘Navel’ orange in 2005. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Cultivar	Severity of AB	Juice (%)	Juice pH	SSC	Acidity
Leng Navel	Nil	54.90	3.44	14.61	0.91
	Moderate	54.99	3.48	14.67	0.83
	Severe	55.37	3.47	14.66	0.90
Mean		55.09A	3.46B	14.65A	0.88
Autumn Gold	Nil	42.42	3.40	12.98	0.86
	Moderate	44.10	3.41	12.95	0.77
	Severe	43.31	3.38	12.61	0.80
Mean		43.28C	3.39C	12.85B	0.81
Washington Navel	Nil	49.48	3.69	14.46	0.84
	Moderate	50.10	3.69	14.47	0.81
	Severe	49.42	3.67	14.38	0.88
Mean		49.67B	3.68A	14.44A	0.84
LSD ($P \leq 0.05$)	Severity of AB	ns (0.67)	ns (0.02)	ns (0.12)	ns (0.02)
	Cultivar	1.95	0.05	0.36	ns (0.02)
	SOAB	x ns (1.63)	ns (0.03)	ns (0.21)	ns (0.04)
	Cultivar				

AB = albedo breakdown. SOAB = Severity of albedo breakdown. Nil = no albedo breakdown. Slight = < 25% of fruit surface affected with AB. Moderate = 25% - <50% of fruit surface affected with AB. Severe = ≥ 50 of fruit surface affected with AB. n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Table 4.4. Effect of different severities of albedo breakdown on ascorbic acid ($\text{mg}\cdot 100 \text{ mL fresh juice}^{-1}$) and individual organic acids including citric, malic, succinic and tartaric acids ($\text{g}\cdot \text{L fresh juice}^{-1}$) in different cultivars of ‘Navel’ oranges in 2005. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Cultivar	Severity of AB	Ascorbic acid	Organic acids			
			Citric	Malic	Succinic	Tartaric
Leng Navel	Nil	59.0	5.69	2.06	1.14a	0.32b
	Moderate	60.4	5.90	2.14	0.90b	0.37b
	Severe	60.1	6.84	2.44	0.84b	0.47a
Mean		60.7A	6.14	2.21	0.96B	0.39A
Autumn Gold	Nil	43.6	5.05	1.96	1.27a	0.21b
	Moderate	45.6	5.45	2.11	1.18ab	0.29a
	Severe	43.5	5.17	2.06	1.05b	0.26ab
Mean		44.3B	5.23	2.04	1.17A	0.25B
Washington Navel	Nil	64.9	4.97	1.66	0.88	0.34
	Moderate	57.1	6.02	1.95	0.80	0.36
	Severe	54.4	7.05	2.22	0.75	0.38
Mean		58.8A	6.01	1.94	0.81C	0.36A
LSD ($P \leq 0.05$)	AB	ns (0.90)	ns (0.32)	ns (0.10)	0.14	0.05
	Severity					
	Cultivar	2.63	ns (0.32)	ns (0.10)	0.14	0.05
	SOAB x Variety	4.56	ns (0.55)	ns (0.16)	ns (0.24)	ns (0.09)

AB = albedo breakdown. SOAB = Severity of albedo breakdown. Nil = no albedo breakdown. Slight = < 25% of fruit surface affected with AB. Moderate = 25% - <50% of fruit surface affected with AB. Severe = ≥ 50 of fruit surface affected with AB. n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

4.4. Discussion

Fruit diameter increased rapidly from 97 ± 5 DAFB to 244 ± 5 DAFB. Then a slow increase in fruit diameter was recorded until commercial harvest during 2005-06 and 2006-07 (Fig. 4.1). Similarly, Bain (1958) reported that in general, orange fruit under subtropical conditions develop in a long period of eight to ten months for ‘Navel’ oranges depending on cultivars. In Western Australia, stage of cell division from mid September to mid November, the fruit grow and develop until mid-winter in July. The orange fruit growth shows a typical sigmoid growth curve which is divided into three stages including cell division, expansion and ripening (Bain, 1958, Iglesias et al., 2007; Hutton et al, 2007).

Albedo breakdown incidence was visible after colour break (244 ± 5 DAFB) and increased slowly until at commercial harvest. Later on, it increased rapidly during 2007 and 2008 (Fig. 4.1). The severity of albedo breakdown also increased with advancement of fruit maturation, ripening and over-ripening. Similarly, higher incidence of albedo breakdown was observed in the tree whose fruit was harvested after the commercial harvest period had lapsed (Jones et al., 1967; Tugell et al., 1993; Dick, 1995; McIntosh, 1998). The total pectin and water-soluble pectin decrease during fruit ripening in sweet orange affecting the fruit texture (Ladaniya, 2007). It has been proposed that degradation of pectin is partially contributing to an increase in albedo breakdown incidence during fruit maturation and fruit over ripening as fruit held on the tree beyond commercial harvest typically soften and are prone to drop (Davies and Albrigo, 1994).

The albedo breakdown incidence and severity in 'Washington Navel' orange varied significantly at different locations within Western Australia. The incidence and severity was lowest at Harvey as compared to other three locations (Fig. 4.2). Possibly, the variation in incidence may be ascribed to the variable climatic factors in these agro-climatic zones of Western Australia. Similarly, the incidence and severity varied from year to year and from location to location and among cultivars of sweet oranges (Jones et al., 1967; Sneath, 1987; Treeby et al., 1995).

Severity of albedo breakdown did not significantly affect fruit quality attributes such as juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and individual organic acids except for a reduction in succinic acid at all the locations in 2005 whilst, location had significantly affected the fruit quality variables in 'Washington Navel' orange.

Various fruit quality variables as mentioned above were not significantly affected in cultivars 'Leng Navel', 'Autumn Gold' and 'Washington Navel' with the severity of albedo breakdown (Tables 4.3 and 4.4). The higher levels of severity of albedo breakdown have reduced the concentrations of succinic acid in the fruit juice and the trend was reverse for tartaric acid (Table 4.4). Citric acid is a major acid contributing to the organic acids in fruit juice, following malic acid. Succinic and tartaric acid are in minor quantities in citrus juice (Davies and Albrigo, 1994; Iglesias et al., 2007; Ladaniya, 2007; Pretel et al., 2004; Clements 1964a; Matsumoto and Shiraishi, 1981). Succinic acid concentration was 5 - 7 folds lower than citric acid in fruit juice

among cultivars at the same location in 2005 (Table 4.4) and it was 4 – 12 folds lower than citric acid in ‘Washington Navel’ among locations in 2006 (Table 4.2). Citric acid concentration was 16 – 20 folds higher than tartaric acid concentration among cultivars in 2005 (Table 4.4). Therefore, succinic acid and tartaric acid are not major acids affecting the fruit quality in ‘Navel’ sweet oranges. Earlier it has also been reported that the albedo breakdown did not affect internal fruit quality in sweet orange (Pelizzo, 1997; Goldie, 1998). Contrarily, Jones and Embleton (1967), Jones et al. (1967) and Sneath (1987) reported that fruit with albedo breakdown had a higher specific gravity, a thinner peel and a significantly higher juice content and lower total acid and ascorbic acid concentration than normal fruit. The exact mechanism of regulation of production of succinic acid and tartaric acid with albedo breakdown is not yet known and warrants further investigations.

The juice content, its pH, soluble solids concentration, ascorbic acid, succinic acid and tartaric acid differ significantly among different cultivars. Similarly, Pretel et al. (2004) reported that significant differences in fruit weight, soluble solids content and titratable acidity among sweet orange cultivars as genetic factors associated with fruit quality.

In conclusion, albedo breakdown incidence and severity was visible and developed slowly after colour break and was coupled with the gradual fruit growth. They then increased rapidly after commercial harvest until 332 ± 5 DAFB in 2005 and 2006. The significantly lowest incidence of albedo breakdown and severity were observed in Harvey as compared to three locations (Gingin, Chittering and Serpentine) in 2006. Irrespective of locations and cultivars, the severity of albedo breakdown did not affect the major fruit quality attributes such as juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid, citric and malic acid whilst it reduced succinic acid in 2005 and 2006 and enhanced tartaric acid in 2005. Locations and cultivars significantly contributed to fruit quality attributes in ‘Navel’ sweet oranges in 2005 and 2006.

CHAPTER 5

Responses of 'Navelina' orange to irrigation levels: water relations, growth, yield and fruit quality with an emphasis on albedo breakdown

Abstract

Albedo breakdown (creasing) is a serious physiological disorder in citrus. I explored its incidence as affected by water status of the tree. I irrigated 'Navelina' orange, in Western Australia, with the following percentages of commercial irrigation: 100% (T100, Control), 125% (T125), 75% (T75), and 50% (T50). T50 had significantly lower incidence of albedo breakdown than the other treatments. I attributed this to a slower growth of endocarp with albedo not being overstretched. Reduced irrigation did not affect rind thickness, dry matter content of rind and pulp although there was a tendency for thicker rind in T50. The improvement in fruit quality was obtained in deficit irrigation in terms of increased soluble solids concentration and acidity levels. Percentage of juice, pH of juice, ascorbic acid and individual organic acids were not affected with irrigation management.

5.1. Introduction

Albedo breakdown in sweet orange [*Citrus sinensis* (L.) Osbeck.] is a physiological disorder with cracks in the internal white tissue (albedo) causing the overlying flavedo to collapse forming random grooves over the surface of the fruit. Sometimes it affects up to 90% of the crop (Bower, 2000). It is triggered by a multitude of environmental and plant factors and their complex interactions. Considering the profound effects of water on plant growth and development, it is expected that albedo breakdown may be affected by water status of the plant and fruit. The published results are so far contradictory and information is needed for clarification. Treeby et al. (2007) applied deficit irrigation (DI) and partial rootzone drying (PRD), over the whole growing seasons of 1999 and 2000, to 'Bellamy' Navel orange grafted on five rootstocks in an orchard at Dareton Primary Industries Institute in the south-western New South Wales, Australia. Both DI and PRD treatments received *ca.* 50% of water given to the control. For DI, irrigation water was applied to the entire rootzone and for PRD to only one side of the tree row at each irrigation time. Albedo breakdown ranged from 20% to 80% in this experiment and was influenced

by water supply, season, and rootstock. There was a significant interaction between rootstock and irrigation treatment. Both DI and PRD resulted in significantly less incidence of albedo breakdown compared to the control. Thickness of the fruit rind was similar among the treatments. Both DI and PRD treatments significantly reduced yield and fruit size and significantly increased soluble solids concentration and titratable acidity.

While Treeby et al. (2007) did not measure plant water status in their research, Gonzalez-altozano and Castel (1999) did for their work on 'Clementina de Nule' mandarin (*Citrus clementina* Hort. ex Tan.). No relationship could be established between leaf water potential and incidence of albedo breakdown in their experiment. They applied reduced irrigation (by replacing 25% and 50% of potential evapotranspiration) at different stages of fruit growth and for the entire growing seasons of 1995 and 1996. For most of the time, leaf water potential in reduced irrigated trees was similar to the fully irrigated control. However, in 1996 the reduced irrigated trees experienced more water stress as evident by a lower leaf water potential. Yet, it was in 1995 that albedo breakdown occurred in 25% of the fruit and was more severe, as assessed visually, in the 25% treatment than in the 50% treatment. In 1996, less than 1% of the fruit experienced albedo breakdown in either of the two reduced irrigated treatments. They also found that yield and fruit quality were not affected by application of reduced irrigation in summer for both 1995 and 1996. However, trees irrigated with applications of 25% and 50% of potential evapotranspiration in autumn produced more small fruit (by 25% and 11%, respectively) than that in control treatment for both seasons. Soluble solids concentration and titratable acidity, without affecting percentage of juice significantly increased with application of 50% potential evapotranspiration in autumn in both 1995 and 1996.

Hutton et al. (2007) applied reduced irrigation up to 33% as compared to full irrigation with three irrigation intervals (3 days, 10 days and 17 days) during summer and autumn in two seasons 1992/1993 and 1993/1994 to 'Valencia' orange trees. Reduced irrigation decreased fruit size and increased both soluble solids concentration and titratable acidity.

In an attempt to resolve the inconclusive results in the literature, I developed the following hypothesis as the basis of our experiment. If development of albedo

breakdown is assumed to be due to a fast growth of endocarp in Stage III (Holtzhausen, 1981), reduced irrigation should slow this growth and decrease the incidence of albedo breakdown if albedo had not been already weakened for some other reasons. I therefore applied DI to 'Navelina' orange, monitored plant water status, and measured albedo breakdown in the fruit as well as other fruit quality attributes.

5.2. Materials and methods

5.2.1. Experimental site and plant material

The experiment was carried out in a commercial orchard located in Bindoon, Western Australia (Latitude 31° 23', longitude 116° 06'). The climate is described as wet winters and hot, dry summers. Total rainfall was 328.4 mm during the experiment (January – July 2006). The soil is gravely pale sandy loam with poor water holding capacity. Except for irrigation, all other cultural practices including nutrition, insect and weed control in all the blocks were the same as the commercial orchard housing the experiment.

Twelve years old 'Navelina' sweet orange trees [*Citrus sinensis* (L.) Osbeck] grafted on 'Swingle citrumelo' (*Poncirus trifoliata* [L.] Raf.) x (*Citrus paradisi* Macf.) rootstock were used for the experiment. They were spaced 6.0 m between rows and 2.0 m within rows with row direction of north - south.

5.2.2. Treatments and experimental design

Four irrigation treatments were applied during the experiment. Control trees received 100% of commercial irrigation (T100) while T50, T75 and T125 received 50%, 75% and 125%, respectively, of the water applied to T100. The drip irrigation lines were installed on both sides of each row. In the commercial orchard, irrigation was applied when tensiometer readings were between 50 and 70 centibars. Approximately 77 liters of water were given daily to each control tree. The experiment started in February 2006 and stopped in July after harvesting.

The experimental design was a randomized block design with four replications. Four trees were irrigated as an experimental unit in which the centre two trees were sampled for measurements.

5.2.3. Parameters determined

Soil volumetric water content, midday stem water potential and stomatal conductance were determined. Albedo breakdown incidence was recorded. Rind, albedo and flavedo thickness and dry matter content of rind and pulp were measured. Fruit growth, fruit drop and trunk diameter were recorded. Average fruit weight, fruit size and yield were measured. Percentage of juice, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and organic acids were determined as fruit quality parameters.

5.2.3.1. Measurement of soil volumetric water content, midday stem water potential and stomatal conductance

Soil volumetric water content (θ) was measured biweekly at depths of 300 mm and 600 mm using a MP 406 moisture probe (ICT International Pty Ltd, Australia). Two measurements were recorded at 50 cm away from the tree trunk on both sides of each orange tree (four measurements per treatment unit). Soil volumetric water content was expressed as percent. The procedure for monitoring soil volumetric water content was described in more details in Section 3.2.

Midday stem water potential (ψ_{md}) was determined from 11.00 to 13.00 using a pressure chamber (Soil Moisture Equip. Corp, Santa Barbara, CA, USA) and expressed as MPa. The detailed procedure for determination of midday stem water potential has been mentioned in Section 3.3.

Stomatal conductance was determined from 11.00 to 13.00 five times (93, 107, 128, 142 and 156 days after full bloom) using a leaf porometer AP4 [(Model Sc-1 (steady state diffusion porometer))] as mentioned in more details in Section 3.4.

5.2.3.2. Determination of albedo breakdown incidence and severity

Albedo breakdown in percent was recorded, on a sample of 100 fruit harvested from 2 metre-squares from one side throughout other side of each tree from the middle canopy. The albedo breakdown incidence and the severity of albedo breakdown were expressed as percentage of fruit. The procedure for determining the incidence and severity of albedo breakdown have been described in more details in Section 3.7.

5.2.3.3. Determination of rind, flavedo and albedo thickness

Ten mature fruit collected at north, east, west and south points of each tree at about 1.5 m high were sampled to measure rind, flavedo, and albedo thickness (mm) as mentioned in more details in Section 3.9.

5.2.3.4. Determination of rind and pulp dry matter content

Five mature fruit collected at north, east, west and south points of each tree at about 1.5 m high were sampled to determine dry matter content. The detailed procedure for determination of dry matter contents of rind and pulp has been described in Section 3.10.

5.2.3.5. Measurement of fruit growth, fruit drop and trunk diameter

Fruit diameter was measured biweekly on 8 fruit marked per tree using an electronic digital calliper (16 fruits per treatment). Fruit drop was determined at two week intervals from five tagged branches per tree (10 branches per treatment unit) and expressed as percent. Trunk diameter was measured at 30 cm above the soil line on two occasions before and after the application of irrigation treatments (73 and 240 days after full bloom, respectively). Two trees per treatment unit were sampled.

5.2.3.6. Determination of average fruit weight, fruit size and yield

Fruit from 2 metre-squares from one side throughout the other side of each tree from middle canopy were harvested and weighed. Fruit size in percent was classified according to fruit diameter into three categories: small (< 64 mm), medium (64-88 mm) and large (>88 mm). All fruit from two whole trees were weighed to measure fruit yield per tree.

5.2.3.7. Determination of other fruit attributes

Five mature fruit selected at north, east, west and south sides of each tree at about 1.5 m high were weighed and juiced using a citrus juicer (Sunbeam citrus juicer, TE 2600, Sunbeam Co. Ltd., China). The freshly extracted juice was used for calculating juice content and determining juice pH, soluble solids concentration, titratable acidity, ascorbic acid and organic acids.

5.2.3.7.1 Juice contents

Juice content was calculated by dividing juice weight by fruit weight as mentioned in more details in Section 3.11.1.

5.2.3.7.2 Juice pH

Juice pH was measured using a bench digital pH meter (Cyberscan pH 510, Eutech Instrumnet Pte Ltd., Singapore) as described in more details in Section 3.11.2.

5.2.3.7.3 Soluble solids concentration

Soluble solids concentration (SSC) in percent was recorded using an infrared digital refractometer (Atago-Palette PR 101, Atago Co. Ltd, Itabashi-Ku, Tokyo, Japan) as mentioned in Section 3.11.3.

5.2.3.7.4 Titratable acidity

Titrateable acidity (TA) was determined by following the titration method to phenolphthalein endpoint as described in more details in Section 3.11.4. Titrateable acidity was expressed as mg citric acid · 100 mL fresh juice⁻¹.

5.2.3.7.5 Ascorbic acid

Ascorbic acid concentration was determined as described in more details in Section 3.11.5. Ascorbic acid concentration was calculated using a standard curve of L-ascorbic acid and expressed as mg ascorbic acid · 100 mL fresh juice⁻¹.

5.2.3.7.6 Individual organic acids

Organic acid concentration was determined by using the high performance liquid chromatography (HPLC) technique. Organic acids concentration was calculated using a standard curve of citric acid, malic acid, succinic acid and tartaric acid and expressed as g · L fresh juice⁻¹. The detailed procedure for determination of individual organic acids has been mentioned in Section 3.11.6.

5.2.4. Statistical analysis

The data were subjected to one way ANOVA using Genstat 9 release 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The least significant difference (Fisher's protected LSD) was calculated at $P \leq 0.05$. To ensure the validity of statistical analysis all the variables of ANOVA were checked.

5.3. Results

5.3.1. Soil water content

Irrigation treatments significantly affected the soil volumetric water content at 300 mm depth on six occasions (128, 142, 156, 171, 184 and 211 DAFB) (Fig. 5.1A).

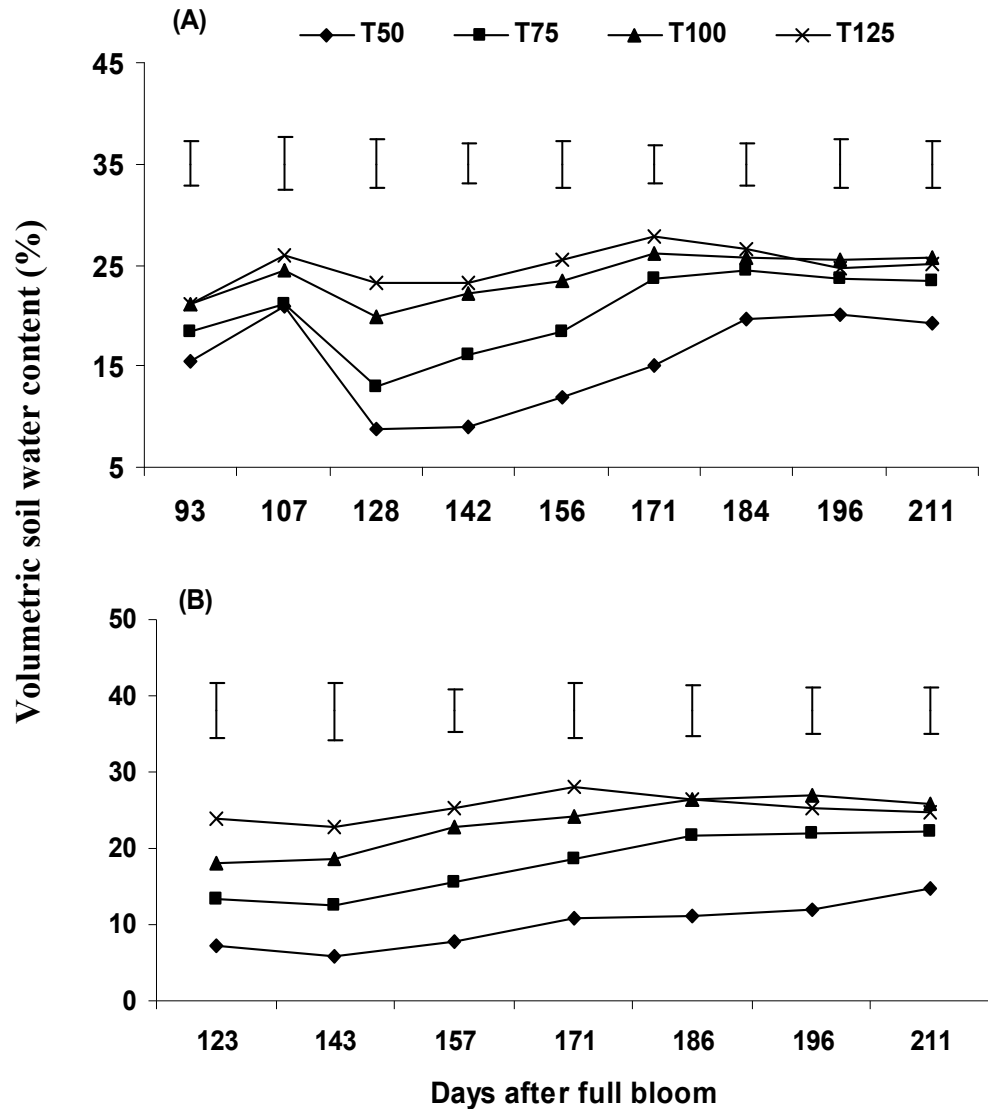


Figure 5.1. Effect of irrigation treatments on volumetric soil water content at soil depth of 300 mm (A) and 600 mm (B) in 'Navelina' orange. Vertical bars represent LSD at $P < 0.05$. $n = 4$ replications

The highest soil volumetric water content at 300 mm was recorded in T125 during the experimental period while T50 had significantly lowest soil volumetric water content. The values of soil volumetric water content at 300 mm depth decreased

rapidly from 107 DAFB reaching the lowest values of 8.74%, 16.03% and 19.96% in T50, T75 and T100 at 128 DAFB, respectively. It then started to rapidly increase until 184 DAFB. After this time, it slowly increased to harvest in T50, T75 and T100 except T125 (Fig. 5.1A). Soil volumetric water content was significantly affected at 600 mm soil depth with the application of irrigation treatments (Fig. 5.1B). It steadily increased from 143 DAFB to 211 DAFB in T50. Such a trend was not observed for T100 and T125. The soil volumetric water content was significantly lowest in T50 at a soil depth of 600 mm. The values in T50 at 600 mm depth increased slowly from 5.90% at 143 DAFB to 14.75% at 211 DAFB (Fig. 5.1B).

5.3.2. Plant water status

Midday stem water potential was significantly affected with the application of irrigation treatments on five occasions (Table 5.1).

Table 5.1. Effect of irrigation treatments on midday stem water potential (MPa) in ‘Navelina’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Treatment	Midday stem water potential (MPa)							
	Days after full bloom							
	91	107	130	142	157	171	186	217
T125	-0.96	-0.77	-1.00a	-1.36a	-1.68a	-1.35a	-1.62a	-2.36
T100	-0.96	-0.78	-0.99a	-1.71b	-1.78a	-1.48ab	-1.69a	-2.45
T75	-0.94	-0.88	-1.04a	-1.85b	-2.01a	-1.63bc	-1.74ab	-2.43
T50	-0.91	-0.89	-1.35b	-2.26c	-2.53b	-1.83c	-1.85b	-2.32
LSD	ns	ns	0.19	0.18	0.35	0.24	0.13	ns
($P \leq 0.05$)	(0.08)	(0.06)						(0.19)

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

The values were lower in T50 at 91, 107 and 217 DAFB (-0.91 MPa, -0.89 MPa and -2.32 MPa, respectively) although there were not significant differences among the treatments. Significantly lowest midday stem water potentials were recorded in T50 at 130, 142, 157, 171 and 186 DAFB (-1.35 MPa, -2.26 MPa, -2.53 MPa, -1.83 MPa and -1.85 MPa, respectively) as compared to T125 and T100. T125 had the highest

midday stem water potential during experimental period although there were no significant differences between T125 and T100 (Table 5.1).

Stomatal conductance was significant lowest in T50 at 107 DAFB ($29.17 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) as compared to other treatments. However, stomatal conductance was non-significant at 93, 128, 142 and 156 DAFB among the treatments (Table 5.2).

Table 5.2. Effect of irrigation treatments on stomatal conductance ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in 'Navelina' orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$. $n = 4$ replications

Treatment	Stomatal conductance ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)				
	Days after full bloom				
	93	107	128	142	156
T125	42.29	39.43a	38.31	33.91	33.53
T100	39.20	34.50b	32.26	30.70	30.68
T75	33.70	34.26b	30.76	29.20	28.43
T50	33.08	29.17c	32.82	31.13	27.03
LSD ($P \leq 0.05$)	ns (4.65)	4.08	ns (2.77)	ns (4.38)	ns (2.48)

ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

5.3.3. Albedo breakdown incidence and severity

Percentage of fruit with moderate incidence of albedo breakdown was significantly lowest (4.8%) in T50 (Table 5.3). There was no significant difference in percentage of fruit with moderate incidence of albedo breakdown among T75, T100 and T125 (24.9%, 29.2% and 24.5%, respectively). Percentage of fruit with severe incidence of albedo breakdown was significantly lower in T50 and T125 (2.8% and 8.8%, respectively) as compared to that in T75 (21.1%) and T100 (16.0%).

The percentage of total albedo breakdown incidence was significantly affected by irrigation treatments. T50 resulted in the significantly lowest percentage of total albedo breakdown incidence (7.5%). There were no significant differences in percentage of total albedo breakdown incidence among T75, T100 and T125 (33.6%, 30.6% and 21.0%, respectively). The severity of albedo breakdown was significantly affected by application of different irrigation treatments. T50 resulted in the

significantly lowest severity of albedo breakdown (5.1%) while there were no significant differences in the severity of albedo breakdown among T75, T100 and T125 (33.6%, 30.6% and 21.0%, respectively) (Table 5.3).

Table 5.3. Effect of irrigation treatments on moderate incidence of albedo breakdown (MAB, % of fruit), severe incidence (SAB), total AB incidence (TAB), and the severity of albedo breakdown (ABS, %) in ‘Navelina’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$. n = 4 replications.

Treatment	Incidence of albedo breakdown(% of fruit)			ABS
	MAB	SAB	TAB	
T125	24.5a	8.8bc	33.2a	21.0ab
T100	29.2a	16.0ab	45.2a	30.6a
T75	24.9a	21.1a	46.0a	33.6a
T50	4.8b	2.8c	7.5b	5.1b
LSD ($P \leq 0.05$)	13.69	12.26	23.09	17.17

5.3.4. Thickness of rind, flavedo and albedo

Thickness of rind was higher in T50 and T125 as compared to T75 and T100. Flavedo was thicker in T50, T75 and T125 as compared to T100.

Table 5.4. Effect of irrigation treatments on thickness (mm) of rind, flavedo and albedo and dry matter content of rind and pulp ($\text{g} \cdot 100 \text{ g fresh sample}^{-1}$) at harvest in ‘Navelina’ orange. n = 4 replications.

Treatment	Thickness (mm)			Dry matter content	
	Rind	Flavedo	Albedo	Rind	Pulp
T125	6.6	2.0	4.7	26.55	21.08
T100	6.4	1.8	4.6	27.59	21.81
T75	6.4	1.9	4.6	28.10	23.09
T50	6.5	1.9	4.6	28.49	22.43
LSD ($P \leq 0.05$)	ns (0.17)	ns (0.09)	ns (0.10)	ns (1.13)	ns (0.56)

ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

However, the differences in rind, flavedo and albedo thickness among all treatments were non-significant (Table 5.4).

5.3.5. Dry matter content of rind and pulp

Dry matter content of rind was higher in T50 as compared to control and other treatments. The highest pulp dry matter content was obtained in T75. However, there were no significant differences in rind and pulp dry matter content among all treatments (Table 5.4).

5.3.6. Fruit growth

The application of reduced irrigation significantly decreased fruit diameter at harvest. Fruit diameter was significantly lowest in T50 (75.19 mm) as compared to that in T100 (79.52 mm) (Fig. 5.2).

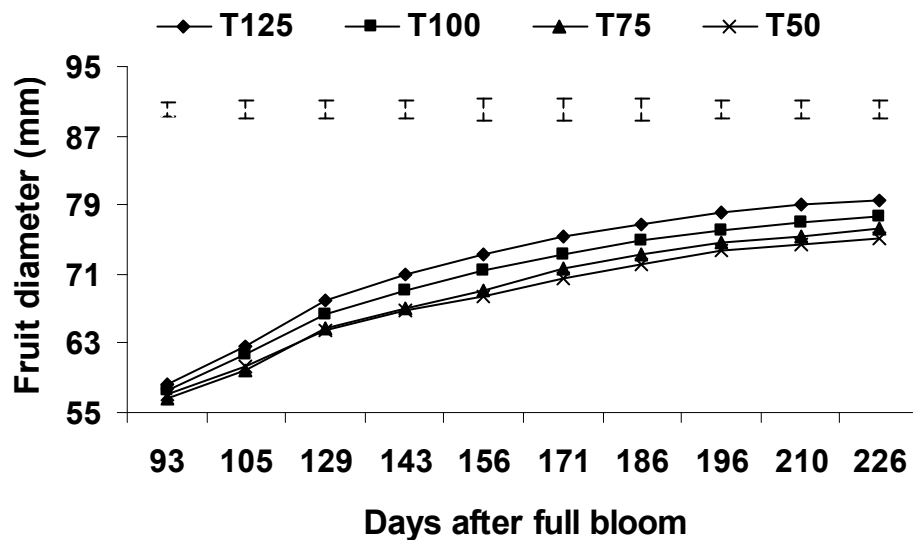


Figure 5.2. Effect of irrigation treatments on fruit diameter (mm) in ‘Navelina’ orange during fruit development and maturation. Vertical bars represent LSD at $P \leq 0.05$. $n = 4$ replications.

Proportion of fruit drop at harvest was higher in T50 (8.97%) than other treatments. However, the percentage of fruit drop was not significantly different among the treatments (Table 5.5).

5.3.7. Trunk diameter

Trunk diameter was not significantly different among the treatments. Trunk diameter was 14.39 cm, 13.95 cm, 13.92 cm and 13.28 cm in T100, T50, T75 and T125, respectively, at 240 DAFB.

Table 5.5. Effect of irrigation treatments on fruit drop in ‘Navelina’ orange.

Treatment	Fruit drop (%)								
	Days after full bloom								
	106	129	143	157	170	186	196	212	226
T125	0	0.00	0.00	0.00	0.00	0.00	0.42	0.42	0.42
T100	0	0.27	0.27	0.27	0.27	0.91	3.27	3.27	3.54
T75	0	0.00	0.00	0.00	0.00	0.00	3.49	3.49	6.27
T50	0	0.79	1.09	1.38	1.38	1.38	5.80	5.80	8.97
LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns
($P \leq 0.05$)	(0.00)	(0.37)	(0.44)	(0.57)	(0.59)	(0.77)	(3.19)	(3.19)	(3.76)

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

5.3.8. Yield, average fruit weight and fruit size

The application of reduced irrigation treatments significantly decreased the yield (Table 5.6). T50 resulted in the significantly lowest yield ($45.50 \text{ kg} \cdot \text{tree}^{-1}$). The yield was higher in T125 ($68.63 \text{ kg} \cdot \text{tree}^{-1}$) compared to yield in T100 ($66.38 \text{ kg} \cdot \text{tree}^{-1}$). However, there were no significant differences in yield between T100 and T125 (Table 5.6). Average fruit weight was significantly decreased with reduced irrigation. T50 resulted in significantly lowest average fruit weight ($183 \text{ g} \cdot \text{fruit}^{-1}$) while the highest average fruit weight was obtained in T125 ($220 \text{ g} \cdot \text{fruit}^{-1}$) (Table 5.6).

Deficit irrigation significantly increased percentage of fruit with small size. T50 significantly increased the percentage of small fruit (28.0%) while T125 significantly reduced the proportion of small fruit (8.7%). There were no significant differences in percentage of fruit with medium size among irrigation treatments. T125 resulted in

the significantly highest percentage of large fruit as compared to other treatments (Table 5.6).

Table 5.6. Effect of irrigation treatments on the total yield ($\text{kg}\cdot\text{tree}^{-1}$), average fruit weight (AFW, g), and distribution of fruit size (%) into small (<64 mm diameter), medium (64-88 mm diameter) and large (>88 mm diameter) for 'Navelina' orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Treatment	Total yield	AFW	Size		
			Small	Medium	Large
T125	68.63a	220a	8.7c	52.6	38.7a
T100	66.38ab	197b	21.6b	54.0	24.4ab
T75	55.00c	197b	24.1ab	59.7	16.2b
T50	45.50d	183b	28.0a	55.5	16.5b
LSD ($P \leq 0.05$)	9.04	0.02	5.98	ns (7.52)	16.50

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

5.3.9. Fruit quality

Table 5.7. Effect of irrigation treatments on percentage and pH of the juice in 'Navelina' orange.

Treatment	Juice (%)	Juice pH
T125	47.3	3.4
T100	45.7	3.4
T75	45.9	3.3
T50	45.4	3.3
LSD ($P \leq 0.05$)	ns (0.76)	ns (0.08)

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Soluble solids concentration and titratable acidity were significantly increased in T50 as compared to control while T125 significantly decreased the soluble solids concentration at harvest (Table 5.8).

Percentage of juice, juice pH, ascorbic acid as well as individual organic acids were not significantly affected by the application of irrigation treatments (Tables 5.7, 5.8 and 5.9).

Table 5.8. Effect of irrigation treatments on soluble solids concentration (SSC, %), titratable acidity (TA, mg·100 mL fresh juice⁻¹) and ascorbic acid (mg·100 mL fresh juice⁻¹) in ‘Navelina’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Treatment	SSC	TA	Ascorbic acid
T125	13.4c	1.30bc	61.32
T100	14.0bc	1.27c	61.32
T75	14.8ab	1.49ab	65.55
T50	15.2a	1.51a	63.82
LSD ($P \leq 0.05$)	1.05	0.20	ns (2.74)

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Table 5.9. Effects of irrigation treatments on individual organic acids (g·L⁻¹ fresh juice) in ‘Navelina’ orange.

Treatment	Organic acids			
	Citric	Malic	Succinic	Tartaric
T125	15.70	5.43	1.28	0.50
T100	14.62	5.10	1.28	0.55
T75	17.77	5.63	1.28	0.53
T50	17.25	5.38	1.28	0.58
LSD ($P \leq 0.05$)	ns (0.88)	ns (0.14)	ns (0.06)	ns (0.02)

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

5.4. Discussion

For most of the measurement occasions, stomatal conductance was lower in T50 and T 75 than in T125 and T100. However, a significant difference occurred only at 107 DAFB (Table 5.2). But on this occasion there were no differences in midday stem water potential among the treatments (Table 5.1). Stomatal closure could therefore have been brought about by chemical signals, such as abscisic acid, originating from the drying soil (Tardieu et al., 1992). Lowering of stomatal conductance and midday stem water potential could have had an impact on photosynthetic rate (not measured) with subsequent reduction of fruit growth as reflected in Fig. 5.2 and discussion below.

Application of reduced irrigation resulted in a significant decrease in total albedo breakdown incidence and the severity of albedo breakdown (Table 5.3). The lower stem water potential was reflected in the development of lower albedo breakdown; in terms of moderate, severe, total albedo breakdown incidence and the severity of albedo breakdown; in T50 than in the other treatments (Table 5.3). Smaller fruit, arising from a large crop load, have been found more susceptible to albedo breakdown because of a thinner rind (Ali et al., 2000; Jones et al., 1967). In my study the crop load, in terms of yield, was lower ($P < 0.05$) in T50 than the other treatments. Rind thickness was the same among the treatments with an average of 6.5 mm. I suggest that the significantly lower albedo breakdown incidence in T50 was not a result of differences in rind thickness but was due to a higher proportion of smaller fruit whose endocarp grew slower in Stage III because of a lower plant water status. This confirms my working hypothesis. I also expect that the medium and large fruit of T50 would have grown at a slower rate in achieving their respective final sizes and therefore being less prone to albedo breakdown. I assumed that there were no significant differences in incidence of albedo breakdown in T75 and T100 due to the same percentage of fruit size, in terms of proportion of small fruit and medium fruit. Rind thickness was not different for these treatments.

Rind thickness at harvest was not significantly affected by deficit irrigation although there was a tendency for thicker rind in T50 (Table 5.4). Similar results were observed by Treeby et al. (2007), Domingo et al. (1996) and Ritermour et al. (2003) who reported that an application of water stress during fruit growth resulted in thicker fruit rinds at maturation in oranges. A slight increase of dry matter content of

rind and pulp was obtained in deficit irrigation treatments although there were no significant differences in dry matter content among treatments (Table 5.4). It may be argued that deficit irrigation may contribute to decreased cellular hydration leading to a reduction of water volume in fruit (Mpelasoka et al., 2001). Similarly, Kilili et al. (1996) reported that deficit irrigation improved the dry matter content in apple fruit at harvest.

Trunk diameter was not affected by irrigation treatments. It may be suggested that trunk diameter will be influenced when soil water potential in tree root zone becomes lower than a certain value that might not have reached in our experiment. A decrease of trunk growth may occur after an application of deficit irrigation in apple, peach and pear (Behboudian and Mills, 1997). Domingo et al. (1996) also reported the same results in “Fino” lemon tree.

Fruit drop was not significantly affected by irrigation treatments. Possibly, the deficit irrigation in this study resulted in the moderate water stress which sufficiently supplied water and nutrients to maintain fruit number on the trees (Kriedemann and Barrs, 1981; Spiegel-Roy and Goldschmidt, 1996). Similarly, a reduction of water supply up to 33% of full irrigation in summer and autumn did not affect the fruit number per tree in ‘Valencia’ orange (Hutton et al. 2007). In contrast, Gonzalez-altozano and Castel (1999) reported that an application of reduced water with 25% and 50% of full irrigation in spring increased ‘June drop’ in ‘Clementina de Nules’ citrus trees.

The significant decrease in fruit weight among irrigation treatments suggested that water stress can cause a decrease in fruit size. Apparently, soil moisture status was highly associated with fruit growth (Kriedemann and Barrs, 1981). Maotani et al. (1977) reported that low levels of dawn leaf water potential (<-0.8 MPa) affect significantly fruit growth in sweet orange. Previous studies have reported that application of deficit irrigation resulted in the smaller fruits in mandarins (Gonzalez-altozano and Castel-altozano, 1999; Verreynne et al., 2001), citrus (Riternour et al., 2003), oranges (Treeby et al., 2007) and pears and apples (Behboudian and Mills, 1997). Similarly, Hutton et al. (2007) found that water stress reduced fruit growth and fruit size by an application of extended irrigation intervals in summer and autumn in oranges. However, it is important to note that a reduction of fruit size as a result of water stress might be advantage for a decrease of albedo breakdown as

albedo breakdown was less likely to occur in fruit of the smaller size (Treeby et al., 1995).

With regard to fruit quality, it is worth mentioning that deficit irrigation did not affect juice content. This confirms previous findings by Verreyne et al. (2001) and Velez et al. (2007) who indicated that water deficit did not influence peel and juice content in citrus. In contrast, Ritermour et al. (2003) reported that water stress anytime during fruit growth and development in citrus may reduce juice content. Significant increase of soluble solids concentration due to deficit irrigation was reported for mandarins (Gonzalez-altozano and Castel, 1999), oranges (Treeby et al., 2007, Hutton et al., 2007) and 'Marisol' Clementine (Verreyne et al., 2001). The decrease of water content in fruit may be associated with an increase of SSC after deficit irrigation (Dorji et al., 2005). Therefore, the lower SSC in full irrigation may be due to an effect of solute dilution (Behboudian and Mills, 1997; Kilili et al., 1996; Kramer and Boyer, 1995; Mpelasoka et al., 2001; van Hooijdonk et al., 2004). It is also suggested that higher SSC in DI fruit may be associated with an increase of conversion of starch into sugar as sugar mainly contributes to SSC (Mpelasoka et al., 2001; Kramer and Boyer, 1995). Mpelasoka et al. (2001) reported that SSC was increased in all DI treatments before early fruit ripening in apples. In this study, deficit irrigation significantly increased acidity in fruit in comparison to control treatment. Gonzalez-altozano and Castel (1999) and Velez et al. (2007) reported that the sugars and acidity ratios were not affected by an application of deficit irrigation in citrus. In contrast, deficit irrigation has advanced fruit maturity in apples and pears (Behboudian and Mills, 1997). Reduction of irrigation water did not affect ascorbic acid at harvest. In contrast, Domingo et al. (1996) reported that deficit irrigation improved ascorbic acid in lemon.

In conclusion, T75 and T50 significantly decreased total yield due to reduced fruit size and resulted in significantly decreased albedo breakdown incidence and the severity of albedo breakdown. The improvement in fruit quality was obtained in deficit irrigation in terms of increased SSC and acidity. Juice content, rind thickness, dry matter content of rind and pulp, ascorbic acid and individual organic acids were not influenced by deficit irrigation.

CHAPTER 6

Different surfactants improve calcium uptake into leaf and fruit of ‘Washington Navel’ sweet orange [*Citrus sinensis* (L.) Osbeck.] and reduce albedo breakdown

Abstract

Albedo breakdown or creasing in sweet oranges is a physiological disorder with cracks in the albedo resulting in puffiness of orange peel causing serious economic losses. Insufficient calcium has been implicated in albedo breakdown development. I tested the efficacy of different surfactants added to aqueous solutions of $\text{Ca}(\text{NO}_3)_2$ for spraying onto the leaves and fruit to reduce albedo breakdown. A solution of 2% $\text{Ca}(\text{NO}_3)_2$ was sprayed either alone or with one of the following surfactants: 0.05% ‘Tween 20’, 0.05% ‘Tween 80’, 0.05% ‘Triton X100’, and 0.05% ‘Tergitol’. Spraying was done five times at intervals of 10 days starting from 81 days after full bloom (DAFB) on ‘Washington Navel’ sweet orange grown in Gingin (Western Australia). Unsprayed trees were treated as control. A randomized block design was used with four replications. Concentrations of Ca in the leaf, rind, and pulp of fruit were determined on 182 and 276 DAFB. The incidence of albedo breakdown was recorded for each tree as a percentage of the fruit. Surfactants enhanced the uptake of Ca in leaf, rind, and pulp of the fruit and reduced albedo breakdown compared to the calcium-only treatment. ‘Tween 20’ was the most effective surfactant in improving Ca uptake and reducing incidence of albedo breakdown as well as improving rind hardness and tensile strength. In conclusion, five foliar sprays of 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’ starting from 81 DAFB at 10-day intervals improved Ca uptake in leaf, rind and pulp of fruit and reduced the incidence of albedo breakdown in ‘Washington Navel’ orange while maintaining the other important fruit quality attributes.

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Different surfactants improve calcium uptake into leaf and fruit of ‘Washington Navel’ sweet orange and reduce albedo breakdown

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6.1. Introduction

Albedo breakdown, also known as creasing, is a physiological disorder with cracks in the internal white tissue (albedo) causing puffiness of orange peel (Treeby and Storey, 2002). The development of albedo breakdown is related to the degradation of pectin that is an important component in the cell walls of plant tissues. The loosening of the connections between cells is the result of this degradation (Jona et al., 1989). Albedo breakdown causes considerable economic losses to the citrus industry as an increase of every one percent albedo breakdown contributes to a decrease in return to orange producers estimated at \$1 million to \$2 million dollars (Goldie, 1998). Up to 15% of fruit was found to be affected by albedo breakdown at some locations in South Africa (Goldie, 1998).

A limited success in reduction of albedo breakdown in sweet oranges has been reported with regulated deficit irrigation (Treeby et al., 2007) and exogenous spray application of gibberellic acid in summer (Embleton et al., 1973; Jona et al., 1989), potassium nitrate, and soil application of potassium sulphate as well as phosphorous (Jones et al., 1967; Bevington et al., 1993). Nutritional factors such as N and K which result in thicker rind reduced incidence of albedo breakdown in sweet oranges (Ali et al., 2000; Bevington et al., 1993; Jones et al., 1967; Embleton et al., 1973; Monselise et al., 1976).

Some authors reported that lower levels of Ca in oranges are associated with albedo breakdown (Storey et al., 2002; Treeby and Storey, 2002). Treeby and Storey (2002) showed that the application of five foliar sprays of either 0.11% or 0.33% calcium starting in December – January period; or the January - February period at an early stage of ‘Navel’ orange fruit growth resulted in a significant decrease of albedo breakdown as calcium sprays increased the Ca levels in the rind and albedo of fruit.

However, the foliar application of Ca has not always increased Ca levels in tissue because Ca is not phloem mobile (Treeby and Storey, 2002). Apparently, the cuticles are the first barrier to penetration of Ca (Harker and Ferguson, 1991; Schonherr, 2001). Calcium penetration into leaf and fruit tissues can be improved with surfactants depending on the concentration and type of a surfactant (Harker and Ferguson, 1991; Schonherr, 2001).

No research work has been reported on the effects of different surfactants in enhancing the uptake of Ca into the leaf and fruit tissues of sweet orange. Thus, the objective of this study was to investigate whether selected surfactants with varying hydrophile-lipophile balance number (HLB) added to an aqueous solution containing $\text{Ca}(\text{NO}_3)_2$ enhance up take of Ca into leaf and fruit tissues and reduce albedo breakdown and affect fruit quality in 'Navel' sweet orange.

6.2. Materials and methods

6.2.1. Experimental site and plant material

The experiment was carried out in a commercial orchard located in Gingin, Western Australia (Latitude 31° 21' S, longitude 155° 55' E). The climate is described as a winter dominant with wet winters and hot, dry summers. Total rainfall was 518 mm during experimental period. The soil is a sandy loam. All the cultural practices including irrigation, insect and weed control in all the blocks were the same except for the experimental treatments.

Twenty-two years old uniform 'Washington Navel' orange trees [*Citrus sinensis* (L.) Osbeck] grafted on [*Poncirus trifoliata* (L.) Raf.] rootstock were used in the experiment. The trees were spaced 7.5 m between rows and 2.7 m within rows with row direction of north – south.

6.2.2. Treatments and experimental design

An aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ was sprayed either alone or with one of the following surfactants: 0.05% 'Tween 20', 0.05% 'Tween 80', 0.05% 'Triton X100', and 0.05% 'Tergitol'. Spraying onto fruit and leaves of the whole tree was applied five times at intervals of 10 days starting from 81 days after full bloom (DAFB) from Dec 5, 2006 to Jan 17, 2007. Unsprayed trees were treated as control. An aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ with or without surfactants was sprayed with a sprayer (The Selecta Trolley Pak Mk II, Australia) at the rate of 1000 L.ha⁻¹ till run off. The rate of a nozzle (Chierici Titisrl, Italy) was 70 L/min under the pressure of 300 KPa.

The experimental design was randomised block with four replications. Single tree was treated as an experimental unit.

6.2.3. Chemicals

Four tested surfactants described below including ‘Tween 20’, ‘Tween 80’, ‘Tergitol’ and ‘Triton X100’ were purchased from Sigma Chemical Company, Missouri, USA.

Table 6.1. Description of various surfactants with varying hydrophile-lipophile balance number (HLB) used in the experiment

Trade name of a surfactant	HLB	Chemical name of a surfactant
Tween 20	16.7	Polyoxyethylene (20) sorbitan monolaurate
Tween 80	15.0	Polyoxyethylene (20) sorbitan mono-oleate
Triton X100	13.5	Polyoxyethylene (10) tetra-methyl-buthylbenzene
Tergitol 15-S-9	13.3	Polyoxyethylene (9) sec-dodecyl ether

6.2.4. Observation recorded

Concentration of Ca in leaf, fruit rind and pulp were determined. Albedo breakdown incidence was recorded. Rind hardness, cohesiveness, adhesiveness, springiness, fracture force, stiffness, rind tensile strength force and fruit compression test were recorded as texture profile analysis. Rind, albedo and flavedo thickness, dry mater content of rind and pulp were measured. Percentage of juice, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and individual organic acids were accessed as fruit quality parameters.

6.2.4.1. *Determination of calcium concentrations from leaf, rind and pulp*

Fully developed six - month old spring flush leaves (25 /tree) from non-fruiting shoot and five fruit per tree were collected for nutrient analysis. The leaves and fruit from each tree were collected from unshaded position at about 1.5 m height at the north, east, south and west points of tree at 101 days after sprays (182 DAFB) and 195 days after sprays (286 DAFB) during the experimental period. All leaves and fruit collected were free of damage from insects or diseases.

Calcium concentrations in leaf, rind and pulp of fruit were analysed by using Radial Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) which operated in simultaneous mode as described in Section 3.6.

6.2.4.2. Determination of albedo breakdown incidence

Fruit from each experimental tree were examined for albedo breakdown. The albedo breakdown incidence was expressed as percentage of fruit. The procedure for determination albedo breakdown incidence has been mentioned in more details in Section 3.7.

6.2.4.3. Texture profile analysis

Textural properties of rind such as hardness, cohesiveness, adhesiveness, springiness, fracture, stiffness, tensile strength and fruit firmness from normal fruit and fruit with albedo breakdown were determined using a texture analyser (TA Plus, AMETEK Lloyd instruments Ltd., Hampshire, UK) as explained in Section 3.8.

6.2.4.3.1 Rind puncture test

Rind sample were cut in the size of 2.5 cm wide x 0.6 cm thick using a slicer (Zylyss Easy slice 2" folding Mandolin slicer, Swiss) to give uniform sections for determining rind puncture test. Two rinds samples were dissected 90 degree apart per fruit. Ten fruit of each fruit group (normal and albedo breakdown fruits) were tested from each tree. Hardness, cohesiveness, adhesiveness, springiness and fracture were determined as detailed in Section 3.8.1.

6.2.4.3.2 Rind tensile strength test

The rind tensile test was carried out to measure the behaviour of the orange rind up to the rind deflection of 10 mm. The rind tensile strength force was calculated at the maximum load and limit points where the rind deflection occurred as mentioned in Section 3.8.2.

6.2.4.3.3 Fruit compression test

Ten fruit of each fruit group (normal or albedo breakdown) with the height of about 8.5 cm were used for each compression test as described in more details in Section 3.8.3.

6.2.4.4. Determination of rind, flavedo and albedo thickness

Ten mature fruit collected at north, east, west and south points of each tree at about 1.5 m high were sampled to determine rind, flavedo and albedo thickness as detailed in Section 3.9.

6.2.4.5. Measurement of fruit quality parameters

Five mature fruit selected at north, east, west and south sides of each tree at about 1.5 m high were weighed and juice squeezed using a citrus juicer (Sunbeam citrus juicer, TE 2600, Sunbeam Co. Ltd., made in China to Sunbeam's specification). The freshly extracted juice was used for determining fruit quality parameters including juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and organic acids as mentioned in Section 3.11.

6.2.5. Statistical analysis

The data were subjected to ANOVA using Genstat 9 release 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The least significant difference (Fisher's protected LSD) at level of $P \leq 0.05$ was used to compare the treatment means for all experimental parameters.

6.3. Results

6.3.1. Ca concentration in leaf, fruit rind and pulp

The spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and 'Tween 20' resulted in significantly higher leaf Ca concentration (2.74%) at 101 days after sprays (DAS) as compared to all other treatments. Other surfactants along with 2% $\text{Ca}(\text{NO}_3)_2$ increased leaf Ca concentration as compared to control and Ca-only treatment but the higher differences among these surfactants were not significant at 101 DAS. The leaf Ca concentration did not differ significantly among treatments at 195 DAS (Table 6.2).

The foliar spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ and all surfactants increased rind Ca concentration as compared to both the calcium-only treatment and control at 101 DAS. Ca concentration in rind was significantly higher with applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and 'Tween 20', 'Tween 80' or 'Tergitol' than that in the control and Ca-only treatment at 101 DAS. The spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and 'Tween 20' almost doubled rind Ca concentration (0.76%) as compared to the control (0.38%) although it was not significantly different from 'Tergitol' treatment (0.66%) at 101 DAS. The rind Ca concentration was higher with the foliar application of 2% $\text{Ca}(\text{NO}_3)_2$ and all surfactants than that in the control and Ca-only treatment at 195 DAS. Among all the surfactants, 2% $\text{Ca}(\text{NO}_3)_2$ application along with 'Tween 20' and 'Tergitol' resulted

in significantly higher rind Ca concentration as compared to control and Ca-only at 195 DAS (Table 6.2).

An aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and any surfactants significantly increased pulp Ca concentration at 101 DAS as compared to control treatment. The significantly highest pulp Ca concentration was observed with the spray application of 2% $\text{Ca}(\text{NO}_3)_2$ and 'Tween 20' (0.25%) although it was not significantly different from other surfactants and Ca-only treatment at 101 DAS. Pulp Ca concentration was higher with an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and all surfactants than that with the control at 195 DAS (Table 6.2). An aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and 'Tween 20' resulted in the significantly highest pulp Ca concentration (0.14%) as compared to control (0.11%), Ca-only (0.12%) and other surfactants except 'Tergitol' (0.13%) at 195 DAS (Table 6.2).

An aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and 'Tween 20' resulted in the highest Ca concentration in the leaf, rind and pulp at 101 DAS and 195 DAS in 'Washington Navel' orange (Table 6.2).

Table 6.2. Effects of different surfactants added into an aqueous spray solution containing 2% Ca(NO₃)₂ on the concentrations of Ca in leaf, fruit rind and pulp tissues at 101 and 195 days after sprays (182 and 276 days after full bloom, respectively) in 'Washington Navel' orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Treatment	Ca (%)					
	Leaf		Rind		Pulp	
	Days after sprays (Days after full bloom)					
	101 (182)	195 (276)	101 (182)	195 (276)	101 (182)	195 (276)
Control	2.09b	2.29	0.38d	0.33c	0.13b	0.11b
Ca(NO ₃) ₂ 2%	2.17b	2.56	0.44d	0.36bc	0.22a	0.12b
Ca(NO ₃) ₂ 2% and Tween 20	2.74a	2.36	0.76 a	0.53a	0.25a	0.14a
Ca(NO ₃) ₂ 2% and Tween 80	2.27b	2.62	0.60bc	0.44abc	0.23a	0.12b
Ca(NO ₃) ₂ 2% and Triton X100	2.32b	2.35	0.55cd	0.40bc	0.24a	0.12b
Ca(NO ₃) ₂ 2% and Tergitol	2.27b	2.51	0.66ab	0.47ab	0.25a	0.13ab
LSD ($P \leq 0.05$)	0.25	ns (0.13)	0.15	0.12	0.04	0.02

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SE).

6.3.2. Albedo breakdown incidence

An aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and all surfactants except ‘Triton X100’ significantly reduced albedo breakdown incidence as compared to both the calcium-only treatment and control at harvest. The trees sprayed with an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’ resulted in the lowest albedo breakdown (43.8%) but this was not significantly higher from ‘Tergitol’ (44.1%), ‘Tween 80’ (53.8%) and ‘Triton X100’ (61.3%) (Fig.6.1).

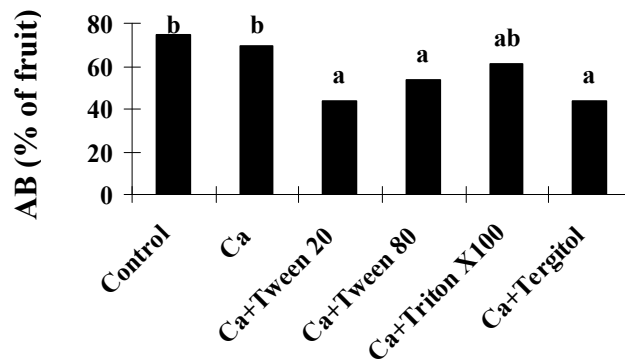


Figure 6.1. Effects of different surfactants added into an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ on the percentage of albedo breakdown incidence (AB, % of fruit) in ‘Washington Navel’ orange. Means followed by different letters on bars are significantly different at $P \leq 0.05$. $n = 4$ replications.

6.3.3. Relationship between leaf, rind and pulp Ca concentration and albedo breakdown incidence

The concentration of Ca in leaf, rind and pulp at 101 DAS showed significant negative correlation ($r = -0.780$; -0.947 and -0.891 , respectively) with albedo breakdown incidence at the harvest. There was not a significantly negative correlation between Ca concentrations in the leaf at 276 DAFB and albedo breakdown incidence at harvest ($r = -0.279$). Albedo breakdown incidence at the harvest was significantly negatively correlated with Ca concentration in rind and pulp ($r = -0.891$ and -0.905 , respectively) at 195 DAS (Table 6.3).

Table 6.3. Relationship between Ca concentrations in the leaf, rind and pulp tissues and albedo breakdown (AB) incidence at harvest

Independent variables		Correlation (r)
101 DAS (182 DAFB)	Leaf vs. AB incidence	- 0.780 **
	Rind vs. AB incidence	- 0.947 **
	Pulp vs. AB incidence	- 0.891 **
195 DAS (276 DAFB)	Leaf vs. AB incidence	- 0.279 ns
	Rind vs. AB incidence	- 0.891 **
	Pulp vs. AB incidence	- 0.905 **

DAS = days after spray, DAFB = days after full bloom. AB = albedo breakdown. n = 24. ns = not significant at $P \leq 0.05$. * * = significantly different at $P \leq 0.01$.

6.3.4. Texture profile analysis of the rind and the fruit

6.3.4.1. Rind hardness and cohesiveness

Rind hardness of normal fruit was higher than that of albedo breakdown fruit. The foliar spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and all surfactants resulted in the higher rind hardness of normal and albedo breakdown fruit than that with Ca-only treatment or control (Table 6.4). An aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’ resulted in the significantly highest rind hardness for both normal and albedo breakdown fruit as compared to all other surfactant treatments, control and Ca-only treatment. The rind hardness of the normal fruit was significantly increased with an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’ (35.35 N), ‘Tween 80’ (26.81 N) and ‘Tergitol’ (27.04 N) as compared to control and Ca-only treatment (Table 6.4). The foliar applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’ (18.83 N) and ‘Tween 80’ (15.01 N) significantly increased rind hardness of fruit with albedo breakdown as compared to ‘Triton X100’, control and Ca-only treatment in ‘Washington Navel’ orange (Table 6.4).

Table 6.4. Effects of different surfactants added into an aqueous spray solution containing 2% Ca(NO₃)₂ on rind hardness (N) and cohesiveness in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Treatment	Rind hardness (N)		Rind cohesiveness	
	Normal	AB	Normal	AB
Control	19.88e	11.62d	0.06e	0.06d
Ca(NO ₃) ₂ 2%	21.88e	13.40d	0.08e	0.06d
Ca(NO ₃) ₂ 2% and Tween 20	35.35a	18.83a	0.14a	0.15a
Ca(NO ₃) ₂ 2% and Tween 80	26.81cd	15.01bc	0.10cd	0.11cd
Ca(NO ₃) ₂ 2% and Triton X100	24.20de	13.52d	0.09de	0.08d
Ca(NO ₃) ₂ 2% and Tergitol	27.04bc	15.00cd	0.10bc	0.12bc
LSD ($P \leq 0.05$)	2.95	1.96	0.02	0.03

AB = albedo breakdown. n = 4 replications.

The foliar applications of an aqueous solution containing 2% Ca(NO₃)₂ and all surfactants resulted in the higher rind cohesiveness of normal and albedo breakdown fruit than that with Ca-only treatment or control. An aqueous spray solution containing 2% Ca(NO₃)₂ and ‘Tween 20’ resulted in the significantly highest rind cohesiveness in both normal and albedo breakdown fruit as compared to all other treatments (Table 6.4). The rind cohesiveness of the normal fruit was significantly increased with an aqueous spray solution containing 2% Ca(NO₃)₂ and ‘Tween 20’ (0.135), ‘Tween 80’ (0.098) and ‘Tergitol’ (0.100) as compared to control and Ca-only treatment (Table 6.4). The foliar applications of an aqueous solution containing 2% Ca(NO₃)₂ and ‘Tween 20’ (0.151) and ‘Tergitol’ (0.117) significantly increased rind hardness of albedo breakdown fruit as compared to control and Ca-only treatment in ‘Washington Navel’ orange (Table 6.4).

6.3.4.2. Rheological properties of rind

Among various parameters of rheological properties of rind, only the adhesiveness of rind of normal and albedo breakdown fruit was significantly affected with the treatments. The spray applications of an aqueous solution containing 2% Ca(NO₃)₂ with any surfactant resulted in higher rind adhesiveness in both normal and albedo breakdown fruits as compared to untreated and Ca-only treatments (Table 6.5). The spray applications of 2% Ca(NO₃)₂ along ‘Tween 20’ significantly increased rind

adhesiveness (0.34 N and 0.22 N, respectively) in both normal and albedo breakdown fruit as compared to control and Ca alone. Rind adhesiveness in normal fruit was higher than in albedo breakdown fruit (Table 6.5). The spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ with ‘Tween 20’ resulted in the highest springiness, fracture force and stiffness of rind in both normal and albedo breakdown fruit (Table 6.5). There were no significant differences in springiness, fracture force and stiffness of rind in both normal and albedo breakdown fruits among treatments. Springiness and stiffness of rind in normal fruit were higher in albedo breakdown fruit (Table 6.5).

6.3.4.3. Rind tensile strength force

The spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and all surfactants increased rind tensile strength force of both normal and albedo breakdown fruits as compared to control and Ca-only treatment (Fig. 6.2). The rind tensile strength force of normal fruit was higher than that of fruit with albedo breakdown.

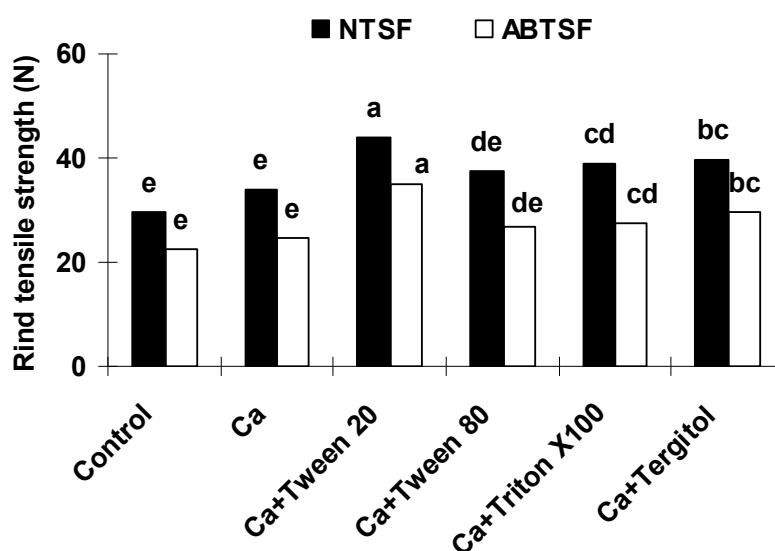


Figure 6.2. Effects of different surfactants added into an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ on rind tensile strength force (N) in ‘Washington Navel’ orange. Means followed by different letters on bars are significantly different at $P \leq 0.05$. $n = 4$ replications. NTSF: normal tensile strength force. ABTSF = albedo breakdown tensile strength force.

Table 6.5. Effects of different surfactants added into an aqueous spray solution containing 2% Ca(NO₃)₂ on adhesiveness (N), springiness (mm), fracture force (N) and stiffness (kgf·mm⁻¹) in 'Washington Navel' orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Treatment	Adhesiveness (N)		Springiness (mm)		Fracture force (N)		Stiffness (kgf·mm ⁻¹)	
	Normal	AB	Normal	AB	Normal	AB	Normal	AB
Control	0.12c	0.10c	1.77	1.60	1.55	0.99	1.06	0.55
Ca(NO ₃) ₂ 2%	0.21bc	0.13bc	1.96	1.67	2.19	2.89	1.11	0.69
Ca(NO ₃) ₂ 2% and Tween 20	0.34a	0.22a	2.07	2.00	4.73	3.86	1.36	0.82
Ca(NO ₃) ₂ 2% and Tween 80	0.25ab	0.19ab	1.84	1.70	4.12	3.20	1.25	0.75
Ca(NO ₃) ₂ 2% and Triton X100	0.24b	0.14bc	1.96	1.59	2.73	3.11	1.14	0.69
Ca(NO ₃) ₂ 2% and Tergitol	0.25ab	0.17ab	2.00	1.79	3.10	3.44	1.21	0.75
LSD ($P \leq 0.05$)	0.10	0.07	ns (0.09)	ns (0.12)	ns (1.39)	ns (0.92)	ns (0.13)	ns (0.07)

AB: albedo breakdown. n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SE).

An aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’ resulted in the significantly highest rind tensile strength force in both normal and albedo breakdown fruit (43.97 N and 35.03 N, respectively) as compared to all other treatments (Fig. 6.2). All surfactants except ‘Tween 80’ along with 2% $\text{Ca}(\text{NO}_3)_2$ significantly increased rind tensile strength force of normal fruit as compared to control and Ca-only treatment but the differences between ‘Triton X100’ (37.63 N) and ‘Tergitol’ (39.50 N) were not significant (Fig. 6.2). The spray application of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’, ‘Triton X100’ and ‘Tergitol’ resulted in significantly higher tensile strength force of rind of albedo breakdown fruit as compared to both the calcium-only treatment and control. There was no significantly higher difference in tensile strength force of rind of albedo breakdown fruit with ‘Triton X100’ (27.41 N) and ‘Tergitol’ (29.68 N) in ‘Washington Navel’ orange (Fig. 6.2).

6.3.4.4. Fruit compression test

Firmness of fruit sprayed with an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’ was significantly highest in both normal and albedo breakdown fruit (352.5 N and 317.1 N, respectively) as compared to all other treatments (Fig. 6.3).

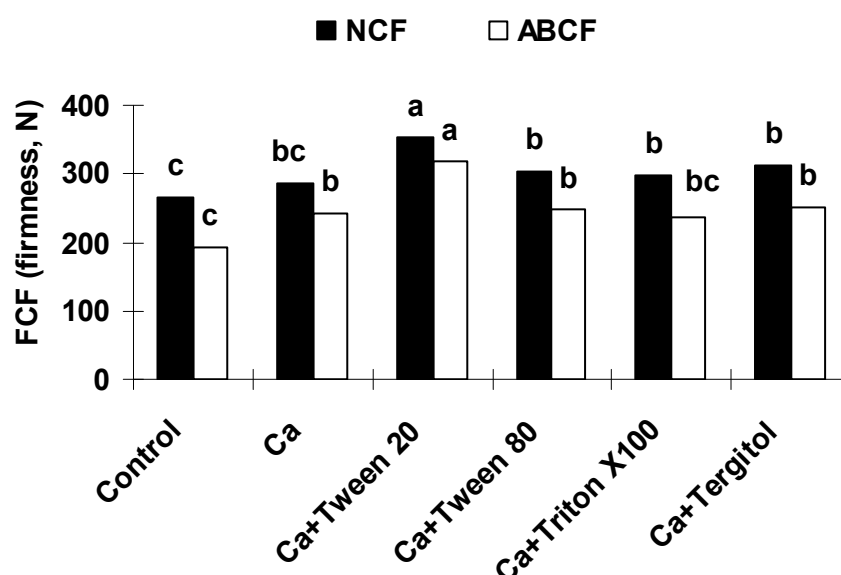


Figure 6.3. Effects of different surfactants added into an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ on fruit firmness (N) in ‘Washington Navel’ orange. Means followed by different letters on bars are significantly different at $P \leq 0.05$. $n = 4$ replications. FCF = fruit compression force. NCF = normal compression force. ABCF = albedo breakdown compression force.

The spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and any surfactant resulted in higher fruit firmness in both normal fruit and fruit with albedo breakdown as compared to unsprayed treatment.

6.3.5. Rind, flavedo and albedo thickness

The spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and any surfactant except ‘Triton X100’ resulted in an increase in fruit rind and albedo thickness as compared to untreated and Ca-only treatments (Table 6.6). The spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ along with ‘Tween 20’ significantly increased rind thickness (5.6 mm) as compared to control (4.3 mm) and Ca-only treatment (4.6 mm).

Table 6.6. Effects of different surfactants added into an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ on rind, flavedo and albedo thickness (mm) in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Treatment	Rind (mm)	Flavedo (mm)	Albedo (mm)
Control	4.3c	1.3	3.0c
$\text{Ca}(\text{NO}_3)_2$ 2%	4.6bc	1.2	3.4bc
$\text{Ca}(\text{NO}_3)_2$ 2% and Tween 20	5.6a	1.5	4.1ab
$\text{Ca}(\text{NO}_3)_2$ 2% and Tween 80	5.2ab	1.2	4.0b
$\text{Ca}(\text{NO}_3)_2$ 2% and Triton X100	4.6bc	1.2	3.4bc
$\text{Ca}(\text{NO}_3)_2$ 2% and Tergitol	5.1ab	1.4	3.7bc
LSD ($P \leq 0.05$)	0.6	ns (0.1)	0.7

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

The spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ with any surfactant did not significantly affect the flavedo thickness as compared to untreated or Ca alone treatment (Table 6.6). An aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ with ‘Tween 20’ or ‘Tween 80’ resulted in significantly thicker albedo (4.1 mm and 4.0 mm, respectively) as compared to control (3.0 mm) (Table 6.6).

6.3.6. Dry matter content of rind and pulp

The spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and any surfactant resulted in increased dry matter content of both exposed and shade rind as compared to control and Ca alone (Table 6.7). The spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ containing ‘Tween 20’ significantly increased dry matter content of exposed side of the rind (35.1%) and shaded side of the pulp (19.9%) as compared to other treatments. An aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and ‘Tween 20’ resulted in higher dry matter of shaded rind and exposed pulp but there were not significant differences in dry matter content of shade rind and exposed pulp among treatments (Table 6.7).

Table 6.7. Effects of different surfactants added into an aqueous spray solution containing 2% $\text{Ca}(\text{NO}_3)_2$ on dry matter content of rind and pulp ($\text{g}\cdot 100\text{ g}^{-1}$) in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Treatment	Dry matter content			
	Rind		Pulp	
	Exposed	Shaded	Exposed	Shaded
Control	32.0b	30.9	16.4	14.4b
$\text{Ca}(\text{NO}_3)_2$ 2%	32.7b	31.0	16.5	14.5b
$\text{Ca}(\text{NO}_3)_2$ 2% and Tween 20	35.1a	33.7	17.2	19.9a
$\text{Ca}(\text{NO}_3)_2$ 2% and Tween 80	33.3b	31.7	16.9	15.6b
$\text{Ca}(\text{NO}_3)_2$ 2% and Triton X100	33.3b	31.9	17.1	15.2b
$\text{Ca}(\text{NO}_3)_2$ 2% and Tergitol	33.4b	32.8	17.1	16.6b
LSD ($P \leq 0.05$)	1.4	(ns) 0.7	(ns) 0.3	2.7

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

6.3.7. Fruit quality

Percentage of juice, juice pH, soluble solids concentration (SSC), titratable acidity, ascorbic acid as well as individual organic acids were not significantly affected with the spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and any surfactant (Tables 6.8 and 6.9).

Table 6.8. Effects of different surfactants added into an aqueous spray solution of $\text{Ca}(\text{NO}_3)_2$ on percentage of juice (%), juice pH, soluble solids concentration (%) (SSC), titrable acidity ($\text{mg citric} \cdot 100 \text{ mL fresh juice}^{-1}$) (TA) and ascorbic acid ($\text{mg} \cdot 100 \text{ mL fresh juice}^{-1}$) in ‘Washington Navel’ orange.

Treatment	Juice		SSC	TA	Ascorbic acid
	%	pH			
Control	52.8	3.50	13.2	0.95	64.3
$\text{Ca}(\text{NO}_3)_2$ 2%	53.6	3.54	12.8	0.98	63.5
$\text{Ca}(\text{NO}_3)_2$ 2% and Tween 20	54.4	3.52	13.0	0.95	64.3
$\text{Ca}(\text{NO}_3)_2$ 2% and Tween 80	55.6	3.49	12.6	0.95	60.5
$\text{Ca}(\text{NO}_3)_2$ 2% and Triton X100	53.2	3.59	12.5	0.83	64.8
$\text{Ca}(\text{NO}_3)_2$ 2% and Tergitol	54.3	3.55	13.0	0.95	65.4
LSD ($P \leq 0.05$)	ns (1.34)	ns (0.05)	ns (0.43)	ns (0.06)	ns (2.58)

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Table 6.9. Effects of different surfactants added into an aqueous spray solution of $\text{Ca}(\text{NO}_3)_2$ on individual organic acids ($\text{g} \cdot \text{L fresh juice}^{-1}$) in ‘Washington Navel’ orange.

Treatment	Organic acids			
	Citric	Malic	Succinic	Tartaric
Control	11.14	4.26	1.76	0.68
$\text{Ca}(\text{NO}_3)_2$ 2%	12.09	4.67	1.97	0.67
$\text{Ca}(\text{NO}_3)_2$ 2% and Tween 20	10.84	4.21	1.85	0.63
$\text{Ca}(\text{NO}_3)_2$ 2% and Tween 80	11.50	4.21	1.66	0.62
$\text{Ca}(\text{NO}_3)_2$ 2% and Triton X100	10.56	4.04	1.69	0.56
$\text{Ca}(\text{NO}_3)_2$ 2% and Tergitol	10.88	4.26	1.68	0.67
LSD ($P \leq 0.05$)	ns (0.38)	ns (0.13)	ns (0.13)	ns (0.07)

n = 4 replications. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

6.4. Discussion

Pre-harvest spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ containing different surfactants increased Ca concentration in leaf, fruit rind and pulp tissues at 101 and 195 days after sprays (Table 6.2). Amongst four surfactants tested, pre-harvest spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ containing 'Tween 20' significantly increased Ca concentration in leaf, fruit rind and pulp tissues at 101 and 195 days after sprays, except for leaf Ca concentration at 195 days after sprays (Table 6.2). The enhanced Ca uptake into leaf, rind and pulp tissues with surfactants may be ascribed to the lower surface tension as previously reported that Ca ions were distributed better on the surface of leaf and fruit due to the lower contact angles of spray solution (Neumann and Prinz, 1974). It may also be argued that surfactants added into the spray solution may have increased the binding capacity of the cuticle for Ca ions consequently improving Ca uptake. The surfactants containing long alkyl chains were known as moiety agents contributing to the improved wetting on the leaf and fruit surfaces and increased the uptake of Ca ions (Harker and Ferguson, 1991; Roy et al., 1996; Saftner et al. 1997). Possibly, the pre-harvest spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ containing different surfactants enhanced Ca uptake into leaf, rind and pulp tissues may be due to the altered sites of adsorption by changing, partially damaging or extracting cuticle wax. The diffusion of the surfactants into the cuticle along hydrophilic-lipophilic interfaces caused the dilation of hydrophilic pores leading to the decreased resistance and increased permeability of the cuticles has earlier been reported (Roy et al., 1996; Harker and Ferguson, 1991).

The efficiency of improving Ca uptake into leaf, rind and pulp tissues varied among surfactants (Table 6.2). The pre-harvest spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ containing 'Tween 20', 'Tween 80' and 'Tergitol' resulted in higher Ca concentration in fruit rind and pulp tissues as compared to all other surfactants (Table 6.2). The value of hydrophilic-lipophilic balance (HLB) mainly contributed to the enhanced concentrations of Ca in leaf and fruit tissues. The penetration of a nutrient through the cuticular membrane was more effective when the optimal HLB values are ranged within 15-17. The higher HLB value of the surfactants was a pathway to enhance the penetration of a nutrient through the cuticular membrane (Wojcik, 2004) as the HLB values of 'Tween 20' and 'Tween 80' were higher than of 'Tergitol' and 'Triton X100'. 'Tergitol' containing alkylbenzen which is a hydrophobic agent

resulted in increased absorption of Ca into fruit (Roy et al., 1996; Saftner et al., 1997).

Pre-harvest spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ containing some surfactants resulted in the higher Ca concentration in the leaf tissue than fruit rind and pulp tissue (Table 6.2). As Ca is not phloem mobile (Treeby and Storey, 2002) and hence the foliar applications of Ca solution did not contribute to the fruit Ca concentration (Saure, 2005). Therefore, an aqueous Ca solution should be sprayed directly to the fruit surface to improve the penetration of Ca into the fruit as reported in apples (Saure, 2005; Harker and Ferguson, 1991; Schlegel and Schonherr, 2002).

The spray applications of an aqueous 2% $\text{Ca}(\text{NO}_3)_2$ solution containing ‘Tween 20’, ‘Tween 80’ and ‘Tergitol’ decreased albedo breakdown incidence compared to the calcium-only treatment and control (Fig. 6.1) mainly due to the increased Ca concentrations in rind and pulp tissues (Table 6.2). My experimental data also show negative significant correlations ($r = -0.947^{**}$ and -0.891^{**} , respectively) between Ca concentrations in the rind at 101 and 195 days after spray and albedo breakdown incidence at harvest. There was significant negative correlations ($r = -0.891^{**}$ and -0.905^{**} , respectively) between pulp Ca concentrations at 101 and 195 days after spray with albedo breakdown incidence at harvest (Table 6.3). Similarly, other researchers have reported that there was lower Ca concentration in the albedo and flavedo of fruit with albedo breakdown than normal fruit of sweet oranges (Storey and Treeby, 2002; Treeby and Storey, 2002; Jone et al., 1967; Bevington et al., 1993; Moulds et al., 1995). In contrast, Lovatt (2000) reported that there was a significant positive correlation between rind N and Ca concentration and albedo breakdown incidence at harvest in ‘Valencia’ orange in California. Bower (2004) reported that the role of calcium was less important in reducing the albedo breakdown than that of molybdenum or sulphur.

It may also be argued that spray applications of an aqueous 2% $\text{Ca}(\text{NO}_3)_2$ solution containing surfactants has significantly improved textural properties of the rind such as hardness, cohesiveness, adhesiveness, tensile strength and fruit firmness (Tables 6.4, 6.5 and 6.6; Figures 6.2 and 6.3) that consequently contributed to decreased albedo breakdown. The disconnection of the adjoining cells in the cell wall at the middle lamella and the lower levels of pectins and hemicellulose in the cell wall seem to be associated with fracture in albedo tissue during the end of stage II in the

fruit development (Storey and Treeby, 1994; Storey and Treeby, 2002; Bower, 2004; Jona et al., 1989).

It is also suggested that significantly increased rind thickness with sprays of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and surfactants may also have partially contributed to the significant reduction of albedo breakdown incidence (Table 6.6). Ali et al. (2000) monitored the relationships among the severity of albedo breakdown, leaf, and rind nutrient concentrations, rind thickness and temperature for two years at eight 'Navel' and 'Valencia' orange orchards in California and found that rind thickness in October showed a significant negative correlation with albedo breakdown incidence at harvest. Similarly, it has been reported that albedo breakdown is more likely to occur on the fruits with thin rind (Bevington et al., 1993; Jones et al., 1967 and Moulds et al., 1995).

The spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and surfactants resulted in the improved textural properties of the rind such as hardness, cohesiveness, adhesiveness, tensile strength force and fruit firmness as well as the thicker rind, and albedo of fruit (Tables 6.4, 6.5 and 6.6; Figures 6.2 and 6.3) in both normal and albedo breakdown fruits. There was also a significantly increased dry matter content in fruit rind and pulp with sprays of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and surfactants as compared to control and Ca-only treatment (Table 6.7). It may be argued that increased Ca concentration in fruit resulted in the higher cell wall strength and thickness as Ca formed cross-links within the pectin polysaccharide matrix resulting in the strong structural rigidity in the cell wall (Easterwood, 2002) and contributing to the fruit firmness (Tucker, 1993; Singh et al., 2007). The maintenance of the cell wall stabilisation and membrane integrity is known to be the roles of calcium in the plant cell (Saure, 2005; Singh et al., 2007). Therefore, increased calcium concentration in fruit resulted in the higher fruit firmness due to the improved fruit texture (Tucker, 1993; Singh et al., 2007; Roy et al., 1996). Similarly, the foliar application of calcium nitrate just before or during fruit colour-break increased the rind resistance to puncturing in 'Fortune' mandarin (Zaragoza et al., 1996). Similar results were recorded by El-Hilali et al. (2004) who demonstrated that the foliar spray solutions containing 1% or 2% of calcium nitrate applied four weeks prior to harvest increased the flesh firmness in 'Fortune' mandarin.

Percentage of juice, juice pH, soluble solids concentration (SSC), titrable acidity, ascorbic acid as well as individual organic acids were not significantly affected with the spray applications of an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ and surfactants (Tables 6.8 and 6.9). Similarly, El-Hilali et al. (2004) reported that pre-harvest spray of 1% or 2% calcium nitrate applied four weeks before harvest did not affect juice content, soluble solids concentration (SSC) and SSC and titrable acidity ratio in the 'Fortune' mandarin. Contrarily, Moss and Higgins (1975) found that the brix and acid ratio was associated with leaf Ca ($r = 0.46^*$) in 'Late Valencia' oranges in New South Wales.

In conclusion, 'Tween 20' was the most efficient surfactant in enhancing Ca uptake into leaf, rind and pulp tissues and reducing albedo breakdown. Pre-harvest five spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ containing Tween 20' commencing from 81 DAFB at 10-day intervals increased Ca concentrations in leaf, fruit rind and pulp tissues, decreased albedo breakdown incidence and improved textural properties of the rind such as hardness, cohesiveness, adhesiveness, tensile strength force, fruit firmness and the thicker rind, and albedo of fruit without affecting the fruit quality attributes as compared to the calcium-only treatment and control.

CHAPTER 7**Foliar application of boron reduces albedo breakdown and improves rind textural properties in ‘Washington Navel’ sweet orange [*Citrus sinensis* (L.) Osbeck.]****Abstract**

Albedo breakdown in sweet oranges is a rind disorder, which is caused by the cracks in the internal white tissues (albedo). I investigated the efficacy of different concentrations and time of foliar application of boron in reducing albedo breakdown and improving textural properties of rind on ‘Washington Navel’ sweet orange. Boron was sprayed at different concentrations (0, 200, 400 and 600 mg·L⁻¹) in 2007 and (0, 200, 400, 600 and 800 mg·L⁻¹) in 2008. The spray was applied as (a) single spray in early summer only (81 days after full bloom) (DAFB), (b) two sprays one in early summer (81 DAFB) and followed by second spray in early winter (233 DAFB) and (c) single spray in early winter only (233 DAFB) in 2007. In 2008, The boron spray was applied as (a) single in early summer (80 DAFB) and (b) two sprays one in early summer (80 DAFB) and followed by second spray in early winter (232 DAFB). The experiment was set out as a randomized block design and included four replications. Concentrations of boron in the leaf, rind, and pulp of fruit were determined on 182 and 276 DAFB in 2007 and 182 and 272 DAFB in 2008. The incidence of AB was recorded for each tree as a percentage of the fruit. Boron concentration in leaf, rind and pulp was increased with the two foliar boron sprays in 2007 while single boron spray in early summer resulted in the significantly higher boron concentration in leaf at 182 DAFB and in fruit rind and pulp at 182 and 272 DAFB in 2008. The foliar boron spray application significantly decreased albedo breakdown incidence and improved rind hardness, cohesiveness, tensile strength force and fruit firmness. In conclusion, the one foliar spray of boron (600 mg·L⁻¹) in early summer significantly increased boron concentration in leaf, rind and pulp of fruit, reduced the incidence of albedo breakdown and improved the rind textural properties in ‘Washington Navel’ while maintaining the other important fruit quality attributes.

7.1. Introduction

Albedo breakdown is a physiological disorder with cracks in the internal white tissue (albedo) causing puffiness in the rind of sweet oranges. The development of albedo breakdown is related to the disconnection of the adjoining cells in the cell wall at the middle lamella. The lower levels of pectins and hemicellulose in the cell wall have been indicated to involve in albedo breakdown due to causing irregular fractures in albedo tissue at the end of stage II in fruit development (Storey and Treeby, 1994; Storey et al., 2002; Bower, 2004; Jona et al., 1989). Albedo breakdown causes a marked economic loss to the citrus industry as it affects 35% - 45% of the total area planted to sweet orange in the world (Monselise et al., 1976). Albedo breakdown also affects up to 15% to 90% of fruit at some locations in South Africa and Australia, respectively (Goldie, 1998).

The incidence of albedo breakdown has been reported to be influenced by rootstock, (Agusti et al., 2003; Treeby et al., 1995; Moulds et al., 1995), regulated deficit irrigation (Treeby et al., 2007), a foliar application of gibberellic acid in summer (Embleton et al., 1973; Jona et al., 1989) and mineral nutrition (Ali et al., 2000; Bevington et al., 1993; Jones et al., 1967; Embleton et al., 1973; Monselise et al., 1976). Jones et al. (1967) reported that the increased soil application rate of N in summer resulted in the significantly lower incidence of albedo breakdown than spring application in 'Valencia' oranges. Albedo breakdown incidence was significantly reduced with the soil phosphorus application to the P-deficient trees (Jone et al., 1967). Two foliar sprays of KNO_3 solution in summer also significantly reduced albedo breakdown from 42.6% to 27.2% in 'Valencia' orange (Jone et al., 1967).

Ali et al. (2000) reported that both rind K and P concentrations were significantly positively correlated with rind thickness in October whilst, there was significant negative correlation with albedo breakdown incidence at harvest on 'Navel' and 'Valencia' oranges in California.

Lower levels of Ca in oranges are associated with albedo breakdown (Storey et al., 2002; Treeby and Storey, 2002) as calcium plays an important role in building the structure and permeability of cell membranes and preserving cell wall stability (Manganaris et al., 2005; Tuna et al., 2007). Treeby and Storey (2002) reported that

albedo breakdown was significantly decreased from 83% to 53% with the application of five foliar sprays of either 0.11% or 0.33% calcium commencing in summer at an early stage of 'Navel' orange fruit growth due to increased Ca levels in the rind and albedo of fruit.

Boron plays an important role in cell wall formation in citrus tree (Haas, 1929; Matoh, 1997; Haas, 1945; Zekri and Obreza, 2003). Physical quality parameters of citrus fruit, especially the rind thickness and smoothing are very sensitive to boron supply as boron also assists in binding calcium to the cell walls (Foroughi et al., 1973; Haas, 1929; Smith and Reuther, 1950, Matoh, 1997). "Hard fruit" is named for the affected citrus fruit which has thick and lumpy rind at boron deficiency level. Tariq et al. (2007) also reported that the rind of citrus fruit softened with the foliar application of boron but no related data to support this observation were published. Most of the research on the role of boron in the citrus trees has been reported in terms of fruit yield and fruit quality (Tariq et al., 2007; Abd-Allah, 2006; Maurer and Taylor, 1999; Nguyen and Nguyen, 2006; Smith, 1955). Smith and Reuther (1950) reported that the foliar application of boron did not significantly affect the yield, fruit size, rind thickness, juice content, percentage of total soluble solids concentration and citric acid in the juice except the ascorbic acid content of the juice in the low boron trees in oranges. In contrast, Tariq et al. (2007) found that foliar application of boron significantly increased the fruit yield, the percentage of juice, fruit size and decreased the rind thickness in sweet oranges.

Apparently, no research work has been reported on the effects of boron on albedo breakdown incidence and textural properties of the rind in citrus. Therefore, I investigated the effects of different boron concentrations and time of its application on incidence of albedo breakdown, textural properties of rind and fruit and fruit quality in 'Washington Navel' orange.

7.2. Materials and methods

7.2.1. Experimental site and plant materials

The experiment was carried out in a commercial orchard located at Gingin, Western Australia (Latitude 31° 21' S, longitude 155° 55' E). The climate is described as winter dominant with wet winters and hot, dry summers. The soil is a sandy loam. All the cultural practices including irrigation, fertiliser application, insect and weed

control in all the blocks were the same except for the experimental treatments of boron.

Twenty two years old uniform ‘Washington Navel’ orange trees [*Citrus sinensis* (L.) Osbeck] grafted on [*Poncirus trifoliata* (L.) Raf.] rootstock were used in the experiment. The trees were spaced 7.50 m between rows and 2.70 m within rows with north-south row direction.

7.2.2. Treatments and experimental design

7.2.2.1. Experiment 1: 2006-2007

An aqueous solution containing four different concentrations of boron (0, 200, 400, or 600 mg·L⁻¹) and a non-ionic surfactant: ‘Tween 20’ (0.05%) was sprayed onto fruit and leaves of the whole tree. The spray was applied (a) single spray in early summer only (December 5, 2006), 81 days after full bloom (DAFB), (b) two sprays, one in early summer (December 5, 2006), 81 DAFB and followed by second in early winter (May 6, 2007), 233 DAFB and (c) single spray in early winter only (May 6, 2007), 233 DAFB.

7.2.2.2. Experiment 2: 2007-2008

The foliar sprays of five different concentrations of boron (0, 200, 400, 600 or 800 mg·L⁻¹) and a non-ionic surfactant: ‘Tween 20’ (0.05%) was applied onto fruit and leaves of the whole tree. Single spray was applied in early summer (December 5, 2007), 80 DAFB and two sprays; first in early summer (December 5, 2007), 80 DAFB and second in early winter (May 6, 2008), 232 DAFB.

For both Experiments 1 and 2, the aqueous solution of boron with surfactant was sprayed using a sprayer (The Selecta Trolleyapak Mk II, Victoria, Australia) at the rate of 1000 L·ha⁻¹ till run off. The nozzle (Chierici Titisrl, Rubiera Italy) was used under the pressure 250 KPa. The rate of nozzle was 70 L/min.

Both experiments were laid out by following a randomised block design with four replications. Single tree was treated as an experimental unit. Unsprayed trees were treated as control.

7.2.3. Chemicals

Solubor (Disodium octaborate tetrahydrate) was purchased from Incitec Pivot Limited, Victoria, Australia. The white powder solubor ($B_8Na_2O_{13} \cdot 4H_2O$) contains 20.5% w/w B.

7.2.4. Parameters measured

Boron and calcium concentration in leaf, fruit rind and pulp were determined twice in 2007 (182 and 286 DAFB) and twice in 2008 (182 and 272 DAFB). Albedo breakdown incidence was recorded. Rind hardness, cohesiveness, fracture, springiness, rind tensile strength force and fruit compression were recorded as texture profile analysis. Rind, albedo and flavedo thickness was determined. Percentage of juice, juice pH, soluble solids concentration, titratable acidity and ascorbic acid were assessed as fruit quality parameters.

7.2.4.1. *Determination of B and Ca concentrations from leaf, rind and pulp*

Fully developed six months old spring flush leaves (25 /tree) from non-fruiting shoot and five fruit per tree were collected for nutrient analysis. The leaves and fruit from each tree were collected from unshaded position at about 1.50 m height at the north, east, south and west points of tree. The leaves and fruits sampled twice in 2007 (182 and 286 DAFB) and twice in 2008 (182 and 272 DAFB). All leaves and fruit sampled were free from damage of insects or diseases.

Boron and calcium concentrations were analysed by using Radial Inductively Coupled Plasma Optical Emission Spectrometry (VISTA – PRO, CCD Simultaneous ICP-OES, VARIAN, Australia) which operated in simultaneous mode as described in more details in Section 3.6.

7.2.4.2. *Determination of albedo breakdown incidence*

All fruit from each tree were harvested and examined for albedo breakdown. The albedo breakdown incidence was expressed as percentage of fruit as mentioned in Section 3.7.

7.2.4.3. *Texture profile analysis*

Textural properties of rind such as hardness, cohesiveness, springiness, fracture force, tensile strength force and fruit firmness from normal fruit and fruit with albedo breakdown were determined using a texture analyser (TA Plus, AMETEK Lloyd

instruments Ltd., Hampshire, UK). A personal computer with Nexygen® software was interfaced to a texture analyser. A 5/16 Magness-Taylor probe, with a 500 N load cell was used for the measurement of textural parameters.

7.2.4.3.1 Rind puncture test

Rind sample from two groups (normal and albedo breakdown fruits) were cut 2.5 cm wide x 0.6 cm thick using a slicer (Zyliss Easy slice 2” folding Mandolin slicer, Swiss) to give uniform sections for determining rind puncture test. Two rinds samples were dissected 90 degree apart per fruit. Ten fruit of each mentioned fruit group were tested from each tree. The detailed procedure for determination of rind puncture test has been mentioned in Section 3.8.1.

7.2.4.3.2 Rind tensile strength test

The rind tensile strength test was determined to measure the behaviour of the orange rind up to the rind deflection of 10 mm. The rind tensile strength force was calculated at the maximum load and limit points where the rind deflection occurred as detailed in Section 3.8.2.

7.2.4.3.3 Fruit compression test

The fruit with the height of about 8.5 cm were used for each compression test. Each fruit was placed between two flat plates with the stem axis perpendicular to the plate. The crosshead speed was 200 mm/min. This test was completed at strain of 25% of fruit height as described in Section 3.8.3.

7.2.4.4. Determination of rind, flavedo and albedo thickness

Ten mature fruits from north, east, west and south points of each tree at about 1.5 m high were sampled to determine rind, flavedo, albedo thickness as mentioned in Section 3.9.

7.2.4.5. Estimation of fruit quality parameters

Five mature fruit selected around the canopy of each tree at about 1.5 m high were weighed. Fruit juice was extracted using a juicer (Sunbeam citrus juicer, TE 2600, Sunbeam Co. Ltd., made in China to Sunbeam’s specification). The freshly extracted juice was used for determining fruit quality parameters including juice content, juice pH, soluble solids concentration, titratable acidity and ascorbic acid as detailed in Section 3.11. Juice content was calculated and expressed as percentage. Juice pH was

recorded using a digital pH meter (Cyberscan pH 510, Eutech Instrumnet Pte Ltd., Singapore). Soluble solids concentrations (SSC) in percent were recorded by measuring the refractive index at 20°C using an infrared digital refractometer (Atago-Palette PR 101, Atago Co. Ltd, Itabashi-Ku, Tokyo, Japan). Titratable acidity (TA) was determined by following the titration method to phenolphthalein endpoint and calculated in milligram citric acid per 100 mL fresh juice. Ascorbic acid concentration was determined following the combined method of Jagota and Dani (1982) and Malik and Singh (2005). Ascorbic acid concentration was calculated using a standard curve of L-ascorbic acid and expressed as mg ascorbic acid per 100 mL fresh juice.

7.2.5. Statistical analysis

The data were analysed by two way analysis of variance (ANOVA) using Genstat 9 release 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The least significant difference (Fisher's protected LSD) was calculated at $P \leq 0.05$. All the assumptions of ANOVA were checked to ensure the validity of statistical analysis.

7.3. Results

7.3.1. Boron concentration in leaf, fruit rind and pulp

The increase in leaf boron concentration at 182, 276 and 272 DAFB was increased with increased boron spray application in 2007 and 2008, respectively (Table 7.1). The increase in leaf boron concentrations was more pronounced in 2008 than 2007. Two boron sprays (summer and winter) resulted in significantly higher leaf boron concentration at 276 DAFB than single spray in winter or summer in 2007 and 2008 (Table 7.1). The single or two foliar spray(s) of boron ($600 \text{ mg}\cdot\text{L}^{-1}$ and $800 \text{ mg}\cdot\text{L}^{-1}$) resulted in highest leaf boron concentration in 2007 and 2008, respectively (Table 7.1). Two boron sprays first in early summer followed by the second spray in early winter resulted in the highest leaf boron concentration ($174.4 \text{ mg}\cdot\text{kg}^{-1}$) at 276 DAFB in 2007 and $192.0 \text{ mg}\cdot\text{kg}^{-1}$ at 272 DAFB in 2008. However, the two boron sprays did not

Table 7.1. Boron concentrations in leaf, fruit rind and pulp tissues influenced by different concentrations and time of application of B in 2007 and 2008 in 'Washington Navel' orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

B (mg L ⁻¹)	Leaf boron concentration (mg·kg ⁻¹)			Rind boron concentration (mg·kg ⁻¹)			Pulp boron concentration (mg·kg ⁻¹)			
	2007	2008	2008	2007	2008	2008	2007	2008	2008	
	Days after full bloom									
	182	276	182	272	182	272	182	276	182	272
0	125.0	140.0b	160.0b	157.5c	33.3c	27.25d	26.75c	18.8	22.75d	21.25c
200	137.5	150.0b	162.5b	170.0b	35.0b	34.50c	34.75b	20.0	25.25c	24.00b
400	142.5	155.0ab	172.5b	177.5b	35.0b	34.75bc	35.00b	19.0	26.50ab	25.00ab
600	147.5	162.5a	175.0b	175.0b	38.0a	35.50b	36.25ab	19.5	27.50a	25.75a
800	-	-	197.5a	197.5a	-	37.25a	37.25a	-	26.00bc	25.25ab
Mean	-	151.9	173.5	175.5	-	33.85	34.00	-	16.2	25.60
0	-	140.0c	160.0b	157.5d	-	27.25b	26.75c	-	15.8b	22.75
200	-	175.0b	165.0b	180.0c	-	24.3b	27.75ab	-	16.8a	22.75
400	-	180.0b	170.0b	200.0b	-	25.0b	27.50ab	-	16.5a	23.25
600	-	202.5a	175.0b	205.0ab	-	26.5a	27.50ab	-	17.0a	25.25
800	-	-	192.5a	217.5a	-	-	31.75a	-	-	23.50
Mean	-	174.4	172.5	192.0	-	24.8	27.70	-	16.5	23.50
0	-	140.0c	-	-	-	23.3c	-	-	15.8	-
200	-	155.0b	-	-	-	24.5b	-	-	16.0	-
400	-	165.0ab	-	-	-	25.0b	-	-	16.0	-
600	-	170.0a	-	-	-	26.8a	-	-	16.3	-
Mean	-	157.5	-	-	-	24.9	-	-	16.0	-
LSD $P \leq 0.05$	ns	14.07	16.68	14.68	1.42	0.87	1.19	ns (0.52)	0.57	1.11
B con.	ns	(6.36)								
Time	12.19	ns	ns	9.28	ns (0.26)	0.75	0.49	ns (0.17)	0.70	1.02
B con. x Time	ns (8.47)	ns (3.64)	ns (7.15)	ns (8.13)	ns (0.52)	1.68	1.10	ns (0.34)	ns (0.54)	ns (0.78)

n = 4 replications. (-) = not available. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

significantly increase leaf boron concentration at 182 DAFB as compared to single spray in summer or winter during 2008 (Table 7.1). The interaction between the number of sprays and different boron concentrations applied for leaf boron concentration was not significant for both years (Table 7.1).

Rind boron concentration was significantly increased with the application of increased boron concentration for both 2007 and 2008. The spray application of boron ($600 \text{ mg}\cdot\text{L}^{-1}$) in 2007 and ($800 \text{ mg}\cdot\text{L}^{-1}$) in 2008 resulted in the highest boron concentration in fruit rind with either single boron spray or two boron sprays (Table 7.1). There were no significant differences in rind boron concentration between single boron spray and two boron sprays in 2007, while single boron spray in early summer significantly increased rind boron concentration as compared to two boron sprays in 2008 (Table 7.1). The interaction between the number of boron spray applications and boron concentration was not significant for rind boron concentration at 276 DAFB in 2007 (Table 7.1). In 2008, the interaction between the number of boron spray applications and boron concentration at 182 and 272 DAFB was significant.

Pulp boron concentration significantly increased with the increased boron applied in 2007 and 2008 except at 182 DAFB in 2007 (Table 7.1). The spray application of boron ($600 \text{ mg}\cdot\text{L}^{-1}$) resulted in the highest pulp boron concentration for both single and double boron sprays for over two years of 2007 and 2008 (Table 7.1). One or two boron sprays resulted in similar values of pulp boron concentration in 2007 while single boron spray in early summer significantly increased pulp boron concentration as compared to two boron sprays in 2008 (Table 7.1). The interaction between the number of boron sprays and boron concentration applied for pulp boron concentration was found to be non-significant in 2007 and 2008 (Table 7.1).

7.3.2. Calcium concentration in leaf, fruit rind and pulp

The exogenous spray application of boron irrespective of concentrations and number of sprays did not significantly influence leaf Ca concentrations at 182 and 276 DAFB in 2007 (Table 7.2). In 2008, spray application of boron ($600 \text{ mg}\cdot\text{L}^{-1}$) as a single and double sprays resulted in highest concentrations of Ca in leaf at 182 and 272 DAFB (Table 7.2). The leaf Ca concentration did not differ significantly between a single and two boron sprays in 2007 and at 272 DAFB in 2008 (Table 7.2). The interaction

between the boron spray number and boron concentration was found to be non-significant for leaf Ca concentration in 2007 and 2008 (Table 7.2).

Rind Ca concentration were significantly increased with increased single B spray application ($200 \text{ mg}\cdot\text{L}^{-1}$ to $600 \text{ mg}\cdot\text{L}^{-1}$) in 2007 and 2008 (Table 7.2). Rind Ca concentration at 276 DAFB did not vary significantly with double or single boron spray(s) in summer or winter in 2007 whilst, single boron spray in early summer significantly increased rind Ca concentration as compared to two boron sprays in 2008 (Table 7.2). The interaction between boron concentrations and the number of sprays was found to be non-significant for rind calcium concentration at 276 DAFB in 2007, however, the interaction was significant in 2008 (Table 7.2).

Pulp Ca concentration at 182 DAFB was not significantly increased with different foliar boron spray treatments during 2007 (Table 7.2). The single spray application of B ($600 \text{ mg}\cdot\text{L}^{-1}$) in early summer resulted in significantly increased Ca concentration in pulp at 182 and 272 DAFB as compared to control and double sprays and single spray in winter (Table 7.2). Pulp Ca concentration significantly increased with the increased boron concentrations up to $600 \text{ mg}\cdot\text{L}^{-1}$. The interaction between different boron concentrations and spray number was not significant for pulp Ca concentration at 276 DAFB in 2007 and significant for 2008 (Table 7.2).

7.3.3. Albedo breakdown incidence

All the spray treatments of B as single spray in early summer, winter or two sprays, first in early summer followed by second in winter reduced the incidence of albedo breakdown in 2007 and 2008 (Table 7.3). The single boron spray ($600 \text{ mg}\cdot\text{L}^{-1}$) in early summer resulted in the significantly lowest albedo breakdown incidence (37.6% and 18.0%) as compared to control (70.4% and 51.8%) and other treatments in 2007 and 2008, respectively (Table 7.3). Summer spray application of B was more effective in reducing albedo breakdown incidence as compared to single spray in winter and double sprays. The interaction between the number of boron spray application and concentration was significant for incidence of albedo breakdown in 2007 only (Table 7.3).

Table 7.2. Ca concentration in leaf, fruit rind and pulp tissues influenced by different concentrations and time of application of B in 2007 and 2008 in 'Washington Navel' orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

B (mg·L ⁻¹)	Leaf Ca concentration (%)			Rind Ca concentration (%)			Pulp Ca concentration (%)				
	2007	2008	2008	2007	2008	2008	2007	2008	2008		
	Days after full bloom										
	182	276	182	272	182	272	182	276	182	272	
0	2.08	2.26	2.45c	2.62b	0.45c	0.37b	0.38c	0.36b	0.22	0.12b	0.14b
200	2.12	2.32	2.58c	2.66b	0.48b	0.38b	0.57b	0.52a	0.23	0.13a	0.29a
400	2.09	2.25	2.70bc	2.74ab	0.50ab	0.39ab	0.56b	0.50a	0.22	0.13a	0.30a
600	2.14	2.35	2.82ab	2.93a	0.53a	0.40a	0.62a	0.54a	0.24	0.13a	0.31a
800	-	-	2.75a	2.90a	-	-	0.60ab	0.52a	-	-	0.30a
Mean	-	2.29	2.66	2.77	-	0.38	0.55	0.49	-	0.13	0.27
0	-	2.26	2.45c	2.62b	-	0.37b	0.38c	0.36	-	0.12b	0.14b
200	-	2.43	2.78b	2.76b	-	0.41a	0.40bc	0.38	-	0.14a	0.15b
400	-	2.26	2.95ab	2.79ab	-	0.38b	0.42ab	0.39	-	0.13a	0.16b
600	-	2.46	3.00a	3.00a	-	0.42a	0.45a	0.40	-	0.14a	0.17a
800	-	-	2.88ab	2.80ab	-	-	0.43ab	0.39	-	-	0.16b
Mean	-	2.35	2.81	2.79	-	0.39	0.42	0.38	-	0.13	0.16
0	-	2.26	-	-	-	0.37b	-	-	-	0.12b	-
200	-	2.29	-	-	-	0.40a	-	-	-	0.14a	-
400	-	2.29	-	-	-	0.40a	-	-	-	0.14a	-
600	-	2.44	-	-	-	0.40a	-	-	-	0.14a	-
Mean	-	2.32	-	-	-	0.39	-	-	-	0.14	-
LSD	ns (0.09)	ns (0.06)	0.20	0.22	0.03	0.02	0.04	0.05	ns (0.01)	0.01	0.02
$P \leq 0.05$											
Time	-	ns (0.05)	0.13	ns (0.14)	-	ns (0.01)	0.03	0.03	-	ns	0.01
											(0.004)
B con.	x	-	ns (0.10)	ns (0.32)	-	ns (0.02)	0.06	0.07	-	ns (0.01)	0.03
Time											0.05

n = 4 replications. (-) = not available. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Table 7.3. Incidence of albedo breakdown influenced by different concentrations and time of application of B in ‘Washington Navel’ orange in 2007 and 2008. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

B (mg·L ⁻¹)	Spray time	Albedo breakdown incidence (% of fruit)	
		2007	2008
0	Summer (only)	70.4b	51.8c
200		43.3a	33.2b
400		41.1a	26.8ab
600		37.6a	18.8a
800		-	18.0a
Mean		48.1	29.7
0	Summer Winter	70.4b	51.8b
200		57.2b	38.8a
400		65.9b	36.8a
600		27.9a	29.2a
800		-	32.8a
Mean		55.4	37.8
0	Winter (only)	70.4b	-
200		45.4a	-
400		47.9a	-
600		40.2a	-
800		-	-
Mean		51.0	-
LSD $P \leq 0.05$	B con.	8.96	10.7
	Time	ns (2.70)	3.30
	B con. x Time	15.51	ns (5.2)

n = 4 replications. (-) = not available. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

7.3.4. Texture profile analysis of the rind and the fruit

7.3.4.1. Rind hardness

All the spray treatments of B as single spray in early summer, winter or two sprays, first in early summer followed by second in winter significantly increased rind hardness of both normal and fruit with albedo breakdown in 2007 and 2008 (Table 7.4). The single spray of boron (600 mg·L⁻¹) in early summer resulted in higher rind hardness of normal fruit (29.00 N and 33.53 N) and with albedo breakdown (18.34 N and 29.11 N) during 2007 and 2008, respectively. The single spray application of B in early summer was more effective in increasing the rind hardness of both normal and fruit with albedo breakdown than single spray in winter and double sprays during both years. In general, normal fruit showed higher rind hardness than fruit with

albedo breakdown in both years, irrespective of the treatments (Table 7.4). There were no significant interactions between time application and boron concentration for rind hardness in 2007 and 2008 except for fruit with albedo breakdown in 2008 (Table 7.4).

Table 7.4. Rind hardness influenced by different concentrations and time of application of B in 2007 and 2008 in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

B (mg·L ⁻¹)	Spray time	Rind hardness (N)			
		Normal		Albedo breakdown	
		2007	2008	2007	2008
0	Summer	21.85b	21.31c	12.90b	12.85d
200	(only)	27.16a	24.56bc	14.64b	15.14d
400		25.38ab	25.58b	14.09b	17.51cd
600		29.00a	33.53a	18.34a	29.11a
800		-	27.18b	-	21.99b
Mean		25.85	26.43	14.99	19.32
0	Summer	21.85b	21.31c	12.90b	12.85b
200	Winter	26.16a	25.16ab	14.61ab	14.71b
400		24.31ab	26.62a	14.00ab	14.04b
600		28.62a	26.40ab	15.43a	18.25a
800		-	24.39abc	-	15.59ab
Mean		25.23	24.78	14.24	15.09
0	Winter	21.85b	-	12.90b	-
200	(only)	25.42ab	-	14.54ab	-
400		23.33b	-	13.45ab	-
600		28.55a	-	14.70a	-
Mean		24.79	-	13.90	-
LSD	B con.	4.65	3.79	1.77	3.48
$P \leq 0.05$	Time	ns (1.4)	ns (0.83)	ns (0.53)	2.20
	B con. x Time	ns (2.8)	ns (1.85)	ns (1.06)	4.93

n = 4 replications. (-) = not available. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

7.3.4.2. Rind cohesiveness

The foliar spray treatments of boron resulted in the significantly increased rind cohesiveness in 2007 and 2008 except for normal fruit in 2008 (Table 7.5). Rind cohesiveness was higher in 2008 than that in 2007. The significantly highest values of rind cohesiveness were observed with the foliar spray application of boron (600 mg·L⁻¹) in both normal and fruit with albedo breakdown in two years of 2007 and 2008 (Table 7.5).

Table 7.5. Rind cohesiveness influenced by different concentrations and time of application of B in 2007 and 2008 in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

B (mg·L ⁻¹)	Spray time	Rind cohesiveness			
		Normal		Albedo breakdown	
		2007	2008	2007	2008
0	Summer (only)	0.05b	0.12a	0.04d	0.08c
200		0.06a	0.12a	0.05c	0.09bc
400		0.06a	0.11b	0.07b	0.07c
600		0.09a	0.13a	0.09a	0.12a
800		-	0.10b	-	0.11ab
Mean		0.06	0.11	0.06	0.09
0	Winter	0.05b	0.12a	0.04c	0.08b
200		0.05ab	0.11a	0.05b	0.08b
400		0.06a	0.11a	0.06b	0.08b
600		0.08a	0.09b	0.09a	0.09b
800		-	0.08b	-	0.11a
Mean		0.06	0.10	0.06	0.09
0	Winter (only)	0.05b	-	0.04b	-
200		0.05b	-	0.05a	-
400		0.06a	-	0.05a	-
600		0.06a	-	0.07a	-
Mean		0.05	-	0.05	-
LSD		B con.	0.01	0.02	0.01
$P \leq 0.05$	Time	ns (0.004)	ns (0.003)	ns (0.004)	ns (0.004)
	B con.x Time	ns (0.01)	ns (0.01)	ns (0.01)	ns (0.01)

n = 4 replications. (-) = not available. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

The rind cohesiveness of both normal and fruit with albedo breakdown was not significantly affected by number of B sprays in 2007 and 2008. The interaction of number of sprays and B concentration was found to be not significant for rind cohesiveness of both normal and fruit with albedo breakdown in 2007 and 2008 (Table 7.5).

7.3.4.3. Rind fracture force

Rind fracture force was significantly increased in both normal and fruit with albedo breakdown with the increased concentration of boron in spray solution in 2007. Whilst, in 2008, spray application of B increased rind fracture force as compared to control, but the difference was not significant (Table 7.6).

Table 7.6. Rind fracture force influenced by different concentrations and time of application of B in 2007 and 2008 in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

B (mg·L ⁻¹)	Spray time	Rind fracture force (N)			
		Normal		Albedo breakdown	
		2007	2008	2007	2008
0	Summer (only)	1.05c	2.95	1.01bc	2.16
200		3.48b	2.84	1.51b	2.17
400		5.25b	3.41	2.79ab	3.24
600		7.95a	2.61	4.07a	3.23
800		-	3.11	-	2.74
Mean		4.43	2.99	2.35	2.71
0	Winter	1.05c	2.95	1.01c	2.16
200		2.69c	2.91	1.72bc	1.85
400		4.02bc	6.93	2.57ab	1.78
600		9.72a	3.48	3.75a	1.85
800		-	2.18	-	2.71
Mean		4.37	3.69	2.26	2.07
0	Winter (only)	1.05b	-	1.01c	-
200		2.24b	-	1.43bc	-
400		5.02a	-	2.57ab	-
600		5.17a	-	3.48a	-
800		-	-	-	-
Mean		3.37	-	2.12	-
LSD	B con.	1.94	ns (0.74)	1.42	ns (0.41)
$P \leq 0.05$	Time	ns (0.58)	ns (0.47)	ns (0.43)	ns (0.26)
	B con.x Time	ns (1.17)	ns (1.05)	ns (0.85)	ns (0.58)

n = 4 replications. (-) = not available. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

The foliar spray application of boron (600 mg·L⁻¹) resulted in significantly highest rind fracture force for both normal fruit and fruit with albedo breakdown in 2007. The single boron spray in early summer was more effective in increasing rind fracture force than single spray in winter and double sprays in 2007 or 2008. The interaction between the time of sprays and boron concentration was found to be non-significant for rind fracture force for normal and fruit with albedo breakdown in 2007 and 2008 (Table 7.6).

7.3.4.4. Rind springiness

Rind springiness in normal and fruit with albedo breakdown increased with the spray solution as compared to control in 2007 and 2008 (Table 7.7).

Table 7.7. Rind springiness influenced by different concentrations and time of application of B in 2007 and 2008 in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

B (mg·L ⁻¹)	Spray time	Rind springiness (mm)			
		Normal		AB	
		2007	2008	2007	2008
0	Summer	1.61b	1.62b	1.37b	1.70b
200	(only)	1.85a	1.63b	1.43b	1.71b
400		1.92a	1.51b	1.65a	1.68b
600		2.02a	1.88a	1.72a	1.85a
800		-	1.79a	-	1.59b
Mean		1.85	1.69	1.54	1.71
0	Summer	1.61b	1.62b	1.37b	1.70a
200	Winter	1.78ab	1.67ab	1.41b	1.63b
400		1.92a	1.65b	1.64a	1.66b
600		1.99a	1.71ab	1.73a	1.62b
800		-	1.79a	-	1.42b
Mean		1.82	1.69	1.54	1.60
0	Winter	1.61c	-	1.37b	-
200	(only)	1.74bc	-	1.38b	-
400		1.87ab	-	1.47b	-
600		1.93a	-	1.66a	-
Mean		1.79	-	1.47	-
LSD	B con.	0.19	0.12	0.16	0.05
$P \leq 0.05$	Time	ns (0.06)	ns (0.03)	ns (0.05)	0.03
	B con. x Time	ns (0.12)	ns (0.06)	ns (0.10)	ns (0.06)

n = 4 replications. (-) = not available. AB = albedo breakdown. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

The single spray application of B (600 mg·L⁻¹) in early summer was more effective in increasing the rind springiness of normal fruit in 2007 and fruit with albedo breakdown in 2008 than single spray in winter and double sprays during both years. Rind springiness in albedo breakdown fruit in 2007 and in normal fruit in 2008 was not significantly different between two boron sprays and single spray in early summer or early in winter. The interaction between the boron concentration and the time of application was found not to be significant for rind springiness in both normal and albedo breakdown fruits in 2007 and 2008 (Table 7.7).

7.3.4.5. Rind tensile strength force

The spray application of boron ($200 \text{ mg}\cdot\text{L}^{-1}$ to $600 \text{ mg}\cdot\text{L}^{-1}$) has increased rind tensile strength force in normal and fruit with albedo breakdown than control in 2007 and 2008 (Fig. 7.1).

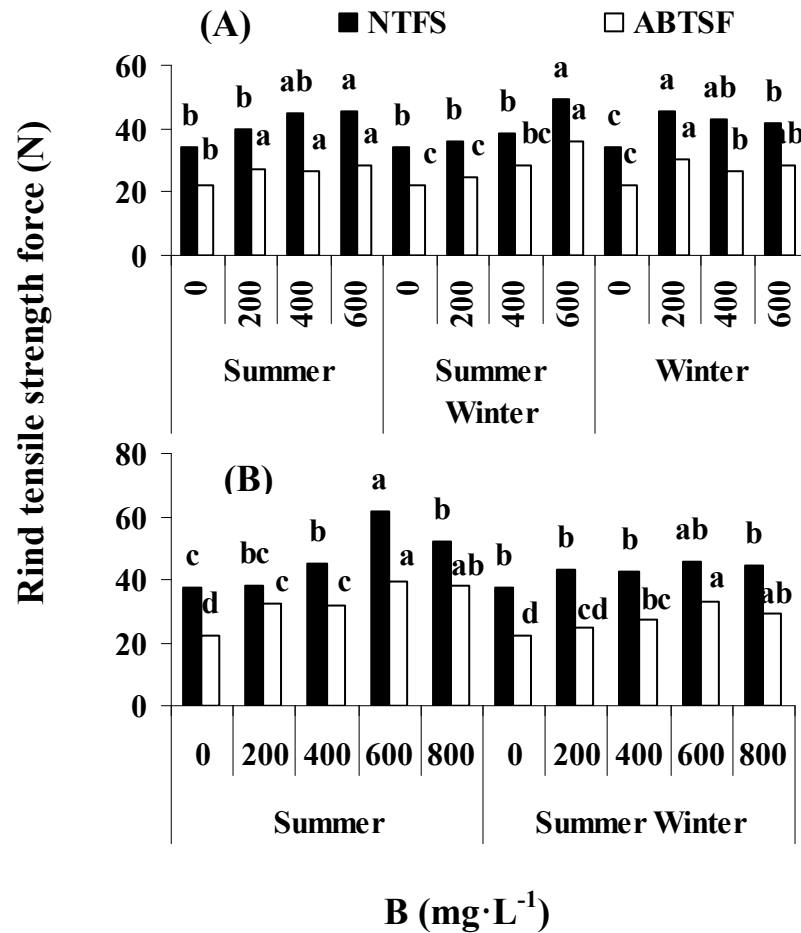


Figure 7.1. Rind tensile strength force influenced by difference concentrations and the time of boron application in 2007 (A) and 2008 (B) in ‘Washington Navel’ orange. Means followed by different letters on bars are significantly different at $P \leq 0.05$. $n = 4$ replications. NTSF = normal tensile strength force. ABTSF = albedo breakdown tensile strength force.

The significantly highest rind tensile strength force was observed with boron ($600 \text{ mg}\cdot\text{L}^{-1}$) spray in early summer and double sprays first in summer and second in winter for both normal fruit and fruit with albedo breakdown in 2007 and 2008 (Fig. 7.1). Rind tensile strength force was not significantly different between two boron sprays and single spray in 2007 while rind tensile strength force was higher with

single boron spray in early summer than two sprays for either normal or fruit with albedo breakdown in 2008 (Fig. 7.1).

7.3.4.6. Fruit compression test

Fruit firmness was significantly improved in both normal fruit and fruit with albedo breakdown with the foliar spray application of boron as compared to control treatment in 2007 and 2008, except for fruit with albedo breakdown in 2008 (Fig. 7.2).

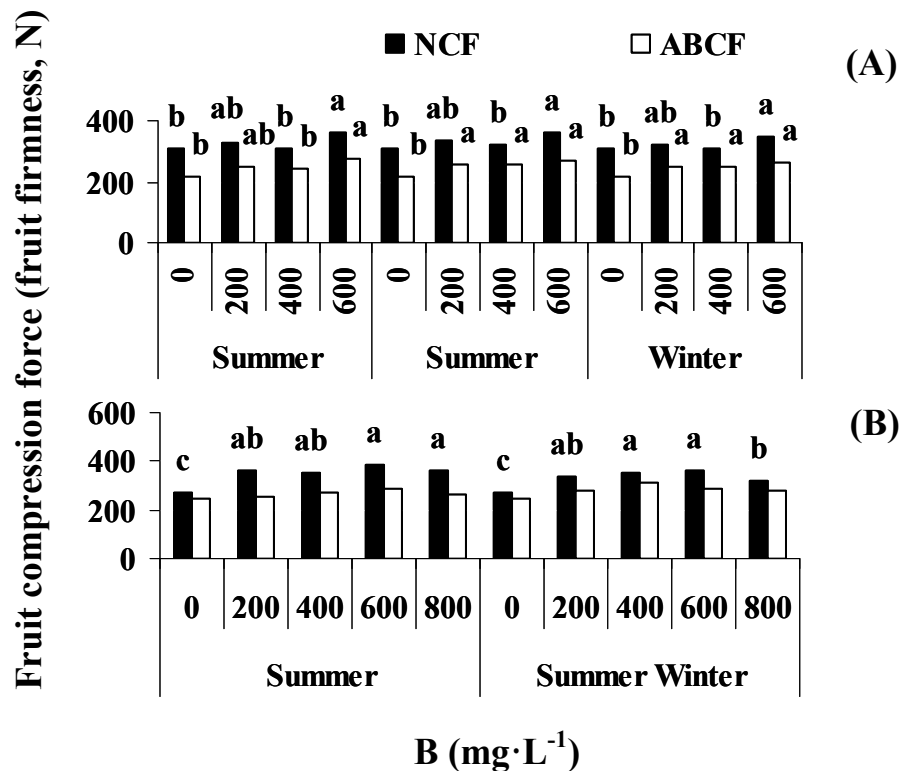


Figure 7.2. Fruit firmness influenced by difference concentrations and the time of boron application (N) in 2007 (A) and 2008 (B) in 'Washington Navel' orange. Means followed by different letters on bars are significantly different at $P \leq 0.05$. $n = 4$ replications. NCF = normal compression force. ABCF = albedo breakdown compression force.

Fruit firmness was not significantly different between single boron spray (early summer or early winter) and two sprays for normal fruit and fruit with albedo breakdown in 2007 and for normal fruit in 2008 while double boron sprays were more effective in fruit firmness than single spray in early summer in albedo breakdown fruit in 2008 (Fig. 7.2). The interaction between boron concentration and

number of boron sprays was not significantly different for fruit firmness for either normal fruit or fruit with albedo breakdown in 2007 and 2008 (Fig. 7.2)

7.3.5. Rind thickness

Table 7.8. Rind, flavedo and albedo thickness influenced by different concentrations and time of application of B in 2007 and 2008 in 'Washington Navel' orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

B (mg·L ⁻¹)	Spray time	Thickness					
		Rind (mm)		Flavedo (mm)		Albedo (mm)	
		2007	2008	2007	2008	2007	2008
0	Summer	5.23c	5.24c	1.58	1.16c	3.64c	4.08
200	(only)	5.74b	5.27bc	1.51	1.22c	4.23b	4.05
400		5.97b	5.61ab	1.57	1.52a	4.40b	4.09
600		6.52a	5.87a	1.66	1.48a	4.86a	4.38
800		-	5.87a	-	1.32b	-	4.55
Mean		5.86	5.57	1.58	1.34	4.28	4.23
0	Summer	5.23b	5.24b	1.58	1.16cd	3.64bc	4.08
200	Winter	5.00b	5.25b	1.52	1.13d	3.48c	4.13
400		5.67ab	5.36ab	1.69	1.23c	3.98b	4.13
600		5.77a	5.61a	1.77	1.52a	4.00a	4.09
800		-	5.51a	-	1.41b	-	4.10
Mean		5.42	5.39	1.64	1.29	3.78	4.11
0	Winter	5.23c	-	1.58	-	3.64b	-
200	(only)	5.48b	-	1.58	-	3.90b	-
400		5.68b	-	1.60	-	4.08ab	-
600		6.02a	-	1.64	-	4.38a	-
Mean		5.60	-	1.60	-	4.00	-
LSD	B con.			ns			ns
		0.46	0.36	(0.07)	0.09	0.42	(0.12)
$P \leq 0.05$	Time	ns	ns	ns	ns	0.36	ns
		(0.14)	(0.08)	(0.06)	(0.02)		(0.08)
	B con. x Time	ns	ns	ns	0.13	ns	ns
		(0.28)	(0.18)	(0.12)		(0.25)	(0.18)

n = 4 replications. (-) = not available. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

The rind thickness was increased with the increased concentration of boron (200 mg·L⁻¹ to 600 mg·L⁻¹) applied as compared to control in 2007 and 2008 (Table 7.8). The foliar application of boron (600 mg·L⁻¹) resulted in the significantly highest rind thickness in 2007 and 2008 (Table 7.8). The single spray application of B (600 mg·L⁻¹) in early summer was more effective in increasing the rind thickness than single

spray in winter and double sprays during both years. The interaction between the time of sprays and boron concentration was found to be non-significant for rind thickness in 2007 and 2008.

The foliar spray application of B significantly increased the thickness of flavedo in 2008 but did not show any significant effects in 2007. The single foliar spray application of boron (400 or 600 mg·L⁻¹) in early summer and two B sprays (600 mg·L⁻¹) (in early summer and followed by winter) resulted in thicker flavedo than control (Table 7.8). Both treatments were equally effective in increasing thickness of flavedo in 2008. The interaction between the time of sprays and boron concentration was not significant for thickness of flavedo in 2007 (Table 7.8).

The foliar spray application of B significantly increased the thickness of albedo in 2007 but did not show any significant effects in 2008 (Table 7.8). The B single spray in summer, winter and two sprays (600 mg·L⁻¹) resulted in thicker albedo than control and all other treatments in 2007. All these three treatments were equally effective in increasing thickness of albedo during 2007 but early summer application resulted in thickest rind (Table 7.8). There was non-significant interaction between boron concentrations and the time of boron application for albedo thickness during 2007 and 2008 (Table 7.8).

7.3.6. Fruit quality

Percentage of juice, juice pH, soluble solids concentration (SSC), titratable acidity and ascorbic acid were not significantly affected with the different treatments of foliar spray application of boron in 2007 and 2008 (Table 7.9).

7.4. Discussion

The boron concentration in the leaf, rind and pulp increased with the increased boron concentration in the spray solution (Table 7.1). Papadakis et al. (2003) reported that boron concentration in all parts of the citrus trees increased linearly with the increasing boron supply. Contrarily, Haas (1945) reported that there was no relation between the boron applied to the soil and the rind boron concentration although the pulp boron concentration may be associated with the soil application of boron.

Table 7.9. Percentage of juice (%), juice pH, soluble solids concentration (%) (SSC) and titrable acidity (mg citric·100 mL fresh juice⁻¹) (TA) influenced by different concentrations and time of application of B in 2007 in 'Washington Navel' orange.

B (mg·L ⁻¹)	Spray time	Percentage of juice		Juice pH		SSC		TA		Ascorbic acid	
		2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
0	Summer	53.93	53.18	3.56	3.54	13.09	13.18	0.91	0.94	60.23	60.92
200	(only)	52.90	51.76	3.68	3.63	12.78	13.28	0.79	0.93	56.52	58.61
400		51.72	52.42	3.62	3.63	13.25	12.93	0.86	0.87	59.19	58.87
600		53.93	50.31	3.75	3.58	13.05	12.93	0.76	0.93	59.38	59.69
800		-	52.37	-	3.70	-	13.13	-	0.81	-	53.45
Mean		53.13	52.01	3.58	3.61	12.98	13.09	0.90	0.89	60.17	58.31
0	Summer	53.93	53.18	3.56	3.54	13.09	13.18	0.91	0.94	60.23	60.92
200	Winter	53.34	52.33	3.56	3.61	13.15	13.33	0.90	0.91	60.27	59.66
400		52.00	52.65	3.62	3.63	12.93	13.33	0.87	0.88	59.75	58.06
600		53.26	52.94	3.60	3.57	12.75	13.30	0.90	0.90	60.41	60.19
800		-	54.30	-	3.60	-	13.05	-	0.83	-	60.79
Mean		53.48	53.08	3.58	3.59	13.21	13.24	0.91	0.89	60.46	59.92
0	Winter	53.93	-	3.56	-	13.09	-	0.91	-	60.23	-
200	(only)	54.67	-	3.58	-	13.23	-	0.94	-	62.23	-
400		54.08	-	3.58	-	13.30	-	0.85	-	60.32	-
600		51.25	-	3.59	-	13.23	-	0.92	-	59.04	-
Mean		53.12	-	3.65	-	13.04	-	0.83	-	58.83	-
LSD	B con.	ns (0.81)	ns	ns	ns	ns	ns	ns	ns	ns	ns
P≤0.05	Time	ns (0.70)	ns (1.54)	ns (0.02)	ns (0.03)	ns (0.12)	ns (0.13)	ns (0.03)	ns (0.04)	ns (1.05)	ns (1.62)
	B con. x Time	ns (1.40)	ns (0.97)	ns (0.02)	ns (0.02)	ns (0.11)	ns (0.08)	ns (0.03)	ns (0.03)	ns (0.91)	ns (1.02)
	Time	ns (2.17)	ns (0.04)	ns (0.05)	ns (0.21)	ns (0.18)	ns (0.06)	ns (1.82)	ns (2.29)	ns	ns

n = 4 replications. (-) = not available. Con. = concentration. ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Boron concentration in leaf was higher than that in the rind and pulp with boron foliar application here (Table 7.1). Possibly, the citrus leaf has ability to retain boron in it after absorption process as the mobilisation of boron from leaf to the other organs is limited (Papadakis et al., 2003). Although the boron absorption and distribution mechanism have not been clearly identified yet, the large difference in boron concentration between leaves and fruits may indicate that the translocation of boron in phloem within the citrus tree may be limited (Boaretto et al., 2006; Boaretto et al., 2008).

The increased boron concentration in leaf with two boron sprays in early summer and early winter as compared to one boron spray in early summer or in early winter in 2007 and 2008 (Table 7.1) suggests that redistribution of boron is restricted in orange trees. As consequence, boron should be applied to the new vegetative parts of the tree (Boaretto et al., 2006). The boron concentration in rind and pulp were similar or higher with single boron spray in early summer or early winter than that with double boron sprays in 2007 and 2008, respectively (Table 7.1). It is proposed that the boron absorption of old fruit was not effective with the foliar application of boron solution. The results of this work are in agreement with the previous results of Boaretto et al. (2006) and Boaretto et al. (2007) who reported that boron should be applied whenever the new vegetative organs are developed as boron is an immobile element.

The calcium concentration in the leaf, rind and pulp increased with the increasing boron concentration ($200 \text{ mg}\cdot\text{L}^{-1}$ to $600 \text{ mg}\cdot\text{L}^{-1}$) in 2007 and 2008 (Table 7.2). Two boron sprays (early summer and early winter) resulted in higher leaf Ca concentration than one spray in early summer or early winter. Ca concentrations in rind and pulp were similar between single spray (early summer or early winter) and double sprays in 2007, whereas, single spray in early summer was more effective in increasing Ca concentration in rind and pulp than double sprays in 2008 (Table 7.2). It has been well known that the boron concentration in the leaf, rind and pulp affect the calcium absorption into these organs of the citrus tree (Haas, 1929; Smith and Reuther, 1950; Papadakis et al., 2003; Haas, 1945). After the calcium concentration in the leaf, rind and pulp reached the maximum value, the calcium absorption declined with the higher boron concentration in the spray solution (Table 7.2). It may be argued that the calcium absorption into the tree was depressed at high boron supply levels (Haas, 1929; Smith and Reuther, 1950). Contrarily, Papadakis et al.

(2003) reported that there was no consistent effect of boron supply on the calcium absorption into the citrus trees. The reason for the declining of the calcium uptake at high boron supply level still remains unclear. However, my results suggest that the boron concentration in the spray solution can not be higher than $600 \text{ mg}\cdot\text{L}^{-1}$ in order to avoid the reducing of calcium absorption, which can cause the paralysing action on the growth process of the trees (Haas, 1929) and increases the albedo breakdown problem of the fruit as discussed below.

The albedo breakdown incidence reduced with the increasing boron supply levels ($200 \text{ mg}\cdot\text{L}^{-1}$ to $600 \text{ mg}\cdot\text{L}^{-1}$). Single boron spray in early summer was more effective in reducing albedo breakdown incidence than one boron spray in early winter in 2007 or double sprays for both 2007 and 2008 (Table 7.3). It may be argued that the significantly higher boron concentration in leaf, rind and pulp may have partially contributed to the reduction of albedo breakdown. It is known that boron is a structural element of the plant cell walls as it plays the key role to form the complexes with rhamnogalacturonan II (RG-II). As a consequence, boron crosslinks two chains of pectic polysaccharides and creates a pectic polysaccharides system in the cell walls. This process improves the cell wall integrity (Matoh, 1997; Goldbach and Wimmer, 2007; Dong et al., 2009).

Calcium concentration in leaf, rind and pulp increased with the increased boron concentration applied ($200 \text{ mg}\cdot\text{L}^{-1}$ to $600 \text{ mg}\cdot\text{L}^{-1}$) resulting in reducing albedo breakdown incidence than control. It is well known that high calcium concentration in the fruit reduces the albedo breakdown incidence (Storey et al, 2002; Treeby and Storey, 2002; Jone et al., 1967; Bevington et al., 1993; Moulds et al., 1995) as mentioned in Chapter 6. The reduction of calcium concentration in the fruit should be the major factor to explain the increasing albedo breakdown incidence at the boron concentration of $800 \text{ mg}\cdot\text{L}^{-1}$ in the spray solution (Table 7.3). These results show that the strong interaction between B and Ca can significantly impact the albedo breakdown incidence in orange fruit.

The significantly improved textural properties of the rind such as hardness, cohesiveness, adhesiveness, fracture force, tensile strength force and fruit firmness (Tables 7.4 and 7.5, Figures 7.1 and 7.2) with the foliar application of boron solution may have contributed to the significantly decreased albedo breakdown as the changes in cell wall cohesion of adjoining cells at the middle lamella result in the formation

of fracture in fruit albedo tissue during the post colour-break period causing albedo breakdown (Storey and Treeby, 1994; Storey et al., 2002).

Possibly, the significantly increased rind thickness with the foliar spray of boron solution is also suggested to be associated with the significant reduction of albedo breakdown (Table 7.5) as discussed in Chapter 6. It has been reported that rind thickness in October significantly and negatively correlated with albedo breakdown incidence at harvest in 'Navel' and 'Valencia' oranges in California (Ali et al., 2000). Similarly, Bevington et al. (1993), Jones et al. (1967) and Moulds et al. (1995) also reported that the fruit with thin rind are more susceptible to albedo breakdown than the fruits with thick rind.

Textural properties of the rind such as hardness, cohesiveness, adhesiveness, fracture force, tensile strength force and fruit firmness were significantly improved with the spray applications of an aqueous boron solution for both normal fruit and fruit with albedo breakdown. Single boron spray in early summer resulted in the highest rind hardness, rind fracture force, rind tensile strength force as compared to single spray in early winter in 2007 or double sprays in 2007 and 2008 (Tables 7.4, 7.5 and 7.6, Figures 7.1 and 7.2). It has been reported that boron may play a part in building structures of the cell walls as B-diester bonding is connecting site for pectic polysaccharides chains (Matoh, 1997; Dong et al., 2009). As a consequence, the integrity, elasticity and tensile strength of cell wall are maintained (Goldbach and Wimmer, 2007; Dong 2009). In contrast, Tariq et al. (2007) reported that the boron foliar application resulted in the softer and thinner rind while Maurer and Taylor (1999) found that rind thickness was not improved with the foliar application of boron in sweet oranges.

It may be argued that increased Ca concentration in fruit with the foliar application of boron may partially have attributed to the improvement of textural properties of rind, fruit firmness and rind thickness. It is well known that the strong structural rigidity in the cell wall is a result of the formation of Ca and B bridges within the pectin polysaccharide chains in the cell wall (Easterwood, 2002; Dong et al., 2009). This process maintains cell wall stabilisation and membrane integrity leading to improvement of the fruit firmness (Tucker, 1993; Singh et al., 2007; Saure, 2005; Dong et al., 2009). Dong et al. (2009) reported that the tissue structure of segment membrane was improved by the foliar combined application of calcium and boron in

‘Cara Cara’ Navel orange. The resistance to puncture of rind and the flesh firmness was improved with the foliar application of calcium nitrate solution just before fruit colour break or four week before harvest in ‘Fortune’ mandarin fruit (El-Hilali et al., 2004; Zaragoza et al., 1996).

Percentage of juice, juice pH, soluble solids concentration (SSC), titrable acidity and ascorbic acid were not significantly affected with the foliar boron application at different concentrations and the number of sprays. These results agree with those who reported that no systematic changes were found in chemical fruit quality parameters such as, juice content, percentage of total soluble solids and citric acid in the juice with the boron application (Smith and Reuther, 1950).

In conclusion, the foliar application of boron resulted in increased boron and Ca concentrations in leaf, rind and pulp. The one foliar boron spray in early summer ($600 \text{ mg}\cdot\text{L}^{-1}$) significantly increased boron and Ca concentrations in leaf, rind and pulp of fruit, reduced the incidence of albedo breakdown and improved textural properties of the rind such as hardness, cohesiveness, fracture force, springiness, tensile strength force as compared to single spray in early winter or double sprays (early summer and early winter) in 2007 and 2008. Rind was significantly thicker while the fruit quality parameters were not affected with one spray in early summer ($600 \text{ mg}\cdot\text{L}^{-1}$) in comparison to one spray in early winter or two sprays in ‘Washington Navel’ for both years.

CHAPTER 8**Albedo breakdown and rind textural properties of ‘Washington Navel’ sweet orange [*Citrus sinensis* (L.) Osbeck.]: the role of ethylene****Abstract**

The role of ethylene in albedo breakdown of Washington Navel’ orange [*Citrus sinensis* (L.) Osbeck] was investigated by determining amount of endogenous ethylene production in normal fruit and those with albedo breakdown during maturation and ripening, and the effects of exogenous applications of ethephon and inhibitors of ethylene biosynthesis. The effects of ethephon and inhibitors of ethylene biosynthesis were also investigated on textural properties of the rind and fruit. Endogenous ethylene production in rind of normal and fruit with albedo breakdown were determined four times during fruit development and maturation (at 263, 269, 283 and 321 days after full bloom (DAFB)). To determine the effects of exogenously applied ethephon and inhibitors of ethylene biosynthesis on albedo breakdown and textural properties of rind and fruit, whole trees (22 years old) were sprayed with different concentrations (0, 100, 200 and 300 mg·L⁻¹) of ethephon and ethylene inhibitors (AVG and CoSO₄) on 227 DAFB. The production of endogenous ethylene was higher in rind of fruit with albedo breakdown than in normal fruit at 269 and 321 DAFB (by 45.00 % and 4.48 %, respectively). The incidence of albedo breakdown was slightly increased (by 5.7%) over control with the exogenous spray application of ethephon (300 mg·L⁻¹). The foliar spray application of AVG (200 mg·L⁻¹) and CoSO₄ (300 mg·L⁻¹) significantly reduced albedo breakdown incidence (by 21.9% and 22.7%, respectively) as compared to control, and improved rind textural properties and fruit firmness. Exogenous application of ethephon did not significantly affect rind hardness, adhesiveness, springiness, fracture force, tensile strength force and fruit firmness as compared to control. Exogenous spray application of AVG was more effective in improving hardness and adhesiveness, springiness of rind and firmness of the normal fruit and those with albedo breakdown than spray application of CoSO₄. Ethylene therefore seems to play a role in incidence of albedo breakdown and the rind textural properties of citrus fruit.

8.1. Introduction

Albedo breakdown, a physiological disorder, causes cracks in the albedo tissues resulting in puffiness of orange peel. The development of albedo breakdown is connected with the increase in content of water-soluble pectins which is associated with an earlier senescence of albedo tissue (Monselise et al., 1976). Albedo breakdown causes serious economic losses in the production of citrus fruit. In Israel, throughout the season a large percentage (from 26% to 60%) of the produced orange did not meet the standards of the fresh market because of this physiological disorder. The sweet oranges planted in different parts of the world are affected by this disorder by 35% to 45% (Monselise et al., 1976). In Australia, albedo breakdown can affect 90% of the citrus fruit in some locations (Goldie, 1998).

Albedo breakdown incidence has been reported to be influenced by rootstocks (Agusti et al., 2003; Treeby et al., 1995; Moulds et al., 1995), plant nutritional status (Jones et al., 1967; Ali et al., 2000; Treeby and Storey, 2002; Storey et al., 2002; Bower, 2004), plant water relations (Sneath, 1987; Agusti et al., 2004; Gonzalez-altozano and Castel, 1999), climate (Ali et al., 2000; Treeby et al., 1995; Shear, 1975; Sneath, 1987), tree age (Moulds et al., 1995), fruit position on the tree (Bevington et al., 1993), and plant growth regulators (Monselise et al., 1976; Jona et al., 1989).

It has also been reported that endogenous levels of gibberellins in the rind of the developing fruit are associated with albedo breakdown (Jones et al., 1967; Monselise et al., 1976). Monselise et al. (1976) and Jona et al. (1989) reported that exogenous spray application of gibberellic acid ($20 \text{ mg}\cdot\text{L}^{-1}$) at an early stage of fruit development (30 to 40 mm in diameter) in July significantly reduced incidence of albedo breakdown. It was subsequently reported that GA_3 ($20 \text{ mg}\cdot\text{L}^{-1}$) applied weekly during cell separation from mid January to mid February at fruit sizes of 30 – 40 mm or during cell division from mid June to mid July when fruit were 60 - 80 mm reduced albedo breakdown more than 50% as compared to untreated treatment (Treeby and Storey, 1994; Bevington et al., 1993; Moulds et al., 1995; Tugell et al., 1993; Dick, 1995; Treeby, 1996). Bevington et al. (1993) and Treeby and Storey (1994) reported that the foliar application of GA_3 ($20 \text{ mg}\cdot\text{L}^{-1}$) during cell separation was more effective in reducing albedo breakdown incidence (by 17%) than the GA_3

sprays during cell division (by 46%) than control in ‘Valencia’ and ‘Navel’ oranges in Australia.

It is well known that ethylene is a plant hormone that plays an important role in basic plant processes such as fruit maturity, ripening, and senescence. Ethylene occurs naturally in fruit and accelerates the fruit softening due to disintegrating cell membranes making them leakier (Rath and Prentice, 2004; Ladaniya, 2007). Ethylene has been reported to regulate fruit colour, flavour, chemical composition and texture in citrus fruits (Ladaniya, 2007; Oetiker and Yang, 1995). The concentrations of ethylene in the atmosphere of internal fruit were higher in late maturing fruit with albedo breakdown ($0.09 \text{ mL}\cdot\text{kg}^{-1}$) than the normal fruit ($0.04 \text{ mL}\cdot\text{kg}^{-1}$) on the same day in ‘Valencia Late’ orange (Monselise et al., 1976).

Exogenous application of ethylene has been reported to increase respiration rate, promote ripening, and improve colour in citrus fruit (Porat et al., 1999; Ladaniya, 2007; Agusti et al., 2002; Burg, 2004; Monselise et al., 1976; Al-Mughrabi et al., 1989). Porat et al. (1999) reported that exogenous application of ethylene ($10 \mu\text{L}\cdot\text{L}^{-1}$) for 60 hours to ‘Shamouti’ oranges did not affect the fruit weight and fruit firmness. The exogenous application of ethephon ($250 \text{ mg}\cdot\text{L}^{-1}$) seven days before harvest increased rind puffing in ‘Satsuma’ mandarin (Burg, 2004, Ladaniya, 2007). In contrast, fruit weight, rind thickness, rind weight, juice percentage, soluble solids concentration, acidity and ascorbic acid were not affected with the foliar application of ethrel (Al-Mughrabi et al., 1989). Moreover, the exogenous application of ethephon did not promote the activity of pectolytic enzyme (PE), which is involved in promoting senescence in sound mature ‘Valencia Late’ orange fruit (Monselise et al., 1976).

Monselise et al. (1976) reported higher levels of endogenous ethylene in the ‘Valencia Late’ orange fruit with albedo breakdown than in normal fruit at late maturity stage. This indicated involvement of ethylene in albedo breakdown of sweet orange fruit. Although their research indicated a role of ethylene in albedo breakdown in sweet orange fruit, but the available information is sporadic and inconclusive. I investigated the endogenous concentrations of ethylene in the rind of normal fruit and those with albedo breakdown during maturation and fruit ripening period as related to development of albedo breakdown. I also investigated the effects

of the ethylene and its inhibitors on the albedo breakdown incidence, rind textural properties and fruit firmness in 'Washington Navel' sweet orange.

8.2. Materials and methods

8.2.1. Experimental site, plant materials

Three experiments were carried out in a commercial orchard located at Gingin, Western Australia (Latitude 31° 21' S, longitude 155° 55' E) in 2008. The climate is described as winter dominant with wet winters and hot, dry summers. The soil is a sandy loam. All the cultural practices including irrigation, fertiliser application, insect and weed control in all the blocks were uniform except for the experimental treatments described below.

8.2.1.1. Experiment 1: Endogenous ethylene in the rind of normal and fruit with albedo breakdown during fruit maturation

'Washington Navel' orange [*Citrus sinensis* (L.) Osbeck] trees of uniform size grafted onto 'Troyer citrange' hybrid rootstock [*Citrus sinensis* (L.) x *Poncirus trifoliata* (L.) Raf.] were used in this experiment. The tree age was 22 years old which were planted in a north – south direction (6.5 m between rows and 1.5 m within rows). Two fruit of each fruit group (normal and albedo breakdown) per tree were collected to determine the production of endogenous ethylene in the rind of the fruit during fruit maturation and ripening (236-321 DAFB). The experimental design was randomised block design with four replications. Single tree was treated as an experimental unit.

8.2.1.2. Experiment 2: Effect of exogenous application of ethephon on the albedo breakdown incidence, rind textural properties and fruit firmness

Aqueous solutions containing various concentrations of ethephon at 0, 100, 200, or 300.mg·L⁻¹ and a surfactant 'Tween 20' (0.05%) were sprayed onto the fruit of whole trees at 227 DAFB. The trees were selected from the same block as experiment 1 and sprayed with a sprayer (The Selecta Trolleyapak Mk II, Victoria, Australia) was used at the spraying rate of 1000 L.ha⁻¹ till run off. The nozzle (Chierici Titistrl, Rubiera, Italy) discharged at rate of 70 L/min. under a pressure of 250 KPa. Fruit showing symptoms of albedo breakdown were counted after harvesting the whole tree. Rind hardness, adhesiveness, cohesiveness, springiness, fracture force and rind tensile strength force and fruit firmness were recorded from

normal fruit and with albedo breakdown as the major parameters of the texture profile analysis.

8.2.1.3. Experiment 3: Effects of ethylene inhibitors on the albedo breakdown incidence, rind textural properties and fruit firmness

Exogenous applications of inhibitors of ethylene including aminoethoxyvinylglycine (AVG), and cobalt sulphate (CoSO_4), at 0, 100, 200 and 300 $\text{mg}\cdot\text{L}^{-1}$ with 'Tween 20' (0.05%) were tested. The treatments were applied as a spray onto the fruit of whole trees which were selected from the same block as experiment 1 at 227 DAFB. The experimental design was completely randomised including two factors viz. chemicals and concentrations. Single tree was treated as an experimental unit and included three replications. All other experimental conditions and observations recorded were similar to Experiment 2.

8.2.2. Chemicals

Ethrel[®] containing 480 $\text{g}\cdot\text{L}^{-1}$ ethephon was purchased from Rhone-Poulenc Rural Australia Pty. Ltd, NSW, Australia. Retain[®] containing 15% w/w aminoethoxyvinylglycine (AVG) was purchased from Valent BioSciences, Sumitomo Chemical Australia Pty. Ltd., NSW, Australia. Cobalt sulphate (Heptahydrate) (CoSO_4) was purchased from Sigma Chemical Company, Missouri, USA.

8.2.3. Parameters measured

Endogenous ethylene production in fruit rind from two fruit groups (normal fruit and with albedo breakdown) was determined as described in details below. Albedo breakdown incidence was recorded at harvest. Rind hardness, adhesiveness, cohesiveness, springiness, fracture force and rind tensile strength force and fruit firmness were recorded from normal and fruit with albedo breakdown as the major variables of the rind texture profile analysis.

8.2.3.1. Determination of endogenous ethylene production

In Experiment 1, two fruit of each group (normal and with albedo breakdown) from one tree were randomly sampled for determination of ethylene from their rind. Fruit were collected four times (263, 269, 283 and 321 DAFB).

Endogenous ethylene was extracted from fruit rind following a partial vacuum method as described by Saltveit (1982). Endogenous ethylene was determined using a gas chromatograph (Agilent Technologies, 6890 N Network GC system, Palo Alto, CA, USA) as described in Section 3.5 and expressed as $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{hour}^{-1}$.

8.2.3.2. Determination of albedo breakdown incidence

Fruits from each whole tree were examined for albedo breakdown as mentioned in Section 3.7. The albedo breakdown incidence was expressed as percentage of fruit.

8.2.3.3. Texture profile analysis

Textural properties of rind such as hardness, adhesiveness, cohesiveness, springiness, fracture force, tensile strength force and firmness from normal fruit and fruit with albedo breakdown were determined using a texture analyser (TA Plus, AMETEK Lloyd instruments Ltd., Hampshire, UK) as described in Section 3.8.

8.2.3.3.1 Rind puncture test

Rind samples from two fruit groups (normal and fruit with albedo breakdown) were cut 2.5 cm wide x 0.6 cm thick using a slicer (Zyliss Easy slice 2" folding Mandolin slicer, Swiss) to give uniform sections for determining rind puncture parameters as detailed in Section 3.8.1.

8.2.3.3.2 Rind tensile strength test

The rind tensile test was carried out to measure the behaviour of the orange rind up to the rind deflection of 10 mm. A rind sample section was carefully removed from each fruit in the size of 2.5 cm wide x 5.0 cm length x 0.6 cm thick using a slicer to give uniform sections. Ten fruit of each fruit group (normal and albedo breakdown) were used for each test. The rind tensile strength force was calculated at the maximum load and limit points where the rind deflection occurred as described in Section 3.8.2.

8.2.3.3.3 Fruit compression test

Ten fruit of each fruit group (normal and albedo breakdown) with the height of about 8.5 cm were used for each compression test as mentioned in Section 3.8.3.

8.2.4. Statistical analysis

The data were subjected to one way or two way ANOVA using Genstat 9 release 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The least significant difference (Fisher's protected LSD) was calculated at $P \leq 0.05$. To ensure the validity of statistical analysis all the assumptions of ANOVA were checked.

8.3. Results

8.3.1. Experiment 1: Ethylene production during fruit maturation and ripening and development of albedo breakdown

Endogenous concentrations of ethylene were higher in rind of fruit with albedo breakdown at 269 and 321 DAFB (45.00 % and 4.48 %, respectively) than in the rind of normal fruit (Fig. 8.1).

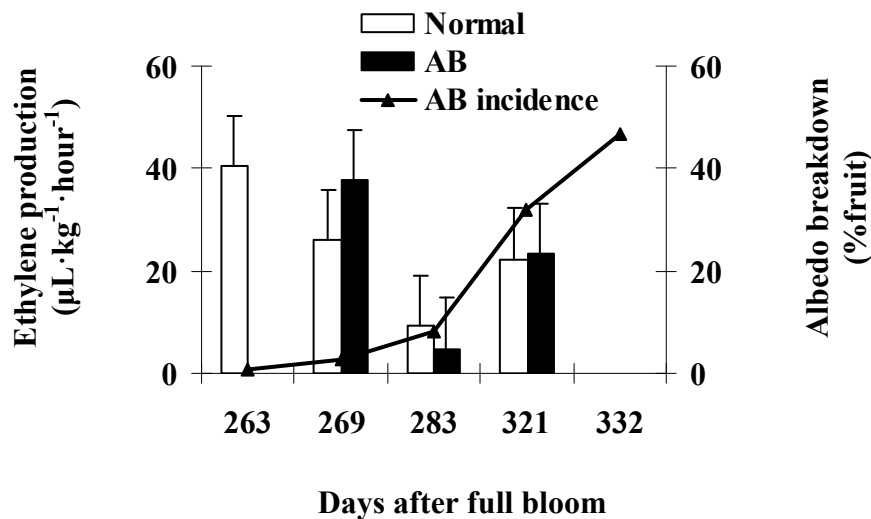


Figure 8.1. Ethylene production in rind of normal fruit and fruit with albedo breakdown and the development of albedo breakdown during fruit growth in 'Washington Navel' orange in 2008. Vertical bars represent LSD at $P \leq 0.05$. $n = 4$ replications. The commercial harvest was at 283 DAFB. AB = albedo breakdown.

The production of endogenous ethylene in rind of fruit with albedo breakdown was lower ($4.80 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{hour}^{-1}$) at 283 DAFB than in the rind of normal fruit ($9.18 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{hour}^{-1}$) (Fig. 8.1). In general, the ethylene production decreased rapidly in rind of normal and albedo breakdown fruit from 263 DAFB to 283 DAFB and commenced to increase after commercial harvest at 321 DAFB.

8.3.2. Experiment 2: Effect of ethephon on albedo breakdown incidence and textural properties of the rind and fruit

8.3.2.1. Albedo breakdown incidence

All the treatments involving exogenous spray application of ethephon at 227 DAFB did not significantly affect the incidence of albedo breakdown (Table 8.1).

Table 8.1. Incidence of albedo breakdown, rind hardness and adhesiveness as influenced by the foliar application of Ethephon in normal fruit and fruit with albedo breakdown in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Concentration (mg·L ⁻¹)	AB (% of fruit)	Rind hardness (N)		Rind adhesiveness (N)	
		Normal	AB	Normal	AB
0	65.0	22.86	14.77	0.41	0.27
100	58.3	26.89	13.76	0.22	0.10
200	53.3	21.64	13.71	0.22	0.13
300	68.7	22.72	14.14	0.25	0.16
LSD ($P \leq 0.05$)	ns (14.1)	ns (1.09)	ns (1.94)	0.13	ns (0.09)

AB = albedo breakdown. n = 3 replications, ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

8.3.2.2. Texture profile analysis of the rind and the fruit

8.3.2.2.1 Rheological properties of rind

Ethephon foliar application at 227 DAFB did not significantly affect the adhesiveness, hardness, cohesiveness, springiness, fracture force and tensile strength force of the rind of normal fruit and fruit with albedo breakdown except for rind adhesiveness in normal fruit (Tables 8.1 and 8.2). Exogenous application of ethephon (100 and 200 mg·L⁻¹) at 227 DAFB significantly decreased the rind adhesiveness in normal fruit as compared to all other treatments.

Table 8.2. Rind cohesiveness, springiness and fracture force as influenced by the foliar application of Ethepon in normal fruit and fruit with albedo breakdown in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Concentration (mg·L ⁻¹)	Rind cohesiveness		Rind springiness (mm)		Rind fracture force (N)	
	Normal	AB	Normal	AB	Normal	AB
0	0.08	0.09	1.61	1.59	3.17	3.35
100	0.10	0.10	1.84	1.65	3.77	2.53
200	0.10	0.13	1.87	1.72	3.15	3.50
300	0.08	0.08	1.85	1.40	2.28	3.24
LSD ($P \leq 0.05$)	ns (0.01)	ns (0.02)	ns (0.06)	ns (0.13)	ns (1.15)	ns (0.63)

AB = albedo breakdown. n = 3 replications, ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Table 8.3. Rind tensile strength force and fruit firmness as influenced by the foliar application of Ethepon in normal fruit and fruit with albedo breakdown in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Concentration (mg·L ⁻¹)	Rind tensile strength force (N)		Fruit firmness (N)	
	Normal	AB	Normal	AB
0	30.32	21.31	335.0	241.6
100	32.54	25.07	287.6	240.5
200	29.83	22.05	292.0	227.1
300	33.38	23.91	300.3	220.2
LSD ($P \leq 0.05$)	ns (1.33)	ns (1.76)	ns (19.4)	ns (13.4)

AB = albedo breakdown. n = 3 replications, ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

8.3.2.2.2 Fruit compression test

The firmness of normal and fruit with albedo breakdown was decreased with the exogenous application of different concentrations of ethephon at 227 DAFB as compared to control, however the differences were not significant among treatments (Table 8.3).

8.3.3. Experiment 3: Effect of ethylene inhibitors on incidence of albedo breakdown and textural properties of rind and fruit

8.3.3.1. Incidence of albedo breakdown

Incidence of albedo breakdown was significantly decreased with exogenous application of ethylene inhibitors at 227 DAFB.

Table 8.4. Incidence of albedo breakdown, rind hardness and adhesiveness as influenced by the foliar application of ethylene inhibitors in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Chemical	Concentration (mg·L ⁻¹)	AB (% of fruit)	Rind hardness (N)		Rind adhesiveness (N)	
			Normal	AB	Normal	AB
AVG	0	88.0b	24.00b	11.78c	0.04	-0.02
	100	71.7a	24.09b	14.54bc	0.14	0.17
	200	68.7a	27.58a	20.62a	0.12	0.05
	300	69.3a	26.78a	16.38b	0.08	0.08
	Mean	74.4	25.61A	15.83	0.09A	0.07A
CoSO ₄	0	88.0b	18.32c	11.78c	0.06	-0.02
	100	81.0b	20.10bc	13.36bc	0.03	-0.01
	200	80.0b	21.33b	15.50b	0.03	0.06
	300	68.0a	25.08a	18.60a	0.05	0.02
	Mean	79.4	21.21B	14.81	0.04B	0.01B
LSD ($P \leq 0.05$)	Chemical	ns (2.83)	1.70	ns (0.46)	0.02	0.06
	Concentration	12.12	2.40	1.97	ns (0.01)	ns (0.03)
	Chem x Cont	ns (5.65)	ns (1.12)	2.79	0.05	ns (0.04)

AB = albedo breakdown. Chem = chemical. Cont = concentration. n = 3 replications, ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

All the treatments of exogenous spray application of AVG at 227 DAFB significantly reduced the incidence of albedo breakdown as compared to control. The exogenous spray application of AVG ($200 \text{ mg}\cdot\text{L}^{-1}$) at 227 DAFB resulted in the lowest albedo breakdown incidence (68.7%) as compared to control and all other treatments. Amongst various spray treatments of CoSO_4 , the spray application of CoSO_4 ($300 \text{ mg}\cdot\text{L}^{-1}$) at 227 DAFB resulted in significantly lowest incidence of albedo breakdown (68.0%) as compared to control (88.0%) (Table 8.4). Both chemicals (AVG and CoSO_4) were equally effective in the reducing albedo breakdown incidence. The interaction between chemical and chemical concentration was found not to be significant for incidence of albedo breakdown (Table 8.4).

8.3.3.2. Texture profile analysis of the rind and the fruit

8.3.3.2.1 Rheological properties of rind

Rind hardness of the normal fruit and with albedo breakdown was significantly improved with the exogenous spray application of AVG 227 DAFB (Table 8.4). The spray of AVG ($200 \text{ mg}\cdot\text{L}^{-1}$) 227 DAFB resulted in the highest rind hardness in both normal and albedo breakdown fruit (27.58 N and 20.62 N, respectively) than all other treatments of AVG. Amongst CoSO_4 treatments, the $300 \text{ mg}\cdot\text{L}^{-1}$ spray 227 DAFB resulted in the significantly highest rind hardness (25.08 N and 18.60 N) for both normal and albedo breakdown fruit, respectively. AVG spray application was more effective in improving rind hardness of normal fruit than foliar spray of CoSO_4 whereas both chemicals did not show significant differences in improving rind hardness of fruit with albedo breakdown (Table 8.4).

Rind adhesiveness was significantly higher with spray application of AVG than CoSO_4 for both normal fruit and fruit with albedo breakdown (Table 8.4). The effects of different concentrations of both chemicals on the rind harness were not significant. The interaction between chemical and chemical concentration for rind adhesiveness were found to be significant in normal fruit whilst it was non-significant for fruit with albedo breakdown (Table 8.4).

Rind cohesiveness remained stable with the exogenous application of ethylene inhibitors in normal fruit whereas it was significantly decreased in fruit with albedo breakdown as compared to control (Table 8.5).

Rind springiness was significantly increased with the spray application of AVG or CoSO_4 in fruit with albedo breakdown in comparison to control (Table 8.5). There were no significant differences in rind springiness with the sprays of ethylene inhibitors among treatments in normal fruit although these sprays resulted in higher rind springiness than control (Table 8.5).

The exogenous application of ethylene inhibitors did not significantly affect rind fracture force in both normal and albedo breakdown fruit (Table 8.5).

Rind tensile strength force was significantly increased with the increased concentrations of AVG or CoSO_4 foliar application in normal and albedo breakdown fruit than control (Table 8.6). The significantly highest rind tensile strength force was found with AVG spray or CoSO_4 ($300 \text{ mg}\cdot\text{L}^{-1}$) in normal fruit while spray of AVG ($200 \text{ mg}\cdot\text{L}^{-1}$) or CoSO_4 ($300 \text{ mg}\cdot\text{L}^{-1}$) resulted in the significantly highest rind tensile strength force in fruit with albedo breakdown (Table 8.6). The interaction between chemical and chemical concentration was found to be non-significant for rind tensile strength force for both normal and fruit with albedo breakdown (Table 8.6).

Table 8.5. Rind cohesiveness, springiness and fracture force as influenced by the application of ethylene inhibitors in normal fruit and fruit with albedo breakdown in 'Washington Navel' orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Chemical	Rind cohesiveness		Rind springiness (mm)		Rind fracture force (N)		
	Concentration (mg·L ⁻¹)	Normal	AB	Normal	AB	Normal	AB
AVG	0	0.20	0.22a	2.18	1.87c	3.08	2.11
	100	0.19	0.18b	2.22	2.04b	2.09	2.19
	200	0.19	0.18b	2.15	2.25a	4.07	4.72
	300	0.22	0.17b	2.32	2.06b	1.52	3.55
	Mean	0.20	0.19	2.22	2.05A	2.69	3.14
CoSO ₄	0	0.21	0.22a	1.93	1.87b	1.82	2.11
	100	0.20	0.17c	2.02	1.74b	3.59	4.02
	200	0.22	0.19b	2.23	1.80b	2.44	3.79
	300	0.17	0.18b	1.98	2.23a	2.14	3.87
	Mean	0.20	0.19	2.04	1.91B	2.50	3.45
LSD	Chemical	ns (0.01)	ns (0.004)	ns (0.10)	0.10	ns (0.58)	ns (0.50)
($P \leq 0.05$)	Concentration	ns (0.01)	0.02	ns (0.14)	0.14	ns (0.41)	ns (0.75)
	Chem x Cont	ns (0.02)	ns (0.01)	ns (0.20)	0.20	ns (0.82)	ns (1.05)

AB: albedo breakdown. Chem: chemical. Cont: concentration. n = 3 replications, ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

Table 8.6. Rind tensile strength force and fruit firmness as influenced by the foliar application of ethylene inhibitors in normal fruit and fruit with albedo breakdown in ‘Washington Navel’ orange. Within each column, means followed by different letters are significantly different at $P \leq 0.05$.

Chemical	Concentration ($\text{mg}\cdot\text{L}^{-1}$)	Rind tensile strength force (N)		Fruit firmness (N)	
		Normal	AB	Normal	AB
AVG	0	40.4b	27.5b	304.0	265.8bc
	100	42.1b	29.7b	324.2	256.9c
	200	46.5b	32.4a	331.7	303.2a
	300	50.0a	31.4b	320.0	296.8ab
	Mean	44.7	30.15	320.0A	280.7
CoSO ₄	0	33.55c	25.65b	261.8b	255.5b
	100	36.61bc	28.74b	275.5b	265.3b
	200	42.17b	29.96b	282.3b	271.7ab
	300	50.14a	36.39a	320.0a	291.5a
	Mean	40.6	30.19	284.9B	271.0
LSD ($P \leq 0.05$)	Chemical	ns (1.88)	ns (0.91)	19.7	ns (4.83)
	Concentration	8.06	3.92	ns (9.12)	20.7
	Chem x Cont	ns (3.76)	ns (1.83)	ns (12.9)	ns (9.65)

AB = albedo breakdown. Chem = chemical. Cont = concentration. n = 3 replications, ns = not significant at $P \leq 0.05$. Values within the bracket represent standard errors of means (SEM).

8.3.3.2.2 Fruit compression test

Exogenous spray application of ethylene inhibitors significantly improved fruit firmness as compared to control except for AVG spray in normal fruit (Table 8.6). Fruit firmness was highest with the foliar spray application of AVG (200 $\text{mg}\cdot\text{L}^{-1}$) in normal fruit and fruit with albedo breakdown (331.7 N and 303.2 N, respectively) (Table 8.6). The spray of CoSO₄ (300 $\text{mg}\cdot\text{L}^{-1}$) resulted in the significantly highest fruit firmness in normal fruit and fruit with albedo breakdown (320.0 N and 291.5 N, respectively) (Table 8.6). AVG spray resulted in the significantly higher fruit firmness than CoSO₄ application in normal fruit. But such effects of this chemical were not recorded on firmness of fruit with albedo breakdown. The interaction

between chemical and chemical concentration was found to be non-significant for fruit firmness of normal and albedo breakdown fruit (Table 8.6).

8.4. Discussion

The ethylene production was higher in rind of the fruit with albedo breakdown than in normal fruit, particularly at 269 DAFB (Fig. 8.1) when albedo breakdown was already visible. This implicates ethylene in albedo breakdown incidence. It may be argued that increased activities of 1-aminocyclopropane-1-carboxylase synthase (ACC synthase) and ACC oxidase may be contributing to the higher concentration of endogenous ethylene in the rind of fruit with albedo breakdown. The activities of ACC synthase and ACC oxidase in the rind of normal and albedo breakdown fruit warrant investigation. Monselise et al. (1976) reported higher concentrations of ethylene in the internal atmosphere of the fruit with albedo breakdown ($0.09 \text{ mL}\cdot\text{kg}^{-1}$) than the normal fruit ($0.04 \text{ mL}\cdot\text{kg}^{-1}$) of 'Valencia Late' orange. Earlier, the positive relationship of ethylene production and wounding or stress in citrus fruit has been reported by Burg (2004) and Ladaniya, (2007). The production of endogenous ethylene markedly increased in the rind of albedo breakdown and normal fruit after commercial harvest coupled with increased incidence of albedo breakdown further suggesting the role of ethylene in albedo breakdown. Ethylene production in rind of fruit with albedo breakdown was significantly lower than in the rind of normal fruit at commercial harvest (283 DAFB, Fig. 8.1). It is likely that the association of advanced fruit maturity and albedo breakdown may have contributed to the lower production of endogenous ethylene in the rind of fruit with albedo breakdown than in normal ones at commercial harvest maturity. It has been reported that fruit with albedo breakdown are more mature than normal fruit on the same tree as indicated by higher ratio of soluble solids concentration to acidity (Jones and Embleton, 1967). My data also support the above hypothesis because there was a higher production of endogenous ethylene in the rind of the developing orange fruit than in the mature fruit (Fig. 8.1).

Spray application of ethephon at 227 DAFB slightly increased the incidence of albedo breakdown without any significant effects on rind textural properties and fruit firmness (Tables 8.1, 8.2 and 8.3). My results suggest auto inhibition of the ethylene production with the exogenous application of ethephon. Similarly, Yang and Hoffman (1984) found that the exogenous application of ethylene significantly

inhibited the ethylene production in flavedo tissue in grapefruit. The exogenous application of ethephon ($250 \text{ mg}\cdot\text{L}^{-1}$) seven days before harvest has also been reported to increase peel puffing in 'Satsuma' mandarin (Burg, 2004; Ladaniya, 2007).

Foliar spray of ethylene inhibitors (AVG and CoSO_4) significantly reduced albedo breakdown incidence and improved the rind textural properties and fruit firmness (Tables 8.4, 8.5 and 8.6). As expected, the ethylene biosynthesis and its action are involved in albedo breakdown and textural properties of sweet orange fruit. It is well known that AVG and Co^{2+} are inhibitors of ethylene biosynthesis at the conversion of S-adenosylmethionine (SAM) to ACC or from ACC to ethylene through the action of the ACC oxidase enzyme (Even-Chen et al., 1982; Hyodo and Nishino, 1981; Ladaniya, 2007). It has also been reported that ethylene is involved in the fruit softening process and stimulates fruit maturation and ripening (Burg, 2004; Oetiker and Yang, 1995; Rath and Prentice, 2004). Possibly, the reduction in the incidence of albedo breakdown and improvement of rind textural properties and fruit firmness with the exogenous spray application of ethylene inhibitors may be attributed to the reduced endogenous ethylene production (not determined) through inhibiting the activities of enzymes involved in ethylene biosynthesis (ACC synthase and ACC oxidase).

In conclusion, the increased ethylene production in the rind of fruit with albedo breakdown than normal fruit at 269 DAFB and reduction of the incidence of albedo breakdown in sweet oranges with the exogenous application of ethylene inhibitors at 227 DAFB suggest the involvement of ethylene in albedo breakdown of sweet orange.

CHAPTER 9

General discussion, conclusion and future research

9.1. Introduction

Albedo breakdown is the rind disorder due to abnormal separation of cells leading to the formation of irregular fractures in the white tissue (albedo) causing the cracks of orange rind (Treeby and Storey, 2002; Jones et al., 1967; Bevington et al., 1993; Sneath, 1987; Storey and Treeby, 1994; Tugell et al., 1993). The development of albedo breakdown was thought to be associated with the increased loss of pectin and in the cellular walls of rind tissue causing the loosening of the connection among cells in sweet oranges (Monselise et al., 1976; Li et al., 2009; Bower, 2000). Albedo breakdown has resulted in significant economic losses to citrus industry in Australia (Sneath, 1987; Treeby and Storey, 1994; Pelizzo, 1997), California (Jones et al., 1967, Ali et al., 2000), Israel (Monselise et al., 1976), Uruguay (Gambetta et al., 2000), South Africa (Bower, 2004) and China (Li et al., 2009) as it causes approximately 15% to 30 % or more of 'Navel' orange fruit rejected at the time of packing.

Fruit quality, particularly albedo breakdown has been attributed to plant water relations (Sneath, 1987; Agusti et al., 2004; Gonzalez-altozano and Castel, 1999; Treeby et al., 2007), genetic factors (Agusti et al., 2003; Treeby et al., 1995; Moulds et al., 1995; Bevington et al., 1993), plant nutritional status (Jones et al., 1967; Ali et al., 2000; Treeby and Storey, 2002; Storey et al., 2002; Bower, 2004) and plant growth regulators (Embleton et al., 1973; Jona et al., 1989; Treeby and Storey, 1994; Tugell et al., 1993; Dick, 1995). However, the research work on the fruit quality with an emphasis on albedo breakdown in sweet oranges is sporadic and inconclusive. The general aim of my research work was to investigate the effects of pre-harvest factors on fruit quality with an emphasis on albedo breakdown in sweet oranges.

9.2. The development of the incidence and the severity of albedo breakdown during fruit maturation and ripening, the effects of severity of albedo breakdown on fruit quality among locations and cultivars of ‘Navel’ sweet oranges

Albedo breakdown has been reported to influence fruit quality (Jones et al., 1967; Jones and Embleton, 1967; Sneath 1987; Treeby and Storey, 1994). In contrast, Goldie (1998) reported that internal fruit quality parameters were not affected by albedo breakdown. I investigated the development of albedo breakdown incidence coupled with fruit maturation and ripening, the influence of location on the incidence and severity of albedo breakdown and the effects of the severity of albedo breakdown among locations and cultivars to fruit quality in ‘Navel’ sweet oranges.

In the first experiment, the incidence of albedo breakdown was determined by assessment of all fruit on the twenty-two years old ‘Washington Navel’ orange trees. The incidence and severity of albedo breakdown became visible after colour break and coupled with the slow growth of fruit and then increased rapidly after the commercial harvest due to the degradation of pectin. Similarly, the incidence of albedo breakdown was higher on the tree in which fruit held longer (McIntosh, 1998; Tugell et al., 1993; Dick, 1995; Jones et al., 1967).

In the second experiment, fruit from ‘Washington Navel’ sweet orange cultivar grown at four commercial orchards at Gingin, Chittering, Serpentine and Harvey, in Western Australia were classified into four categories of albedo breakdown. The lowest incidence and severity of albedo breakdown at Harvey as compared to three different locations proposed the association of climate factors in these agro-climate zones in albedo breakdown. Similarly, the difference of incidence and severity of albedo breakdown was from year to year and from location to location and among cultivars of sweet oranges (Jones et al., 1967; Sneath, 1987; Treeby et al., 1995). The severity of albedo breakdown did not affect fruit quality in terms of juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and the individual organic acids except for the reduction in succinic acid. The highest soluble solids concentration, the lowest titratable acidity, ascorbic acid and the individual organic acids of fruit collected in Gingin have been suggested as being the results of climatic, physiological trees and soil characteristics (Davies and Albrigo, 1994; Pretel et al., 2004).

In the third experiment, fruit from three ‘Navel’ sweet orange cultivars including ‘Leng Navel’, ‘Autumn Gold’ and ‘Washington Navel’ at the Westralian Fruits in Gingin was classified into three categorises of the albedo breakdown. The major fruit quality attributes such as juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and the individual organic acids were not affected by the severity of albedo breakdown among three cultivars while the decreased succinic acid and the increased tartaric acid were observed with the increased severity of albedo breakdown. However, succinic and tartaric acid contributed to the minor amount of organic acids in citrus juice (Karadenis, 2004; Shaw and Wilson, 1983). The lowest pH juice, soluble solids concentration, titratable acidity and ascorbic acid in ‘Autumn Gold’ orange cultivar as compared to ‘Leng Navel’ and ‘Washington Navel’ suggested genetic factor mainly contributed to fruit quality. Similarly, the fruit weight, soluble solids concentration and titratable acidity were significantly different among sweet orange cultivars (Pretel et al., 2004; Kahn et al., 2007).

9.3. Responses of ‘Navelina’ oranges to irrigation levels: water relations, growth, yield and fruit quality with an emphasis on albedo breakdown

The water management has been reported to be associated with the water status of plant and fruit, the average fruit weight and other quality attributes (Gonzalez-altozano and Castel, 1999; Verreynne et al., 2001; Ritermour et al., 2003; Hutton et al., 2007) and the albedo breakdown incidence (Treeby et al., 2007; Gonzalez-altozano and Castel, 1999). In order to improve the inconclusive results in the literature, I investigated the effects of application of deficit irrigation on plant water status, albedo breakdown incidence and fruit quality parameters in ‘Navelina’ sweet oranges.

Twelve years old ‘Navelina’ sweet orange trees were irrigated at an early stage of fruit development with the following percentages of commercial irrigation including 100% (T100, control), 125% (T125), 75% (T75) and 50% (T50). T50 resulted in the significantly lowest fruit diameter, average fruit weight and yield in ‘Navelina’ sweet oranges. My data indicated that the application of deficit irrigation decreased volumetric soil water content at soil depths of 300 mm and 600 mm, the midday stem water potential and also the stomatal conductance leading to the reduction in fruit growth as reflected the impact on photosynthesis rate.

The significantly lowering of albedo breakdown; in terms of moderate, severe, total albedo breakdown and the severity of albedo breakdown, in T50 as compared to other irrigation treatments may be the results of the higher proportion of smaller fruit in which slower growth of endocarp in stage III occurred due to a lower plant water status. Consistent with my results, albedo breakdown was highly associated with the smaller fruit which had a thinner rind than medium and large fruit (Jones et al., 1967, Ali et al., 2000). A slower rate of fruit growth in medium and large fruit in T50 to reach their respective final fruit sizes may partially contribute to the reduction in albedo breakdown. My data were in the agreement with the findings of those who reported that albedo breakdown seems to be attributed to the formation of cracks in white albedo tissues underneath the rind due to the quick increases in fruit size after first eight weeks of fruit development (Tugell et al., 1993). The same percentage of small fruit and medium fruit in T75 and T100 may be suggested contributing to non-significant differences in the incidence of albedo breakdown between two treatments.

The tendencies of a slight increase in rind thickness and the dry matter content of rind and pulp with the application of deficit irrigation were observed in my investigation. These results were proposed due to a reduction in water volume in fruit causing the decreased cellular hydration (Mpelasoka et al., 2001; Kilili et al., 1996).

The highest proportion of small fruit in T50 in comparison to other irrigation treatments indicated that the decreased irrigation application was highly associated with the plant water status resulting in the reduction of fruit size. Similarly, fruit was smaller with the application of deficit irrigation in mandarins (Gonzalez-altozano and Castell, 1999; Verrenynne et al., 2001), sweet oranges (Treeby et al., 2007; Hutton et al., 2007) and pears and apples (Behboudian and Mills, 1997). However, slower rate of fruit growth at stage III of fruit development to reach the probably final fruit sizes was possible a benefit of decreased irrigation application to reduce albedo breakdown as small fruit were less likely to be susceptible with albedo breakdown than medium fruit (Treeby et al., 1995).

The reduction in irrigation water volume applied increased soluble solids concentration and titratable acidity in 'Navelina' sweet oranges. It proposed that the conversion of starch to sugar was increased due to water stress during fruit maturation (Mpelasoka et al., 2001; Kramer and Boyer, 1995). Possibly, the dilution of solute also may involve to the increase in soluble solids concentration with the

application of full irrigation (Bebhoudian and Mills, 1997; Kili et al., 1996; Kramer and Boyer, 1995). Consistent with my data, higher soluble solids concentration was observed due to the application of deficit irrigation in sweet oranges (replacing 50% of water applied to control) (Treeby et al., 2007) and mandarins (water supply at 25% and 50% of potential evaporation) (Gonzalez-altozano and Castel, 1999) and partial root zone drying applied cover the whole growing season to 'Bellamy' Navel oranges (Treeby et al., 2007). In my investigation, the application of deficit irrigation did not affect ascorbic acid concentration in the juice. In contrast, ascorbic acid was increased with the decreased water volume applied in lemons (Domingo et al., 1996).

9.4. Different surfactants improve calcium uptake into leaf and fruit of 'Washington Navel' sweet orange [*Citrus sinensis* (L.) Osbeck.] and reduce albedo breakdown

The involvement of calcium in albedo breakdown has also been indicated in some previous reports (Treeby and Storey, 1995; McIntosh, 1998; Lovat, 2000). The foliar application of 2% $\text{Ca}(\text{NO}_3)_2$ aqueous solution starting at an early stage of fruit development reduced albedo breakdown. However, the Ca uptake into fruit with the foliar spray of calcium solution was limited as Ca is not a mobile element (Treeby and Storey, 2002). Using surfactants to improve the Ca penetration into fruit has been reported in apples (Saure, 2005; Harker and Ferguson, 1991; Schlegel and Schonherr, 2002) and mango (Singh et al., 2000). In this experiment, I explored the role of different surfactants added to the foliar spray aqueous solutions containing 2% $\text{Ca}(\text{NO}_3)_2$ in the enhancement of Ca uptake into leaf and fruit, the reduction in albedo breakdown and the improvement of the rind textural properties and fruit firmness in 'Washington Navel' sweet orange.

Twenty-two years old uniform 'Washington Navel' trees were sprayed with an aqueous solution of 2% $\text{Ca}(\text{NO}_3)_2$ either alone or with one of the following surfactants: 'Tween 20' (0.05%), 'Tween 80' (0.05%), 'Triton X100' (0.05%) and 'Tergitol' (0.05%). Higher Ca concentration in leaf, rind and pulp tissues was the results of five sprays of 2% $\text{Ca}(\text{NO}_3)_2$ solutions containing different surfactants commencing at 81 DAFB with 10 day intervals. This suggested surfactants added to an aqueous solution of $\text{Ca}(\text{NO}_3)_2$ can be used to enhance the Ca uptake into leaf and fruit due to the lower surface tension between droplet and surface of leaf and fruit leading to better distribution of Ca ion on the surface of leaf and fruit. The efficiency

of surfactants on enhancing the Ca uptake into leaf and fruit may partially attribute to increased binding capacity of the cuticle for Ca ion due to the improved wetting on the leaf and fruit of surfactants. Similarly, the Ca uptake into fruit was increased by first dipping apples into different surfactants and followed with pressure-infiltrating with a 2% CaCl_2 solution (Roy et al., 1996). The efficiency among four tested surfactants to enhance the Ca uptake was different. The concentration of Ca in leaf, rind and pulp was higher with the foliar sprays of 2% $\text{Ca}(\text{NO}_3)_2$ containing 'Tween 20', 'Tween 80' and 'Tergitol'. This suggested that the efficiency of improving the Ca uptake was greatly associated with the value of hydrophilic-lipophilic balance (HLB) of surfactants. My data were in agreement with those who reported that surfactants which have a higher HLB value were more effective on the Ca uptake into leaf and fruit (Wojcik, 2004). Among four used different surfactants, I found that 'Tween 20' was the most effective in enhancing the uptake of Ca into leaf and fruit in 'Washington Navel' sweet orange.

The reduced incidence of albedo breakdown with the spray applications of 2% $\text{Ca}(\text{NO}_3)_2$ solution containing 'Tween 20', 'Tween 80' or 'Tergitol' in comparison to the calcium-only treatment and control was mainly due to the increased Ca concentrations in leaf, rind and pulp. The Ca concentration in rind and pulp showed a significant negative correlation with the albedo breakdown incidence. Similarly, it has been reported that the albedo and flavedo of fruit with albedo breakdown had the lower Ca concentration than those in normal fruit of sweet oranges (Storey et al., 2002; Treeby et al., 2002; Jone et al., 1967). In contrast, the albedo breakdown incidence was highly associated with the higher Ca concentration in rind of 'Valencia' sweet orange (Lovatt, 2000). Improving textural properties of rind and fruit with the sprays of different surfactants added into an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ may partially contribute to the lower incidence of albedo breakdown. The lower albedo breakdown incidence may be due to the increased rind thickness with the foliar application of 2% $\text{Ca}(\text{NO}_3)_2$ containing different surfactants. My data were in agreement with those who reported that fruit with thinner rind were more susceptible with albedo breakdown than fruit with thicker rind (Bevington et al., 1993; Jones et al., 1967; Ali et al., 2000; Moulds et al., 1995).

The addition of different surfactants into aqueous solutions of 2% $\text{Ca}(\text{NO}_3)_2$ increased the textural properties of rind and fruit, rind thickness and the dry matter

content in ‘Washington Navel’ sweet orange. These data suggest that increased Ca concentration in rind and pulp resulted in the higher cell wall strength and thickness as Ca contributes to the strong structural rigidity in the cell wall and maintains the cell wall stabilisation because of the Ca cross-link bridge within the pectin poly saccharide matrix. Similarly, increased Ca concentration in fruit with the foliar application of Ca solution resulted in the higher fruit firmness (Tucker, 1993; Singh et al., 2007; Roy et al., 1996; Zaragoza et al., 1996; El-Hilali et al., 2004).

The foliar applications of 2% $\text{Ca}(\text{NO}_3)_2$ containing different surfactants did not affect fruit quality parameters such as percentage of juice, juice pH, soluble solids concentration, ascorbic acid and individual organic acids in ‘Washington Navel’ sweet orange. Consistent with my data, juice content and soluble solids concentration and titrable acidity ratio was unchanged with the foliar spray of calcium nitrate solution (1% or 2%) in the ‘Fortune’ mandarin (El-Hilali et al., 2004).

‘Tween 20’ added into an aqueous solution containing 2% $\text{Ca}(\text{NO}_3)_2$ was the most effective in increasing the Ca concentration in leaf, rind and pulp, reducing albedo breakdown and improving textural properties of rind and fruit in ‘Washington Navel’ sweet orange.

9.5. Boron foliar application reduces albedo breakdown and improves rind textural properties in ‘Washington Navel’ sweet orange [*Citrus sinensis* (L.) Osbeck.]

Boron plays a key role in forming plant cell and maintaining the calcium in a soluble form to insure its proper utilizations in citrus trees (Zekri and Obreza, 2003). The thickness and firmness of rind were negatively attributed to the exogenous boron application (Foroughi et al., 1973; Haas, 1929; Tariq et al., 2007; Matoh, 1997). In contrast, fruit size, rind thickness, juice content, soluble solids concentration and citric acid was not affected with the foliar application of boron (Smith and Reuther, 1950). I investigated the effects of the boron foliar application on the incidence of albedo breakdown, the textural properties of rind and fruit and fruit quality in ‘Washington Navel’ sweet orange.

In the first experiment, different concentrations of boron (0, 200, 400 or 600 $\text{mg}\cdot\text{L}^{-1}$) were sprayed on twenty-two years old ‘Washington Navel’ orange uniform trees. The single spray was applied in early summer at 81 DAFB or in early winter at 233

DAFB. Two sprays were applied first in early summer and followed by second in early winter. In the second experiment, boron sprays (0, 200, 400, 600 or 800 mg·L⁻¹) were applied on twenty-two years old 'Washington Navel' orange uniform trees. The single spray was applied in early summer at 80 DAFB. Two sprays were applied first in early summer and second in early winter (232 DAFB).

The increased concentration in spray solution of boron resulted in the increased boron concentration in leaf, rind and pulp. Similarly, the increased linearly boron concentration in all parts of the citrus tree has been reported to be the results of the increasing boron supply (Papadakis et al., 2003). I found that the higher boron concentration in leaf than those in rind and pulp may be partially attributed to the limited mobilisation of boron from leaf to other organs. Boaretto et al. (2006) and Boaretto et al. (2008) reported that the movement of boron within citrus tree was mainly via the xylem. Two boron sprays increased the boron concentration in leaf as compared to the single spray in early summer or in early winter while one boron spray in early summer or in early winter resulted in the similar or higher boron concentration in rind and pulp in comparison to two boron sprays. My experimental results suggest the limited mobility of boron within organs of citrus trees. As consequence, boron should be applied frequently to the new vegetative parts of the tree (Boaretto et al., 2006 and Boaretto et al., 2008).

The Ca concentration in the leaf, rind and pulp was also increased with the foliar application of the increased boron concentration (200 mg·L⁻¹ to 600 mg·L⁻¹) in 2007 and 2008. Two boron sprays increased the Ca concentration in the leaf, rind and pulp than one boron spray in early summer or in early winter. My data suggest that the absorption of calcium was highly associated with the boron concentration in the leaf, rind and pulp of sweet oranges. Similarly, the binding of calcium to the cell wall and the movement of calcium into the apple fruit was improved with the assistance of boron (Shear, 1975; Zude et al., 1997).

The foliar sprays of boron (200 mg·L⁻¹ to 600 mg·L⁻¹) decreased the incidence of albedo breakdown. The single boron spray in early summer resulted in the lower incidence of albedo breakdown than one boron spray in early winter or two sprays. This proposed that the reduction in the albedo breakdown with the range of boron supply was attributed to the increased boron concentration in leaf, rind and pulp as boron plays a key role in improving the cell wall integrity (Match, 1997; Goldbach

and Wimmer, 2007; Dong et al., 2009). Possibly, the increased Ca concentration in rind and pulp within this range of boron supply partially contributes to the lower incidence of albedo breakdown as mentioned earlier in Section 9.4. The higher incidence of albedo breakdown at the higher concentration of boron ($800 \text{ mg}\cdot\text{L}^{-1}$) may be associated with the reduction in the Ca concentration in the fruit. My experimental data suggest a strong effect of relationship between Ca and B on the albedo breakdown incidence in sweet oranges. Improving the textural properties of rind and fruit and increasing rind thickness with the foliar application of boron may have contributed to reduce the incidence of albedo breakdown as discussed in Section 9.4.

The spray application of an aqueous solution containing boron increased the textural properties of rind and fruit in 'Washington Navel' sweet orange. The rind hardness, rind fracture force and rind tensile strength force were highest with one boron spray in early summer in comparison to one boron spray in early winter or two sprays. Possibly, boron may be involved in the maintaining the integrity, elasticity and tensile strength of cell wall due to the B-diester bonding within pectic polysaccharide chains (Matoh, 1997; Goldbach and Wimmer, 2007; Dong et al., 2009). My results were contrary to those who reported that the foliar boron sprays decreased the thickness and firmness of rind (Tariq et al., 2007) or did not affect rind thickness (Maurer and Taylor, 1999). Possibly, the increased Ca concentration in fruit due to spray of boron may have contributed to the improving the textural properties of rind and fruit as mentioned earlier in Section 9.4.

The foliar application of boron sprays did not affect the fruit quality attributes such as juice content, juice pH, soluble solids concentration, titratable acidity and ascorbic acid. Similarly, juice content, soluble solids concentration and citric acid were not affected with the boron application in sweet oranges (Smith and Reuther, 1950).

In conclusion, one spray of boron ($600 \text{ mg}\cdot\text{L}^{-1}$) in early summer significantly increased boron concentration in leaf, rind and pulp of fruit, reduced the incidence of albedo breakdown and improved textural properties of the rind such as hardness, cohesiveness, fracture force, springiness, tensile strength force and fruit firmness with insignificant effects on other fruit quality parameters.

9.6. Albedo breakdown and rind textural properties of ‘Washington Navel’ sweet orange [*Citrus sinensis* (L.) Osbeck.]: the role of ethylene

Ethylene has been reported to be involved in regulating fruit senescence, chemical composition and texture in citrus fruit. Exogenous application of ethylene did not affect fruit weight and fruit firmness in ‘Shamouti’ oranges (Porat et al., 1999) whereas rind puffing was increased with the foliar spray of ethephon solution in ‘Satsuma’ mandarin (Burg, 2004; Ladaniya, 2007). The preliminary results showed increased endogenous concentration of ethylene in fruit with albedo breakdown than normal fruit suggested an association of ethylene with albedo breakdown. I investigated the role of ethylene in albedo breakdown and its effects on textural properties of rind and fruit in ‘Washington Navel’ sweet orange.

In the first experiment, endogenous ethylene was determined in the rind of fruit with albedo breakdown and normal fruit and the incidence of albedo breakdown was also monitored during fruit maturation. Higher endogenous production of ethylene in rind of fruit with albedo breakdown than those in the rind of normal fruit suggested the involvement of ethylene in albedo breakdown. Similarly, fruit with albedo breakdown produced higher ethylene production than normal fruit in ‘Valencia’ sweet orange (Monselise et al., 1976). The lower endogenous production of ethylene in rind of the fruit with albedo breakdown than the rind of normal fruit at commercial harvest implicated ethylene in ripening and senescence. The rind of the developing orange fruit showed the higher production of endogenous ethylene than the rind of the mature fruit in my experiment. Fruit with albedo breakdown have been reported to be more mature than normal fruit on the same tree (Jones and Embleton, 1967).

In the second experiment, the spray applications of ethephon (0, 100, 200 or 300 mg·L⁻¹) were applied on the whole twenty-two years old ‘Washington Navel’ orange trees at 227 DAFB. The slightly increased incidence of albedo breakdown without affecting any rind textural properties and fruit firmness with the application of ethephon seems to implicate the auto inhibition of ethylene production. Similarly, the inhibition of the ethylene production in flavedo tissue of grapefruit was associated with the exogenous application of ethylene (Yang and Hoffman, 1984).

In the third experiment, ethylene inhibitors including AVG and CoSO₄ (0, 100, 200 or 300 mg·L⁻¹) were sprayed onto the whole twenty-two years old ‘Washington

Navel' orange trees at 227 DAFB. The decreased incidence of albedo breakdown and the improved rind textural properties and fruit firmness suggested the involvement of ethylene in albedo breakdown. The elevated levels of ethylene in the rind of fruit with albedo breakdown and reduction of albedo breakdown with inhibitors of ethylene suggest its association with albedo breakdown

9.7. Conclusions

- The incidence and the severity of albedo breakdown increased rapidly after commercial harvest. The albedo breakdown incidence and severity was lowest in the fruit harvested from Harvey in comparison to among four selected locations. Irrespective of cultivars and locations, the severity of albedo breakdown did not affect the major fruit quality parameters such as juice content, soluble solids concentration, titratable acidity, ascorbic acid, citric acid and malic acid except for decreasing succinic acid and increasing tartaric acid.
- The application of reduced water supply (50% and 75% water supply of control trees) decreased the incidence and the severity of albedo breakdown due to the reduction in fruit size and yield.
- The application of deficit irrigation increased soluble solids concentration and titratable acidity and did not affect the rind thickness, the dry matter content of fruit as well as the other fruit quality parameters such as juice content, ascorbic acid and the individual organic acids in 'Navelina' sweet orange.
- Different surfactants added into aqueous solutions containing 2% $\text{Ca}(\text{NO}_3)_2$ increased the Ca concentration in leaf and fruit, reduced the incidence of albedo breakdown and improved the rind textural properties and fruit firmness of sweet oranges without affecting the fruit quality parameters including juice content, juice pH, soluble solids concentration, titratable acidity, ascorbic acid and the individual organic acids.
- Among four tested surfactants, 'Tween 20' was the most effective in reducing albedo breakdown and improving the rind textural properties and fruit firmness without affecting the other fruit quality parameters.
- Single spray of boron ($600 \text{ mg}\cdot\text{L}^{-1}$) in early summer increased the concentration of boron and calcium in leaf, rind and pulp, reduced the incidence of albedo breakdown and improved rind textural properties and

fruit firmness without affecting any fruit quality parameters as compared to the one boron spray in early winter or two sprays in ‘Washington Navel’ sweet orange.

- The higher ethylene production in the rind of fruit with albedo breakdown than normal fruit and the reduced incidence of albedo breakdown with the exogenous application of ethylene inhibitors suggest the association of ethylene with albedo breakdown in sweet oranges.
- **Recommendations to the citrus industry:**
 1. The surfactant ‘Tween 20’ (0.05%) should be added into pre-harvest five spray applications of 2% Ca(NO₃) aqueous solution commencing from 81 DAFB at 10-day intervals under Western Australian conditions due to its beneficial effects on increasing Ca concentrations in leaf, fruit rind and pulp tissues, decreasing albedo breakdown incidence and improving textural properties of the rind.
 2. The one foliar spray of boron (600 mg·L⁻¹) in early summer should be applied to control albedo breakdown under Western Australian conditions due to the increased boron concentration in leaf, rind and pulp of fruit, the reduced incidence of albedo breakdown and the improved textural properties of the rind .

9.8. Future research

This research provides basic information on the development of albedo breakdown during fruit maturation and ripening, the severity of albedo breakdown and the pre-harvest factors affecting fruit quality in sweet oranges with an emphasis on albedo breakdown. Future research work on albedo breakdown may focus on the following areas:

1. The application of deficit irrigation has been shown to reduce albedo breakdown. It has been proposed that the higher proportion of small fruit is important involving in the reduction in albedo breakdown. However, the reduction in yield was a disadvantage of the method. It also has been suggested that the rate of fruit growth in first eight weeks during fruit development is contributed to the initial of the albedo breakdown development than final fruit size. Therefore, the

application of deficit irrigation in reducing albedo breakdown without affecting yield in Western Australian conditions need to be investigated in more detail.

2. Exogenous ethylene inhibitors including AVG and CoSO_4 have been shown to reduce the incidence of albedo breakdown. This implicates the involvement of ethylene in albedo breakdown. However, I did not determine the endogenous production of ethylene and the levels of ACC synthase and ACC oxidase along with the application of AVG and CoSO_4 . Therefore, the study of the relation among endogenous production of ethylene, the activities of these enzymes and the foliar application of the ethylene inhibitors will provide a better understanding on the role of ethylene in albedo breakdown.
3. Several pectolytic and cellulolytic enzymes such as polygalacturonase (PG), pectin methylesterase (PME), pectinlyase (PL) appear involving in albedo breakdown development. Therefore, the activities of these enzymes during fruit development, matuarion and ripening in relation of albedo breakdown needs to be investigated.

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