Abstract:

Citrus is a leading fruit after grapes in the world. The genus citrus is divided into different groups which include sweet oranges, mandarins, lime, lemons and grapefruit. Among these groups oranges are the prominent group of citrus which is grown on large scale in Australia. Albedo breakdown, also known as creasing, is a preharvest physiological disorder that affects the albedo of citrus fruit causing creases on the surface of the fruit. It is a recurrent problem in Navel and Valencia oranges and can cause individual orchard losses which often exceed 50%. Although the contributing factors are known, the physiological basis of creasing development is unresolved and the current control measures do not prevent creasing satisfactory. Hence, better control measures and further understanding of the physiology of creasing development is required. The study will focus particularly on the role of PAs in normal, creased and rind pitting fruits of oranges and their effect on pre and postharvest physiology of oranges.

**Objectives:**

* To identify the position of bearing fruit on the tree and creasing incidence and severity on navel oranges
* To check the effect of light intensity and pruning on incidence of creasing oranges.
* To indentify the endogenous level of PAs in albedo, flavedo and pulp in normal and creased fruits at different stages of fruits.
* To exploration the effect of exogenous application of PAs on endogenous PAs and creasing rind texture properties.
* To check the effects of PAs biosynthesis inhibitors on albedo breakdown.

**Background:**

Citrus fruits are one of the largest fruit crop in the world. About 30% of citrus is processed to obtain various products, mainly juice. Similarly, the citrus industry is also the second largest fruit processing industry, surpassed again by the grape industry, which mainly produces wine. Although citrus fruits have been consumed since ancient times, citrus processing, as it is known today, was not possible until thermal treatment (to inactivate enzymes and microorganisms) and concentration processes were commercially available. Since then, the citrus industry has developed rapidly, becoming prominent.

Citrus (*Citrus sinensis* L.) is one of the major commercial fruit crop that is widely consumed both as fresh fruit and juice. Its global demand is attributed to its high vitamin. C content and its antioxidant potential (Gorinstein et al., 2001). Citrus is mainly cultivated in the subtropical and tropical regions of the world between 40˚ north and south latitude in over 137 countries on six continents (Ismail and Zhang, 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). Major citrus producing areas in Australia are New South Wales, South Australia, Victoria, Western Australia, Queensland and Northern Territory. In citrus production the major share is New South Wales which produce 264315 tonnes of citrus on an area of 5296 ha. But the production per hectare of New South Wale (49.91 ha) is less than the South Australia (70.69 ha) (ABS- 2008). In Australia Grape fruit, Lemon and limes, mandarins and oranges are grown while the major share in production is (78 %) oranges and (12%) mandarins with smaller share of lemons, grapefruit and limes. However, the recurring incidence of albedo breakdown is a major problem affecting the fruit quality in ‘Navel’ oranges particularly the appearance of fruit.

**Creasing:**

Creasing, also known as albedo breakdown is a physiological disorder that affects the albedo (white part) of citrus fruit. The physiological basis of creasing development is unknown, but several hypotheses have been proposed. Visual symptoms are observed as creases on the surface of the fruit. The albedo, also known as the mesocarp, is part of the outer layer of a citrus fruit called the rind. It is usually colourless or sometimes tinted and is surrounded by the flavedo, the outer coloured portion of the rind. The thickness and consistence of the albedo varies with the species. It is usually 1-2 mm thick in some fruit and it merges undetectably into the flavedo towards the outside (Grierson, 2006). Albedo development was categorized into three distinct stages for ‘Valencia’ oranges by Bain (1958), referred to as stage I, II and III (Storey and Treeby, 1994). During the first two to three months after flower development, the albedo tissue undergoes cell division (stage I), after which cell division ceases and growth in the albedo tissue is limited to cell enlargement and differentiation which continues for six months (stage II). From the end of stage II until harvest time, is the last stage of growth, and is called the maturation stage (stage III) (Storey and Treeby, 1994).

During early fruit development, the albedo tissue consists of a loose network of thin-walled parenchyma cells with numerous large air spaces as part of the inner mesocarp (Grierson, 2006; Jones et al., 1967). As the fruit enlarges, these cells stretch resulting in the development of bulges on the cell walls. These bulges become cylindrical arms as they continue to elongate, and thus the albedo cells become deeply lobed (Grierson, 2006; Jona et al., 1989; Storey and Treeby, 1994) forming an intricate network with large intercellular spaces (Jones et al., 1967). The albedo tissue is composed of cellulose, soluble carbohydrates, flavanoids, amino acids, proteins and pectic substances (Nagy et al., 1985). Creasing is normally detectable at maturity (Gambetta et al., 2000; Jona et al., 1989; Monselise et al., 1976) or post colour break (Storey et al., 2002) and tends to increase as fruit matures (du Plessis and Maritz, 2004; Embleton et al., 1973; Nagy et al., 1982). However, Abadalla et al. (1984) reported that as early as the end of the flowering phase, the anatomic initiation of creasing could be recognised. Visual symptoms of creasing development are manifested as separations of cells at the middle lamella of the albedo tissue (Treeby et al., 2000), resulting in fractures in the albedo and collapse of the flavedo showing creases on the surface of the fruit (Storey and Treeby, 1994; Treeby et al., 1995). However, for creasing to develop many cell separations should arise in the albedo tissue (Storey and Treeby, 1994). Creasing is associated with increased polygalacturonase (PG) activity resulting in a higher content of water soluble pectin in the creased fruit (Jona et al., 1989; Monselise et al., 1976). Incorporation of amino acids into proteins is usually higher in creased tissues compared to non-creased tissues (Monselise et al., 1976). Imbalances in hormone levels (especially GA3) are also associated with creased fruit (Jones et al., 1967; Monselise, 1973). A decrease in cell wall pectin (Jona, 1983), hemicellulose and cell wall polysaccharides is observed in the rind of creased fruit compared to non-creased fruit (Jona et al., 1989).

**Polyamines:**

Polyamines, mainly diamine putrescine (put), triamine spermidine (Spd) and tetraamine spermine (Spm), are polycationic compounds of low molecular weight that are found in all living organisms (**Cohen, 1998**). Polyamines have been proposed to be a new category of plant growth regulators and are purported to be involved in a large spectrum of physiological process, such as stress tolerance, embryogenesis, cell division, morphogenesis, development and plants growth (Evans and Malmberg 1989; Flores et al., 1989; Galston and Kaur-Sawhney 1990) early fruit development and leaf senescence and stress responses (Tiburcio et al., 1990; Rastogi and Davies, 1991). Furthermore, plant showing altered levels of PAS display abnormal phenotypes that can be retured to wild type by the application of PAs (Kumar et al., 1996).

**Role of polyamines in fruit set**:

Polyamines, natural compounds involved in many plant growth and development processes, are universally present in all places of plant cells (Valero et al., 2002, Galston, 1983). It has been proposed that polyamines are one of indispensable members of several internal components required for flower initiation and floral organ morphogenesis (Evans and Malmberg 1989; Galston et al. 1997). Several lines of evidence have revealed the possible correlation of polyamines with flower induction, initiation and floral organ development (Tiburcio et al. 1988; Applewhite et al. 2000; Huang et al. 2004). Furthermore, utilization of some special mutants adds new avenues for probing interrelationship between polyamines biosynthesis and floral development. The influence of polyamines in increasing fruit set has been observed in apple (*Malus demestica* BorK.) and ‘comice’ pear (*pyrus communis* L.) (Costa and Bagni, 1983; Cosat et al., 1986; Crisosto et al., 1988, 1992), and particularly in Comice pear , putrescine enhanced pollen tube ovule penetration and delayled senescence without affecting flower ethylene production (Crisosta et al., 1988, 1992). *In vitro* pollination test revealed that higher pollen germination was present in the stigma of Japanese flowers sprayed with 1.0 mM of putrescine compared to the control. Putrescine did not affect post-pollination ethylene production in the styles (Franco-Mora et al., 2005).

**Role of polyamines in fruit development:**

Fruit development is induced by the growth regulators (Gillaspy et al., 1993). In peas and tomato, induction of parthenocarpy fruit development by gibberellic acid (GA3) and other plant growth regulators results in changes in the levels of polamines (PAs), and in the expression of genes of PAsd biosynthesis (Cabonell and Navarro, 1989, Pérez-Amador et al., 1995). There is evidence suggesting that PAs may have role in early fruit development in several species (Costa and Bagni, 1983; Rugini and Mencuccini, 1985; Crisosto et al., 1988; Evan and Malmber, 1989; Ege-Cortines and Mizrahi, 1991). Changes in PAs content have been correlated with fruit growth and woody crops, suggesting that PAs biosynthesis is associated with post-fertilization growth and development of ovary tissues (Slocum and Galston, 1985). In tissues of apple fruit (Biasi et al., 1988), Avacado fruit (Kushad et al., 1988), peach fruit (Kushad, 1998) and grapeberry (Shiozaki et al., 2000), levels of free PAs are relatively high during the first week after full bloom, and decreasing gradually shortly afterwards.

**Role of polyamines in postharvest physiology of citrus:**

In citrus species, fruit growth and development are complex process that must be coordinate in time, and their studies have included the changes in endogenous levels of one or more plant hormones such as polyamines and abscisic acid (ABA) (El-Otmani et al., 1995).The presence of ABA in citrus fruit ripening is well documented, and several authors suggest that ABA plays an essential role in fruit development and that changes in the endogenous levels are directly related to the ripening process (Goldschmidt, 1976; Nooden, 1988; Valero et al., 1992). ABA may be involved in colour development, since the content of ABA in flavedo increases throughout the process of citrus fruit development (Aung et al., 1991). Suresh et al showed a relationship between ABA and PAs because ABA decreases the putrescine and ADC (Arginine decarboxylase) activity. However, ABA did not seem to affect polyamines levels or their rates of changes during avocado ripening (Cutting et al, 1990).

Information available about the role of endogenous polyamine level for citrus is limited, and most of the work has focused on the study of chilling injury (CI). Cold storage of mandarin, grapefruit, and oranges fruits increases the level of putrescine, and the magnitude of these responses was different in each cultivar (Yuen et al., 1995). ODC and ADC had the greatest activities in fully developed but unripe mandarin and were accompanied by increases in putrescine and less significantly, spermidine (Nathan et al., 1984). Increased putrescine levels during the ripening of storage have also been reported (Hasdai. 1986). Since PAs are implicated in fruit development and ripening and may also influence the postharvest physiology of fruits (Kramer and Wang, 1989), there are some reports on the influence of treatments with several polyamines, such as putrescine, spermidine and their role in fruit physiology. Exogenous application of polyamines retarded softening of apples and strawberries during storage (Kramer et al., 1991; Ponappa et al., 1993).

**Significance:**

**Australia is one of the major orange producing countries which produce 606 thousand tons with value $166.505 million in 2009. Australia production of citrus was 503637 T with value of $440 million in 2007/08. Citrus was largest fresh fruit export with annual export earnings of $180 million with the majority was oranges. The reduction of 1% due to albedo breakdown in oranges will wipe out $1-$2 million potential return to growers. Hence, the success of this research may lead to development of technology which may reduce incidence of albedo breakdown. The success of this research will also contribute to basic understanding the role of gibberellins and causation of albedo breakdown.**

**Experiments # 1:**

**Relationship between the position of fruit on the tree and incident of creasing oranges:**

The aim of this experiment is to determine the position of creasing appearance on the tree. Two different varieties will be selected from a commercial orchard. Each tree (canopy) will be divided in different three sections i.e. upper, middle and bottom section of tree canopy and each main section of tree will be divided into three sub-sections and each section again divided in four parts. Each tree will be divided into 36 sectors, *viz*. north, south, east and west. In each sector, fruit will be harvested from four different sub-sectors, from the inside and outside of the top part of the tree canopy as well as the inside and outside of the middle part and bottom part of the tree canopy. A total of thirty six positions will be used and these thirty six positions will be replicated four times. Analysis of variance will be performed using the computer program SAS (StatisticalAnalysis System) Enterprise Guide 3. Duncan multiple range test at *P*= 0.05 will be used to test the treatment effects. The correlation between albedo mineral content and creasing incidence or severity will be demonstrated with the Pearson’s correlation coefficients and only r values ≥ 0.5 will be considered physiological significant.

**Experiments # 2:**

**Identification and quantification of comparative level of endogenous polyamines in creased and non creased fruits:**

The aim of this experiment is to characterise the different types and concentrations of PAs in albedo, flavedo and pulp of sweet orange fruits determined during fruit development and ripening. Three cultivars of sweet orange will be used including Washington Navel, Navelina and Valencia. The fruit samples will be collected at the different stages i.e. before colour break, after colour development and ripening stage. Albedo, flavedo and pulp immediately separated and stored in -80 °C until used. The samples then freeze dry for four days and ground for PAs determination by using HPLC.

**Experiments # 3:**

**Exogenous application of polyamines on different stages of fruit development**

The aim of this experiment is to investigate the effects of exogenous application of PAs on endogenous PAs, incidence of albedo breakdown, fruit quality, and textural properties of the rind. Two cultivars of early season (Washington Navel) and late season (Lane Late Navel) will be use for this experiment. Treatments will be use were control (untreated), Put (01, 02 mM, Spd 01, 02 mM and spm 01, 02 mM). Treatment of fruit will be commence before colour break and golf ball size or 30mm – 50mm in diameter aqueous solution containing different concentrations of Put, Spd and Spm and 0.1% of surfactant (Tween 20) will be sprayed onto every single fruits. The experiment will be conducted in split plot design including single tree as an experimental unit and will be replicated 4 times. A total of (48\*2=96) trees will be use in this experiment. At harvest incidence of albedo breakdown will be recorded. 30 samples of healthy and creased fruits will be taken from each tree for further determination. The textural properties of the rind and fruit quality of healthy and creased fruit will be determined. This experiment will be repeated using the best concentration and number of spray on other cultivars

**Experiments # 4: Effect of PAs inhibitors on endogenous PAs; in relation to creasing oranges.**

This experiment will be conducted to manipulate the mechanism of the manipulation of endogenous PAs and their relationship with albedo breakdown. From the findings of experiment 2 and 3 we will test the most effective treatments in up and down regulating incidence of albedo breakdown and control. PAs inhibitor (DMFO and DMFA) will be used as a treatment. Treatment of fruit will commence at golf ball stage (mid December). Untreated trees will serve as control. Single tree as an experimental unit and will be replicated 4 times. At harvest incidence of albedo breakdown will be recorded. The textural properties of the rind with and without albedo breakdown will be determined. This experiment will be on Washington Navel sweet orange and Navelina.

**Experiments # 5:**

**Effect of methylglyoxal bis-(guanylhydrazone) on polyamine and ethylene biosynthesis on oranges fruit after harvest.**

Citrus fruit without any injury will be selected and stored at 4˚C for 16h and then transferred to 24˚C. The fruits will be randomly divided into two lots for the pressure infiltration of 5 mM MGBG or distilled water (control) using an aspirator ( A-3S, Tokyo Rikakikai Co. Ltd., Tokoyo Japan0 at 101 Kpa for 3 min. Fifty fruit will be used per treatment. After infiltration, the fruits will be placed on paper and allowed to dry naturally. Then fruits will be placed in the kraft box and kept at 24 ˚C for 32 days. Five fruit from each treatment will be sampled at each sampling point. The sampled fruit will be first used for measurement of ethylene production (Khan and Singh, 2007). Then, the flesh of these fruits will be pooled and immediately frozen in liquid nitrogen and stored at -80˚C for subsequent measurement of polyamines, 1-aminocyclopropane-1-carboxylic acid (ACC) as describe by (Khan and Singh, 2007a) and RNA isolation. The data will be subjected to one way ANOVA test using the “Data Analysis” tool in Excel (Microsoft Japan).

**Experiments #6: Postharvest treatment with PAs (Put) on endogenous polyamines, Abscisic acid and quality of oranges fruits.**

At ripening stages 1500 fruit will be picked from mature trees. Once in the laboratory 1200 Washington navel fruits will be selected in accordance with their colour and weight. They will be randomized and divided into 6 lots of 40 fruits for the following treatments: Control (Distilled water), 1 mM putrescine and 2 mM putrescine. Tween 20 (0.2%) will be added to all solutions to improve the absorption of the chemicals. The treatment will be performed by two method one for simply dipping in distal water and other vacuum infiltration by placing fruit in 5 L of solution and applying 200 mmHG for 8 min. Following infiltration the fruits will be placed on Kraft paper and allowed to dry before storage at 15 ˚C in a temperature controlled chamber, in permanent darkness and with relative humidity of 90%. After 1, 2, 3 and 4 week, 5 fruits for each treatment will be sampled. They will be weighed and the color will be measured Hunter Lab system Colour flex 45˚ /0˚and fruit firmness will be determined by using an electronic pressure tester (Model EPT-1). For each 3 individual dishes (flavedo, albedo and pulp) will be sampled, which will be immediately frozen in liquid Nitrogen and then will be stored at -80 C˚ until polyamines will be analysed. Polyamines will be analysed according to the method of Flores and Galston (1982). Two factor ANOVA will be made to determine the effect of treatment and storage time on polyamines, ABA, weight loss, firmness and color level. Mean comparisons will be calculated by using HSD Tukey test. All analyses will be performed with SAS statistical software packages.

**Experiment # 7:**

**Cellular wall metabolism in citrus fruit pericarp and its relation to creasing fruit rate**

The Washington navel and Valencia oranges will be chosen on the basis of their difference of the creasing fruit incidence. Fruit will be sampled from four different directions (east, west, north and south) of each tree crown after post petal fall to fruit harvesting at a 10 day interval. Five single tree replication will be used for each species and the number of total fruit (C0) and the phased (10 days interval) fruit creasing rate (Cn) of each individual tree will be recorded at each time of sampling. An evaluation of creasing rate (C) will be calculated by the this equation

C= (C1+------------+n/C0) ×100%. Cellular wall will be extracted and fractionated from peel by using the methods of Ahmad and Labavitch (1980) and Ray (1987). Three replications will be used for each cellular wall extraction and fraction. Tissue blocks of approximately 2mm3 will be taken from normal sweet oranges peel of both cultivars and fixed for 3 days on a rotator in 4% (v/v) glutaraldyde containing citric acid-Na-phosphate buffer (pH 7.0) in 5 cm centrifuge tubes. Tissue will be washed three times over 1 h in the buffer solution, then dehydrated in ethanol series (30,50,70,80,95,and 100%) followed by two changes of propyleneoxide to ensure complete dehydration and finally embedded in epoxy resin series (3;1. 1;1 and 1:3) (Spurr, 1969). Alternatively, tissues will be postfixed in 2% aqueous **uranly** acetate and lead citrate before viewing with TECNAT G212 TEM (Made in nether land).

The measurement will be taken for the above experiments are as followed:

1. To identify the position of creasing incidence on bearing tree.
2. Effect of pruning and light intensity on creasing oranges
3. Identification and quantification of comparative endogenous level of polyamines in creasing and non-creasing fruits.
4. Incidence and severity of albedo breakdown will be measured and categorised according to the severity (Treeby and Storey, 2002).
5. Fruit firmness – peel tensile test, puncture resistance and fruit compression test using texture analyser
6. Cell wall-degrading enzymes – Exo-polygalacturonase (Exo-PG), Endo-polygalacturonic acids (Endo-PG), Pectinaseterase (PME) and Endo-1,4-β-D-glucanase (EGase) (Li et al., 2009)
7. Sugar and organic acids using HPLC
8. Titratable acidity.
9. Total soluble solids content (TSS) digital refractometer
10. Total antioxidant activity and ascorbic acid using spectrophotometer
11. Weather data – temperature, relative humidity using Tiny Tag Data logger

**Ethical issues**

The proposed research will not relate to any ethical issue.

**FACILITIES AND RESOURCES**

**Materials:** Washington navel orange trees will be used from commercial orange farm (Westralian Fruits) located at Gingin.

**Facilities:** All equipments are available at Horticulture Laboratory, Muresk Institute, Curtin University of Technology except the LC-MS system at Chemistry Centre, Western Australia.

**Budgets:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Items** | **Cost (AU$)** | | |
|  | 2011 – 12 | 2012 – 2013 | 2013 – 2014 |
| **Chemicals** | 3500 | 3000 | 3000 |
| **Lab Consumables** | 3500 | 4000 | 4000 |
| **Travel** | 2000 | 2000 | 2000 |
| **Fruit** | 2500\* | 2500\* | 2000\* |
| **Total/Year** | 9000 | 9000 | 9000 |
| **Grand total** | 2009 – 10 | 2010 – 2011 | 2011 – 2012 |

**\***in kind from Westralian Fruits

**Resources:** Curtin University of Technology.

**DATA STORAGE:**

Data collected will be stored in electronic files on a computer and CDs for a five year periods. SAS 9.1 (SAS Institute Inc., Cary, NC, USA)will be used to calculate analysis of variance (ANOVA) and least significant difference (Fisher’s protected LSD) at level of P≤0.05.

**TIME LINE:**

The proposed research will take approximately 3 years to complete beginning from August 2010.

|  |  |  |
| --- | --- | --- |
| Year | Duration | Activities |
| 2010 | August – October | Searching information and writing literature review |
|  | November-December | Preparing and presenting research candidacy |
| 2010/11 | December – November | Experiment 1 |
|  |  | Experiment 2 |
|  |  | Experiment 3 |
|  |  | Experiment 4 |
|  |  | Experiment 5 |
|  |  | Data analysis and writing |
| 2011/12 | December - November | Experiment 1 |
|  |  | Experiment 2 |
|  |  | Experiment 3 |
|  |  | Experiment 4 |
|  |  | Experiment 5 |
|  |  | Data analysis and writing |
| 2012/13 | July - February | Writing and correcting thesis |
|  | March | Thesis submission |

Abadalla, K.M., A.M. Badawi, and A.A. Tewefik. 1984. Anatomical aspects of creasing development

in citrus rind. Proc. Intl. Soc. Citricult. 1:267–271.

Achilea, O., Y. Soffer, D. Raber, and M. Tamim. 2002. “Bonus-NPK” Highly concentrated, enriched

potassium nitrate, an optimal booster for yield and quality of citrus fruits. Acta Hort. 594:461–466.

Ali, A., L. L. Summers, G.J. Klein, and C.J. Lovatt. 2000. Albedo breakdown in California. Proc.

Intl. Soc. Citricult. 3:1090–1093.

Alabadί, D., Carbonell, J. 1998. Expression of ornithine decarboxylase is transiently increased by

pollination, 2, 4-dichlorophenoxyacetic acid, and gibberllic acid in tomato ovaries. Plant

Physiol. 118: 323-328.

Alva, A.K., M. Jr. Mattos, S. Paramasivam, B. Patil, H. Dou, and K. Sajwan. 2006. Potassium

management for optimizing citrus production and quality. Intl. J. Fruit. Sci.6 (1):3–43.

Anonymous, 2009. Export standards and requirements: Part 2: Oranges and Seville oranges. 20

February 2009.  [www.nda.agric.za/docs/PlantQuality/default.htm](http://www.nda.agric.za/docs/PlantQuality/default.htm).

Biasi, R., Bagni, N. And Costa, G. 1988. Endogenous polyamines in apple and their relationship

to fruit set and fruit growth. Physiol {Plant. 73: 201-205.

Bain, J.M. 1958. Morphological, anatomical and physiological changes in the developing fruit of the

‘Valencia’ orange, *Citrus sinensis* (L) Osbeck. Austral. J. Bot. 6:1–23.

Bar-Akiva, A. 1975. Effect of foliar application of nutrients on creasing of ‘Valencia’ oranges.

Hortscience 10:69–70.

Bevington, K.B. 1973. Effect of gibberellic acid on rind quality and storage of coastal navel oranges. Austral. J. Exp. Agric. Animal Hus. 13:196–199.

Bower, J.P. 2000. Prediction and physiology of creasing. Proc. Intl. Soc. Citricult. 3:1089. Bower, J.P. 2004. The physiological control of citrus creasing. Acta Hort. 632:111–115.

Bramlage, W.J. 1994. Physiological role of calcium in fruit. p. 101–107. In: A.B. Petersen,

R.G. Stevens (eds) Tree fruit nutrition. Good fruit grower, Yakima, WA.

Carpita, N. and M. McCann. 2000. The cell wall, p. 52–108. In: Buchanana, B.B., W. Gruissem and

R.L. Jones (eds). Biochemistry and molecular biology of plants. Amer. Soc. of Plant Physiologists, Rockville, Maryland.

Carbonell, J., Navarro, J. L. 1989. Correlation of spermine levels with ovary senescense and fruit set and development in *Pisum sativum*. Planta. 178: 482-487.

Costa G, Bagni, N. 1983. Effect of polyamine on fruit on fruit set of apple. HortScience. 18; 59-61.

Crisosto, C H., Lombard, P B., Sugar, D., Sugar, D., Polito VS. Putrescine influence ovule senescence, fertilization time, and fruit set in ‘ Comice’ pears. J. Am. Soc. Hort. Sci1. 113: 708-712.

Coggins, C.W. Jr. 1969. Gibberellin Research on Citrus Rind Ageing Problems. Proc. First Intl. Citrus Symp. 3:1177–1185.

Coggins, C.W. Jr. 1981. The influence of exogenous growth regulators on rind quality and internal quality of citrus fruits. Proc. Intl. Soc. Citricult. 1:214–216.

Coggins, C.W. Jr. and W.W. Jones. 1977. Growth regulators and coloring of citrus fruits. Proc. Int.

Soc. Citricult. 2:686–688.

du Plessis, S.F. and J.G.J. Maritz. 2004. Factors affecting the occurrence of creasing in citrus fruit.

Proc. Intl. Soc. Citricult. 2:528–530.

Embleton, T.W., W.W. Jones, and C.W. Jr. Coggins. 1973. Aggregate effects on nutrients and

gibberrellic acid on ‘Valencia’ orange crop value. J. Amer. Soc. Hort. Sci.92:281–285.

Egea-Cortines, M., Mizrahi, Y. 1991. Polyamine in cell division, fruit set and development, an seed

germination. In: Slocum RD, Flores HE, Editors. Biochemistry and physiology of polyamines

in plants. Boca Raton, Fl, USA: CRC Press. P. 143-158.

Evans, PT., Malmber, RL. 1989. Do ployamines have roles in plant development. Annu. Rev. Plant

Physiol. Plant Mol. Biol. 40: 235-269.

Epstein E. and A. Bloom. 2005. Mineral nutrition of plants: Principles and perspectives. 2nd ed.

Sinauer associates Inc, Plumtree Road, Sunderland.

Evans P. T and Malmberg R. L. 1989. Do polyamines have roles in plant development? Ann. Rev. Plant Physiol. Plant Mol. Biol. 40: 235-269.

El-Flores H. E., Protacio C. M and Sings M.W. 1989. Primary and secondary metabolism of

polyamines in plants. Recent. Adv. Phytochem. 23: 329-393.

Fourie, S. and G.F.V. Joubert. 1957. The effect of potash and phosphate on yield and “creasing” of

navel oranges in the Citrusdal area. The Citrus Grower, February p. 1–3.

Gambetta, G., H. Arbiza, A. Ferenczi, A. Gravina, L. Orlando, V. Severin, and A. Telias. 2000.

Creasing

of ‘Washington’ navel oranges in Uruguay: Study and control. Proc. Intl. Soc. Citricult. 2:453–455.

Galston Aw. 1983. Polyamines as modulators of plant development. Biosciences. 33: 382-388.

Galston A. W and Kaur-Sawhney R. K. 1990. Polyamines in plant physiology. Plant Physiol. 94: 406-410.

Galston, A. W. 1983. Polyamines as modulstors of plant development. Bioscience. 33: 382-388.

Galston, A. W., Kaur-Sawhney, R., Altabella, T., Tiburcio, A. F. 1997. Plant polyamines in reproductive activity and response to abiotic stress. Bot Acta. 110: 197-207.

Garcia-Luis, A., M. Agusti, V. Almela, E. Romero, and J.L. Guardiola. 1985. Effect of gibberellic acid on ripening and peel puffing in ‘Satsuma’ Mandarin. Scientia Hort. 27:75–86.

Gilfillan, I.M., J.A. Stevenson, E. Holmden, C.J. Ferreirra, and A. Lee. 1980. Gibberellic acid of reducing creasing in navels in the Eastern Cape. Citrus Sub-Trop. Fruit J. 605:11–14.

Gilfillan, I.M., J.A. Stevenson, and W. Koekemoer. 1974. Gibberellic acid reduces creasing in late-season navels. Citrus Sub-Trop. Fruit J. 518:4–5.

Gilfillan, I.M., J.A. Stevenson, J.P. Wahl, and E.A. Holmden. 1981. Control of creasing in Navels with gibberrelic acid. Proc. Intl. Soc. Citricult. 1:224–226.

Gilfillan, I.M. and J.G.M. Cutting. 1992. Creasing reduction in navel oranges: lower efficacy of gibberellic acid in spray mixtures containing petroleum oil. Proc. Intl. Soc. Citricult. 1:527– 529.

Goldschmidt, E.E. 1983. Asymmetric growth of citrus fruit peel induced by localised application of gibberellins in lanolin paste. Scientia Hort. 21:29–35.

Goldschmidt, E.E. and S.K. Eilati. 1970. Gibberellin treated Shamouti oranges: effects on colouration and translocation within peel of fruits attached to or detached from the tree. Bot. Gaz. 131 (2):116–122.

Gonzalez-Altozano, P. and J.R. Castel. 1999. Regulated deficit irrigation in Clementina de Nules citrus trees I. Yield and Fruit quality effects. J. Hort. Sci. Biotech. 74(6):706–713. Gillaspy, G., Ben-David, H.and Gruissem W. 1993. Fruts: a developmental perspective. Plant Cell. 5: 1439-1461.

Kushad MM. 1998. Changes in polyamine levels in relationship to the double-sigmoidal growth curve of peaches. J. Am. Soc. Hortic. Sci. 123:950-955.

Greenberg, J. and E.E. Goldschmidt. 1988. The effectiveness of GA3 application to citrus

fruits. Proc. Intl. Citrus Congr. 6:339–342.

Greenberg, J. and E.E. Goldschmidt. 1989. Acidifying agents, uptake, and physiological activity of

gibberellin A3 in citrus. Hort. Sci. 24(5):791–793.

Greenberg, J., Y. Oren, and G. Eshel. 1992. Gibberellin A3 (GA3) on ‘Minneola’ Tangelo: Extension

of the harvest season and improvement of fruit quality. Proc. Intl. Soc. Citricult. 2:456–458.

Grierson, W. 2006. Anatomy and physiology. p. 1–21. In: W.F. Wardowski, W.M. Miller, D.J. Hall,

and W. Grierson. Fresh Citrus Fruits, 2nd ed. (eds). Florida Science Source, Inc.

Haas, A.R.C. 1950. The relation of phosphorus to creasing and puffing in Valencia oranges. The

Calif. Citograph, May, p. 277–278, 298–300.

Hansch, R. and R.R. Mendel. 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn,

Fe, Ni, Mo, B, Cl). Current opinion in Plant Biol. 12: 259–266.

Hirschi, K.D. 2004. The calcium conundrum. Both versatile nutrient and specific signal. Plant

Physiol. 136:2438–2442.

Holtzhausen, L.C. 1981. Creasing: formulating a hypothesis. Proc. Intl. Soc. Citricult. 1:201–204.

Hopkins, W.G and N.P.A. Huner. 2004. Plant and Inorganic Nutrients p. 241–257. Introduction to

plant physiology. 3rd ed. John Wiley and Sons, Inc. Publishers.

Jona, R. 1983. Cell wall influence on the quality of various crops analysed histochemically Acta

Hort.138:247–254.

Jona, R., R. Goren, and M. Marmora. 1989. Effect of gibberellin on cell wall components of creasing

peel in mature ‘Valencia’ orange. Scientia Hort. 39:105–115.

Jones, W.W., T.W. Embleton, M.J. Garder, and C.B. Cree. 1967. Creasing of orange fruit. Hilgardia

38 (6):231–244.

Kruger, F.J., M. Penter, R. Masheve, and N.K. Combrink. 2005. The use of fruit mineral content as a tool

to investigate the epidemiology of citrus rind disorders. S. A. Fruit J. 4(2):54–59.

Tiburcio, A. F., Kaur-Sawhney, R., Galston. A. W.1990. Polyamines metabolism. Miflin B.

J., Lea, P. J, editors. The biochemistroy of plants, intermediately nitrogen metabolism, vol.

16. New York, USA: Acadamic Press; 283-325.

Costa, G. And Bagni, N. 1983. Effect of polyamines on fruit-set of apple. HortScience. 18:

59-61.

Costa, G., Biasi, R. And Bagni, N. 1986. Effect of putrescine on fruiting performance of

apple (cv. Hi Early). Acta Hort. 179: 355-361.

Crisosto, C. H., Lombard, P. B., Richardson., D. G and Tetley, R. 1992. Putrescine extends

effective pollination period in “ Comice” pear (*Pyrus cummunis* L.) irrespect of post-anthesis

ethylene levels. Sci. Hort. 49: 211-221.

Crisosto, C. H., Lombard, P. B., sugar, D., Polito, V. S. 1988. Putrescine influence ovule

senescence, fertilization time, and fruit set in ‘comice’ pear. J. A.m. Soc. Hort. Sci. 113: 708

712. Galston, A. W., Haur-Sawhney, R. 1995. Polyamines as endogenous growth regulators.

In: Davies, P. J. (Ed.), Plant hormones. Physiology, Biochemistry and Molecular Biology. Kluwer, Dordrecht. Pp. 158-178.

Valero, D., Martίnez-Romero, D., Serrano, M. 2002. The role of polyamines in the improvement of

the shelf life of fruit. Trends Food Sci. Tech. 13: 228-234.

Le Roux, J.C. and P.A. Crous. 1938. Effect of fertilizer on “Creasing” of Mediterranean Sweet oranges. Farming in S.A. 13:66–68.

Marschner H. 1995. Functions of mineral nutrients:Macronutrient and micronutrients. p. 229–396.

Mineral nutrition of higher plants. 2nded. Academic press Inc. San Diego.

Miller, J.E. and P.J. Hofman. 1988. Physiology and Nutrition of Citrus Fruit Growth with Special Reference to the Valencia: A Mini Review. Proc. Intl. Citrus Congr. 2:503–510.

Monselise, S.P. 1973. Fruit quality in citrus and the effect of growth regulators. Acta Hort. 34:457–465.

Monselise, S.P. 1979. The use of growth regulators in citriculture: A review. Scientia Hort. 11:151–162.

Monselise, S.P., E.E. Goldschmidt, and A. Golomb, 1981. Alternate bearing in citrus and ways of control Proc. Intl. Soc. Citriculture.1:239–242.

Monselise, S.P., M. Weiser, N. Sharif, R. Goren, and E.E. Goldschmidt. 1976. Creasing of orange peel-physiology and control. J. Hort. Sci. 51:341–351.

Nagy, S., M. Marshall, W.F. Wardowski, and R.L. Rouseff. 1982. Post harvest creasing of ‘Robinson’ tangerines. Proc. Fla. State Hort. Soc. 95:237–239.

Nagy, S., M. Marshall, W.F. Wardowski, and R.L. Rouseff. 1985. Post harvest creasing of Robinson tangerines as affected by harvest date and pectin esterase activity and calcium content. J. Hort. Sci. 60:137–140.

Obreza T.A. and M. Zekri, and S.H. Futch. 2003. Plant nutrients for citrus trees. Soil and Water Sci. Dept., Florida Coop. Ext. Serv., Inst. of Food and Agricultural Sci., Univ. of Florida. January 2003. SL 200. <  [http://edis.ifas.ufl.ed](http://edis.ifas.ufl.edu./)u.>.

Obreza T.A., M. Zekri, and S.H. Futch. 2008. Nutrition of Florida citrus trees, P. 16–17 In: Obreza T.A. and K. T. Morgan (eds). 2nd ed. Soil and Water Sci. Dept., Florida Coop. Ext. Serv., Inst. of Food and Agricultural Sci., Univ. of Florida. January 2008.<  [http://edis.ifas.ufl.ed](http://edis.ifas.ufl.edu./)u.>.

Peryea, F.J. 1994. Preharvest calcium spray evaluation, p. 123–124.In: A.B. Petersen, R.G. Stevens

(eds) Tree fruit nutrition.Good fruit grower, Yakima, WA.

Rouse, R.E. and M. Zerki. 2006. Preharvest factors that influence fresh fruit quality. p. 49–66. In:

W.F. Wardowski, W.M. Miller, D.J. Hall, and W. Grierson. Fresh Citrus Fruits, 2nd ed. (eds). Florida

Science Source, Inc.

Salisbury, F.B. and C.W. Ross. 1992. Growth and Development and Hormones and Growth

Regulators p. 329–406. Plant physiology. 2nd ed. Wadsworth Publishing Company, Belmont

California.

Saure, C.M. 2004. Calcium translocation to fleshy fruit: its mechanism and endogenous control

Scientia Hort. 105:65–89.

Schlegel, T.K. and J. Schonherr. 2002 Penetration of calcium chloride into apple fruits as affected by

stage of fruit development. Acta Hort. 594:527–533.

Stafford, L.M. 1972. Influence of rootstocks on Navel orange yield and tree growth at Mildura,

Victoria. Austral. J. Exp. Agric. Animal Hus. 12:203–208.

Storey, R. and M.T. Treeby. 1994. The Morphology of epicuticular wax and albedo cells of orange

fruit in relation to albedo breakdown. J. Hort. Sci. 69:329–338.

Storey, R. and M.T. Treeby. 2002. Nutrient uptake into navel oranges during fruit development. J.

Hort. Sci. Biotech. 77:91–99.

Storey, R., M.T. Treeby, and D.J. Milne. 2002. Crease: another Ca deficiency-related fruit

disorder? J. Hort. Sci. Biotech. 77:565–571.

Talon, M., F.R. Tadeo, W. Ben-Cheikh, A. Gomez-Cadenas, J. Mehouchi, J. Perez-Botella, and E.

Primo-Millo. 1997. Hormonal regulation of fruit set and abscission in citrus: classical concepts and new evidence. Acta Hort. 463:209–217.

Taiz, L. and E. Zeiger. 2002. Mineral nutrition, p. 67–86. Plant physiology. 3rd ed. Sinauer Associates

Inc. Publishers.

Treeby, M.T., R.E. Henriod, K.B. Bevington, D.J. Milne, and R. Storey. 2007. Irrigation management

and rootstock effects on navel orange (Citrus sinensis (L) Osbeck) fruit quality. Agricultural Water Mgt. p 24–32.  [http://www.sciencedirect.com/>](http://www.sciencedirect.com/).

Treeby, M.T., D.J. Milne, R. Storey, K.B. Bevington, B.R. Loveys, and R. Hutton. 2000. Creasing in

Australia: Causes and control. Proc. Intl. Soc. Citricult. 3:1099–1103.

Treeby, M.T. and R. Storey. 2002. Calcium Spray treatment for ameliorating albedo breakdown in

navel oranges. Austral. J. Exp. Agric. 42:495–502.

Treeby, M.T., R. Storey, and K.B. Bevington. 1995. Rootstock, seasonal, and fruit size influences

on the incidence and severity of albedo breakdown in Bellamy navel oranges. Austral J.

Exp. Agric. Animal Hus. 35:103–108.

Tugwell, B.L., W.L. Chvyl, G. Moulds, and J. Hill. 1996. Control of albedo rind breakdown with

gibberellic acid. Proc. Intl. Soc. Citricult. 2:1147–1149.

Tiburcio, A. F., Kaur-Sawhney, R., Galston, A. W. 1990. Polyamine metabolism. Miflin , B. J., Lea,

P. J. Editors. The biochemistry of plants, intermediary nitrogen metabolism. Vol. 16. New York,

USA: academic Press. P. 283-325.

Van Staden, J.V. and E.L. Cook. 1986. Cytokinin and fruit production. Acta Hort. 179:73–81. Vermeulen, H., D. Jordaan, L. Korsten, and Korsten J. 2006. Private handling standards, handling and hygiene in fruit export supply chains: A preliminary evaluation of the economic impact parallel standards. Univ. of Pretoria. Working paper: 2006–01.

Verreynne, J.S. 2006a. Evaluation of alternative means of controlling creasing (albedo breakdown).p.

366–370. CRI Group annual research report, 2006. Citrus Res. Intl., Nelspruit.

Verreynne, J.S. 2006b. Relationship of bearing position on a tree and the incidence and severity of

creasing/albedo breakdown. 370–380. CRI Group annual research report, 2006. Citrus Res. Intl.,

Nelspruit.

Verreynne, J.S. and Z.P. Phiri. 2008. Evaluation of alternative means of controlling creasing (albedo

breakdown) p. 464–469. CRI Group annual research report for January 2007 to March 2008. Citrus

Res. Intl., Nelspruit.

White, P.J. and M.R. Broadley. 2003. Calcium in plants. Annal. Bot. 92:487–511.

Bower, J.P. 2004. The physiological control of citrus creasing. Acta Hort. 632:111-115.

du Plessis, S.F. and J.G.J. Maritz. 2004. Factors affecting the occurrence of creasing in citrus fruit.

Proc. Intl. Soc. Citricult. 2:528–530.

Fourie, S. and G.F.V. Joubert. 1957. The effect of potash and phosphate on yield and “creasing” of navel

oranges in the Citrusdal area. The Citrus Grower, February p. 1–3.

Gambetta, G., H. Arbiza, A. Ferenczi, A. Gravina, L. Orlando, V. Severin, and A. Telias. 2000.

Creasing of ‘Washington’ Navel Oranges in Uruguay: Study and Control. Proc.Intl. Soc. Citricult.

2:453–455.

Gilfillan, I.M., J.A. Stevenson, J.P. Wahl, and E.A. Holmden. 1981. Control of creasing in Navels

with gibberrelic acid. Proc. Intl. Soc. Citricult. 1:224–226.

Holtzhausen, L.C. 1981. Creasing: formulating a hypothesis. Proc. Intl. Soc. Citricult. 1:201–204. Jones, W.W., T.W. Embleton, M.J. Garder, and C.B. Cree. 1967. Creasing of orange fruit.

Hilgardia 38 (6):231–244.

Kruger, F.J., M. Penter, R. Masheve, and N.K. Combrink. 2005. The use of fruit mineral content as a tool

to investigate the epidemiology of citrus rind disorders. S. A. Fruit J. 4(2):54–59.

Le Roux, J.C. and P.A. Crous. 1938. Effect of fertilizer on “Creasing” of Mediterranean Sweet

oranges. Farming in S.A. 13:66–68.

Marschner, H. 1995. Macronutrients. Mineral nutrition of higher plants. 2nd ed. Academic Press Inc.

San Diego.

Nagy, S., M. Marshall, W.F. Wardowski, and R.L. Rouseff. 1982. Post harvest creasing of ‘Robinson’

tangerines. Proc. Fla. State Hort. Soc. 95:237–239.

Storey, R., M.T. Treeby, and D.J. Milne. 2002. Crease: another Ca deficiency-related fruit disorder? J.

Hort. Sci. Biotech. 77:565–571.

Storey, R. and M.T. Treeby. 2000. Seasonal changes in nutrient concentrations of navel orange fruit.

Scientia Hort. 84:67–82.

Treeby, M.T. and R. Storey. 2002. Calcium Spray treatment for ameliorating albedo breakdown in

navel oranges. Austral. J. Exp. Agric. 42:495–502.

Treeby, M.T., D.J. Milne, R. Storey, K.B. Bevington, B.R. Loveys, and R. Hutton. 2000. creasing in

Australia: Causes and control. Proc. Intl. Soc. Citricult. 3:1099–1103.

Treeby, M.T., R. Storey, and K.B. Bevington. 1995. Rootstock, seasonal, and fruit size influences

on the incidence and severity of albedo breakdown in Bellamy navel oranges. Austral. J. Exp. Agric. Animal Hus. 35:103–108.

KHushad, M. M. 1998. Changes in polyamine leves in relationship to the double-sigmoidal growth

curve of peaches. J. Am. Soc. Hortic. Sci. 123: 950-955.

Khushad M. M., Yelenosky, G.aAnd Knigth, R. Interrelation ship of polyamine and ethylene

biosynthesis during avocado fruit development. PlantPhysiol. 87: 463-475.

Pérez-Amador M. A. Carbonell, J., Granell, A. 1995. Expression of arginine decarboxylase is

induced during early fruit development and in young tissues of Psium sativum (L). Plant Mol.

Biol.28: 997-1009.

Rugini, E and Mencuccini, M. 1985. Increased yield in the olive with putrescine treatment.

HOrtScience. 20 :10-103.

Shiozaki, S., Ogata, T and Horiuchi, S. 2000. Endogenous polyamine in the pericarpand the seed of

grape berry during development and ripening. Sci. Hortic. 83: 33-41.

Slocum , R. D and Galston, A. W. 1985. Changes in po9lyamine biosynthesis associated with post-

fertilization growth and development in tobacco ovary tissues. Plkant physiol. 78: 323-328.