Studies to reduce the incidence of chilling injury in Navel orange fruit

by
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DECLARATION

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SUMMARY

Citrus fruit exported from South Africa to markets such as the USA and China undergo a mandatory 24 day exposure of -0.6 °C during shipment to kill any insect larvae in the fruit, however, this protocol causes chilling injury (CI). The aim of this study was firstly to determine the influence of various preharvest factors on chilling sensitivity. In addition, Near-Infrared (NIR) spectroscopy was tested as a potential management tool to identify variation in CI susceptibility of fruit and lastly the efficacy of thiabendazole (TBZ) applied in the packline to reduce CI was determined. Various factors influence the susceptibility of a navel orange fruit to CI including cultivar, micro-climate, harvest date, fruit size and rind colour. In this study it was found that 'Washington' was more susceptible to CI compared to 'Navelina' navel orange. Fruit from the coldest part of Citrusdal (Tharakama) had the highest incidence of CI, which concurred with literature. The incidence of CI was overall less when fruit were harvested in the middle of the commercial harvest window; however, the internal maturity at harvest does not appear to be related to the sensitivity of orange fruit to CI. Near infrared (NIR) spectroscopy was tested as a potential tool to predict fruit quality parameters in relation to CI. Analysing the NIR data with principal components analysis (PCA), score plots were obtained that separate fruit in clusters from the inside and outside of the canopy positions as well as different sizes and rind colours (green vs. orange). However, analysing data with partial least square regression (PLS) using fruit quality parameters (firmness, rind colour and mass), the NIR spectra obtained with the integrated sphere did not provide a good prediction model for CI index. Thiabendazole (TBZ) is reported to reduce the incidence of CI of citrus fruit and this fungicide was applied in a semi-commercial packline in the wax as well as the drench. The results of the application of different fungicides from the TBZ chemical group indicated that the TBZ dip treatments had the highest efficacy in reducing both the incidence and severity of CI and in addition were more effective when applied in warm (40 °C) than cold water (10 °C). Applications at the commercial recommended rate (20mL.L^{-1} and half of the commercial recommended rate were both effective in reducing the incidence of CI. Wax application was effective in reducing the incidence of CI however, the application of TBZ in the wax reduced the incidence of CI even more. For the successful reduction of CI incidence in commercial shipments of citrus fruit the focus should not be on a single factor but rather a strategy that encompasses pre-harvest factors that would influence rind quality as well as specific postharvest technologies know to decrease the impact of CI.

OPSOMMING

Sitrus vrugte ondergaan 'n verpligte 24 dae blootstelling aan -0,6 °C om moontlike insek-larwes te dood gedurende die uitvoer na markte soos die VSA en China, maar hierdie protokol veroorsaak koueskade. Die doel van hierdie studie was eerstens om die invloed van verskillende voor-oes faktore op koueskade-sensitiwiteit van nawel lemoene te bepaal. Daarbenewens is naby-infrarooi (NIR) spektroskopie as 'n potensiële tegniek getoets om variasie in koueskade-sensitiwiteit van nawel lemoene te identifiseer, en laastens is die effektiwiteit van thiabendazole (TBZ) toediening in die verpakkings lyn, om koueskade te verminder, ondersoek. Verskillende faktore soos kultivar, mikroklimaat, oesdatum, vruggrootte en skilkleur beïnvloed die koueskade-sensitiwiteit van sitrus. Hierdie studie het bevind dat die 'Washington' meer sensitief is vir koueskade as die 'Navelina' nawels. Vrugte afkomstig uit die koudste deel van Citrusdal (Tharakama) het die hoogste voorkoms van koueskade. In die algemeen was vrugte ge-oes in die middel van die kommersiële-venster die minste koueskade-sensitief, maar interne rypheid hou nie verband met koueskade-sensitiwiteit nie. Naby-Infrarooi (NIR) spektroskopie is getoets as 'n potensiële instrument om vrugkwaliteit parameters te voorspel met betrekking tot koueskade. Deur ontleding van die NIR data met behulp van 'Principal Components Analysis' kon vrugte groepeer word volgens posisie (binne vs. buite blaredak), groottes en skilkleur. Deur 'Partial Least Square Regression' verdere data ontleding en met inagneming van vrugkwaliteit parameters (fermheid, skil kleur en massa), kon die NIR spektra wat verkry was egter nie 'n goeie voorspelling model vir koueskade verskaf nie. TBZ verminder die voorkoms van koueskade van sitrusvrugte na dit toegedien was in 'n semi-kommersiële verpakkingslyn in die waks, 'drench' of baddens. Die toediening van verskillende swamdoders van die TBZ chemiese groep in baddens, het aangedui dat die TBZ doop behandeling effektief was om die voorkoms van koueskade te verminder. Daarbenewens was TBZ meer effektief in verlaging van koueskade as dit toegedien word in warm (40 °C) as koue (10 °C) water, asook teen die volle (20mL.L^{-1}) en die helfte van die aanbevole kommersiële dosis. Wakstoediening was effektief in die vermindering van die voorkoms van koueskade en byvoeging van TBZ in die waks het die effektiwiteit verhoog. Die suksesvolle vermindering van koueskade tydens kommersiële verskeping van sitrusvrugte moet egter nie fokus op 'n enkele faktor nie, maar op 'n strategie wat bestaan uit voor-oes faktore wat die vrugskil kwaliteit beïnvloed, sowel as spesifieke na-oes tegnologieë en hanteringsprotokolle wat bekend is vir die vermindering van koueskade.

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GENERAL INTRODUCTION

With the increase in urbanization, globalisation and the production of agricultural products also in marginal regions, fruit and other agricultural food products are transported and shipped around the world. Therefore management of the cold chain and the transport of fresh quality fruit at destination are essential. There are major limitations in the postharvest maintenance of fruit since storing fruit at higher temperatures will enhance deterioration and water loss while storing fruit at lower temperatures may lead to the development of chilling injury (CI).

The South African citrus industry export citrus fruit to different countries including UK, Europe, Middle East, Russia, China, Japan, Far East and the United States. Citrus fruit exported from South Africa to the USA, Korea, Thailand and China undergoes a cold treatment for 24 days at -0.6 °C preceded with precooling at the same temperature, and fruit to Japan for 12 days at -0.6 ˚C. This temperature treatment is used to sterilize insect larvae of major pest in citrus such as false codling moth *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) and Mediterranean fruit fly *Ceratitiscapitata* (Wiedemann) (Diptera: Tephritidae) (White and Elson, 2004; EPPO, 2007). *C. leucotreta* is a major phytosanitary threat, and thus, regulatory procedures such as cold treatment and strict inspection before and after harvest are enforced by several countries such as the USA and China (Hofmeyer et al., 2005). However, this cold treatment may cause CI, which is a physiological disorder induced by very low temperatures. CI is an external quality defect on affected fruit, and symptoms vary including scalding, watery breakdown, rind pitting, soft glazed continuing lesions of mandarins, sunken tissue and damage to the stylar end of lemons (Reuther et al., 1989). Certain cultivars are more susceptible to CI; for example, mandarin is more susceptible compared to Navel and Valencia orange fruit in the following order: mandarin > navel > Valencia (Lafuente et al., 2003). Commercial experience in South Africa showed lemons and grapefruit to be even more susceptible.

Various factors influence the CI susceptibility of fruit including microclimate, cultivar, harvest date and management practices. Previous research in major citrus growing areas such as Spain, Australia and Florida (USA) have investigated the effects of harvest date and different sites on CI susceptibility (Gonzalez-Aguilar et al., 2000; Lindhout, 2007; Purvis, 1979); however, no research has been reported about the CI susceptibility of fruit from different microclimates, harvest dates and cultivars of navel orange in South Africa. Other factors which could also affect the incidence of CI were investigated including canopy position, rind colour and fruit size. Although the physiological mechanism of CI is not fully understood, there are different postharvest treatments i.e. warm water, thiabendazole (TBZ) and waxing of fruit which have been shown to reduce the development of CI (Schirra et al., 2000; Schirra and Mulas, 1995; Lindhout, 2007).

The aim of this study was to investigate the physiology of postharvest CI of navel oranges.

The specific objectives were:

- To determine the effect of harvest date, cultivar and microclimate on CI of navel orange fruit.
- The possible use of near infra-red spectroscopy to predict CI susceptibility of citrus fruit as influenced by fruit characteristics i.e. canopy position, fruit size and colour.
- To investigate the effectiveness of postharvest dip treatments with TBZ and the application of TBZ in a commercial packline system on the CI incidence of navel orange fruit.

References

EPPO (European and Mediterranean Plant Protection Organization), 2007. Data sheets on quarantine pests. Ceratitiscapitata. http://www.eppo.org/QUARANTINE/insects/Ceratitis capitata/CERTCA ds.pdf.\ Accessed 20/06/2011.

Gonzalez- Aguilar, G.A., Zacarias, L., Perez-Amador, M.A., Carbonell, J. and Lafuente, M.T. 2000. Polyamine content and chilling susceptibility are affected by seasonal changes in temperature and by conditioning temperature in cold stored 'Fortune' mandarins. Physiol. Plant. 108: 140-146.

Hofmeyer, J.H., Carpenter, J.E. and Bloem, S. 2005. Developing the sterile insect technique for *Cryptophlebialeucotreta* (Lepidoptera: Tortricidae): Influence of Radiation Dose and Release Ratio on Fruit Damage and Population Growth in Field Cages. J. Econ. Entomol. 98(6): 1924-1929.

Lafuente, M.T. Zacharius, L., Martinez-Tellez,M.A. Sanches-Ballesta, M.T. and Granell, A. 2003. Phenylalanine ammonia-lyase and ethylene in relation to chilling injuryas affected by fruit age in citrus. Postharvest Biol. Technol. 29: 309-318

Lindhout, K., 2007. Physiology of chilling related postharvest rind breakdown of Navel oranges (*Citrus Sinensis* (L.) Osbeck). PhD dissertation, La Trobe University, Bundoora, Australia.

Purvis, A. C., Kawada, K. and Grierson, W. 1979. Relationship between mid-season resistance to chilling injury and reducing sugar level in grapefruit peel. HortScience 14: 227-229.

Reuther, W., Calavan E.C. and Carman, G.E. 1989. The citrus industry Postharvest disorders and diseases. University of California, Division of Agricultural Sciences, Berkley, USA.

Schirra, M., D'hallewin, G., Cabras, P., Azngioni, A., Ben-Yehoshua, S. and Lurie, S. 2000. Chilling injury and residue uptake in cold-stored 'Star Ruby' grapefruit following thiabendazole and imazalil dip treatments at 20 and 50 °C. Postharvest Biol. Technol. 20: 91–98.

Schirra, M. and Mulas, M. 1995. Improving storability of 'Tarocco' oranges by postharvest hot-dip fungicide treatment. Postharvest Biol. Technol. 6: 129-138.

White, I.M., Elson, M.M., 2004. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International—The Australian Center for International Agricultural Research, Eastbourne, UK.

LITERATURE REVIEW: POSTHARVEST CHILLING INJURY OF CITRUS FRUIT

1. Postharvest chilling injury of fruit

1.1 Freezing versus chilling injury

Temperatures above freezing (0 °C) and up to 12 °C are used to extend the shelf life of fruits and vegetables. At these temperatures, the rate of respiration and other metabolic processes of the fruit are lowered, thereby reducing deterioration of fruit. However, certain postharvest horticultural products show evidence of physiological dysfunction when exposed to non-freezing temperatures below 10 °C to 12 °C, which is known as chilling injury (CI) (Lyons, 1973). CI occurs if fruit are exposed to temperatures above freezing whereas freezing injury is a different phenomenon where there is the formation of ice crystals (Wilkinson, 1970). The different types of CI can manifest differently on various species i.e. internal browning on apples, rind spotting and pitting in citrus and damage to the vascular bundles in avocados (Wilkinson, 1970). The most common symptoms of CI are surface pitting, external discolouration, and necrotic areas (Table 1) (McGlasson et al., 1979). In this review, the focus will be on postharvest chilling injury and specifically of citrus fruit.

1.2 Time versus temperature

The incidence of CI of fruit depends on the temperature, duration which the fruit is exposed to the cold treatment, the species, cultivars of single species and the environment in which the plants were grown (Lyons, 1973). For most fruits, there is a threshold temperature and time interaction that will be influenced by region and cultivar.

Incidence of CI symptoms will take weeks to appear if fruit is stored at temperatures between 10-12 °C, but for certain species incidence of CI will occur faster (Lyons, 1973). An example is banana which will show symptoms of CI after a brief exposure to a temperature below 10 °C (Von Loesecke, 1950). With other species such as *Episcia, Achimines* and *Gloxinia,* the incidence of CI will occur after a few hours of exposure to temperature of between 0 and 5 °C (Seible, 1939 as cited by Lyons, 1973).

The lowest temperature limits differ significantly between species; for example, temperate fruit could be stored between temperatures of 0-4 °C, whereas 8 °C is suitable for subtropical fruits and around 12 °C for tropical bananas (Table 1) (Wilkinson, 1970). Recovery from the incidence of CI can occur when the fruit is exposed to certain potential damaging temperatures for a short period and below a threshold (Wilkinson, 1970).

Table 1. Chilling sensitivity of different tropical and subtropical fruits (after McGlasson et al., 1979).

Produce	Approximate lowest safe temperature (°C)	Symptoms
Avocado	11	Pitting, browning of pulp and vascular strands
West Indian other varieties	5	Vascular strand
Banana	12	Browning streaking to blackening of skin
Citrus	5-10	Pitting of flavedo, pitted areas turn brown
Lanzone	10-12	Browning of skin
Litchi	2-5	Loss of red colour
Mango	5	Dull skin, brown patches, abnormal ripening
Papaya	6	Pitting of skin water soaking of flesh, abnormal ripening

The specific physical and chemical symptoms vary between different species. For example, injury can take a form of surface lesions in cucumbers (Eaks and Morris, 1962), inhibition of ripening of tomato fruit (Rugkong et al., 2011) and discolouration of banana fruit (Von Loesecke, 1950). The symptoms of CI also vary within fruit tissue but in general result in damage to the function and the death of cells.

CI in citrus is unique because of the thickness of rind tissue and also presence of certain phytotoxic oils. Symptoms of CI in citrus can be different according to cultivar and severity and include pitting, browning of the rind, scalding, sunken lesions and staining (Reuther at al., 1989). Different symptoms of CI in citrus tend to be associated with various temperature ranges. At very low temperature disorders such as scald and watery breakdown occur whereas at warmer storage temperature pitting occurs (Grierson, 1986).

Obenland et al. (1997) and Underhill et al. (1995) reported that in lemon fruit various types of damage occur to the rind tissue due to chilling temperatures. Obenland et al. (1997) reported damage to epidermal and the sub-epidermal layer without damage to oil glands; however, Underhill et al. (1995) found a disruption to oil glands and collapse of parenchyma cells in lemon fruit. CI in citrus fruit is mostly associated with localised browning of the epidermis. Tissue browning occurs locally around oil glands and a release of oil gland contents in the surrounding cells (Underhill, 1995). These symptoms may develop only when the fruit is moved from a chilling to ambient temperature (Ladaniya, 2008).

2. Postharvest chilling injury of citrus fruit

2.1 Steri-preconditioning in SA citrus industry in relation to CI.

Storage of fruit at low temperature is the primary technology used to reduce the rate of fruit respiration, decay development, water loss as well as other associated physiological processes. In addition to these advantages, cold treatment can also be used to sterilize insect larvae of major pests in citrus during shipment, such as false codling moth *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) and Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (White and Elson, 2004; EPPO, 2007) in fruit destined for markets enforcing such a protocol. Due to *C. leucotreta* being regarded as a major phytosanitary threat for a number of agricultural commodities, regulatory procedures are enforced by several countries, including the United States, when receiving citrus from South Africa. These regulatory procedures involve, inter alia, strict inspection of export produce at various times before and after harvest and the application of a stringent cold treatment designed to disinfest fruit during the export and shipping process (Hofmeyer et al., 2005). All citrus fruit exported from South Africa to USA and China undergoes a mandatory 24 day temperature exposure at -0.6 °C plus a 3 day pre-cooling at the same temperature. This temperature protocol is known to cause CI to the flavedo (coloured part of the fruit rind) of all citrus cultivars. In addition, CI symptoms can occur at temperatures as high as 12 °C in susceptible cultivars (Schirra and Mulas, 1995; Martínez-Téllez and Lafuente, 1997).

2.2 The anatomy of the navel orange fruit rind

The rind of navel orange fruit consists of different tissue layers. The pericarp (rind) consists of flavedo and albedo whereas the endocarp (carpels) is the edible portion. The external coloured portion of the pericarp is the flavedo. Flavedo consists of cuticle covered epidermis and a few parenchyma cells next to the epidermis followed by the epicarp, hypoderm and the outer mesocarp (Spiegel-Roy and Goldschmidt, 1996).

Embedded inside the flavedo are oil glands, which contain different essential oils (Fig. 1 and 2) (Spiegel-Roy and Goldschmidt, 1996). These essential oils in navel fruit are a mixture of alcohols, aldehydes, hydrocarbons and acid esters which are phytotoxic to surrounding cells and can contribute to development of CI symptoms and other disorders on the fruit rind (Knight et al., 2002). Oil glands are not uniform in size and shape, including spherical, ovoidal to pyriform and can be at different depths in the flavedo due to the size of the gland (Knight et al., 2001). Oil glands are formed through a process which is called lysigeny and schizogeny. Lysigeny is the disintegration of cells that occurs when new structures for example oil glands are differentiated (Buchanan et al., 2000). Schizogeny is a process where cells will separate and won`t degenerate to create oil glands. During the process of shizogeny boundary cells are formed. These boundary cells also consist of secretory parenchyma which is responsible for the synthesis of oil and consist of a permeable membrane which helps with the movement of oils in and out of the gland (Knight et al., 2002). Microscopically studies by Lindhout et al., (2005) indicated that the integrity of the cells and the oil gland cavity is related to different macroscopic symptoms which occur in the fruit rind during exposure to a stress such as potential chilling temperatures.

The albedo consists of large deep layered cells which have very large intercellular spaces. The albedo occupy more than 60-90% of the fruit volume when the fruit is in early stage of development but later the albedo will become thinner and the portion of the albedo will decline (Spiegel-Roy and Goldschmidt, 1996).

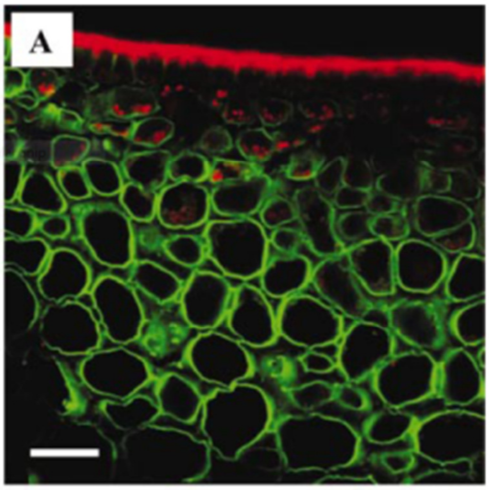


Figure 1. Rind oil localisation using confocal microscopy. Two colour optical sections of fresh tissue stained with Calcofluor white for cell walls (green) and Nile red for lipid (red) (Knight et al., 2002).

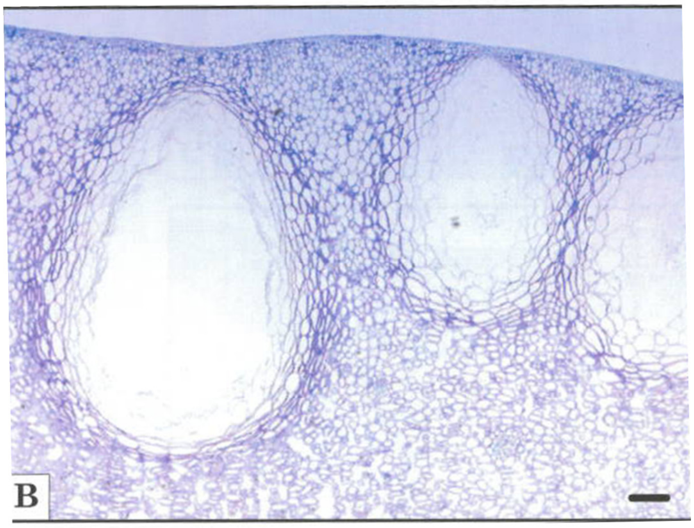


Figure 2. Flavedo tissue of fruit not exhibiting symptoms of chilling injury. The structural integrity of cells, tissue and oil gland cavities is uncompromised (adapted from Lindhout , 2007).

2.3 Chilling injury symptoms of citrus fruit rind

Symptoms of CI in navel orange can be described with different terms i.e. scalding, rind pitting, watery breakdown and is influenced by cultivars susceptible to different temperature ranges (Reuther et al., 1989). Lindhout et al. (2005) described and arranged these symptoms in navel oranges using 7 different types (Table 2). Type 1 symptoms begin with positive staining and have some association with the stomata and the beginning of pitting. Type 1 and 7 did not begin with the oil glands and did not begin with the release of the oils. Type 2 begins as a colourless collapse of the tissue. Type 3 indicated the release of oil because the tissue under the glands collapses. Type 4 show the collapse of cells above the oil gland cavity. The phytotoxic oils from the gland can enter the rind tissue and cause the damage. Type 5 shows the collapse and browning of the tissue around the calyx of the fruit (Lindhout, 2007). However, the lesions as illustrated in Type 6 corresponds to Phomopsis stem-end rot (*Phomopsis citri*) as described by Lesar (2013).

2.4 Physiological factors involved in the mechanism of chilling injury

2.4.1 Cell membrane

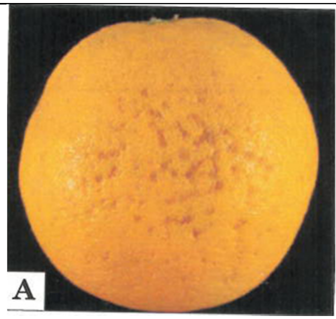
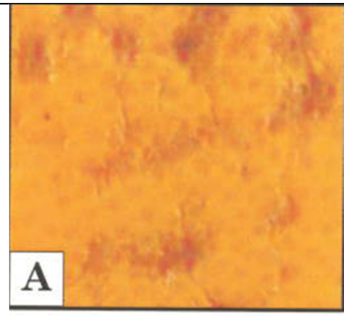
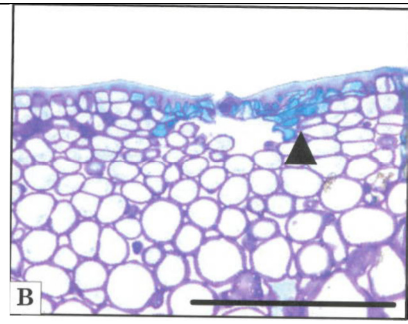
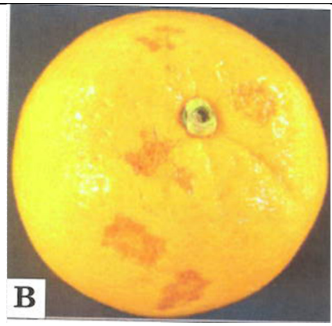
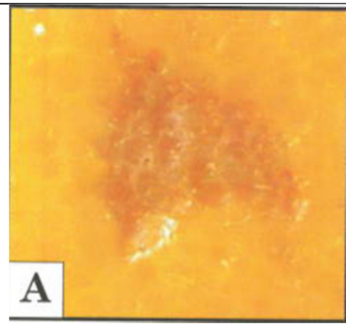
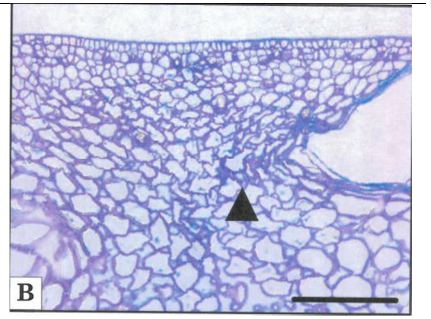
Membranes consist of lipids, which are capable of undergoing liquid to gel phase transitions. According to the bulk lipid phase transition theory developed by Lyons (1973), CI is the consequence of lipid phase transition resulting in a change in the fluidity of the membranes.

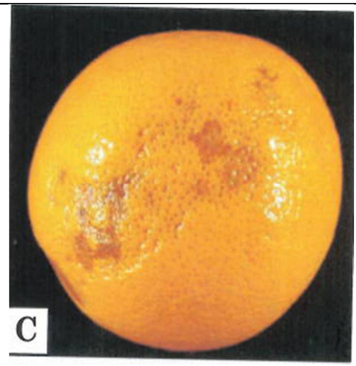
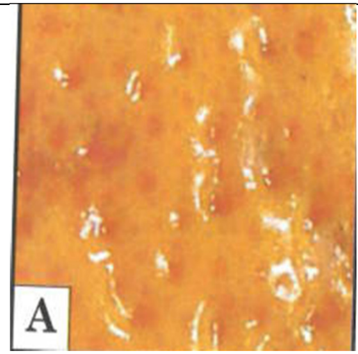
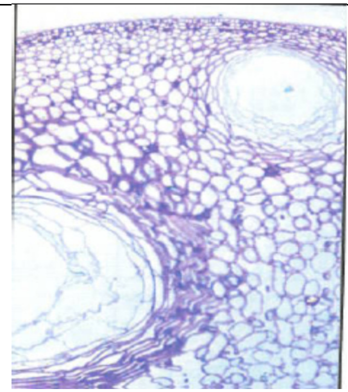
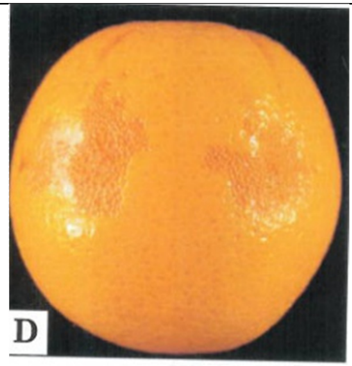
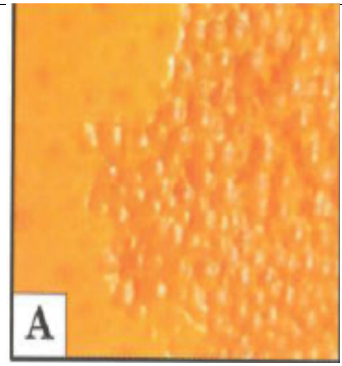
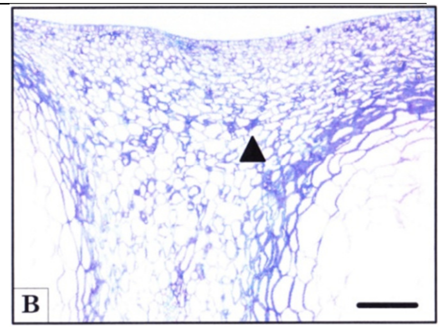
The membrane will change from a more flexible liquid crystalline structure to a solid gel structure. Changes in membrane permeability during cold storage are associated with a decrease in functionality of the membrane in fruit (Marangoni et al., 1996).

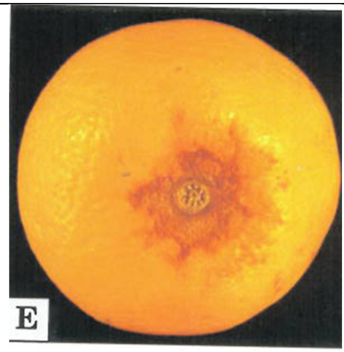
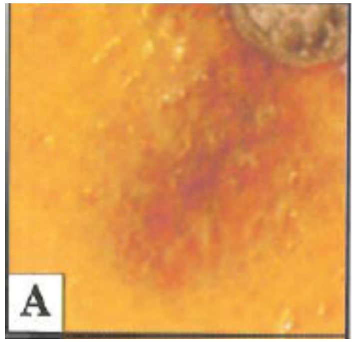
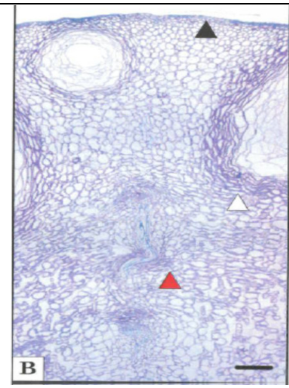
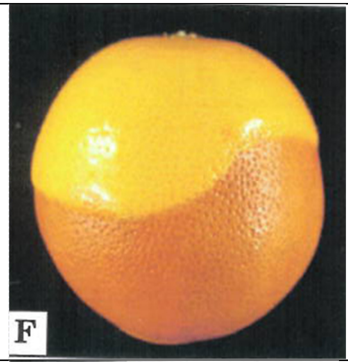
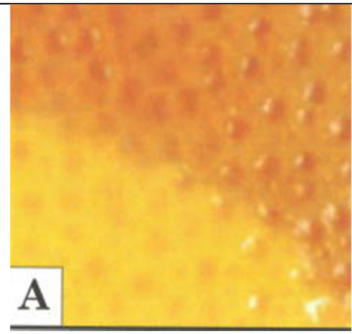
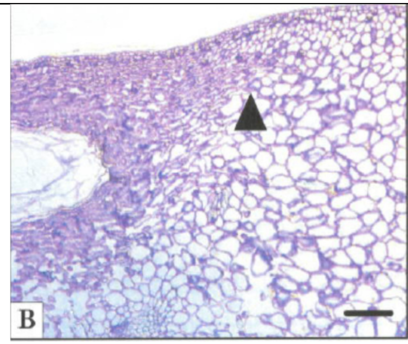
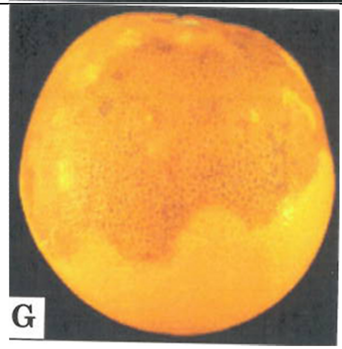
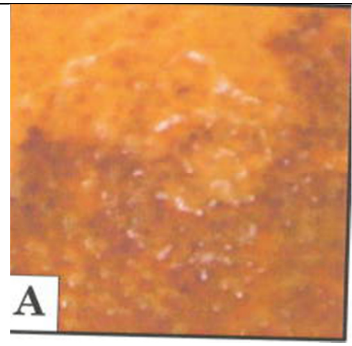
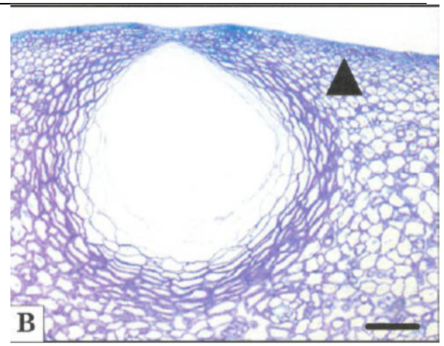
However, new research results have shown that membranes do not undergo bulky phase transitions as a single unit since membranes contain different lipid domains. Certain lipid domains will undergo a phase transition from liquid to gel. Only 2-5 % of the lipids do undergo lipid phase transition; however, this is still considered a major change in chilling sensitive species (Raison and Orr, 1986). Therefore if a shift occurs too far to the liquid or to the gel phase, it could result in a change in the membrane permeability and the lipid domains, which are gelled, will be more prone to leakage (Somerville et al., 2000).

The theory by Lyons (1973) also suggested that the effect of low temperature cause an increase in the micro viscosity of the matrix due to a decrease in the mobility of the lipids, a decrease in the rotation movements of the aliphatic chains of fatty acids and a decrease in the desaturation of fatty acids (Sevillano et al., 2009; Wolfe, 1978). Sharom et al. (1994) reported a membrane phase transition is associated with CI; however, it is not a direct, but an indirect effect

Table 2. Macroscopic symptoms of chilling related injuries of Navel orange fruit. (Adapted from Lindhout et al, 2005 , Lindhout, 2007)

Type CI	Description	Macroscopic images		Light microscopic image
1	Staining for polyphenolics in the epidermal and subepidermal cells, which is associated with stomata. Start of pitting and advancing will cause collapse of oil glands.			
2	Large lesions that have a defined edge. Injury begins as a colourless collapse of the tissue. Hypodermal tissue between oil glands is first affected.			

Type CI	Description	Macroscopic images		Light microscopic image
3	Collapse of the individual oil glands which cause pits in the tissue. These pits cause surface browning and result in large darkened lesions.			
4	Pebbly appearance which is caused by the collapse of the tissue around the large oil glands. Hypodermal and epidermal tissue total collapse.			

Type CI	Description	Macroscopic images	Light microscopic image
5	Collapse and browning of the tissue around the calyx of the fruit. An uneven distribution of tissue damage is still seen in the lower albedo.	 	
6	Affected tissue is extremely sunken and becomes hard and brown. The injury has a defined edge. The tissue remains soft and a distinctive honey-like odour is produced. Extensive damage affects the albedo layer.	 	
7	Superficial silvery discolouration in the early stages. The discolouration darkens over time and is accompanied by dehydration of the rind. Epidermal and sub-epidermal layers collapse.	 	

of fruit exposed to low temperatures. The phase transition only intensified when it was exposed to ambient temperatures.

As the lipid will eventually lose mobility and the tail will extend, this will cause stress in hydrophobic bonds. This will affect the cation concentration in nearby water which will affect electric forces on the charged or dipolar proteins. The protein in membranes caught in regions of fluidity and solidity will cause energy of the activated complex to differ and if forces are great it will cause a conformational change in membrane bound proteins (Wolfe, 1978). These changes in membrane secondary responses, such as electrolyte leakage, loss of metabolic energy and disintegration of the photosynthetic system will eventually lead to cell death (Lyons, 1973).

The phase transition will also increase activation energy of membrane enzymes which will cause a slower reaction rate and imbalances with enzymes which are not membrane bound (Lyons, 1973). Rate of reactions of the enzyme system, for example glycolysis, will decrease constantly with a temperature coefficient (Q_{10}) and activation energy of two and the reaction rate of membrane enzymes in the mitochondrion will also decrease with a temperature coefficient of two. If the temperature is below the critical temperature where phase transition occurs the activation energy of the enzyme reactions will increase and this results in imbalance between different systems (Lyons, 1973). This is one of the reasons for the sudden increase in secondary metabolites such as pyruvate, acetaldehyde and ethanol in 'Marsh'grapefruit (Pantastico et al., 1967 as cited by Lyons, 1973). These different secondary effects will eventually lead to development of CI symptoms (Lyons, 1973).

The most significant changes in lipid composition are lipid peroxidation, degradation of phospholipids and also an increase in sterol-phospholipid ratio which will change the fluidity of membranes (Lee et al., 2005; Parkin and Kuo, 1989). One of the most important factors in the membrane is the degree of saturation of lipid membrane, which is used to assess the functionality of membranes. Fatty acid desaturation results in an increase in lipid fluidity (Marangoni et al., 1996). Glycerol-3-phosphate acyltransferase (GPAT) enzyme is associated with changes in fatty acid composition where it is localised inside the mature protein. Depending on type of fatty acid that the isoform uses as substrate, it can stimulate either CI sensitivity or tolerance. Saturated fatty acid will induce CI susceptibility and unsaturated will induce CI tolerance (Murata et al., 1992).

Sterols are less flexible than most of the other membrane lipids and will therefore disturb the phase transition from a liquid to gel phase transition of phospholipids at low temperatures. Sterols are important for maintenance of membrane fluidity (Bach and Benveniste, 1997). Critical low temperatures modify membrane function and biophysical properties which results in injury developing in sensitive tissue. However, with cold acclimation, the membrane will alter its composition and even though the membrane is exposed to low temperatures it will not change its biophysical properties and will not develop CI (Marangoni et al., 1996) (Fig. 3).

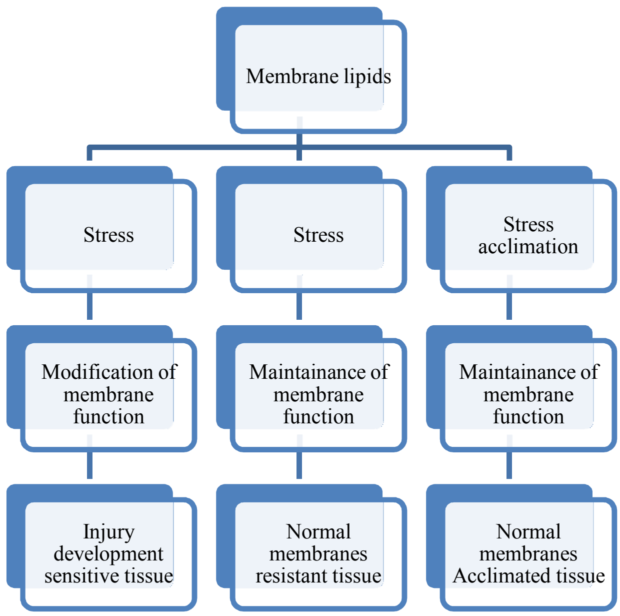


Figure 3. Membrane injury or acclimation resulting from stress (adapted from Marangoni et al, 1996; Leshem 1992).

.4.2 Oxidative stress

Reactive oxygen species (ROS) and lipid peroxidation

Reactive oxygen species are different and reduced forms of oxygen, i.e. hydrogen peroxide, hydroxyl radicals and superoxide which have extra electrons that can oxidise uncontrollably and cause oxidative destruction of the cell. Oxidative stress in tissue occurs when there is an increase in reactive oxygen species and is considered as by-products from respiration, photosynthesis and oxidative phosphorylation (Mittler, 2002). These reactive oxygen species increase during different stresses such as irradiation (Barka et al., 2000), microbial attack (Devlin and Gustine, 1992) and senescence (Borrel et al., 1997).

During low temperature stress, it is known that there is an increase in ROS level in the plant. It was reported by Rao et al. (1998) that in apples ROS increased in scald susceptible apples, but there was not a significant increase in resistant species. Uncoupling of the electron transport chain in oxidative phosphorylation which occurs in the mitochondrion will also be affected. The changes to membrane proteins due to low temperature stress will cause an alteration in electron transfer and cause ROS to increase. ROS's great toxicity levels affect the biomolecule and the membrane in the plants (Mittler, 2002).

ROS are potentially damaging molecules from which certain lipid hyperdroperoxides are formed through different reactions (Shewfelt and Purvis, 1995). This formation of lipid hydrogen peroxides result in change in membrane fluidity and integrity and this will result in certain membrane bound proteins to cease or protein will not function properly (Shewfelt and del Rosario, 2000). The key enzyme which regulates lipid peroxidation in membrane is lipoxygenase (LOX) (Lee et al., 2005).

Antioxidants

Plants are protected against ROS by upregulating synthesis of various antioxidant species such as the enzymatic and the non-enzymatic types, β-carotene, lycopene, α tocophorol and also other water soluble reducing agents such as ascorbic acid and glutathione. In citrus rind improvement some of the enzymatic antioxidants are superoxide dismutase (SOD) catalase (CAT) and ascorbate peroxide (APX) and also glutathione reductase (GR) (Shewfelt and del Rosario, 2000). Sala et al., (1998) observed that there was an increase in SOD activity in chilling sensitive and tolerant mandarin cultivars. In addition Lindhout, (2007) reported that chilling tolerant navel orange cultivars had a higher activity of CAT, APX and GR.

Shewfelt and Purvis (1995) suggested that with lipid peroxidation it is important to not only focus on a single event, but rather on the overall reactions between oxidants and the defence mechanism in order to understand the balance between oxidants and the defence mechanism. ROS is normally produced in the cell by metabolic processes, but antioxidants defences will scavenge these molecules and will result in no incidence of CI. However, if there is a sudden increase in ROS levels due to stress from low temperatures it could cause very high levels of ROS and the antioxidant mechanism cannot scavenge for these high levels of ROS (Shewfelt and Purvis, 1995).

2.4.3 Biochemical changes as protection against chilling injury

Carbohydrates are energy sources for plant cells but can have a direct or indirect effect on the protection of sensitive plants at chilling temperatures. Soluble carbohydrates can either decrease the osmotic potential of the cell or stabilise certain cell membranes and enzymes by binding to the molecule (Purvis, 1990).

Purvis et al. (1980) suggested that the protective measure of soluble carbohydrate is not due to the level of a particular soluble carbohydrate, since grapefruit from the interior and exterior has the same amount of total soluble carbohydrates, but the interior and exterior fruit still differ in the sensitivity to low temperatures. CI is thought to be more involved in carbohydrate metabolism than in levels (concentration) of sugars. Sugars play a major role in the metabolism as respiratory substrate.

Some studies showed that carbohydrate metabolism is involved in protecting of tissue against chilling stress; however, there are conflicting reports of the influence of carbohydrates on CI. Purvis and Grierson (1982) reported that increase of reducing sugars in the rind correlated with a decrease in chilling sensitivity of grapefruit when the temperature was below 10 °C. Similarly Purvis et al. (1979) reported a strong correlation between reducing sugars and resistance to incidence of CI by stabilising the membrane. However, Holland et al. (1999) reported that sugar accumulation in winter didn`t induce resistance to CI, but may suggest that increase in reducing sugars content in the rind of 'Fortune' mandarin could be related to an osmotic hardiness to actually cope with environmental stresses. Increase in the reducing sugars was not effective enough to protect fruit against CI after storage.

In various cold-sensitive plants, it is observed that there is an increase in ethylene synthesis together with the incidence of CI when the plant is exposed to low temperatures. The precursors of ethylene biosynthesis and activity of the different enzymes are also stimulated. There is a strong stimulation of ethylene biosynthesis, especially in fruit such as grapefruit, when fruit is transferred from low temperature to room temperature (Schirra et al., 1997). There is also an increase in biosynthesis of phenylalanine ammonia-lyase (PAL) activity in combination with ethylene production in citrus when stored at low temperatures. PAL is the initial rate-controlling enzyme in the phenylpropanoid pathway. In addition this enzyme has been reported to protect the plant against different environmental stresses through the different phenylpropanoid products. Induction of ethylene as well as PAL activity is induced by cold temperature and is not a cause for CI in citrus fruit (Lafuente et al., 2003).

2.5 Factors affecting the incidence of chilling injury in citrus

2.5.1 Macroclimate and microclimate

The most important geographical aspects that could influence temperature in a specific area are the latitude, altitude and degree of continentality. In general, it is considered that species whose natural habitat is closer to the equator are more sensitive to chilling while those from higher latitudes are not (Patterson and Reid, 1990).

The growing site and harvest date are considered one of the main factors affecting fruit susceptibility to CI. Lindhout (2007) observed differences in CI of citrus navel oranges at various sites investigated in Australia. The sites were located in Sunraysia district in New South Wales where peak flowering occurred mostly uniform in the district (±4 days variation) and it is suggested that the observed difference in maturity and colour indices were related to environmental factors post-flowering. Therefore while Iraak and Dareton fruit of the same variety are the same age in terms of days after anthesis the Dareton fruit were possibly physiologically older and more suceptible to CI (Lindhout, 2007). Cultivar and site differences therefore have to be considered prior to orchard planting to avoid high incidence of CI in exported fruit.

In *Malus domestica* a reduced susceptibility to CI was related to high amounts of preharvest low temperatures (Bramlage and Weiss, 1997). Late season high temperatures resulted in a high incidence of CI but the physiological reasons are not understood for the specific effect (Bramlage and Weiss, 1997).

Some fruit withstand postharvest low temperatures better when conditioned by preharvest low temperatures, but these preharvest temperatures still have to be above the temperature which induces CI. Different examples of those are *Capsicum annuum, Ocimum basilicum,* and *Citrus paradise* (Lang and Cameron 1997, Harding et al., 1957 and McColloch, 1962 as cited by Lang and Cameron 1997). However, Gonzalez-Aguilar et al. (2000) reported that 'Fortune' mandarin fruit harvested in mid-season, the cooler months of the year, has a higher incidence of CI. It is suggested in literature that heat shock proteins are induced by high temperatures and play a role in the tolerance of fruit to low and high temperature stress (Lopez-Matas, 2004).

2.5.3 Cultivar

Citrus cultivars differ in susceptibility and severity to CI. 'Fortune' mandarin has a higher susceptibility to CI, if it is stored at a temperature of 2 °C than 'Valencia' and 'Navelina' orange (Lafuente et al., 2003).. 'Fortune' mandarin could only be stored for 28 days at 2 °C in contrast to navel and 'Valencia' oranges that could be stored for 60 days (Fig. 4). However, the physiological and molecular responses which are related and cause these differences are not understood (Lafuente et al., 2003).

Lindhout (2007) indicated that different navel orange cultivars have a strong influence on type of CI symptom expressed. Lindhout (2007) reported that 'Navelina' orange fruit developed only the type 7 CI symptoms, 'Navelate' orange fruit develop only type 2 and 'Roberts' fruit expressed mostly type 3 CI (Table 2). The susceptibility to CI varies between the different varieties, for example 'Navelina' fruit show strong tolerance to chilling temperatures while 'Thomson' was moderately sensitive and

'Navelate' and 'Roberts' fruit where highly sensitive to chilling temperatures. (Lindhout, 2007)

2.5.4 Harvest date

The maturity of fruit is used commercially to determine the harvesting date, however, fruit age can be defined in three ways i.e. day after anthesis, physiological age and commercial maturity (total soluble solids vs. total titratable acids) (Lindhout, 2007). Days since anthesis is the period after full bloom whereas physiological age focus on days since fruit set and maturity parameter are based on organoleptic properties of fruit specifically for consumer preferences. The days since anthesis (the days after full bloom) and the physiological age of the fruit, are thought to have a great influence on chilling susceptibility of fruit, however environmental factors could also influence fruit age (Lindhout, 2007).

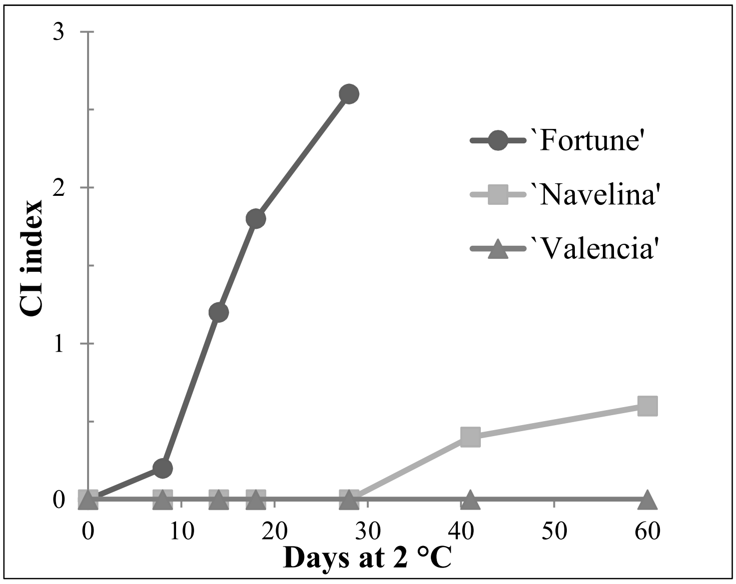


Figure 4. Changes in CI index of 'Fortune' mandarin and 'Valencia' late and 'Navelina' orange fruit held at 2 °C. Results of CI index are means of three replicate samples of 20 fruit (redrawn from Lafuente et al., 2003)

Gonzalez-Aguilar et al. (2000) showed that 'Fortune' mandarin fruit harvested early (November/ December) and late (April) in the season in Spain, had a similar, although low incidence of CI (Fig.5). Fruit harvested in mid-season, the cooler months of the year, had a higher incidence of CI. These seasonal variations in CI incidence during the course of the year followed a bell-shaped curve depending on temperature (Fig.5). Schirra et al. (2000) also reported that the later grapefruits are harvested the lower the CI incidence. Schirra et al. (200) reported that the susceptibility of 'Star Ruby' grapefruit from Southern Sardinia in Italy was significantly dependant on the harvest date and the postharvest dip stored for 6 weeks at 2 ºC. CI incidence was highest in fruit harvested in November–February (cooler part of year), lower in April and negligible in June (warmer part of year).

CI of 'Tarocco' orange from southern Sardinia, in Italy was also dependent on the fruit maturity stage and CI incidence was higher in the earlier harvested, immature fruit. In the Mediterranean countries the 'Tarocco' orange is harvested in January and February, with a high incidence of CI, however, when fruit were left until March the incidence of CI reduced, but resulted in high abscission and dropping rate (>80%) (Schirra et al., 1997). In contrast Lindhout, (2007) reported that 'Thomson' navel orange fruit CI incidence were higher in the two latest harvest dates in Australia during cooler parts of the year.

Purvis et al. (1979) reported that if grapefruit were harvested in Florida in midseason (February – March), it developed the lowest incidence of CI and corresponded with the coolest part of the year and a high amount of reducing sugars in the rind. This pattern is the inverse of what was observed in Spain were the fruit harvested during the coolest part of the year (midseason) had the highest incidence of CI (Fig. 5).

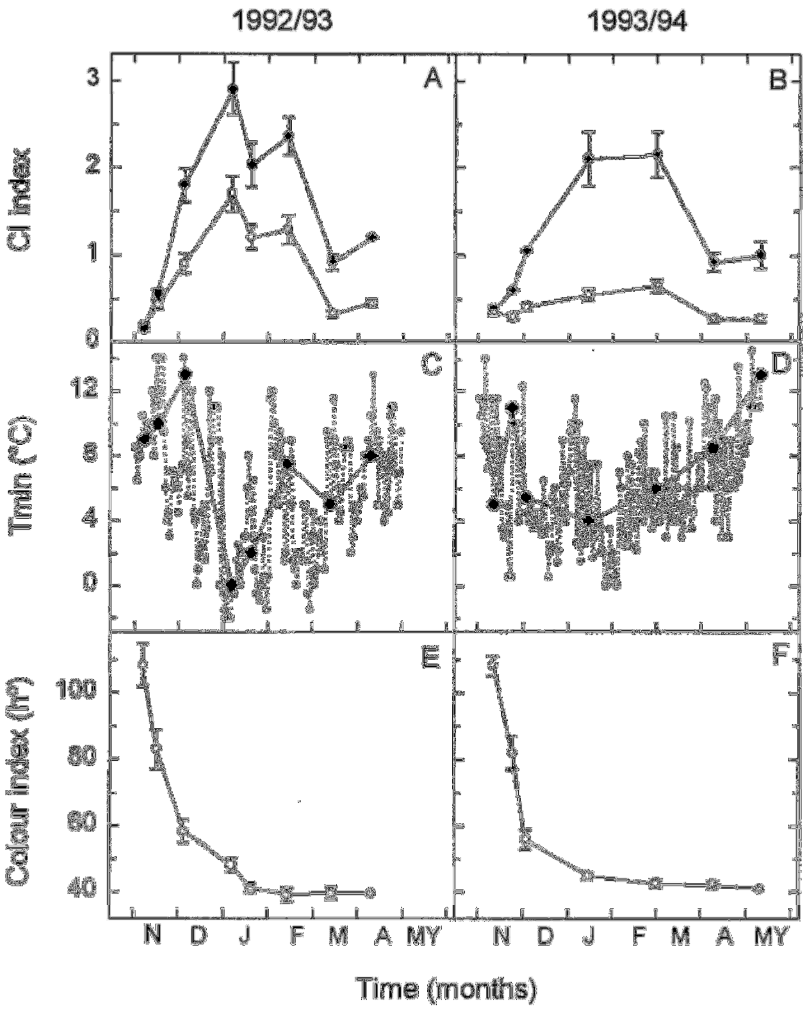


Figure 5. Seasonal changes in chilling susceptibility (A, B), mean minimum field temperatures during the growing period (C, D) and fruit colour (E, F) of 'Fortune' mandarin (adapted from Gonzalez- Aguilar et al., 2000).

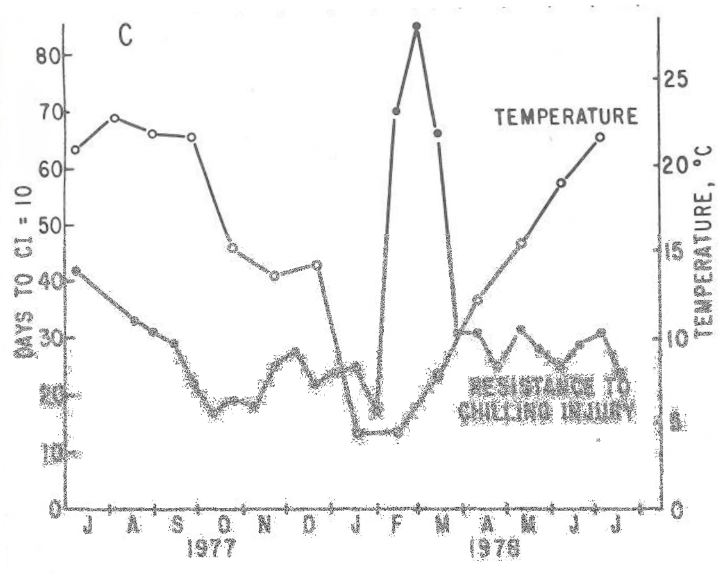


Figure 6. Resistance to chilling injury determined by holding grapefruit at 4.4 °C for 30 days. Chilling injury resistance is the days in storage before the onset of Chilling injury is apparent (Adapted from Purvis et al., 1979).

2.5.5 Variation between trees and fruit

Canopy position and light distribution

Canopy position results in a difference in susceptibility to CI. In addition differences in the internal quality were observed from the fruit from the sunlit area of the canopy and interior canopy positions (Syvertesen and Albrigo, 1980). Purvis et al. (1979) and Nordby and McDonald (1995) reported that 'Marsh' grapefruit from the exterior canopy were more susceptible to CI than fruit fruit from the interior of the canopy which could be due to other abiotic or environmental factors.

Fruit in different parts of the tree canopy are exposed to different microclimates during growth and maturation. One of the important biochemical and physiological regulators is light quality and light intensity (Purvis et al., 1980). Light responses are mediated by response of photoreceptors. Different light colours will selectively activate different photoreceptors and will activate different genes. Higher light intensities could cause higher photosynthetic rate and more carbohydrates; however, Purvis (1980) reported that there was no significant difference in the levels of the soluble carbohydrates, reducing sugars and sucrose in the rind of the exterior and the interior canopy. The surface temperature of the fruit that is exposed to the sun could also alter the metabolism of the rind and can be about 10-15 °C higher than the fruit from interior canopy and could alter the metabolism of the rind.

Sink and source and carbohydrate allocation

Starch is the most important storage carbohydrate in the majority of tree organs and fruit and will be metabolised into fructose, glucose and sucrose (Cameron, 1932). Photosynthates have to be allocated from the source to different sinks and all actively grown organs are considered a strong sink. However, there is competition for photosynthates among different organs i.e. fruit–shoot as well as among fruit-fruit (Goldschmidt and Koch, 1996). Competition between shoot elongation and leaf expansion will generally not occur in citrus because shoot elongation and leaf expansion occur before anthesis (Erner, 1989). Higher leafy inflorescence will cause higher rate of fruit set and persistence. In citrus, competition between fruit is more evident than in other fruit trees and the reduction in fruit numbers on a specific tree is due to carbohydrate status of the specific tree (Goldschmidt et al, 1992). The allocation of these photosynthates to different storage compartments is also thought to be less important than allocation to different organs (Fishler et al., 1983).

Changes occur in the sucrose, fructose and glucose and starch concentrations during maturation or ripening of fruit. In many fruits breakdown of starch to glucose, fructose or sucrose is considered a characteristic ripening event. In mandarins Tadeo et al. (1987) found an increase of sugars, an increase in the sucrose (non-reducing), in the juice as well as an increase of the reducing sugars (fructose, glucose) in the rind during ripening of the fruit. Starch content in the flavedo may vary year by year and Holland et al., (1999) reported that the relationship between incidence of CI and starch content of 'Fortune' mandarin fruit did not show that starch content has an effect on the CI tolerance.

Pigments

The most important pigments in the rind of citrus fruit are chlorophyll and carotenoids. Chlorophyll dominates in the rind of immature fruit during growth (Ladaniya, 2008). There is an increase in synthesis of carotenoids in the chromoplast during ripening while there is a loss of chlorophyll as the chloroplast will change to chromoplast. Carotenoids are long chain components which include carotenes and xanthophylls and the highest amount of carotenoids is in the rind 50-75% (Curl and Bailey, 1956). Pigment synthesis is governed by several factors, for example xanthophyll synthesis is encouraged by a daytime low temperature (20 ºC) followed by a low temperature in the night (7 ºC) soil temperature (12 ºC). Carotenogensisis an endergonic process and is stimulated with ethylene whereas light is not necessary for synthesis of these colour pigments (Ladaniya, 2008).

Pigments are considered to have a protective function against various pre- and postharvest stresses for example photoprotection in apples (Merzlyak and Chivkunova, 2000). In citrus chlorophyll is broken down and carotenoids accumulate in the rind. The amount of carotenoid and therefore colour has an effect on CI, as it was reported that yellow grapefruit was more resistant to CI than green grapefruit (Grierson, 1974 as cited by Purvis et al., 1979).

3. Control strategies to reduce chilling injury in citrus fruit

3.1 Preharvest factors

3.1.1 Cultivar choice

As previously discussed, certain citrus cultivars are more susceptible to CI (Lafuente et al., 2003). Therefore cultivar choice depends on different factors in a growing area as well as on differences in susceptibility of the cultivar to specific diseases and injuries. In general citrus fruit sensitivity to CI follows the following pattern: limes > lemons > white grapefruit > pink grapefruit > Navel orange > 'Valencia orange > mandarin. However, in a cultivar group large variation can still occur (Ladaniya, 2008; Cronjé pers. Comm).

Rootstock choice is influential. Reynaldo (1999) reported that 'Ruby Red' grapefruit budded on a rough lemon rootstock and not on *Citrus ambylecarpa,* had significant lower losses due to CI and decay at a temperature range of 4–12 °C.

3.1.2 Light

Adequate light is important for good internal and external fruit quality. Fruit from the exterior part of the canopy usually has a better quality, inside heavily shaded fuit have lower TSS than outside exposed fruit. Insufficient light contribute to the reduced TSS concentration of inside fruit (Sites and Reitz, 1949). However, excess light will result in sunburn which could affect the quality of the fruit and increase in CI as seen in 'Star Ruby' grapefruit (Cronjé pers. Comm.). Insufficient light will result in very small fruit and fruit that are more prone for other diseases and disorders (Kays, 1999). Therefore pruning including topping and hedging to avoid crowding is important for the optimum flowering and fruit quality. A heavy fruit crop tends to deplete carbohydrates and result in small crop for the next year. Pruning after a heavy crop additionally stimulates vegetative growth and reduce fruit yield and quality the next year. Pruning after a light crop and before expected heavy crop can increase fruit size and help reduce alternate bearing (Rouse and Zekri, 2006; Tucker et al., 1991)

3.1.3 Mineral nutrients

A balanced nutrient management plan is very important for producing good quality fruit. Phosphorous deficiency can result in a very thick rind with a hollow core which is particular in sweet oranges. In addition it will result in the fruit rind to become very rough and the acid content in the pulp will cause delayed maturity (Ladaniya, 2008). Phosphorous increases the number of green fruit and reduce the rind thickness (Rouse and Zekri, 2006). Generally, it is considered that an excess of nitrogen with inadequate irrigation can result in lower yields with lower TSS per ha and will reduce the storage potential of a fruit (Ladaniya, 2008, Rouse and Zekri, 2006).

Potassium (K) is an essential mineral in citrus production; if there is a deficiency of this element it will cause a reduction in yield as deficiency will result in smaller fruit with a soft and thin rind that could increase the incidence of decay ((Rouse and Zekri, 2006) An excess of K will result in reduction of fruit split in 'Valencia' orange and will increase fruit size (Bar-Akiva, 1975) in addition to coarse textured fruit with a very high acid content (Ladaniya, 2008).

Calcium (Ca) is one of the nutrients which are commonly associated with postharvest disorders as it is important to delay developments of any physiological or pathological disorder (Slutzky, et al., 1981). Ca delays ripening and also improves fruit quality (Slutzky, et al., 1981). It is important to consider not only its availability in the soil but also its availability in the plant and the distribution of Ca throughout the plant and interaction with other elements. Ca is considered one of the most immobile elements. The most common symptoms which occur when there is a Ca deficiency are the disintegration of cell walls and collapse of these cells (Slutzky, et al., 1981).

Ezz and Awad, (2009) reported that mineral analysis of fruit tissue can be a good indicator of the physiological changes and reported a higher Ca/Mg ratio (10.76) in grapefruit damaged by low temperatures compared to healthy grapefruit (9.25). Slutzky et al., (1981) reported that levels of Ca in the stained areas where higher than in healthy areas. The concentration in the flavedo increased with CI. The question therefore remains why? Ezz and Awad (2009) suggested that Ca in the albedo could react with other organic acids in the albedo and can thus become more mobile which could then move to the flavedo and to areas with high incidence of CI.

However in general it was reported by Slutzky et al., (1981) and by Ezz and Awad, (2009) that at the end of the storage period in the flavedo of healthy fruit with no incidence of CI there was a higher Ca content. These results could suggest that the higher Ca content in the flavedo could be one of the reasons why the fruit had no incidence of CI (Slutzky et al., 1981). More detail of the different nutrients are discussed in (Cronjé, 2009) and did not fall within the scope of this study.

3.2 Postharvest factors

3.2.1 Plant growth regulators

Jasmonic acid (JA) is an oxylipin plant growth regulator. This growth regulator is responsible for expression of different genes which would encode for other specific defence biomolecules (Crozier, 2000). Sasaki-Sekimoto et al. (2005) confirmed that JA induces antioxidants such as glutathione and cysteine and dehydroascorbate reductase in *Arabidopsis*. These different coordination systems of the metabolic pathways will provide resistance against the different environmental stresses. Meir et al, (1996) observed a reduction in incidence of CI when fruit, such as avocado, grapefruit and bell pepper, was treated with methyl jasmonate (MA) which is a volatile methyl ester of JA. The decreases in CI only occur at certain range of doses. Methyl jasmonate is very ineffective when it is applied in very high dosages. Droby et al. (1999) reported that postharvest application of JA (10 µmol. L^{-1}) on grapefruit stored for 6 weeks at 2 °C was the most effective in reducing incidence of CI and green mould decay. When JA is applied at very low concentrations, it will enhance the resistance of grapefruit and thus reduce the incidence of CI. These are naturally occurring compounds used in very low doses and are more environmental friendly, therefore it could reduce the application of fungicides to control decay of fruit (Droby et al., 1999).

Salicylic acid (SA) is known for resistance against certain pathogen attacks and also regulates the activity of alternative oxidase proteins which will alter the alternative oxidase pathway (Rhoads and McIntosh, 1992). Jasmonates, salicylates and ethylene is also known to interact with certain ROS i.e. H_2O_2 or nitric oxide which will alter certain processes in the plant such as the systemic acquired resistance, the hypersensitive response and even programmed cell death (Overmeyer et al., 2003). In tomatoes the occurrence of CI was reduced by encoding of different defence biomolecules and also occurred for fruit which was treated with volatile MA or with SA (Ding et al., 2002).

3.2.2 Temperature treatments

Intermitting warm temperatures during storage life of fruit reduces the occurrence of CI. The interruption of chilling exposure has to be done before the development of CI (Schirra and Cohen, 1999). Intermitting warm temperatures will vary between different varieties growing conditions and fruit maturity stage. Schirra and Cohen (1999) reported that the use of intermitted warming during storage of 'Olinda' oranges reduced the occurrence of CI. The onset of CI was reduced by nearly 10-12 weeks. In this experiment 'Olinda' oranges were stored for cycles of 3 weeks at 3 °C followed by two weeks with temperature of 15 °C. These temperature changes were made gradually to minimize moisture condensation or weight loss.

Porat et al. (2000) and Lurie (1998) reported that heat treatments such as hot water dips and hot water brushing reduce the incidence of CI with 'Star Ruby' grapefruit. Ghasemnezhad et al. (2008) reported a reduction in CI with 'Fortune' mandarin particularly when it was treated with a temperature of 47.5 °C for 2 min or 5 min and at 50 °C for 2 min before storage for 8 weeks at 2 °C. Temperatures of 47.5 °C and 50 °C for 2 or 5 min causes rind browning due to heat damage (Fig. 7 and Fig. 8)

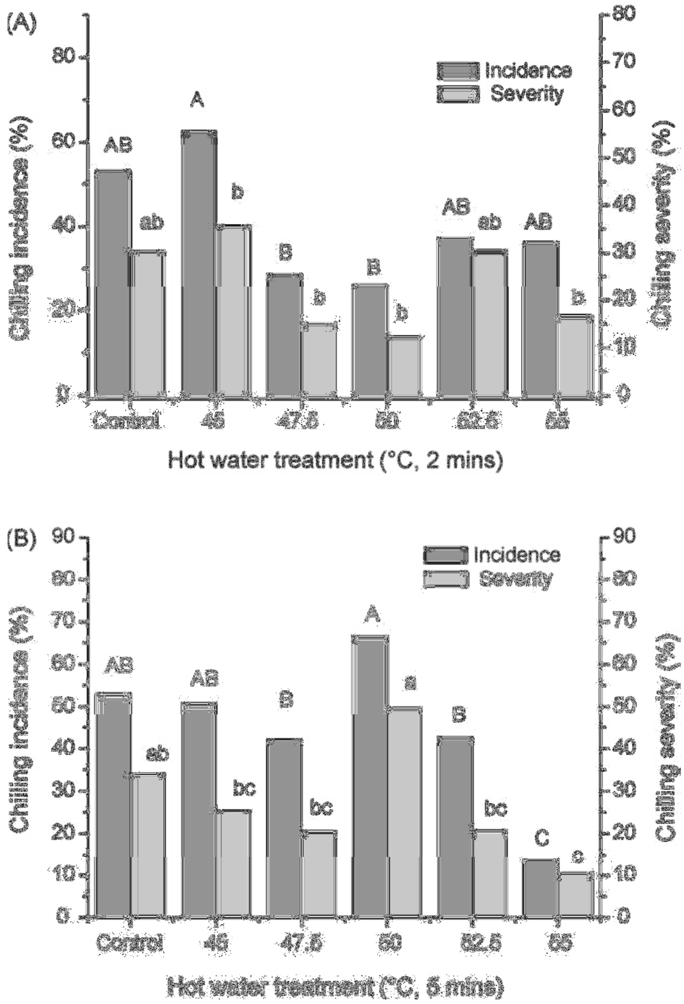


Figure 7. Chilling injury incidence (proportion of fruit with any level of damage) and severity (proportion of fruit with unacceptable level of damage) in 'Satsuma'mandarin fruits immersed in hot water (45–55 ◦C) for (A) 2 min or (B) 5 min and stored at 2 ◦C for 8 weeks. (Ghasemnezhad et al., 2008)

Figure 8. Visual assessment of 'Satsuma' mandarin fruit treated in different hot water dipping treatments after 8 weeks at 2 °C (Ghasemnezhad et al., 2008).

Porat et al., (2000) reported that 'Star Ruby' grapefruit treated with hot water dips and temperature conditioning was effective in reducing CI, however, some of the treatments had an effect on other postharvest quality parameters. Short postharvest treatments with hot water dips (2 to 3 min, 53 °C) didn`t affect internal and external qualities but long term conditioning treatments (3 d 21 °C or 7 d 16 °C) decreased the weight and caused changes in rind colour and increase in the TSS:acid ratio. These change caused a reduction in the fruit quality, especially the increase in weight loss and the increase in the TSS: acid ratio.

The protective effect of heat treatments induces defence mechanism against incidence of CI for example it will induce heat shock proteins (HSP), which is a chaperone. Molecular chaperones are basic proteins which would bind to the folded protein and will help to promote the correct folding of the specific substrate. Heat shock proteins may even be induced when JA or JS methyl salicylic vapour is applied (Ding et al, 2002).

The accumulation of heat shock proteins are not the only chilling tolerance response (Sapitnitskaya et al., 2007). Heat shock can also activate other defence pathways. Sala and Lafuente, (1999) reported an increase in CAT, an antioxidant which is one of the most important enzymes involved in heat induced chilling tolerance of 'Fortune' mandarin. Furthermore there was an increase in the activity of other antioxidants such as SOD and APX.

Dehydrins which are proteins inducing dehydration are also expressed through induction of heat treatments and chilling. Dehydrins have been shown to protect the plant from incidence of CI (Lanham et al., 2001, Porat et al., 2000, Close 1996).

3.2.3 Fungicides

Certain postharvest fungicides, such as thiabendazole (TBZ) reduce the incidence of CI. Application of thiabendazole was effective in reducing the incidence of CI with 'Tarocco' oranges (Schirra and Mulas, 1995) and 'Star Ruby' grapefruit (Schirra et al., 2000). Schirra et al. (2000) reported that higher concentration of 1200 $mg.L^{-1}$ TBZ (15 °C) as dip did not alleviate incidence of CI but a lower concentrations such as 200 $mg.L^{-1}$ (50 °C) was beneficial to reduce incidence of CI.

Limited research was done on the mechanism of how this specific fungicide reduces the incidence of CI. CI susceptibility of citrus fruit is thought to be related to the efficiency of the antioxidant system in the plant and TBZ treatment may be related to the antioxidants properties of fruit (Lindhout, 2007).

3.2.4 Postharvest wax application of citrus fruit

The cuticle is a layer of epidermal wax on citrus fruit which reduces moisture loss. This cuticle layer can be damaged easily and form micro-cracks, which can cause moisture loss (Cohen et al, 1994). Artificial wax, applied postharvest is a way to seal fruit and to reduce moisture loss which also occurs during incidence of CI. Gas exchange and transpiration of fresh produce are restricted by waxing fruit but waxing also reduces the incidence of CI and oleocellosis (Wang 1993, Wild 1993, 1998).

The type of postharvest wax applied would determine if it is effective in reducing CI. Hagenmaier (2000) reported that polyethylene-candelilla wax was better in reducing incidence of CI than shellac or wood resin which is a higher gloss wax. Candilla wax coatings tend to have low gloss but this seems to have been compensated for by mixing it with other polyethylene wax. The gloss of all these waxes decreases with storage time but it was faster for the high gloss waxes than for the candilla wax. The high gloss coatings gave a high gloss in the beginning but decreased fast and the polyethylene-candilla waxed fruit had a better gloss and flavour after storage. The weight loss was lower for fruit treated with polyethylene- candilla wax than for the high gloss coating (Hagenmaier, 2000). The reduction of CI in this treatment could therefore be the result of a reduction in moisture loss or dessication from the rind. Lindhout, 2007 reported that the application of wax was effective in reducing the incidence of CI in navel orange fruit.

3.2.5 Modified atmospheric packaging

The use of plastic films as packaging will help to maintain a certain higher relative humidity and will change the concentrations of CO_2 and O_2. This high relative humidity helps to reduce water loss of fruit. Water loss is one of the major contributions to incidence of CI. Porat et al. (2004) reported that use microfilms such as extended microfilm and polyethylene reduced development of CI by 71 % and 35 %, respectively in ‘Shamouti’ oranges which were stored at a 2 °C for 6 weeks and afterwards placed at room temperature.

Reduction of fruit moisture loss with higher relative humidity is beneficial for export of quality fruit. Henriod (2006) reported that moisture control technology was very effective in reducing moisture loss and consisted of a three-layer packaging design (Fig. 9). The material on the outside contained a polyethylene film which is bonded to wick paper which is water absorbing. The third layer was a loose hydrophobic vapour permeable cellulose fabric. These liners of moisture control technology reduced moisture loss by 88% if compared with cartons which were not packed in cartons with liners. Exported fruit cartons will lose about 0.5 kg water per carton when there is no moisture control technology applied which will cause a moisture loss of 104.4 tonnes of moisture for 3000 pallets. Fruit which was treated with this moisture control technology had a moisture loss of only 12.2 tonnes for 3000 pallets. This moisture control technology also significantly reduced the incidence of CI. This relative high humidity can induce pathogen growth, but using fungicides will prevent growth of pathogens. The wick layer in the modified atmospheric packaging prevents the direct contact of moisture with fruit which also prevent fungal growth (Henriod, 2006).

Modified atmospheric packaging may have certain negative aspects such as the stimulation of anaerobic respiration and development of off-flavours, but with citrus it was observed that modified atmospheric packaging will extend shelf life of fruit. Citrus fruit is very sensitive to environments which contain less than 5 % O_2 and even more than 5-10% CO_2 (Davis et al., 1973). Porat et al. (2004) indicated that if the concentration inside the packaging is more than 7-8% CO_2 and if it is less than 14-15 % O_2 it will cause anaerobic respiration which will lead to formation of off-flavours which will affect fruit quality. It is very important to choose the most efficient type of plastic film perforation which will modify atmospheric concentrations of CO_2 and O_2 in the package to such an extent that it will reduce incidence of CI.

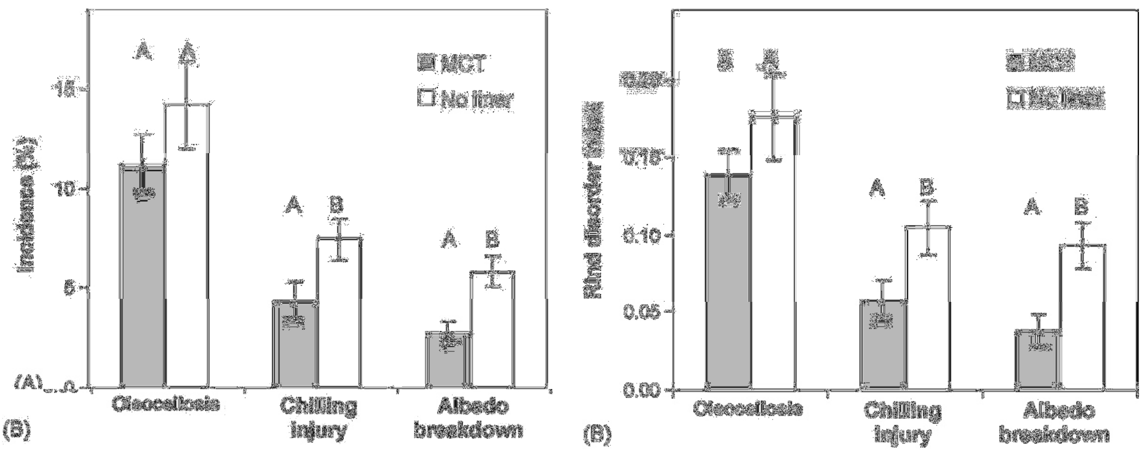


Figure 9. The mean incidence (A) and severity index (B) of three rind disorders expressed in 'Washington' navel orange fruit packed in cartons with and without moisture control technology (MCT) after being exported by sea from Australia to the USA in 2004. Bars with the same letters for each rind disorder were not significantly different at $P < 0.05$ (Henriod, 2006).

4. Conclusion

Chilling injury is a postharvest disorder that develops during the export of navel orange fruit from South Africa due to sterilisation protocol to USA and China. Research has been done on specific factors that will affect the incidence of chilling injury such as harvest date, pre-harvest temperatures, variation in the orchard on other citrus fruit, for example grapefruit in Spain, Florida and Italy (Mediterranean areas). However, no research has been reported about the CI susceptibility of fruit from different microclimates, harvest dates and cultivars of navel orange fruit in South Africa. Other factors including canopy position, rind colour and fruit size, which also affect the incidence of CI, were investigated for grapefruit, however, not for navel orange fruit. Research has been done on postharvest treatments which reduce the development of CI including warm water, TBZ and waxing of certain grapefruit and orange cultivars, but was not applied together in a semi-commercial packline. This study will investigate the various factors in South Africa that influence the CI incidence (cultivar, microclimate, harvest date) and various ways to reduce the incidence of CI.

References

Bach, T.J. and Benveniste, P. 1997. Cloning of cDNAs or genes encoding enzymes of sterol biosynthesis from plants and other eukaryotes: heterologous expression and complementation analysis of mutations for functional characterization. Prog. Lipid Res. 36: 197-226.

Bar-Akiva, A. 1975. Effect of potassium nutrition on fruit splitting in Valencia orange. J. Hort. Sci .50: 85–90.

Barka, E.A., Kalantari, S., Makhlouf, J. and Arul, J. 2000. Effects of UV-C irradiation on lipid peroxidation markers during ripening of tomato (*Lycopersiconesculentum* L.) fruits. Aust. J. Plant Physiol. 27: 147-152.

Borrel, A., Carbonell, L., Farras, R., Puigparellada, P. and Tiburcio, A.F. 1997. Polyamines inhibit lipid peroxidation in senescing oat leaves. Physiologia Plantarum 99: 385-390.

Bramlage, W.J. and Weiss, S.A. 1997. Effects of temperature, light and rainfall on superficial scald susceptibility in apples. HortScience 32: 808–811.

Bunchanan, B.B., Gruissem, W. and Jones, R.L. 2000. Biochemistry & molecular biology of plants. John Wiley & Sons. Rockville, USA. pp 1050-1051

Cameron, S.H. 1932. Starch in young orange tree. Proc. Am. Soc. Hortic. Sci. 29:110-114.

Close, T.J. 1996 Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. Physiologia Plantarum 97: 795-803.

Cohen, E., Shapiro, B., Shalom, Y. and Klein, J.D. 1994. Water loss: a non-destructive indicator of enhanced cell membrane permeability of chilling injured citrus fruit. J. Amer. Soc. Hort. Sci. 119: 983-986.

Cronjé, P.J.R. 2009. Postharvest rind breakdown of 'Nules clementine mandarin' *(Citrus Reticulate* Blanco) PhD dissertation. Stellenbosch University, South Africa.

Crozier, A., Kamiya, Y., Bishop, G. and Yokota, T. 2000. Biosynthsis of hormones and elicitor molecules. In: Buchanan, B.B., Gruissem, W. Jones, R.L. (eds.). Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, USA.

Curl, A.L. and Baily, G.F. 1956. Orange carotenoids. I. Comparison of Valencia orange peel and pulp. J. Agric. Food Chem. 4: 156–159.

Davis, P.L., Row, B. and Bruemmer, J.H., 1973. Biochemical changes in citrus fruits during controlled-atmosphere storage. J. Food Sci. 38: 225–229.

Devlin, W.S. and Gustine, D.L. 1992. Involvement of the oxidative burst in phytoalexin accumulation and the hypersensitive reaction. Plant Physiol. 100: 1189-1195.

Ding, C.K., Wang, C.Y., Gross, K.C. and Smith, D.L. 2002. Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to CI in tomato fruit. Planta 214: 895-901.

Dongo, T., R. Xia, Z. Xiao, P. Wang and W. Song, 2009. Effect of preharvest application of calcium and boron on dietary fibre, hydrolases and ultrastructure in 'Cara Cara' navel orange (*Citrus sinensis* L. Osbek) fruit. Scientia Horticulture, 121:272-277Droby, S., Porat, R., Cohen, L., Weiss, B., Shapiro, B., Philosoph-Hadas, S. and Meir, S. 1999. Suppressing green mold decay in grapefruit with postharvest jasmonate application. J. Amer. Soc. Hort. Sci. 124: 184-188.

Eaks, I.L. and Morris 1962. Deterioration of cucumbers at chilling and nonchilling temperatures. Proc. Amer Soc. Hort. Sci. 69:388-399.

EPPO (European and Mediterranean Plant Protection Organization), 2007. Data sheets on quarantine pests. *Ceratitis capitata*. http://www.eppo.org/QUARANTINE/insects/*Ceratitis capitata*/CERTCA ds.pdf.\ Accessed 20/06/2011

Erner, Y. 1989. Citrus fruit set: carbohydrate, hormone and leaf mineral relationship. In: Wright, C.J. (ed) Manipulation of fruiting. Butterworth and Co. pp. 233-242.

Ezz, T.M. and Awad R.M. 2009. Relationship between mineral composition of the flavedo tissue of 'Marsh' grapefruit and chilling injury during low temperature storage. Res. J. of Agr. Biol. Sci. 5(6): 892-898.

Fishler, M., Goldschmidt, E.E. and Monselise, S.P. 1983. Leaf area and fruit size in girdled grapefruit branches. J. Am. Soc. Hortic. Sci. 108: 218-221.

Ghasemnezhad, M., Marsh, K., Shilton, R., Babalar, M. and Woolf, A. 2008. Effect of hot water treatments on CI and heat damage in 'Satsuma' mandarins: Antioxidant enzymes and vacuolar ATPase, and pyrophosphatase. Postharvest Biol. Technol.48: 364-371

Goldschmidt, E.E, Harpaz, A., Gal, S., Rabber, D. and Gelb, E. 1992. Simulation fo fruitlet thinnings effects in citrus by a dynamic growth model Proc. Int. Soc. Hortic. 1: 515-519.

Goldschmidt, E.E. and Koch, K.E. 1996. Citrus. In Zamski, E., Schaffer, A.A. (eds). Photoassimilate distribution in plants and crops. Marcel Dekker Inc. New York.

Gonzalez-Aguilar, G.A., Zacarias, L., Perez-Amador, M.A., Carbonell, J. and Lafuente, M.T. 2000. Polyamine content and chilling susceptibility are affected by seasonal changes in temperature and by conditioning temperature in cold stored 'Fortune' mandarins. Physiol. Plant. 108: 140-146.

Grierson, W. 1974. Chilling injury in tropical and subtropical fruit: V effect of harvest date, degreening, delayed storage and peel color on chilling injury of grapefruit. Proc Trop. Region Amer. Soc. Hort. Sci. 18:66-73

Grierson, W. 1986. Physiological disorders. In. Wardowski, W., Nagy, S. Grierson, W. (eds.).Fresh citrus fruits. AVI Publishing Company , Westport, USA.

Hagenmaier, R.D. 2000. Evaluation of a polyethylene-candellila coating for 'Valencias' oranges. Postharvest Biol. Technol. 19: 147-154.

Henriod, R. E. 2006. Postharvest characteristics of Navel oranges following high humidity and low temperature storage and transport. Postharvest Biol. Technol. 42: 57-64.

Hofmeyer, J.H., Carpenter, J.E. and Bloem, S. 2005. Developing the sterile insect technique for *Cryptophlebialeucotreta* (Lepidoptera: Tortricidae): influence of radiation dose and release ratio on fruit damage and population growth in field cages. J. Econ. Entomol. 98(6): 1924-1929.

Holland, N., Sala, J.M., Menezes, H.C. and Lafuente, M.C. 1999. Carbohydrate content and metabolism as related to maturity and chilling sensitivity of cv. 'Fortune' mandarins. J. Agric. Food Chem. 47: 2513-2518.

Kays, S.J. 1999. Preharvest factors affecting appearance. Postharvest Biol. Technol. 15: 233–24.

Knight, T.G., Klieber, A. Sedgley and M. 2002. Structural basis of the rind disorder oleocellosis in 'Washington' Navel orange (*Citrus sinensis* L. Osbeck). Ann. Bot. 90: 765-773.

Knight, T.G., Klieber, A. and Sedgley, M. 2001. The Relationship between Oil Gland and Fruit Development in 'Washington' Navel Orange. Ann. Bot. 88: 1039-1047.

Ladaniya, M.S. 2008. Citrus fruit: biology, technology and evaluation. Academic press. pp154.

Lafuente, M.T. Zacharius, L., Martinez-Tellez,M.A. Sanches-Ballesta, M.T. and Granell, A. 2003. Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. Postharvest Biol. Technol. 29: 309-318

Lang, D.L. and Cameron, A.C. 1997. Pre- and postharvest temperature conditioning of greenhouse-grown sweet basil. HortScience 32: 114–116.

Lanham, P.G., Kemp, R.J, Jones, H. and Brennan, R.M. 2001. Expression of dehydrin like genes in response to chilling in leaves of black current, *Ribesnigrum* L. J. Hort. Sci. Technol. 76: 201-207.

Lee, S.H., Ahn S.J., Im,Y.J., Cho, K., Chung, G.C., Cho, B.H. and Han, O. 2005. Differential impact of low temperature on fatty acid unsaturation and lipoxygenase activity in fig leaf gourd and cucumber roots. Biochem. Biophys. Res. Commun. 330: 1194–1198.

Lesar, K. 2013. Compendium of postharvest citrus diseases – illustrated. Citrus Resaerch International, Nelspruit, South Africa.

Leshem, Y.Y., 1992. Plant membranes: a biophysical approach to structure, development and senescence. KIuwer Academic Publishers, Dordrecht.

Lindhout, K., 2007. Physiology of chilling related postharvest rind breakdown of Navel oranges (*Citrus Sinensis* (L.) Osbeck). PhD. dissertation, La Trobe University, Bundoora, Australia.

Lindhout, K., Treeby, M.T. and Parish, R.W. 2005. Chill Out: Chilling-Related Injuries in Navel Oranges. Acta Hort. 687.

Lopez-Matas, M.A., Nú nez, P., Soto, A., Allona, I., Casado, R., Collada, C., Guevara, M.A., Aragoncillo 2004. Protein cryoprotective activity of a cytosolic small heat shock protein that accumulates constitutively in chestnut stems and is up-regulated by low and high temperatures. Plant Physiol. 134:1708–1717.

Lurie, S. 1998. Postharvest heat treatments. Postharvest Biol. Tecnol. 14: 257-269.

Lyons, J.M. 1973. Chilling injury in plants. Ann. Rev Plant. Physiol**.** 24: 445–466.

Marangoni, A.G., Palma, T. and Stanley, D.W. 1996. Membrane effects in postharvest physiology. Postharvest Biol. Technol. 7: 193-217.

Martínez-Téllez, M.A. and Lafuente, M.T. 1997. Effect of high temperature conditioning on ethylene, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase activities in flavedo of chilled 'Fortune' mandarin fruit. J. Plant Physiol. 150: 674-678.

McColloch, L.P. 1962. Chilling injury and alternaria rot of bell peppers. U.S. Dept. Agr. Mktg. Res. Rpt. 536: 1-5.

McGlasson, W.B., Scott, K.J. and Mendoza, Jr D.B. 1979. The refrigerated storage of tropical and subtropical products. Int. J. Refrig. 2: 199–206.

Meir, S., Pholosoph-Hadas, S., Lurie, S., Droby, S., Ackerman, M. Zauberman, G., Shapiro, B., Cohen, E. and Fuchs, Y. 1996. Reduction of chilling injury in stored avocado, grapefruit and bell pepper by methyl jasmonate. Can. J. Botany. 74: 870-874.

Merzlyak, M.N. and Chivkunova, O.B. 2000 Light-stress-induced pigment changes and evidence for anthocyanin photoprotection in apples. J. Photochem. Photobiol. B: Biol. 55: 155–163.

Mittler,R. 2002. Oxidative stress antioxidants and stress tolerance. Trends Plant Sci. 7: 405-410.

Murata, N., Ishizakinishizawa, Q., Higashi, S., Hayashi, H., Tasaka, Y. and Nishida, I. 1992. Genetically engineered alteration in the chilling sensitivity of plants. Nature 356: 710–713.

Naradisorn, M., A. Klieber, M. Sedgley, E. Scottand A.J. Able, 2006. Effect of pre harvest calcium application on grey mould development and postharvest quality in strawberries. Acta. Hort., 708: 147- 150.

Nordby, H.E. and McDonald, R.E. 1995. Variations in chilling injury and epicuticular wax composition of white grapefruit with canopy position and fruit development during the season. J. Agric. Food Chem. 43: 1828-1833.

Obenland, D.M., Margosan, D.A., Houck, L.G. and Aung, L.H. 1997. Essential oils and chilling injury in lemon. Hortscience 32: 108-111.

Overmeyer, K., Brosche, M. and Kangasjarvi, J. 2003. Reactive oxygen species and hormonal control of cell death. Trends Plant Sci. 8: 335-342.Pailly, O., Tison, G. and Amouroux, A., 2004.Harvest time and storage conditions of ‘Star Ruby’ grapefruit (*Citrus paradise* Macf.) for short distance summer consumption. Postharvest Biol. Technol. 34: 65–73.

Pantastico eb, Grierson W, Soule J 1967. chilling injury in tropical fruits: i. bananas (*Musa paradisiaca* var. Sapientum cv. Lacatan). Proc. of the Trop. region American Soc. of Hort. Science 11: 83–91.

Parkin, K.L. and Kuo, S.J. 1989. Chilling-induced lipid degradation in cucumber (*Cucumis sativa* L.cv. Hybrid C) fruit. Plant Physiol. 90: 1049–1056.

Patterson B.D. and Reid, M.S. 1990. Genetic and environmental influences on the expression of chilling injury. In, Wang, C.Y. (eds.) Chilling injury of horticultural crops. CRC press, Florida, USA. pp 211-222.

Porat, R., Pavoncello, D., Peretz, J., Ben-yehoshua, S. and Lurie, S. 2000. Effects of various heat treatments on the induction of cold tolerance and on the postharvest qualities of 'Star Ruby' grapefruit. Postharvest Biol. Technol. 18: 159-165.

Porat, R., Weiss, B., Cohen, L., Daus, A. and Aharoni, N. 2004. Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. Postharvest Biol. Technol. 33: 35-43.

Purvis, A.C. 1980. Influence of canopy depth on susceptibility of 'Marsh' grapefruit to chilling injury. HortScience 15: 731-733.

Purvis A.C. 1990. Relation of chilling stress to carbohydrate composition. In, Wang,C.Y. Chilling injury of horticultural crops. CRC press, Florida, USA. pp 211-222.

Purvis, A. C. and Grierson, W. 1982. Accumulation of reducing sugar and resistance of grapefruit peel to chilling injury as related to winter temperatures. J. Am. Soc. Hortic. Sci.107:139-142.

Purvis, A. C., Kawada, K. and Grierson, W. 1979. Relationship between mid-season resistance to chilling injury and reducing sugar level in grapefruit peel. HortScience 14: 227-229.

Raison, J.K. and Orr, G.R.1986. Phase transitions in liposomes formed from the polar lipids of mitochondria from chilling sensitive plants. Plant Physiol. 81: 807-811.

Rao, M.V., Watkins, C.B., Brown, S.K. and Weeden, N.F. 1998. Active oygen species metabolism in 'White Angel' x 'Rome beaty' apple selections resistant and susceptible to superficial scald. J. Amer. Soc. Hort. Sci. 123: 299-304.

Reuther, W., Calavan E.C. and Carman, G.E. 1989. The citrus industry. Postharvest disorders and diseases. University of California, Division of Agricultural Sciences, Berkley, USA.

Reynaldo, I.M. 1999. The influence of rootstock on the post-harvest behaviour of 'Ruby Red' grapefruit. Cultivos-Tropicales. 20(2): 37–40.

Rhoads, D.M. and McIntosh, L. 1992. Salicylic acid regulation of respiration in higher plants: alternative oxidase expression. The Plant Cell 4: 1131-1139.

Rouse, R.E., Zekri, M. Preharvest factors that influence fresh fruit quality. Chapter 3 In. Wardowski, W., Nagy, S. Grierson, W. (eds.).Fresh citrus fruits. AVI Publishing Company , Westport, USA.

Rugkong, A., McQuinn, R., Giovannoni, J.J., Rose, J.K.C. and Watkins, C.B. 2011. Expression of ripening-related genes in cold-stored tomato fruit. Postharvest Biol. Technol. 61: 1-14.

Sala, J.M. 1998. Involvement of oxidative stress in chilling injury in cold stored mandarin fruits. Postharvest Biol. Technol. 13: 255–261.

Sala, J.M. and Lafuente, M.T. 1999. Catalase in the heat-induced chilling tolerance of cold stored hybrid Fortune mandarin fruits. J. Agric. Food Chem. 47: 2410-2414.

Sapitnitskaya, M., Maul, P., McCollum, G.T., Guy, C.L., Weiss, B., Samach, A. and Porat, R. 2007. Postharvest heat and conditioning treatment activate different molecular responses and reduce chilling injuries in grapefruit. J. Exp. Bot. 57: 2943-2953.

Sasaki-Sekimoto, Y., Taki, N., Obayashi, T., Aono, M., Matsumoto, F., Sakuri, N., Sizuki, H., Hirai, M.Y., Noij, M., Saito, K., Masuda, T.Takamiya, K., Shibata, D. and Ohta, H., 2005. Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerances in Arabidopsis. The Plant Journal 44: 653-668.

Schirra, M. and Cohen, E. 1999. Long-term storage of 'Olinda' oranges under chilling and intermittent warming temperatures. Postharvest Biol. Technol.16: 63-69.

Schirra, M., Agabbio, M., D'hallewin, G., Pala, M. and Ruggiu, R., 1997. Response of 'Tarocco' oranges to picking date, postharvest hotwater dips, and chilling storage temperature. J. Agric. Food Chem.45: 3216-3220.

Schirra, M., D'hallewin, G., Cabras, P., Azngioni, A., Ben-Yehoshua, S. and Lurie, S. 2000. Chilling injury and residue uptake in cold-stored 'Star Ruby' grapefruit following thiabendazole and imazalil dip treatments at 20 and 50 °C. Postharvest Biol. Technol. 20: 91–98.

Schirra, M. and Mulas, M. 1995. Improving storability of 'Tarocco' oranges by postharvest hot-dip fungicide treatment. Postharvest Biol. Technol. 6: 129-138.

Seible, D. 1939. Ein Beitrag zur Frage der Kälteschäden an Pflanzen bei Temperaturen über dem Gefrierpunkt. Beitr.Biol.Pflanz., 26: 289–330.

Sevillano, L., Sanchez-Ballesta, M.T., Romojaroc, F. and Floresc, F.B. 2009. Mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. J. Sci. Food Agric. 89; 555–573.

Sharom, M., Willimot, C. and Thompson, E. 1994. Chilling injury induces lipid phase changes in membranes of tomato fruit. Plant. Physiol.105: 305-308.

Shewfelt, R.L. and del Rosario, B.A. 2000. The role of lipid peroxidation in storage disorders of fresh fruit and vegetables. HortScience 35: 575-579.

Shewfelt, R.L. and Purvis, A.C. 1995. Toward a comprehensive model for lipid peroxidation in plant tissue disorders. HortScience 30: 213-218.

Sites, J.W. and Reitz, H.J. 1949. The variation in individual 'Valencia' oranges from different locations of tree as a guide to sampling methods and spot picking for quality.Part1 Soluble solids in the juice. Proc. Am. Soc. Hortic. Sci. 54: 1-9.Slutzky, B., Gonzales-Abreu, A. and Berdam, I. 1981. Chilling injury related to mineral composition of grapefruit and limes during cold storage. Proc. Int. Soc. Citriculture, 12: 779-782.

Somerville, C., Browse J., Jaworski J.G. and Ohlrogge, J.B. 2000. Lipids. In. Buchanan, B.B., Gruissem W.J., Jones, R.L. (eds.). Biochemistry and molecular biology of plants. American society of Plant physiologists, Rockville, USA. pp 456-527.

Spiegel-Roy, P. and Goldschmidt, E.E. 1996. Biology of citrus. Cambridge Univeristy Press. UK. pp. 88-92.

Syvertesen, J.P. and Albrigo, L.G. 1980. Some effects of grapefruit tree canopy position on microclimate, water relations fruit yield and juice quality. J. Amer. Soc. Hort. Sci. 105: 454-459.

Tadeo, J. L., Ortiz, J. M. and Estelles, A. 1987. Sugar changes in ‘Clementine’ and orange fruit during ripening. J. Hortic. Sci.62: 531-537.

Tucker, D.P.H., Wheaton, T.A. and Muraro, R.P. 1991. Citrus tree spacing and pruning SP-74. University of Florida, IFAS Printing, Gainesville.

Underhill, S.J.R., McLauchlan, R.L. and Eaks, I.L. 1995. ‘Eureka’ lemon chilling injury. HortScience 30: 309-312.

Von Loesecke, H.W. 1950. Bananas : chemistry, physiology, technology. Interscience; New York. USA, pp.55-66.

Wang, C.Y. 1993. Approaches to reduce chilling injury of fruits and vegetables. Hort Reviews 15: 63-95.

White, I.M. and Elson, M.M., 2004. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International—The Australian Center for International Agricultural Research, Eastbourne, UK

Wild, B.L. 1998. New method for quantitatively assessing susceptibility of citrus fruit to oleocellosis development and some factors that affect its expression. Aust. J. Exp. Agr. 38: 279-285.

Wild, B.L. 1993. Reduction of chilling injury in grapefruit and orange stored at 1 °C by prestorage hot dip treatments, curing and wax applications. Aust. J. Exp. Agr. 33: 279-285.

Wilkinson, B. G. 1970. Physiological disorders of fruit after harvesting. Chapter 3. In: Hulme, A.C, The biochemistry of fruits and their products. Academic Press; London pp.531-541.

Wolfe, J. 1978. Chilling injury in plants- the role of membrane lipid fluidity. Plant Cell Environ. 1: 241-247.

PAPER 1:
INFLUENCE OF CULTIVAR, LOCATION AND HARVEST DATE ON CHILLING INJURY OF NAVEL ORANGE FRUIT

Abstract

Citrus fruit exported from South Africa to the USA, Korea, Thailand and China undergoes a mandatory 24 day temperature exposure at -0.6 °C plus a 3 day pre-cooling at the same temperature. This temperature protocol is known to cause chilling injury (CI) to the flavedo of fruit. Various factors could influence the susceptibility of a citrus fruit to CI i.e. citrus type microclimate, harvest date etc. The aim of the study was to determine if different navel orange cultivars, harvest dates as well as different sites in Citrusdal, have an influence on the susceptibility of navel orange fruit to CI. 'Washington' navel was more susceptible to CI while 'Navelina' navel was more tolerant. Harvest date, i.e. rind maturity of the fruit, influence CI susceptibility of navel orange fruit. The incidence of CI was overall less when fruit were harvested in the middle of the commercial harvest window; however, the internal maturity (TSS:TA) at harvest does not appear to be related to the sensitivity of orange fruit to CI. Incidence in CI differ between the different areas in the Citrusdal valley; at the top of the valley (Tharakama), the coldest part with highest rainfall, both cultivars had highest incidence of CI compared to lower down (Hexrivier and Ouwerf) where it is warmer and dryer. It therefore could be construed that the low preharvest temperature negatively affects the postharvest incidence of CI. For future prospects, it can be useful to determine an aspect that could be related to rind maturity or condition detecting optimum harvest date to decrease CI.

Introduction

Fruit are stored at low temperatures to reduce the rate of fruit respiration and to reduce other physiological processes which lead to spoilage. Cold treatment is used to sterilize insect larvae of certain pest in citrus such as false codling moth *Cryptophle* *bialeucotreta* (Meyrick) (Lepidoptera: TortriCIdae) and Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (White and Elson, 2004; EPPO, 2007). All citrus fruit exported from South Africa to the USA, Korea, Thailand and China undergoes a mandatory 24 day temperature exposure at -0.6 °C plus a 3 day pre-cooling at the same temperature. This temperature protocol is known to cause CI to the flavedo of the citrus fruit.

Various factors could influence the susceptibility of a citrus fruit to CI i.e. type, microclimate, harvest date etc.. Differences in CI susceptibility exist between citrus type for example 'Fortune' mandarin has a higher susceptibility compared to 'Valencia' and 'Navelina' navel' oranges. 'Fortune' mandarins could only be stored for 28 days at 2 ºC whereas 'Navelina' and 'Valencia' orange could be stored for 60 days (Lafuente et al, 2003).

Microclimate and the harvest date (fruit maturity) are considered some of the main factors affecting fruit susceptibility to CI. Lindhout, (2007) observed significant differences in CI incidence between Dareton and Iraak site in Australia. Fruit maturity is also known to influence fruit CI susceptibility and Gonzalez-Aguilar et al. (2000) observed in Spain, that 'Fortune' mandarin fruit harvested early (November/ Desember, NH) and late (April, NH) in the season had a similarly, low incidence of CI. In contrast fruit that was harvested in mid-season had a higher incidence of CI.

The lipid phase transition of membranes in flavedo cells is thought to result in CI syptoms. Certain lipid domains will undergo phase transition from liquid to gel phase. These changes in the membrane fluidity were shown in plants to cause a cascade of other secondary responses such as electrolyte leakage and the disintegration of the photosynthetic system which could lead to cell death (Lyons, 1973; Raison and Orr, 1986). Reactive oxygen species, potential damaging molecules from which lipid hypdroperoxides are formed, are also thought to be involved in the development of CI (Shewfelt and Purvis 1995). The formation of lipid hydrogen peroxides in the flavedo cells causes the membrane fluidity and integrity to change and will result in membrane bound proteins not to function properly (Shewfelt and del Rosario, 2000).

The most accurate method to measure lipid peroxidation is to quantify the primary hydroperoxide products; however, it is a complex and difficult process. Malondialdehyde (MDA) is formed through auto-oxidation and enzymatic degradation of polyunsaturated fatty acids in cells. This is a secondary product of oxidation and this product reacts with two molecules of thiobaritiric acid (TBA) with an absorbance maximum at 532 nm (Kapus 1985; Janero 1990). This is used as a method to estimate the amount of lipid peroxidation in biological membranes. This method has certain lack of specificity but is considered as a reliable estimator of lipid peroxidation (Heath and Packer, 1968). There has been concern that non-MDA substances may influence the readings which is an overestimation of the lipid peroxidation. The method is therefore adjusted to compensate for the interference of carbohydrates at 440 nm (Du and Bamlage, 1992) and anthocyanin and other interference compounds at 532 nm (Hodges et al, 1999)

The aim of the study was to determine if different navel orange cultivars (*Citrus sinensis*), harvest dates as well as different sites in Citrusdal, have an influence on the susceptibility of navel orange fruit to CI. Three null hypothesis were tested i.e. that there is no difference in incidence of CI between two navel orange cultivars, that different localities in the same production area do not influence CI susceptibility and that different harvest dates do not influence CI incidence.

Materials and methods

Plant material

Three areas which were separated by an average of 20 km in the Citrusdal valley were chosen as the source for sampling orange fruit in the study. The three areas represent climatically different areas in the same valley and therefore served to create potential contrast in CI susceptibility due to climate difference. 'Washington' and 'Navelina' navel orange fruit were harvested at 4 farms in Citrusdal which differ in the amount of rainfall as well as temperature. Two farms, Hexrivier and Brakfontein, were at the bottom of the valley and were the warmest with the lowest rainfall. Tharakama, situated at the top of the valley received the highest amount of rainfall and had the lowest average temperature, whereas Ouwerf farm situated in the middle of the valley fell between these two ambient climates (Table 1 and Fig. 1).

Fruit were harvested at three different sample dates in 2011, early (2 weeks before commercial harvest, 11 May), commercial harvest (25 May) and two weeks later (9 June). For each treatment 60 fruit were harvested to ensure enough fruit for four evaluation dates during cold storage and for each evaluation date three replicates of five fruit were used. Fruit were stored at -0.6 °C for 35 days and was evaluated on day 1, 7, 21 and 35 for CI symptoms and ethylene production. The flavedo was peeled for the estimation of lipid peroxidation by using thin fruit peelers and frozen in liquid nitrogen where after it was stored at -80ºC and freeze dried before it was finely ground prior to the estimation of the peroxidation of lipids.

In 2012 fruit were harvested at the same sites and trees, and the same harvest dates were used. Fruit were stored for longer, i.e. 40 d, to ensure higher levels of CI to create greater contrast between treatments.

Data collection

Rind colour attribute, lightness (L*), chroma (C), and hue angle (H) were measured using a Minolta chroma meter (Model CR200; Minolta Camera Osaka, Japan). Colour measurements were taken at 2 sides of fruit to include lighter and darker rind colour of fruit. The lightness, gave an indication of dark vs. light, chroma represent intensity of a colour (higher value increase intensity) and hue angle determine which colour is measured (0 red to purple, 90º yellow and 150º green).

Fruit size (height and diameter) was determined using a digital calliper Mitutoyo (Mitutoyo Corporation, Kawasaki, Japan). For internal quality, fruit were juiced using a citrus juicer (Sunkist®, Chicago, USA). Juice was filtrated through a muslin cloth and juice content (%) was determined by dividing the weight of the juice by the total fruit weight. The total soluble solids (TSS) °Brix from the extracted juice was determined using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan). Titratable acidity (TA) expressed as citric acid content was determined by titrating 20 mL of the extracted juice against 0.1 N sodium hydroxide using phenolphthalein as an indicator.

CI incidence were determined in 2011 after 7, 21 and 35 d of storage at -0.6 °C and after 1 week at shelf life (20 °C) the fruit was evaluated for any CI symptoms. In 2012 the fruit were stored for 40 d. The CI data was collected to illustrate incidence and expressed as percentage CI, as well as severity of the injury express as CI index (Fig. 2). The incidence data was collected as in yes/no whereas the severity fruit was scored from 0 to 3 and calculated with the following equation:

1. $$\text{Chilling injury index (0-3)} = \sum \frac{[\text{Chilling injury (scale 0-3)} \times \text{no. of fruit in each class}]}{\text{Total number of fruit in replicate}}$$

During 2011 *ethylene production* was measured.in the cold room (-0.6 °C) at all the evaluation dates 1, 7, 21, 35, as well as after 1 week shelf life. In order to measure ethylene each of the replicates consisting of 5 fruits were placed in sealed bottles for 5 hours. One sample per replicate was drawn out of the glass bottle via plastic septum into an airtight syringe which was measured with a flame ionization gas chromatograph (Varian, Model 3300, Varian instrument Group, Palo Alto, California, USA). The volumes of the oranges as well as the weight were determined to calculate the ethylene production ($\mu l.g^{-1}.h^{-1}$).

Lipid peroxidation was determined with the modified method of Heath and Packer (1968). This method consists of the malondialdehye (MDA) assays, which can be used to estimate the amount of lipid oxidation in biological membranes and biological systems from values determined by spectrophotometry:

2. $$\text{MDA equivalents (nmol.mL}^{-1}) = [(A_{532}-A_{600})/155000]\ 10^{6}$$

In this formula 532 nm represents the maximum absorbance, 600 nm the correction for nonspecific turbidity, and 155000 the molar extinction co-efficient for MDA. Du and Bramlage (1992) improved the accuracy of the method for the interference of TBA-sugar complexes and Hodges et al. (1999) improved the accuracy further for interference of other compounds i.e. anthocyanin by subtracting absorbance at 532 nm of a solution of plant extract incubated without TBA.

For this experiment during 2011, freeze dried material (0.1g) of the flavedo was added to 25 mL of 80% ethanol (g $DW.mL^{-1}$) and placed on the shaker for two min and followed by centrifugation at 3000g for ten min. Afterwards one mL of the sample aliquot was added to a glass Kmax tube with one mL of either the solution with no TBA [containing 20% Trichloratic acid and 0.01% butylated hydroxytoluene (TCA)] or solution with TBA [containing the same as the solution with no TBA plus 0.65% TBA]. The samples were mixed and heated at 95ºC for 25 minutes on a heatblock (Multiblok , Lab Line instruments, I ll., USA). There after the samples were placed on ice for 10 minutes and centrifuged for 10 min at 3000g. The absorbance of the samples were read at 532, 600, 440 nm (Ultraspec 3000, Pharmacia Bitech Cambridge, UK). The calculation to detect the MDA equivalents were as follows:

3. $$[(Abs\ 532_{+TBA})-(Abs\ 600_{+TBA}) - (Abs\ 532_{-TBA} - Abs\ 600_{-600}) = A$$

$$[(Abs\ 440_{+TBA} - ABS\ 600_{+TBA})\ 0.0571] = B$$

$$\text{MDA equivalents } (nmol.mL^{-1}) = (A-B/157000)\ 10^{6}$$

As part of the experiment two methods by Heath and Packer (1968) and the Hodges et al. (1999) were tested and compared in the estimation of MDA equivalents. The results indicated that in the method by Heath and Packer (1968) an overestimation of the MDA occurred (Fig.3). The same trend is observed in both methods; however, the modified method of Hodges is more accurate for quantifying TBA-MDA levels in tissue containing sugars, anthocyanins and other interfering compounds. The estimation of lipid peroxidation in tissues normally contains sugars, anthocyanin or other interfering compounds which would absorb at 532nm and it could also up regulate these through environmental stresses (Hodges et al. 1999).

The aim of this experiment was to estimate differences between the treatments i.e. cultivar, harvest time and location and not the precise estimation of the MDA equivalents in the flavedo. This biochemical analysis was done to offer a possible explanation of the differences in chilling susceptibility between treatments. Therefore only the method of Heath and Packer (1968) was used in this study (equation 2).

Statistical analysis

Data of the CI index (ranked data) was analysed by using the one way Anova and two way Anova (test interaction) using SAS (SAS v.6.12; SAS Institute, Cary, VC, USA). Each treatment was compared with the other using Fischer least significance differences (LSD). The *p*-values illustrate the significance differences in the figures presented. If *p*-value is smaller than 0.05 there is significant differences between treatments. Data that was not analysed by Anova was given with the standard deviation.

Results

Cultivar difference in CI sensitivity

There was no significant difference between incidences of CI for the 'Washington' and 'Navelina' navel oranges harvested in 2011. However, 'Washington' navel orange had a 10% higher CI incidence than 'Navelina' navel (Fig. 4). The low incidence of CI probably leads to a result of no-significance. In 2012 the fruit was stored for 40 days which resulted in significant difference in CI incidence between the two cultivars, with 'Washington' more susceptible to CI than 'Navelina' navel fruit. Although tree variation in suceptibility and amount of trees used in this experiment may have also been a contributing factor.

Influence of microclimate on CI

No significant difference was found in CI incidence and CI index for 'Navelina' oranges between the various sites in 2011 and 2012 (Fig. 5). However, the same pattern is evident from both seasons with 'Navelina' orange fruit from Tharakama (cold area), being more susceptible compared to lower down in the valley.

There was no significant difference between the different sites for 'Washington' navel oranges in 2011 even though the highest incidence of CI per region was recorded at

Tharakama (Fig. 6). In 2012 there was a difference between fruit from Hexrivier and Brakfontein (both at the bottom of the valley). However, the same trend of high CI susceptibility from fruit from higher up in the valley was seen with a significant difference in CI incidence between Hexrivier and Tharakama.

Harvest date

Analysis of the CI incidence for the sites and harvest date show that the interaction between the sites and harvest date was not significant. Analysing the CI index from 2012 for different harvest dates at each farm separately indicate significant differences between different harvests dates (Fig. 7). CI incidence and the CI index follow the same trend for each site and harvest date. ‘Washington’ navel orange fruit from Tharakama had a difference in CI between early and late harvested fruit (late fruit lowest and early fruit highest) and at Hexrivier significant difference in CI occurred between all the harvest dates with the lowest incidence of CI at commercial harvest date. At Brakfontein and Ouwerf there were no significant differences between the different harvest dates; however, the same trend occurred with the lowest incidence of chilling injury at commercial harvest date (Fig. 8).

Combining the CI data of each cultivar from different sites for analysis to determine the difference between the three different harvest dates in both 2011 and 2012 indicated that there were no significant difference in CI incidence (Fig. 7 and 9) . However, for both cultivars it was evident that the commercial harvest date had the lowest incidence of CI and it translated to 17 % and 15 % less CI compared to fruit harvested early and late respectively

‘Navelina’ navel orange fruit had no significant differences between the different harvest dates at the different sites (Fig. 10). However, the same trend as seen in ‘Washington’ navel can be seen with fruit harvested on the commercial harvest date being the least susceptible.

Ethylene production

Ethylene production rate ($\mu L.g^{-1}.h^{-1}$) for fruit measured at ambient conditions after harvest was high but decreased toward day seven when placed in cold storage and increased again from day 21 (Fig.11). 'Navelina' navel orange fruit harvested early at Ouwerf and Tharakama showed a steady increase in ethylene production from day seven until day 35 corresponding with incidence of CI on day 35 and commercially harvested fruit from Ouwerf had no increase in the ethylene production at day 35 corresponding with no CI incidence. Although 'Navelina' late harvested at Ouwerf fruit showed an increase in ethylene production without incidence of CI (Fig.11). Furthermore commercially harvestd fruit at Tharakama had no increase in ethylene but showed CI incidence (Fig. 11). 'Washington' navel orange fruit harvested early and late at Brakfontein had a spike in ethylene production on day 21 and a low and high incidence of CI on day 35, respectively. However for the commercial harvested fruit there was an increase in ethylene production at day 35 with low incidence of CI. 'Washington' navel fruit harvested early and late at Tharakama show a high and moderate increase in ethylene production at day 35 with a high incidence of CI, however the commercial harvested fruit also show an increase in ethylene production at day 35 with no incidence of CI (Fig 11).

Maturity index

The maturity index for the four or three different sites differed significantly for 'Washington' navel and 'Navelina' navel fruit (Fig.12, 13). The TSS and TA of the 'Washington' navel harvested at Hexrivier were significantly lower compared to Brakfontein, Tharakama and Ouwerf. Tharakama and Brakfontein had the highest TSS. The maturity index (TSS:TA ratio) of 'Washington' navel fruit between the different harvest dates did not differ significantly at Hexrivier, Ouwerf and Tharakama however at Brakfontein the late harvested fruit significantly differ from the early and commercial harvested fruit (Table 2). The maturity index of 'Navelina' navel fruit didn`t differ significantly at Hexrivier and Tharakama between the different harvest dates however at Ouwerf the late harvested fruit had a significantly higher TSS:TA ratio compared to the early and commercial harvested fruit (Table 3).

Lipid peroxidation

There was no specific trend for the MDA estimation with CI, however lipid peroxidation seemed to be linked to maturity rather than CI. Some of the graphs have an increase in the MDA concentration at day 35 and some of the graphs a decrease in the concentration. The different harvest dates also vary at the different sites. However at Hexrivier the late harvested 'Washington' navel fruit had a higher MDA concentration from day 1 to 21 and Ouwerf and Brakfontein also show a higher MDA concentration at day 7 and 21 when fruit are harvested late. At Tharakama the late and commercial harvested fruit had a higher MDA concentration at day 1 and 7 compared to early harvested fruit however at day 35 the early and the commercial harvest date has a higher MDA concentration (Fig.14). The 'Navelina' navel fruit at Hexrivier show a higher MDA concentration when harvested late and commercially from day 1-21 and at Ouwerf the late and commercial harvested fruit had a higher MDA concentration compared to the Early harvested fruit (Fig. 15).

Discussion

Susceptibility to CI varies between navel orange cultivars while 'Washington' was more susceptible than 'Navelina'. Similar results were reported by Lindhout (2007), where 'Navelina' navel demonstrated low CI severity compared to 'Thomson' navel, moderately sensitive, and 'Roberts' navel orange fruit with a very high sensitivity to CI temperatures.

The variation in CI susceptibility of 'Washington' navel between the different sites showed that the top of the valley (cold area) had the highest incidence of CI for fruit harvested in 2011 and 2012 even though Brakfontein at the bottom of the valley had a high incidence of CI in 2012. Gonzalez-Aguilar et al. (2000) reported that 'Fortune' mandarin harvested in mid-season, the cooler months of the year, had a higher incidence of CI. The difference in CI susceptibility between harvest dates could be influenced by two factors i.e. ambient temperature and fruit maturity.

In Florida, grapefruit that were harvested in the midseason had the lowest incidence of CI compared to early or late harvested fruit (Purvis et al., 1979) and were thought to be a result of immaturity and over maturity early and late in the season. In contrast, Gonzalez-Aguilar et al. (2000) reported that in Spain 'Fortune' mandarin fruit harvested early and late in the season had similar, although low incidence of CI. The fruit that were harvested in the midwinter had the highest incidence of CI. This increase in CI was thought to be a result of low temperature stress while fruit was still on the tree. CI in this study at the bottom of the valley Hexrivier, Brakfontein and Ouwerf follows in general a similar pattern to Florida, with the early and late fruit being more susceptible, however, Tharakama show similarity to Purvis et al, 1979 where fruit harvested at the coldest site has the highest incidence of CI.

Fruit that are harvested late could be over mature and more susceptible to CI. Coggins (1969) reported that during maturation in citrus fruit, the albedo and flavedo cells enlarge and can become very vacuolated, the tissue can become structurally weak and spongy and the epicuticular wax could have more micro cracks consequently resulting in less resistance to the escape of water vapour from the rind. Schirra and D'hallewin (1997) reported that 'Fortune' mandarin harvested in the mid-season and dipped in warm water (53 °C) reduced the incidence of CI and no heat damage occur, but early and late harvested fruit were susceptible to heat damage, indicating the effect of fruit maturity on the rind physiological condition. The immature fruit was also more susceptible to CI which could be related to rind pigments not synthesising to a threshold level needed for oxidative stress protection. Dou (2004) reported that 'Rio Red' grapefruit has a higher lycopene and B-carotene concentration with a consistently lower incidence of CI compared to the other cultivars. Lower temperature is needed for Gibberellin (GA) to be inhibited which influence chloroplast breakdown and the carotenoid production where there is conversion of chloroplast into a chromoplast. Carotenoids scavenge for ROS free radicals such as the Xanthophyl cycle which is involved in non-photochemical quenching. Violaxanthin is converted into Zeaxanthin (Taiz and Zeiger, 2010). Binding of protons and Zeaxanthin to light harvesting antenna proteins is thought to cause conformational changes that lead to quenching and heat dissipation (Taiz and Zeiger, 2010; Demmig-Adams and Adams, 1992) which reduces the oxidation of membranes.

The measurement of malondialdehyde did not show a specific trend in relation to CI development and cold storage duration. However in this study, duration of cold storage could increase MDA due to increase in the lipid peroxidation with incidence of CI. Certain fruit harvested early, late or commercially at different sites show an increase in MDA concentration but others show a decrease when stored for 35 days (Fig.14, Fig.15). An explanation could be that fruit variation could have contributed to the high amount of variation between the measurements. However some of the sites did show that the late harvested fruit had a higher lipid peroxidation compared to the early harvested fruit. Late harvested fruit rind is older or more senesced and is more prone to lipid peroxidation. It could be that there are still other compounds in citrus rind that interfered with the measurements which are not excluded. For future experiments it is advisable that high performance liquid chromatography (HPLC) should be used to measure the specific lipid peroxidation in the rind of the orange.

The internal maturity at harvest does not appear to be related to the development of CI in navel orange fruit. The TSS of the different sites increased or did not change between successive harvests for the different sites while CI incidence decreased towards commercial harvest and increase at late harvest in 2011. Similarly Lindhout (2007) also reported that maturity does not appear to be related to CI. The TSS and the maturity index increased and TA decreased between two sites in Australia (Dareton and Iraak) but the CI only increased significantly in fruit harvested from Dareton site. This result reinforced the concept of the rind and the pulp being physiologically independent.

Ethylene production is known to have a positive relationship with increased incidence of CI. Lafuente et al. (2003) reported that the increase in ethylene and phenylalanine ammonia-lyase (PAL) is a cold induced response which is stimulated when the fruit develop CI. PAL is the initial rate controlling enzyme in the phenolpropanoid pathway which synthesise phenolic compounds which are known to have antioxidant properties. There was a corresponding increase in CI incidence with a spike in the ethylene production for some of the early and late harvested fruit however certain fruit show an increase in ethylene with no incidence of CI. Late harvested 'Navelina' navel from Tharakama did not show CI or an increase in ethylene production which concur with Lafuente et al. (2003) which reported that 'Navelina' navel' stored at 2 °C for 60 days did not show CI or an increase in ethylene production and PAL activity. However, at Tharakama, the late harvested 'Navelina' orange fruit had an increase in ethylene production at day 35 with no incidence of CI.

Lafuente et al. (2003) reported that there was an increase in the PAL activity of 'Fortune' mandarins and 'Valencia' but not any increase with navel oranges when stored at chilling temperatures. PAL is a defensive response against chilling stress but for certain citrus fruits it isn`t directly related to chilling tolerance. The induction of ethylene as well as PAL activity is induced by cold temperatures and not a cause of CI (Lafuente et al., 2003).

To conclude, there is distinction in CI susceptibility between the two different cultivars with 'Navelina' being more susceptible than 'Washington' navel orange. In commercial handling, particular attention should be given to 'Washington' navel because of its high susceptibility to CI. For the different sites in Citrusdal the highest incidence of CI was at Tharakama (coldest part) at the top of the valley with the lowest average temperature during the year. The incidence of CI was overall less when fruit were harvested at the commercial harvest date. For future prospects the estimation of lipid peroxidation of the citrus rind could be done using high performance liquid chromatography (HPLC) procedure. The identification of an biochemical product that could be related to rind maturity could be useful in detecting optimum harvest date to decrease CI.

References

Coggins, C.W. Jr. 1969. Giberellin research on citrus rind ageing problems. Proc. 1st Int. Citrus Symp. 3: 1177-1185.

Deming-Adms, B. and Adams, W.W., III. 1992. Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:599-626.

Dou, H. 2004. Effect of coating application on chilling injury of grapefruit cultivars. Postharvest Biol. Technol. 39: 558-561 Du, Z. and Bramlage, W.J. 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J. Agric Food Chem. 40: 1566-1570.

EPPO (European and Mediterranean Plant Protection Organization), 2007. Data sheets on quarantine pests. *Ceratitiscapitata*. Accessed 20/06/2012 http://www.eppo.org/QUARANTINE/insects/*Ceratitis capitata*/CERTCA ds.pdf.

Gonzalez-Aguilar, G.A., Zacarias, L., Perez-Amador, M.A., Carbonell, J. and Lafuente, M.T. 2000. Polyamine content and chilling susceptibility are affected by seasonal changes in temperature and by conditioning temperature in cold stored 'Fortune' mandarins. Physiol. Plant. 108: 140-146.

Heath, R.L. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125: 180-198.

Hodges, M.H., DeLong, J.M., Forney, C.F. and Prange, R.K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207: 604-611.

Janero, D.R. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biol Med 9: 515-540

Kapus, H. 1985. Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. Chapter 3 In: Sies, H. (ed.) Oxidative stress. Academic Press, London, UK, pp 273-310.

Lafuente, M.T. Zacarias, L., Martínez-Téllez, M.A., Sanches-Ballesta, M.T. and Granell, A. 2003. Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. Postharvest Biol. Tec. 29: 309-318.

Lindhout, K. 2007. Physiology of chilling related postharvest rind breakdown of Navel oranges (*Citrus sinensis* (L.) Osbeck). PhD dissertation, La Trobe University, Bundoora, Australia.

Lyons, J.M. 1973. Chilling injury in plants. Ann. Rev Plant. Physiol**.** 24,445–466

Purvis, A.C., Kawada, K. and Grierson, W. 1979. Relationship between mid-season resistance to chilling injury and reducing sugar level in grapefruit peel. HortScience.14: 227-229.

Raison, J.K. and Orr, G.R. 1986. Phase transitions in liposomes formed from the polar lipids of mitochondria from chilling sensitive plants. Plant Physiol.81: 807-811.

Schirra, M. and D'hallewin, G. 1997. Storage performance of 'Fortune' mandarins following hot water dips. Postharvest Biol. Technol. 10: 229-237.

Shewfelt, R.L. and del Rosario, B.A. 2000. The role of lipid peroxidation in storage disorders of fresh fruit and vegetables. HortScience. 35: 575-579.

Shewfelt, R.L. and Purvis, A.C. 1995. Toward a comprehensive model for lipid peroxidation in plant tissue disorders. HortScience. 30: 213-218.

Taiz, L. and Zeiger, E. 2010. Plant physiology.5th ed. Sinauer Asssociates. Sunderland USA .pp 190-191,252

White, I.M. and Elson, M.M. 2004. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International—The Australian Centre for International Agricultural Research, Eastbourne, UK.

Tables and Figures:

Table 1. Detailed information of the different sites in the Citrusdal valley that were used in 2011 and 2012 to sample Navel orange fruit.

Location in valley	**Farm and climate (avg. values over year)**	**Cultivar**	**Row orientation**	**Plant density (m)**	**Year planted**	**Rootstock**	**Irrigation**	**Avg. yield (ton/ha)**
Top	Tharakama 32°85'S, 19°09'E Avg. rainfall: 550 mm Avg. max: 27 °C Avg. min: 9 °C Elev. 233 m	Washington	North-South	7.6 × 3.8	1950	Rough lemon (*C. jambhiri* Lush)	Micro	30-35
		Navelina	North-South	6 × 3	1950	Rangpur lime (*C. limonia* Osbeck)	Drip	35
Middle	Ouwerf 32°65'S 19°05'E Avg. rainfall: 300 mm Avg. max: 28.1 °C Avg. min: 10.2 °C Elev. 180 m	Washington	North-South	6 × 3	1976	Rough lemon	Micro	50
		Navelina	North-South	6 × 4	1995	Rough lemon	Drip	38
Bottom	Brakfontein 32°.51'S, 18°99' E	Washington	East-West	5.7 × 3	1982	Rough lemon	Drip	40
	Hexrivier 32°.47' S, 18°97'E Avg. rainfall: 250 mm Avg. max: 29.2 °C Avg. min: 11 °C Elev. 151 m	Washington	North-South	6.1 × 6.1	1931	Rough lemon	Micro	24
		Navelina	North-South	5.5 × 5.2	1996	Rough lemon	Drip	25

Table 2. TSS:TA average of 'Washington' navel orange fruit harvested at 3 harvest dates early (11 May 2011) commercial (25 May 2011) and late (9 June 2011) at 4 different sites Hexrivier and Brakfontein (the bottom of the valley), Ouwerf (middle of the valley) and Tharakama (top of the valley).

	TSS:TA			
	Hexrivier	Brakfontein	Ouwerf	Tharakama
Early	10.96	9.53a	10.09	12.12
Comm.	11.24	10.08a	10.50	12.16
Late	11.59	13.74b	11.00	12.23
p-value	0.102	0.0001	0.208	0.987

Table 3. TSS:TA average of 'Navelina' navel orange fruit harvested at 3 harvest dates early (11 May 2011) commercial (25 May 2011) and late (9 June 2011) at 3 different sites Hexrivier (the bottom of the valley), Ouwerf (middle of the valley) and Tharakama (top of the valley).

	TSS:TA		
	Hexrivier	Ouwerf	Tharakama
Early	11.32	9.74b	11.29
Comm.	11.16	13.04a	10.07
Late	12.17	13.83a	
p-value	0.06	0.004	0.102

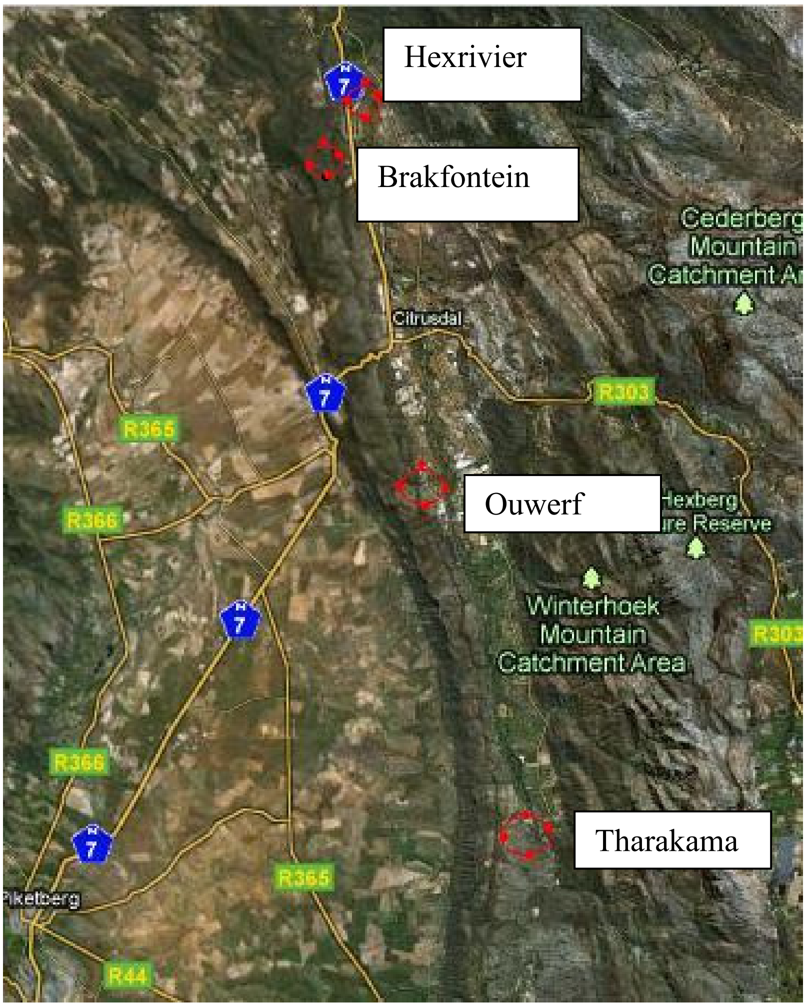


Figure 1. Map of the four different sites where 'Washington' and 'Navelina' navel were harvested. Hexrivier and Brakfontein (bottom of the valley), Ouwerf (middle of the valley) and Tharakama (top of the valley) in Citrusdal

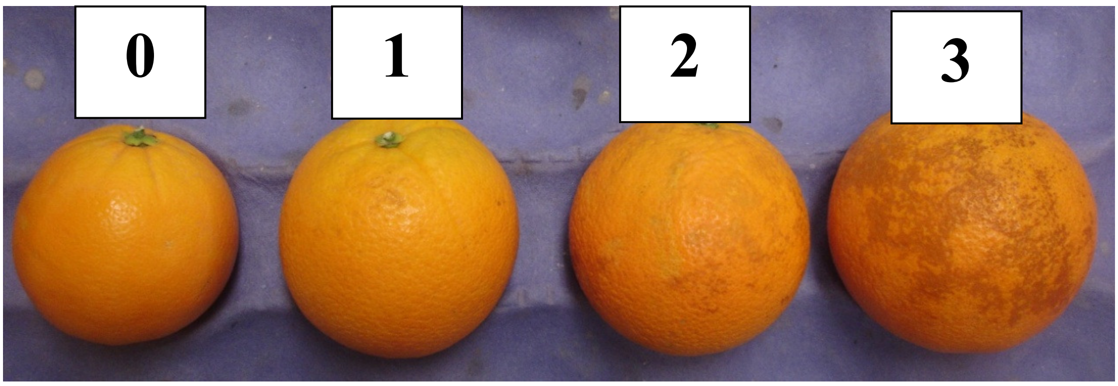


Figure 2. Chilling injury (scalding) scoring of 'Washington' navel orange according to severity.

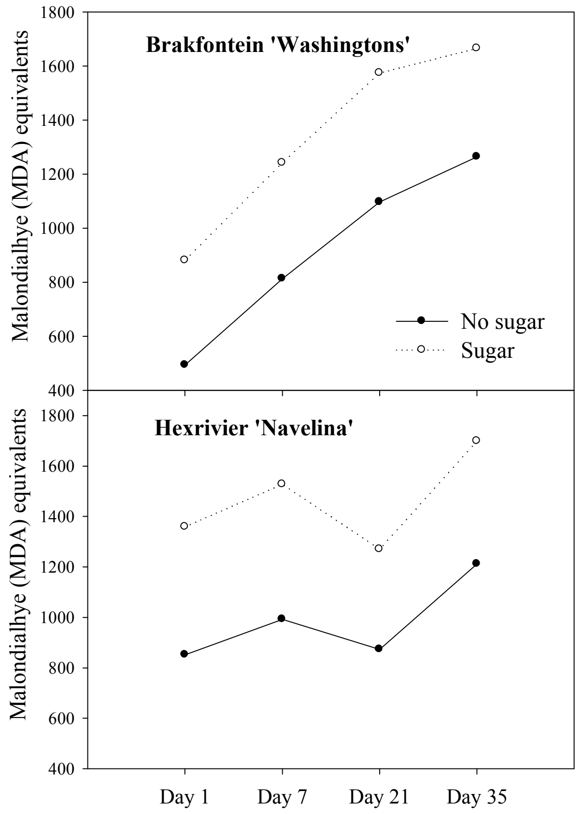


Figure 3. MDA equivalents in the flavedo of ‘Washington’s’ and ‘Navelina’ navel orange fruit after harvest and after storage at -0.6 °C for 7, 21 and 35 days. MDA equivalents were measured according to Heath and Packer (1968) (sugar) and Hodges et al. (1999)(no sugar).

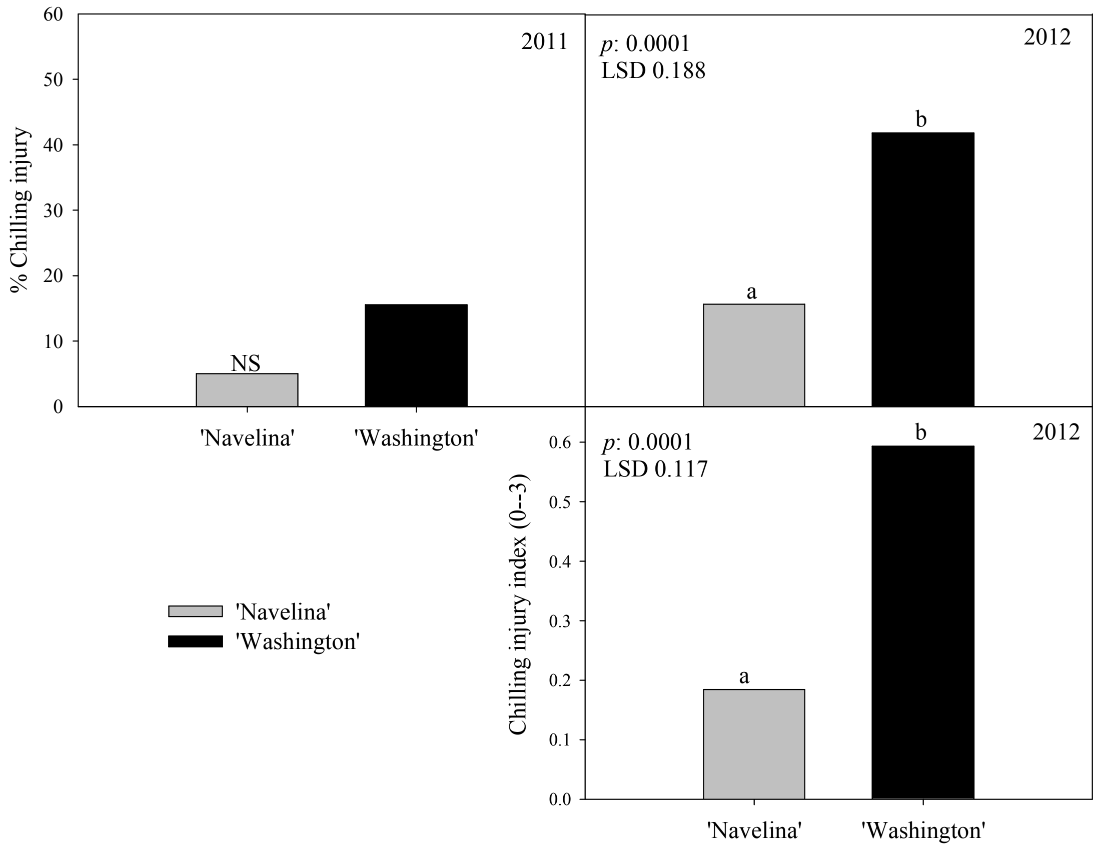


Figure 4. Incidence (% CI) and chilling injury severity index (0-3) of 'Washington' and 'Navelina' navel orange fruit harvested during 2011 and 2012 and stored at -0.6 °C for 35 d (in 2011) and for 40 d (in 2012), followed by one week at 20 °C. Different letters indicate significant differences at 95% level ($p \leq 0.05$).

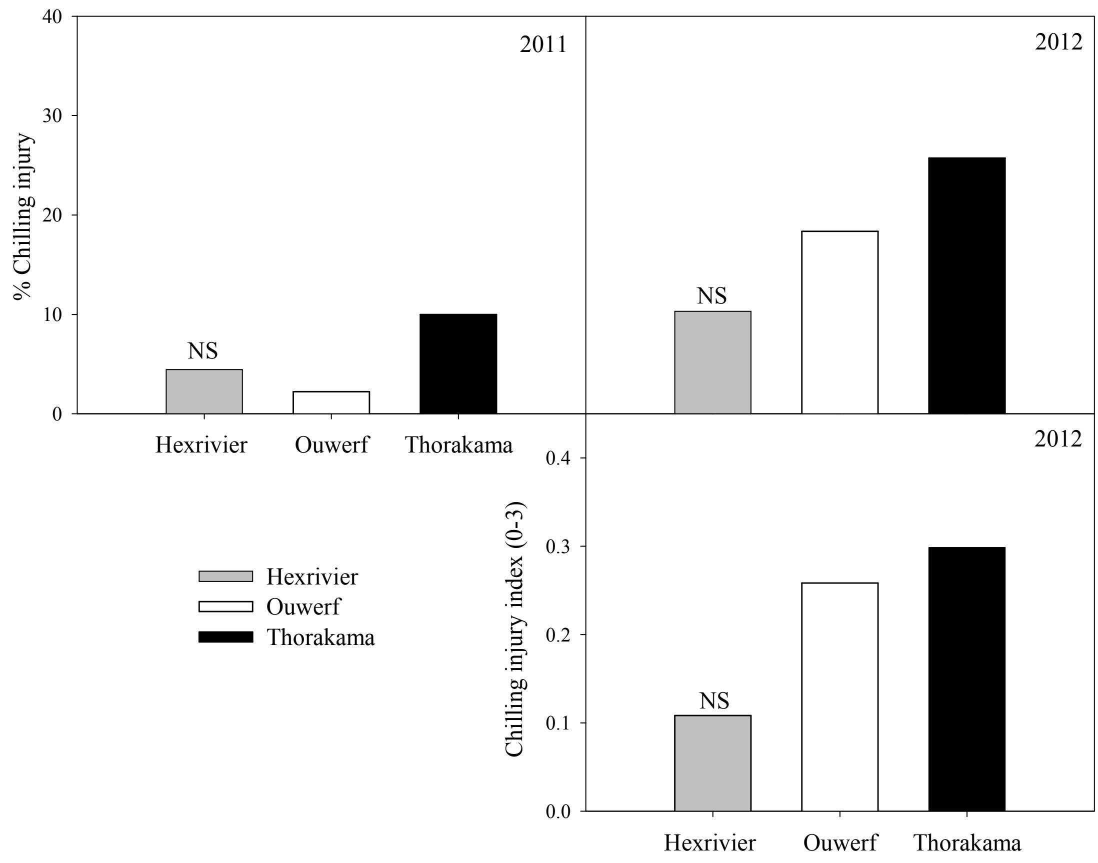


Figure 5. Incidence (% CI) and chilling injury severity index (0-3) of 'Navelina' navel orange fruit harvested during 2011 and 2012 at different sites in Citrusdal viz. Hexrivier (bottom of the valley) Ouwerf (middle of the valley) and Tharakama (top of the valley) and stored at -0.6 °C for 35 d (in 2011) and for 40 d (in 2012), followed by one week shelf life at 20 °C ($p \leq 0.05$).

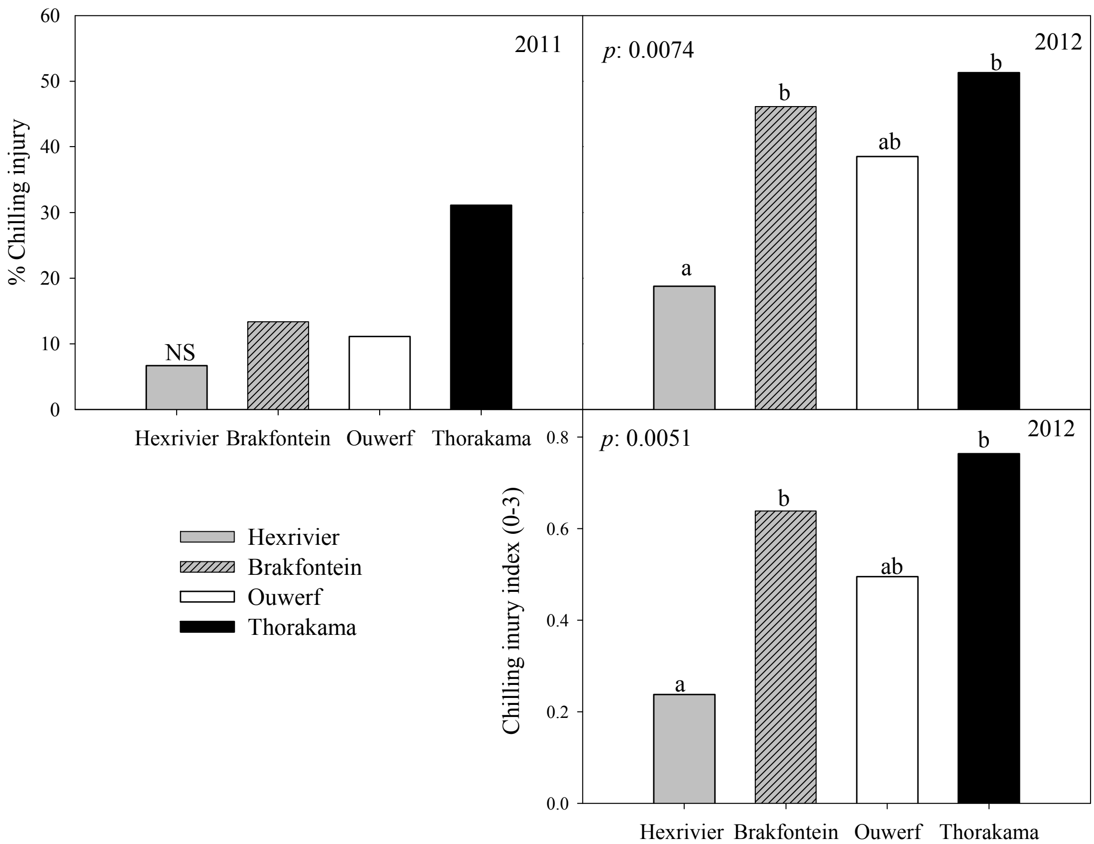


Figure 6. Incidence (% CI) and chilling injury severity index (0-3) of 'Washington' navel orange fruit harvested during 2011 and 2012 at different sites in Citrusdal viz. Hexrivier and Brakfontein (bottom of the valley), Ouwerf (middle of the valley), Tharakama (top of the valley) fruit was stored at -0.6 °C for 35 d (in 2011) and for 40 d (in 2012), followed by one week shelf life at 20 °C ($p \leq 0.05$).

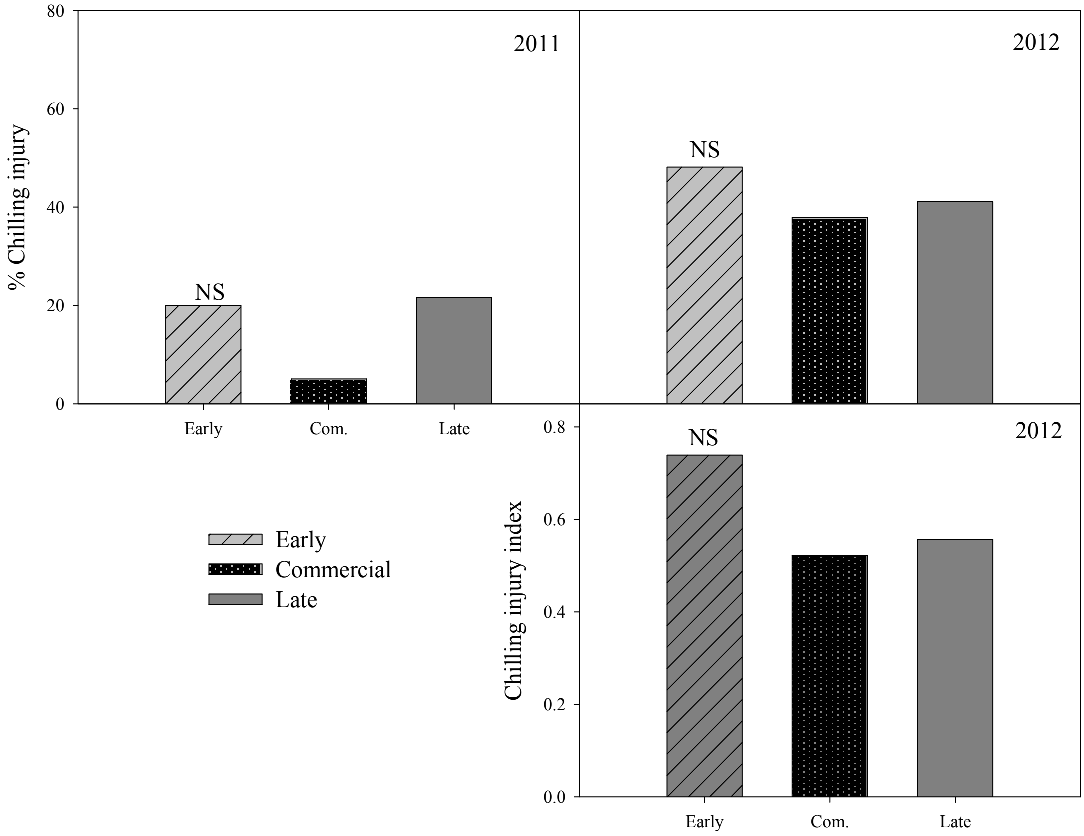


Figure 7. Incidence (% CI) and chilling injury severity index (0-3) of 'Washington' navel orange fruit harvested during 2011 and 2012 at 3 different dates, early (11 May), commercial (25 May) and late (9 June) stored at -0.6 °C for 35 d (in 2011) and for 40 d (in 2012), followed by one week at 20 °C ($p \leq 0.05$).

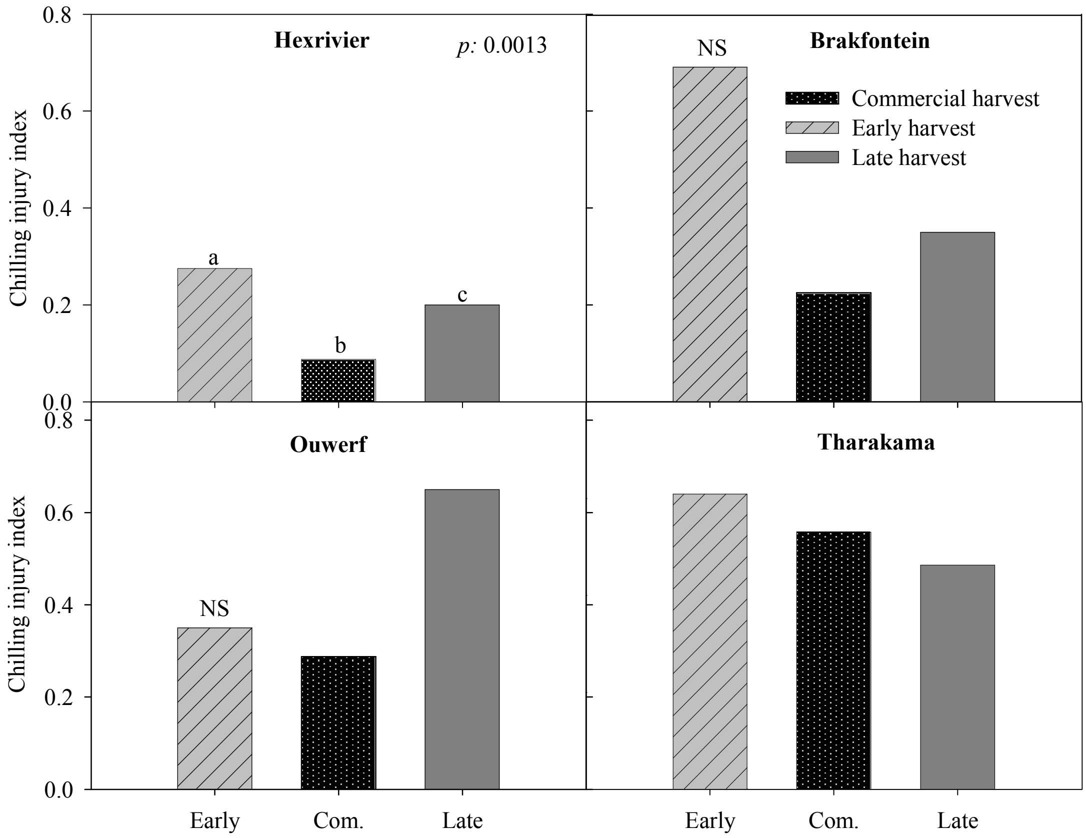


Figure 8. Chilling injury index (0-3) of 'Washington' navel orange fruit harvested at four different sites in Citrusdal during 2012: Hexrivier, Brakfontein (bottom of the valley) Ouwerf (middle of the valley) and Tharakama (top of the valley) and at 3 different dates i.e. early (11 May), commercial (25 May) and late (9 June) which was stored for 40 d at -0.6 °C, followed by one week at 20 °C ($p \leq 0.05$). Hexrivier p: 0.0013; LSD 0.0563.

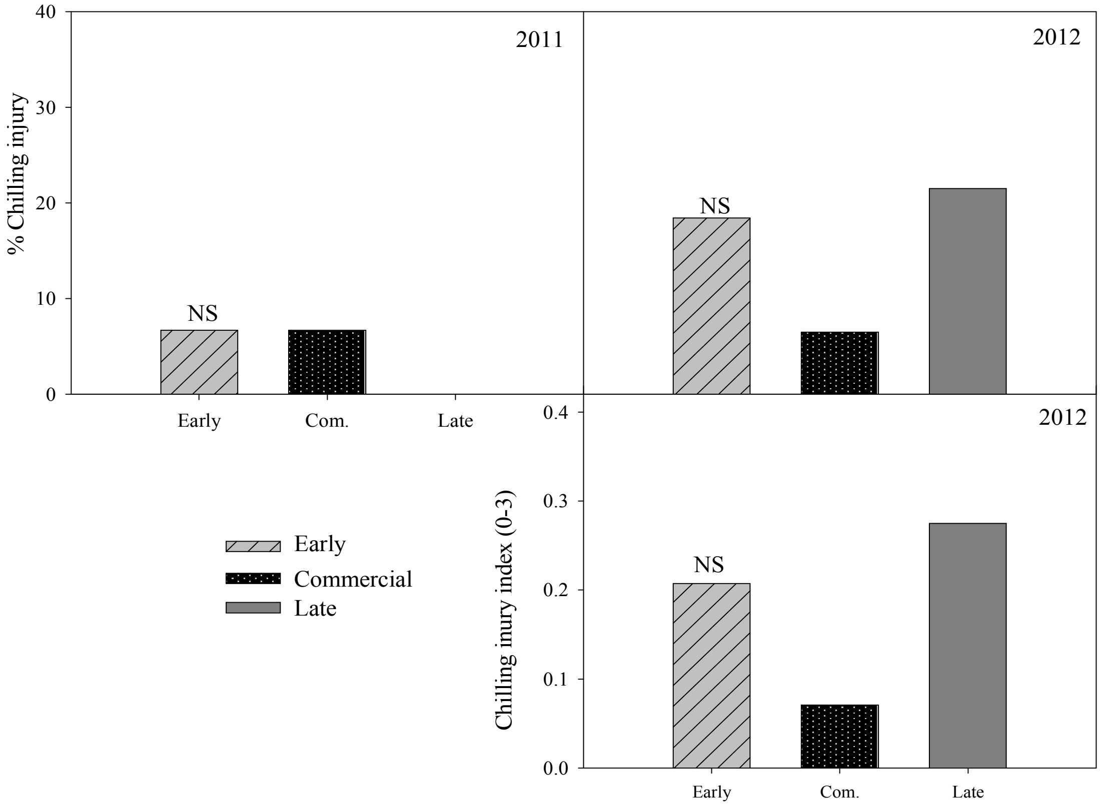


Figure 9. Chilling injury index (0-3) and chilling injury incidence (% CI) of 'Navelina' navel orange fruit harvested during 2011 and 2012 at 3 different dates, early (11 May), commercial (25 May) and Late (9 June) stored at -0.6 °C for 35 d (in 2011) and for 40d (in 2012), followed by 1 week at 20 °C ($p \leq 0.05$).

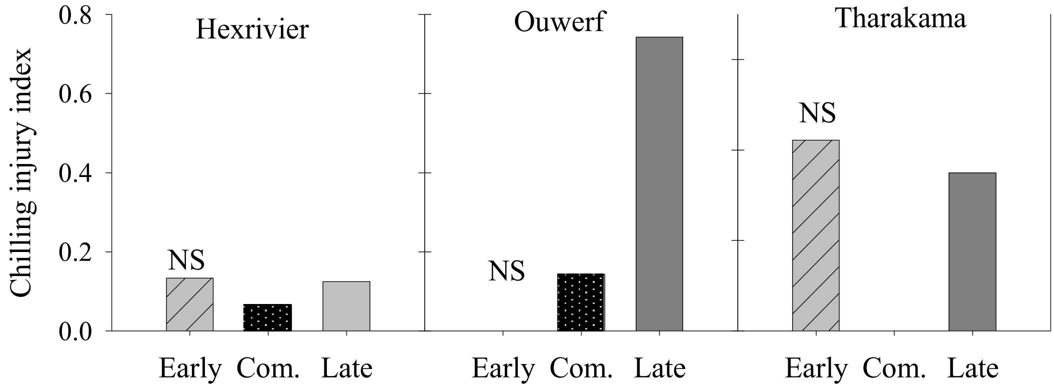


Figure 10. Chilling injury index (0-3) in 2012 of 'Navelina' navel orange fruit harvested at three different sites, Hexrivier, (bottom of the valley) Ouwerf (middle of the valley) and Tharakama (top of the valley) and at 3 different dates, early (11 May), commercial (25 May) and Late (9 June) which was stored for 40 d at -0.6 °C, followed by one week at 20 °C ($p \leq 0.05$).

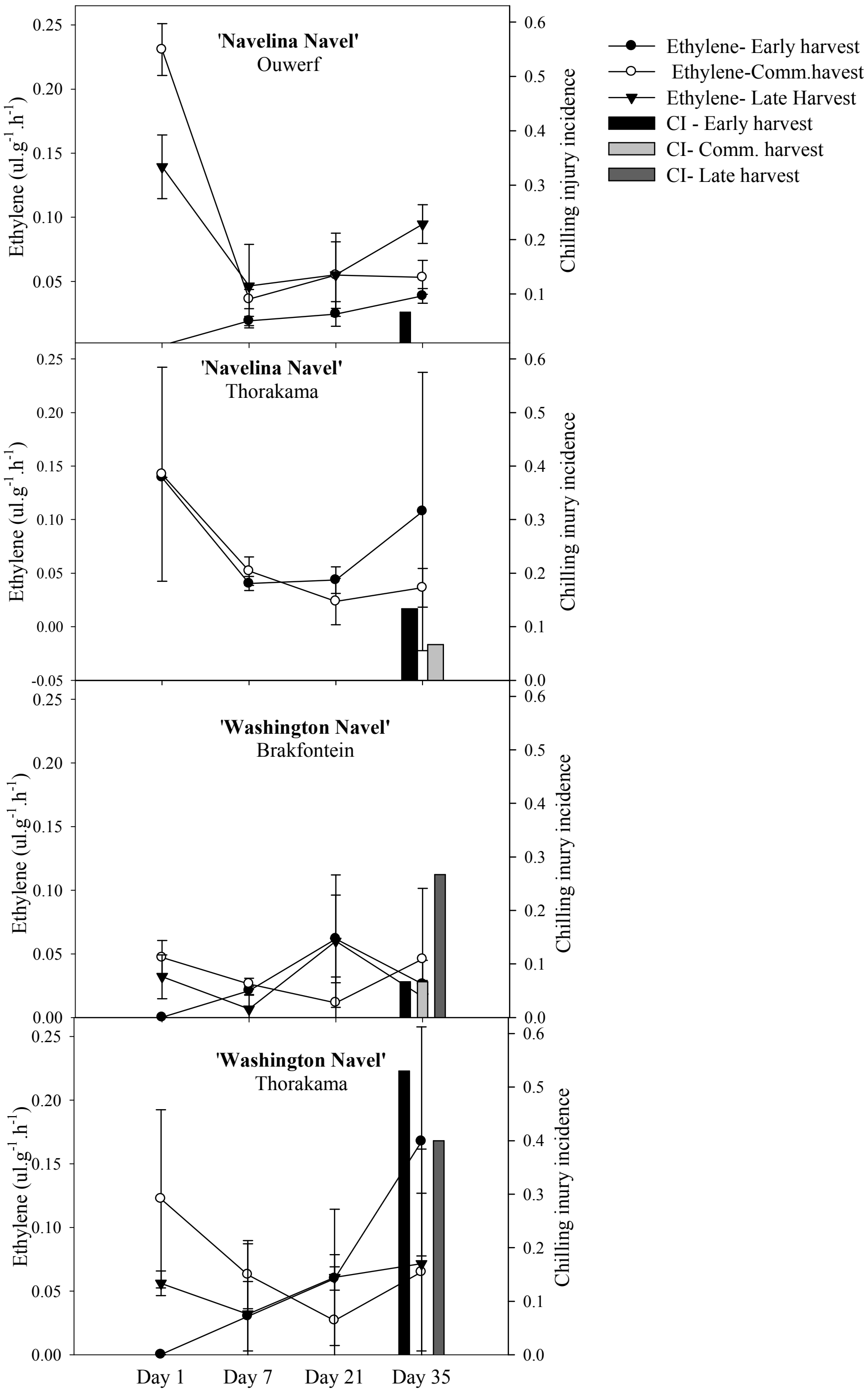


Figure 11. Ethylene production ($\mu L.g^{-1}.h^{-1}$) and chilling injury severity index after cold storage and chilling injury incidence (% CI) of 'Navelina' navel orange fruit from Ouwerf and Tharakama and 'Washington' navel orange fruit from Brakfontein and Tharakama harvested in 2011 in Citrusdal at day 1, day 7, day 21 and day 35 of storage at -0.6 °C.

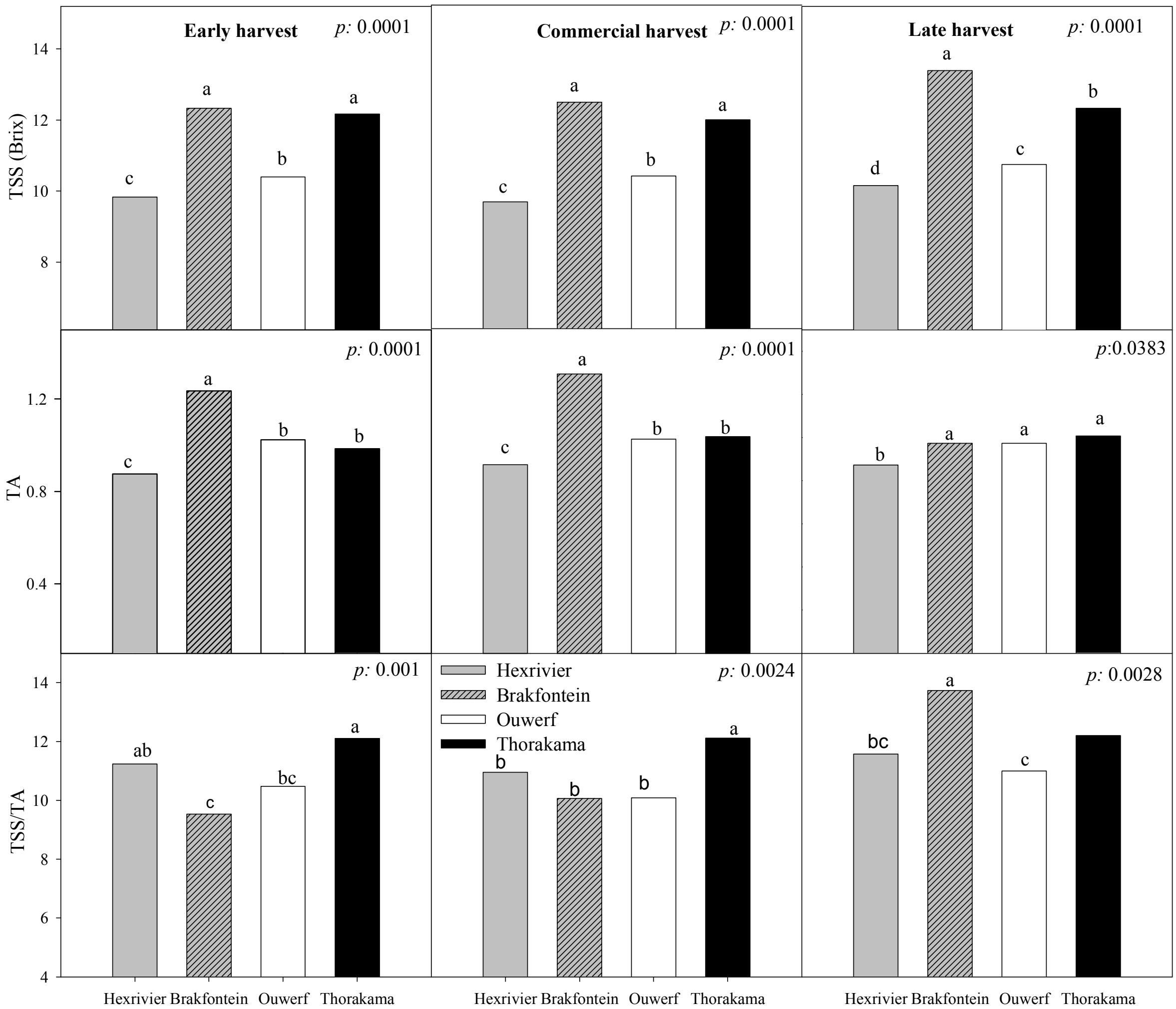


Figure 12. Maturity index (TSS and TA) of 'Washington' navel orange fruit harvested at 3 harvest dates (11, 25 May and 9 June 2011) at 4 different sites Hexrivier, Brakfontein (both bottom of the valley), Ouwerf (middle of the valley) and Tharakama (top of the valley)

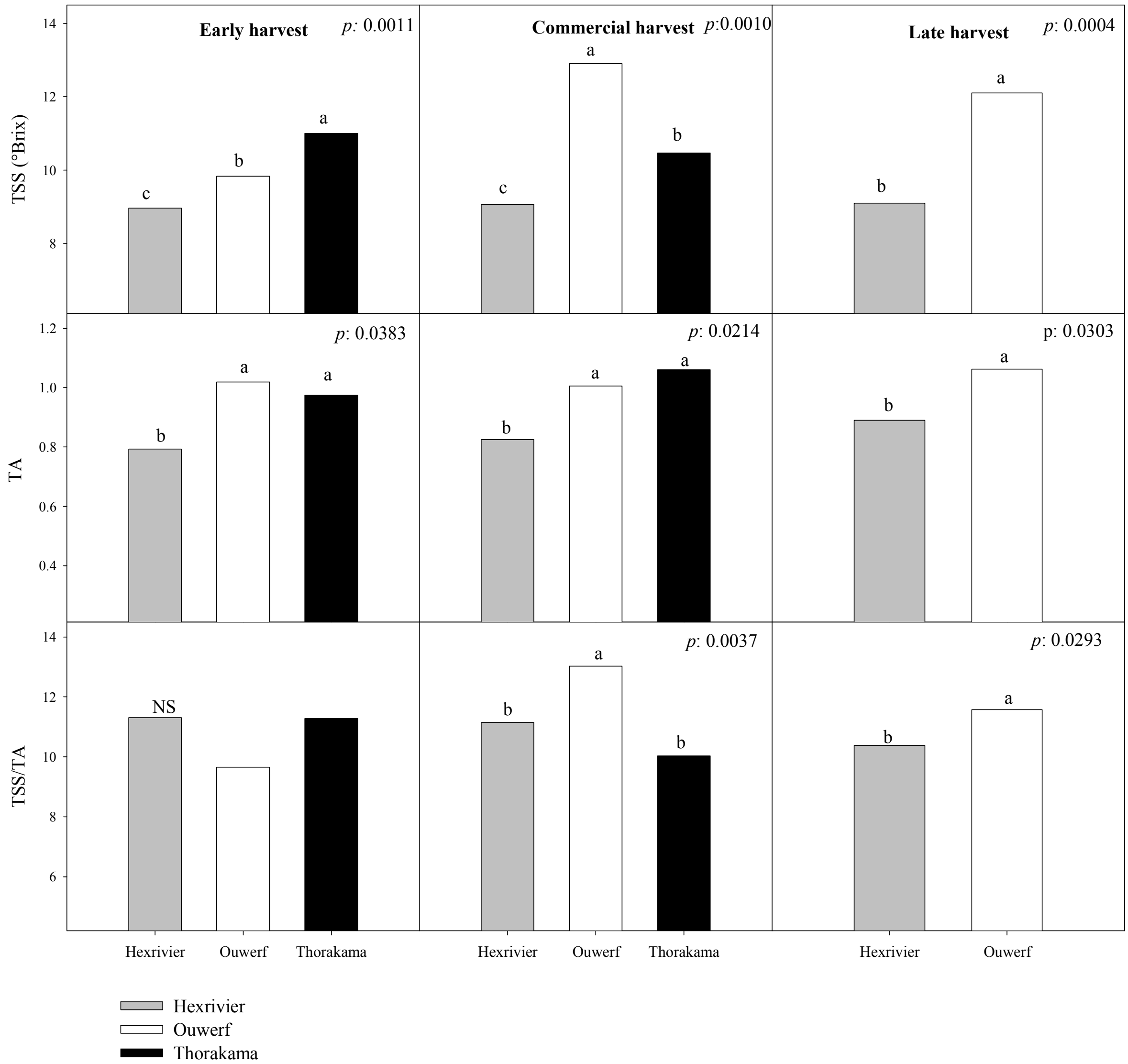


Figure 13. Maturity index (TSS and TA) of 'Navelina' navel orange fruit harvested at 3 harvest dates (11, 25 May and 9 June 2011) at 3 different sites Hexrivier (bottom of the valley), Ouwerf (middle of the valley) and Tharakama (top of the valley).

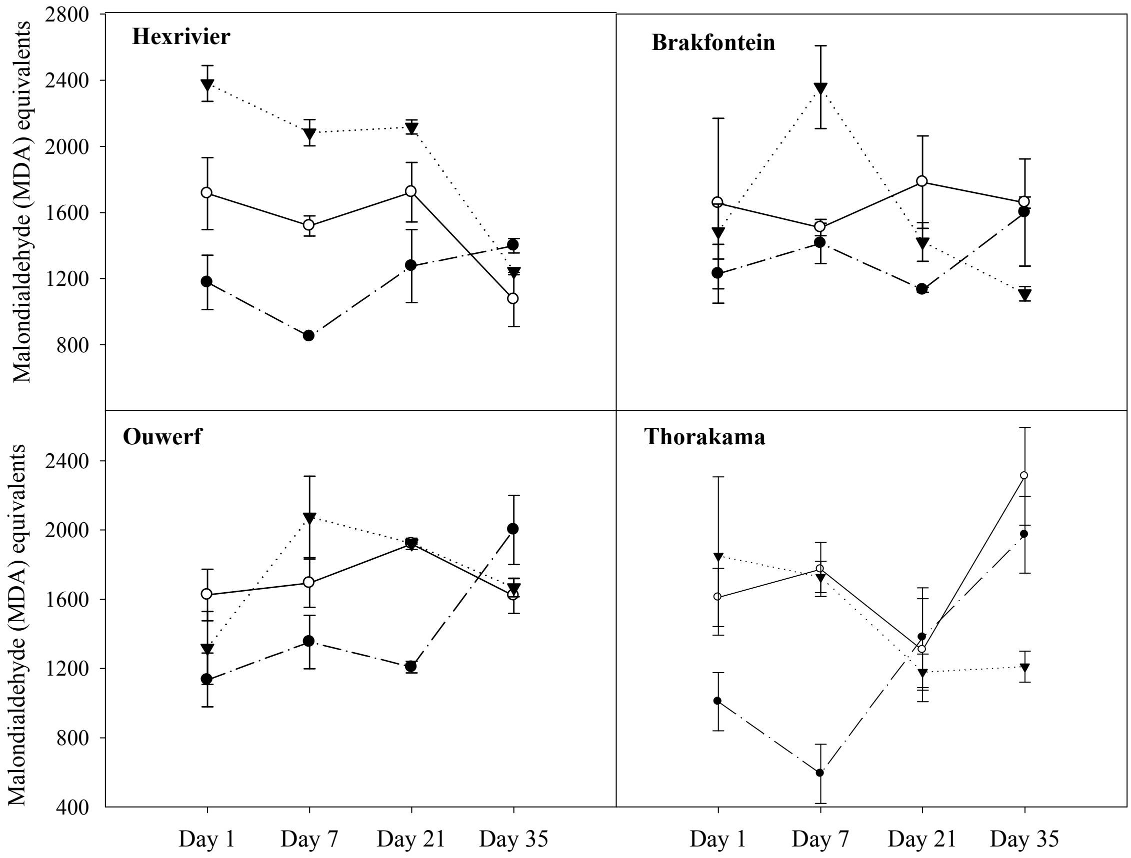


Figure 14. Lipid peroxidation indicated by malondialdehyde concentration (nmol.g DW^{-1}) in flavedo of 'Washington' navel orange fruit rind harvested early (11May 2011), commercial (25 May 2011) and late (9 June 2011) at four different sites in Citrusdal i.e. Hexrivier, Brakfontein, Ouwerf and Tharakama. Fruit was stored for 35 d at -0.6 °C, followed by one week at 20 °C.

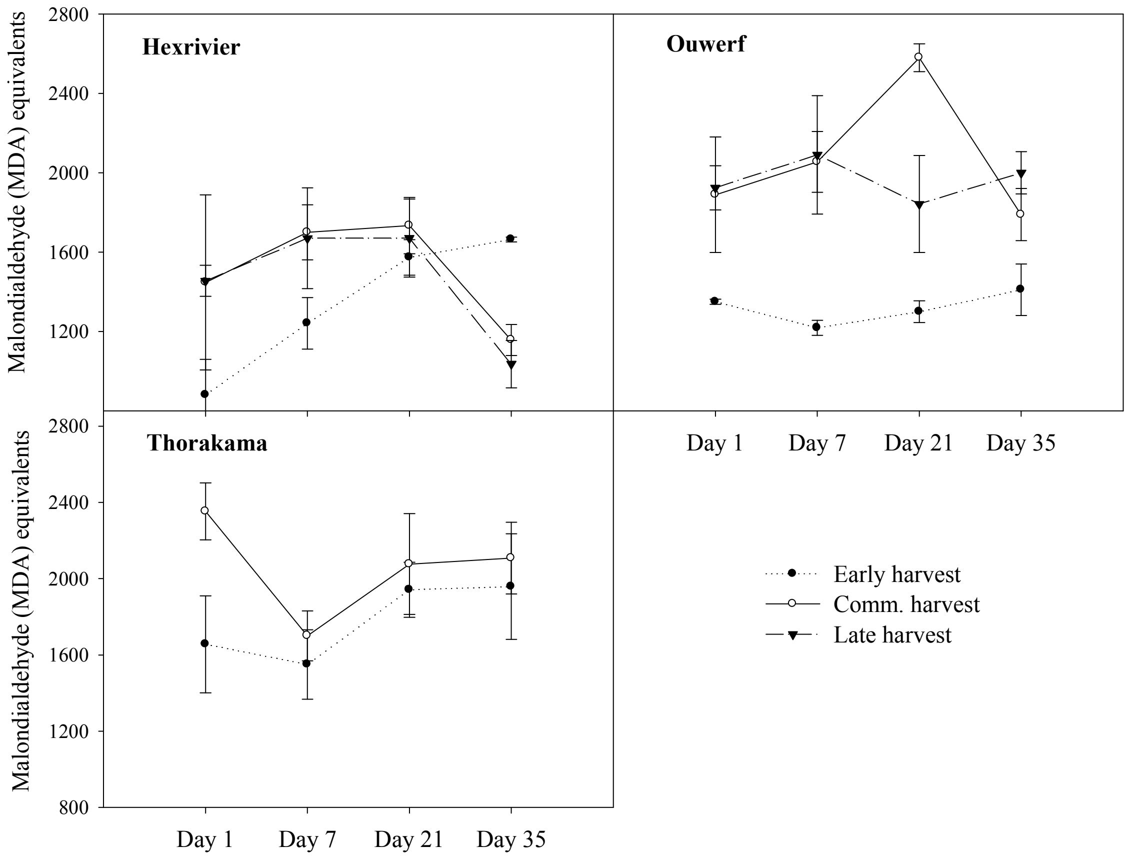


Figure 15. Lipid peroxidation indicated by malondialdehyde concentration (nmol.g DW^{-1}) in flavedo of ‘Navelina’ navel orange fruit rind harvested early (11May 2011), commercial (25 May 2011) and late (9 June 2011) at three different sites in Citrusdal i.e. Hexrivier, Ouwerf and Tharakama. Fruit was stored for 35 d at -0.6 °C, followed by one week at 20 °C.

PAPER 2:
PRELIMINARY STUDY ON THE USE OF NEAR-INFRARED SPECTROSCOPY TO PREDICT CHILLING INJURY SUSCEPTIBILITY OF NAVEL ORANGE FRUIT

Abstract

Previous research on citrus fruit using near infrared (NIR) spectroscopy have focused on the internal quality attributes and not on rind quality and it's association with disorders such as chilling injury (CI). Postharvest CI of citrus fruit is influenced by various factors and the aim of the study was firstly to determine the impact of fruit position in canopy, fruit size and rind colour on CI susceptibility of navel oranges. The second aim was to determine if NIR could predict the external quality parameters in relation to CI. 'Washington' navel orange fruit were harvested from outside and inside canopy positions, as well as in large and small categories and lastly according to rind colour (green vs. orange). The second set of fruit comprised of large (±82 mm) and small (± 65 mm) and lastly orange coloured fruit with average hue angle of 67 and green with average hue angle of 73 of the same size. For each fruit the mass, colour and firmness was determined. NIR spectra were obtained using a Brüker Multi Purpose Analyser (MPA) FT-NIR spectrometer which measures diffuse reflectance with a spectral range of 800nm-2500nm. Reference physical data of the fruit was obtained after 35 days of storage at -0.6 °C and included CI index, hue angle, chroma, lightness, mass and firmness. Principal component analysis (PCA) was used to analyse spectral data to identify clusters in the PCA score plots. Partial least squares (PLS) regression was applied to spectral data to develop prediction models for each parameter. The canopy position, colour and size affected the incidence of CI index. Smaller fruit are more susceptible to CI compared to bigger fruit. PCA plots display clusters which identified the different treatments. The prediction models for colour parameters and firmness were unacceptable using integrated sphere NIR measurements from a wavelength between 800-2500nm. Using these parameters fruit firmness, colour parameters, mass and the NIR spectrum with the integrated sphere did not provide a good prediction model for CI index.

Introduction

The position of citrus fruit in the dense leafy canopy influences internal (TSS, TA and juice percentage) (Sites and Reitz, 1949, Syvertsen and Albrigo, 1980) as well as external quality (colour) (Cronjé et al., 2011). Purvis et al. (1979) reported that 'Marsh' grapefruit located inside the canopy developed less chilling injury (CI) than to outside position and this could be due to abiotic or environmental factors (Purvis and Grierson, 1982). Similarly Nordby and McDonald (1995) reported that 'Marsh' grapefruit CI was more pronounced from outside fruit compared to inside fruit.

Pigments in citrus fruit rind and rind of other fruit types are considered to have a protective function against various pre- and postharvest stresses, for example as photoprotection in apples against sunburn (Merzlyak and Chivkunova, 2000). During ripening of citrus, chlorophyll in the rind breaks down and carotenoids accumulate. The amount of carotenoid, visibly seen as the orange colour, has an effect on rind condition, and it has been previously reported that yellow grapefruit is more resistant to CI than green grapefruit (Grierson, 1974 as cited by Purvis et al., 1979). Although fruit from the inside which is mostly greener has a higher incidence compared to fruit from the outside which is orange and yellow.

Aesthetic appearance is an important parameter of fruit quality and value in the market. Fruit with any lesions (wind, insects) or defects (oleocellosis and creasing) on the rind at harvest can be graded in the packhouse; however, fruits which develop physiological disorders such as rind breakdown or CI during shipment cannot be sorted, resulting in costly rejection in the market (Blasco et al 2007). The challenge therefore is to develop a non-destructive method to assist in the sorting of the fruit before symptoms of postharvest rind disorder develop. Near infrared spectroscopy (NIR) has been used extensively to non-destructively determine parameters of solid and liquid samples. One of the advantages of NIRS is its ability to measure multiple quality attributes, simultaneously (Nicolaï et al., 2007; Cozzolino et al., 2006)

The spectroscopic instrument consists of a radiation source, a wavelength selector, sample containers which can be in different modes and a radiation detector (Hanssens, 2011).

The interaction of radiation, from the NIR light source, with the sample results in NIR spectra which include a reflection of the chemical and the physical properties in the sample. Spectroscopic data can give information about the presence of different chemical components and the microstructure of tissue non-destructively (Nicolaï et al., 2007). The absorbance is measured and estimated through the detector which results in an absorption curve. Fruit consists of 80-90% water, thus water absorption in the NIR is fairly broad and there is peak water absorption at 970, 1200, 1450, 1950 and 2250 nm (Williams and Noris, 2001). The absorption curve of near infrared spectra of citrus is similar to other fruit such as kiwi fruit (McGlone and Kawano, 1998).

Interactions of samples with electromagnetic radiation could cause different types of quantum changes and specific light energy causes specific molecular vibrations (Skoog et al., 2004). The absorption of electromagnetic radiation in the infrared region causes a change in configuration due to molecular rotations and vibrations (Griffiths and de Haseth, 2007). Different spectral responses in specific region could be an indication for certain chemical functional groups which is in a molecule (Pasquini, 2003). NIR consists of broadband which arises from overlapping absorption of different vibrational modes and combinations of C-H, O-H, N-H and S-H (Osborne 2000). C-H and O-H is responsible for strong absorption bands in the NIR spectral region (Nicolaï et al., 2009). Fruit absorption bands are wide and complex and consist of different chemical O-H C-H bonds in water and sugar molecules. As explained by Magwaza et al. (2012a) which discusses the recent developments and application of Vis/NIR spectroscopy to non-destructively evaluate internal and external fruit quality.

For citrus fruit quality evaluation with NIR, three different data acquisition modes may be used (reflectance, transmittance and interactance) (Schaare and Fraser 2000). Reflectance does not contain a lot of information about internal quality because it has a limited penetration depth (Krivoshiev et al., 2000). It is easier to obtain light levels of reflected radiation compared to transmittance (Fu et al., 2007). Transmission mode is used to assess the internal quality of the fruit (Kawano et al., 1993 as cited by Magwaza et al 2012a). However, Gómez et al. (2006) and Cayuela (2008) had good results for predicting internal quality using reflectance mode with mandarins and oranges. The transmittance measurements are influenced by fruit size and therefore the light quantity that penetrates through the fruit could be low, which made it difficult to measure accurate transmittance (Kawano et al., 1993 as cited by Magwaza et al 2012a).

Different wavelength ranges are used by researchers to define different quality parameters which range from visible spectrum to NIR. McGlone et al., (2003) reported that for mandarin fruit internal quality assessment i.e. total soluble solids (TSS) and total acids (TA) the optimal wavelength is 650-1100 nm. The size and maturation of the fruit, and different growing conditions result in a difference in the distribution and the level of attributes (TSS, TA) (Guthrie et al., 2005). To minimize the effect of variation within the fruit, the NIR assessment should be done at different positions on the fruit. Guthrie et al. (2005) reported that for reference sampling for internal quality mandarin fruit one position at equatorial site will represent the entire fruit. However, one position at equatorial site will be insufficient for the detection of rind disorders the information from the pulp distort the information from the rind (first 10 mm of fruit).

In order to develop a NIR spectrometric technique to determine certain fruit quality parameters non-destructively, the first step is to develop calibration equations which relate the spectral data with quality traits (physical measured data in the laboratory). Statistical methods such as the partial least squares regression (PLS) method is commonly used in quantitative spectroscopy to correlate spectroscopy data X with related physiochemical data or measured data Y (Haalan and Thomas, 1988). Thereafter principal component analysis (PCA) plots are applied to identify clusters and outliers in the PCA scores plot. PCA transforms the data to a new coordinate system with the greatest variance by any projection to lie on the first coordinate.

Visible-NIR spectrometric technique has been used to measure the soluble solid content (SSC) of intact navel orange fruit (Cen et al. 2006; Gómez et al., 2006; Liu et al. 2010). The previous research using NIR on citrus has focused on the internal quality attributes and not on the rind physiological disorders (Liu et al., 2010). However, recently Zheng et al. (2010) reported that the oleocellosis sensitivity in citrus fruit could be predicted by NIR using the reflectance mode before the bruising. In contrast NIR was successfully used in detecting surface bruising in apples (Geeola et al., 1994). However, the challenge is in detecting correlation between rind condition and susceptibility to physiological disorder such as CI.

It is hypothesised that canopy position influence, size, firmness and colour which further contribute to susceptibility to CI and that NIR spectroscopy could be used to quantify the quality of the rind which would enable separation of susceptible vs. non susceptible.. The aim of the study was firstly to determine the effect of fruit canopy position, size and rind colour on CI susceptibility. The second aim was to investigate if NIR spectroscopy could be used to separate these aspects of navel orange fruit after harvest in order to differentiate CI sensitive vs. non-sensitive fruit. .

Materials and Methods

Plant material

'Washington' navel, orange (*Citrus senensis* L. Osb) planted in 1982 on 'Rough Lemon' rootstock (*Citrus jambhiri* Lush) were harvested on 25 July 2011 at Brakfontein (32°.51'S 18°99' E) in Citrusdal. The tree density was 5.7 m by 3 m and the crop yield 40 ton.ha^{-1}. For the second season, on 25 July 2012, 'Washington' navel oranges planted in 1950 on 'Rough Lemon' rootstock (*Citrus jambhiri* Lush) were harvested at Tharakama (32°85'S 19°09'E) in Citrusdal. For this experiment 240 'Washington' navel orange fruit were harvested in the following manner for the trial: 40 fruit harvested at the outside of the canopy (10-30 cm inside the canopy) and 40 fruit inside the canopy (> 30 cm inside the canopy) with the average same size and colour. The second set of fruit comprised of 40 large (± 82 mm) and 40 small (± 65 mm) fruit from the outside of the canopy with the same colour and lastly 40 orange coloured fruit with average hue angle 66 and 40 green with average hue angle 73 of the same size (Fig. 1). In 2011 all samples fruit mass, colour and firmness was determined after harvest before NIR spectra was acquired from fruit harvested. After measurements, the fruit was stored for 35 d at -0.6 °C and 1 week shelf life before, measuring mass, colour, firmness and determination of the CI index. Only the CI index, weight loss and colour parameters were determined for navel orange fruit harvested in 2012.

Data collection

Fruit mass was determined before and after storage using an Elec checking scale NBK-30(Model NWH 10422, UWE South Africa). The colour attributes (Light, Chroma, Hue°) were measured using a Minolta chroma meter (Model CR200; Minolta Camera Osaka, Japan). Colour measurements were taken at two positions of the fruit i.e. the lighter and a darker side. Lightness is a value from 0-100 (0= white and 100= black), Chroma represents the intensity of colour and hue angle is colour which was measured in degrees (0 red to purple, 90º yellow and 150º green).

After storage for 35 d fruit was scored for CI (Fig. 2) according to this index and after 2 weeks of shelf life:

$$\text{Chilling injury index (0-3)} = \sum \frac{[\text{Chilling injury (scale 0-3)} \times \text{no. of fruit in each class}]}{\text{Total number of fruit in replicates}}$$

Texture properties were measured using a TA.XT plus Texture Analyser (stable Micro systems, Surrey, England) with extra software (Exponent Version 5.0.9.0). The load cell of texture analyser contains a force sensor which moves down to the sample at a constant velocity. The analyser was calibrated with a calibration weight of 10 kg. A cylindrical flat head aluminium probe which was 39 mm in length and 35 mm in diameter was used for the compression load cell. The load cell moved at a constant velocity to the sample at 2 mm per second, when the probe come in contact with the fruit the fruit was compressed for 6 mm. The sensor in the load cell measures the force that is needed to compress the fruit (Hanssens, 2011).

NIR spectra were taken using the integrated sphere which is an accessory on the Brüker Multi Purpose Analyser (MPA) FT-NIR spectrometer (Brüker Optik GmbH, Etlingen, Gremany) and is used to measure diffuse reflectance of highly scattering solid media. The integrating sphere consists of a 50 mm wide sample cup holder for the measurements of homogenous samples. Fruit was placed on top of the holder cup and the NIR beam is directed into the sphere from where it is directed in through optical window to the sample. The beam scatters off the sample and the light that is reflected enters the sphere and is directed to the detector. The sphere is gold coated to ensure that all light beams are collected and directed to the detector. The wavelength region which was scanned was from 780 nm to 2500 nm with a resolution of 8 cm^{-1}. For each fruit 64 scans were taken per spectrum with a resolution of 16 (Hanssens, 2011).

Each fruit was evaluated from 6 different positions. The first spectrum was taken at the top and the second at the bottom, the third to sixth were taken with equatorial positions (with 90º difference between consecutive positions) were directed toward the source.

Statistical analysis

Data of the CI index (ranked data) was analysed by using the one way Anova using SAS (SAS v.6.12; SAS Institute, Cary, VC, USA). Each treatment was compared with the other using Fischer least significance differences (LSD). The *p*-values illustrate the significance differences in the figures presented. If *p*-value is smaller than 0.05 there is significant differences between treatments. Correlations were done between the CI index and the physical parameters (colour, firmness and mass) using Statistica V.10 (Statsoft Inc. Tulsa, UK). To determine if it is possible to measure the physical properties with NIR before storage and to classify the Navel orange according to susceptibility to CI the following analyses were done.

Principal component analyses (PCA) were obtained using Unscrambler 9.7 (CAMO software AS, Norway) which transforms the data to a new coordinate system such that the greatest variance by any projection comes to lie on the first coordinate. This is a manipulation of the data where the aim is to present the variation in different variables using a small number of factors. With PCA the variables are transformed into principal components (Cozzolino et al., 2011). The use of principal components allows viewing the nature of data in small number of dimensions, which consist of matrices with many variables (Beebe et al., 1998). Partial least squares regression (PLS) method is commonly used in quantitative spectroscopy to correlate spectroscopy data X with related chemical measured data Y. Partial least square regression was applied to spectral data to develop prediction models for firmness, hue angle, chroma, lightness and mass. Partial least squares were obtained from Unscrambler 9.7 (CAMO software AS, Norway) and OPUS version 6.5 (Bruker Optik GmbH, Ettlingen, Germany). Before the Calibrations the reflectance data were transformed to absorbance, mean normalised and optionally treated by scatter correction using Unscrambler 9.7. Preprocessing or scatter correction is a mathematical manipulation of the data before the primary analysis and is used to remove or reduce irrelevant sources of variation (Beebe et al., 1998). For the validation of PLS a test set validation was done where 40% of the data was used for validation. To assess the accuracy of the calibration performance root mean square of calibration (RMSEE), root mean square error of prediction (RMSEP), and the residual predictive deviation (RPD) were calculated (Williams and Sobering, 1996). A good model should have a lower RMSEE, RMSEP and a higher correlation coefficient (r) or coefficient of determination (r^2) and RPD value. An RPD between 1.5 and 2 indicate that the model can discriminate low from high values; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible and a value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy (Nicolaï et al., 2007).

Results

Chilling injury and external quality aspects

The results showed significant differences between the CI index obtained directly after storage compared to CI index after a week of shelf life (Fig. 3). Fruit from the outside had a significantly higher CI index compared to fruit from the inside in 2011; however, in 2012, there was no significant difference between the treatments. Smaller fruit were more susceptible to incidence of CI compared to large fruit (Fig 4).There were significant differences between orange and orange/green 'Washington' navel oranges. Fruit with better rind colour (low hue angle) were more susceptible to CI and had a 41.2 % higher CI index compared to the orange /green fruit in 2011 (Fig. 5).

Negative correlations existed between CI and the different physical parameters viz. mass (before & after cold storage), diameter and length of the fruit which indicated that smaller fruit has a higher susceptibility to CI than larger fruit. Fruit firmness, before and after storage, had no correlation with the incidence of CI (Table 1).

The hue angle (before & after cold storage) had a negative correlation which indicates that greener fruit (with a higher hue angle) had a lower susceptibility to CI than orange coloured fruit. Orange fruit with an average of 66° hue had a 42% higher incidence in CI than green fruit with a higher average hue angle of 72.8°. The other colour attributes i.e. chroma (C) also had negative correlations with CI, indicating that fruit with more vivid colour had lower incidence of CI.

Fruit NIR spectra

Typical diffuse absorbance spectra of fruit acquired by the FT-NIR spectrometer NIR are shown in Figure 6. The wavelength range of 800 nm to 2500 nm was applied to develop the calibration models. In the wavelength from 800 nm to 1800 nm the spectra curves begin to ascend until 1450 nm and then reduces up to 1670 nm, and rise again. There are two large absorption peaks at 1210 nm and 1450 nm, which are associated with bonds of C-H and overtones of bond O-H in H_2O, respectively.

Principal component analysis (PCA)

Individual spectra from the six positions taken on fruit and the average spectra were tested to develop PCA and PLS models. Average spectra of the fruit showed better models than individual spectra at one position of the fruit. PCA was performed on the NIR spectra to compare the spectral characteristics of fruit from different canopy positions, sizes and colours. A PCA score plot, similar to a map of the samples, indicates how the different observations are explained by the principal components. Different observations which have the same characteristics would be grouped together in a score plot (Hanssens, 2011). The data distribution in the PCA score plot displayed two clusters in each PCA score plot for the green and orange fruit with an 87.5% level of accuracy (Fig.7) for the inside and outside canopy fruit with an 86% level of accuracy (Fig.8), for the large and small fruit with an 86% level of accuracy (Fig.9). These clusters provided good discrimination between the different treatments.

The first two principal components for the orange and green fruit accounted for 100% which 97% of the variance is explained by the principal component 1 and 3% by Principal component 2. There was a clear distinction between the green and orange fruit with principal component 2 using a spectral range from 1513 nm- 2500 nm (Fig. 7). This spectral range was used because it results in better discrimination between the green and orange fruit. The first two principal components for the inside and outside fruit accounted for 100% of the X (NIR spectra) variance, 91% of the variance is explained by the principal component 1 and 7% by Principal component 2. There was a clear distinction between the inside and outside fruit with principal component 2 using a spectral range from 800 nm-1600 nm (Fig. 8). The first two principal components for the large and small fruit accounted for 98% of the X characteristics variance, which 88% of the variance is explained by the principal component 1 and 10% by principal component 2 (Fig. 9). There is clear distinction between the big and small fruit using principal component 1 using a spectral range from 800nm- 1800nm.

PLS prediction models

The NIR data were subjected to several pre-processing methods but the spectral pre-processing did not increase the accuracy of the prediction models. Prediction models were obtained by using the outside and inside fruit for prediction, the large and small fruit for second prediction and the green and orange for the third prediction for the different physical properties. Table 2 shows a summary of statistics for calibration and validation results for the different physical properties with PLS models. The prediction models for firmness and hue angle, chrome and lightness parameters were not reliable with a very low validation predictive R^2-values smaller than 30 % and an RPD level below or just above 1.5 indicating that the model cannot discriminate between values (< 1.5) or can only discriminate between very high and low values (Nicolaï, 2007). Mass prediction had higher R^2 value indicating that it could be possible to determine the mass of the fruit with the NIR but due to only the 6 different positions which the NIR beam enters the fruit it was not possible to determine precise mass of the fruit.

Discussion

Citrus fruit which were harvested during 2011 from outside the canopy had a significant higher CI index compared to fruit from inside the canopy. Similar Nordby and McDonald, (1995) reported that CI was more pronounced on outside compared to inside grapefruit. Purvis, (1984) also indicated that the sun exposed side of grapefruit was more prone to CI compared to the shaded side of the same fruit. Due to the high light intensities, in Citrusdal (the fruit growing area in this study), the fruit exposed to the sunlight, outside the canopy, could result in the fruit being more prone to sunburn thereby increasing fruit susceptibility to CI (CRI annual report, 2011). High sunlight lead to an excess in photosystem 1 reduction and the rate of fixation of CO_2 cannot be maintained and $NADP^+$ is reduced, this will cause oxygen to compete for the electrons of photosystem 1 and will cause the formation of ROS and eventually cause lipid peroxidation and alteration in the membrane. (Sevillano, et al. 2009). Free radicals and ROS and lipid peroxidation will increase and the membrane doesn`t have the ability to recover. However, fruit that were harvested in 2012 had no significant difference in CI index between fruit from the inside and from the outside of the canopy. This could be due to better pruning of the trees which will result in better light distribution throughout the canopy and a higher photosynthetic rate in both the canopy and fruit rind (Cronjé, 2009).

Smaller fruit had a higher incidence of CI compared to larger fruit. There is competition for the photosynthates among different organs i.e. fruit to shoot as well as among fruit resulting in larger or smaller fruit (Goldsmith and Koch, 1996). In citrus, the competition between fruit is more evident, than in other fruit trees. The reduction in the fruit numbers on a specific tree is due to the carbohydrate status of the specific tree (Goldschmidt et al, 1992). Larger fruit are considered to be a stronger sink during fruit development and could have a higher nutrient and carbohydrate status. In comparison to smaller fruit with low sink capacity which consists of less carbohydrate and nutrients. Some studies showed that carbohydrate metabolism is involved in the protection of t tissue against the chilling stress. Purvis et al., (1979) reported a strong correlation between the reducing sugars and the resistance to the incidence of CI by stabilising the membrane. In grapefruit reducing sugars were correlated with decrease in CI sensitivity of the fruits during the season (Purvis and Grierson, 1982). Larger fruit is better sink which has more reducing sugars and carbohydrate reserves and is thus probably less prone to CI.

Fruit with green/orange rind colour were less susceptible to chilling injury than orange coloured fruit. In contrast, Grierson (1974) as cited by Purvis et al. (1979) reported that yellow grapefruit were more resistant to CI compared to green grapefruit. It was thought that the orange fruit will be less susceptible to CI but the results show the opposite. An explanation therefore can be that chlorophyll can protect the fruit against CI index. Pigments are considered a protective function against various pre- and postharvest stresses, for example photo protection in apples (Merzlyak and Chivkunova, 2000). Could be due to the ethylene that senesced the membrane with the colour changes. Better coloured fruit could also be over mature, which could lead to a higher susceptibility to CI. As previously discussed the internal maturity at harvest does not appear to be related to the development of CI, but the rind condition does affect the incidence of chilling injury.

PCA plots models were successfully used to identify the different preharvest treatments. NIR models were used to discriminate among large/small fruit, inside/outside fruit and green/orange fruit without the use of different physical measurements. PLS models for firmness and colour were unacceptable due to a very low explained variance. Similarly Hanssens (2011) reported that the model for the compression force or firmness wasn't able to predict, possibly due to the small variation in firmness or robust measurement not picking up small differences in range where it is required. The variation in the firmness/hardness has to be more representative and then it will be possible to have a prediction model for firmness. If fruit is stored at cold temperatures and warm temperatures, a larger variation in firmness could be evident and then it may be possible to have a better prediction model for firmness.

The colour parameter (hue angle, chroma, lightness) models were not acceptable, probably due to the NIR wavelength used (800 nm-2500 nm) which is above the visible range (450-700 nm). As recently reported by Magwaza et al. (2012b) models for the colour parameters were developed using the visible range of the spectrum. Fruit mass gave a better prediction model compared to the firmness and the colour parameters. However, on a sorting line, cheaper and more robust techniques are already currently being used to detect the mass and dimensions (L×h) of the fruit.

To conclude, canopy position, colour and size affected incidence of CI index. Smaller fruit were more susceptible to CI, thus classifying fruit according to size in the packhouse can reduce the incidence of CI. PCA plots models display clusters separating these treatments. It was possible to apply NIR spectral range using PCA plots to distinguish between fruit from different canopy positions, different sizes and different colours. Reliable prediction of colour parameters and firmness were difficult to obtain using integrated sphere NIR measurements from a wavelength between 800-2500 nm, but a better prediction model for colour parameters could possibly be obtained using a broader spectrum 450-2500 nm. With the different parameters firmness, colour parameters, mass and the NIR spectrum using an integrated sphere, a good prediction model for the CI index could not be obtained. For future prospects the changes in biochemical product of the rind for example carotenoids and lipid peroxidation could be assayed and perhaps used for a prediction model for CI index if it could be reliably identified via NIR.

References

Beebe, K.R., Pell, R. J. and Seashol, M. B. 1998. Chemometrics: A practical Guide. New York, USA: John Wiley & Sons, inc. pp 33.

Blasco, J., Aleixos, N., and Moltó, E. 2007. Computer vision detection of peel defects in citrus by means of a region oriented segmentation algorithm. J. Food Eng. 81: 535–543.

Cayuela, J. A. 2008. Vis–NIR soluble solids prediction in intact oranges (*Citrus sinensis* L.) cv. Valencia Late by reflectance. Postharvest Biol. Technol. 47: 75–80.

Cen, H., He, Y. and Huang, M. 2006. Measurements of soluble solids contents and pH in orange juice using chemometrics and Vis–NIRS. J. Agric. Food Chem. 54: 7437–7443.

Cozzolino, D., Cunkar, W., Shah, N. and Smith, P. 2011. Multivariate data analysis applied to spectroscopy: Potential application to juice and fruit quality. Food Research International.

Cozzolino, D., Cynkar, W., Janik, L., Dambergs, R. G. and Gishen, M. 2006. Analysis of grape and wine by near infrared spectroscopy — A review. J. Near Infrared Spectrosc, 14: 279−289.

CRI annual report, 2011 Citrus Research International, Nelspruit, South Africa.

Cronjé, P.J.R. Barry, G.H. and Huysamer, M. 2011. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60:192–201.

Cronjé, P.J.R. 2009. Postharvest rind breakdown of 'Nules clementine mandarin'*(Citrus Reticulate* Blanco) PhD dissertation. Stellenbosch University, South Africa.

Fu, X., Ying, Y., Lu, H. and Xu, X. 2007. Comparison of diffuse reflectance and transmission mode of visible-near infrared spectroscopy for detecting brown heart of pear. J. Food Eng., 83: 317–323.

Geeola, F., Geeola, F. and Peiper, U.M. 1994. A spectrophotometric method for detecting surface bruises on 'Golden Delicious' apples. J Agric. Engng. Res. 58: 47–51.

Goldschmidt, E.E, Harpaz, A., Gal, S., Rabber, D.and Gelb, E. 1992. Simulation for fruitlet thinnings effects in citrus by a dynamic growth model Proc. Int. Soc. Hortic. 1: 515-519.

Goldschmidt, E.E. and Koch, K.E. 1996. Citrus. Chapter 3 In Zamski, E., Schaffer, A.A. (eds.). Photoassimilate distribution in plants and crops. Marcel Dekker Inc. New York, 1996.

Gómez, A. H., He, Y. and Pereira, A. G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using vis–NIR spectroscopy techniques. J. Food Eng. 77: 313–319.

Grierson, W. 1974. Chilling injury in tropical and subtropical fruit: V effect of harvest date, degreening, delayed storage and peel color on chilling injury of grapefruit. Proc Trop. Region Amer. Soc. Hort. Sci. 18:66-73

Griffiths, P. and de Haseth, J. 2007. Fourier transform infrared spectrometry - second edition. Hoboken, New Jersey, USA: John Wiley and Sons Inc.

Guthrie, J. A., Walsh, K. B., Reid, D. J. and Liebenberg, C. J. 2005. Assessment of internal quality attributes of mandarin fruit. 1. NIR calibration model development. Aust. J. of Agric. Res., 56: 405–416.

Haalan, D. and Thomas, E. 1988. Partial least squares method for spectral analysis. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. Anal. Chem. 60: 1193–1202.

Hanssens. A. 2011. Non-destructive measurement of quality parameters in oranges using near infrared (NIR) spectroscopy. MSc dissertation, Katholieke University of Leuven, Belgium, Leuven.

Krivoshiev, G. P., Chalucova, R. P. and Moukarev, M. I. 2000. A possibility for elimination of the interference from the peel in nondestructive determination of the internal quality of fruit and vegetables by vis–NIR spectroscopy. Lebensm-Wiss University of technology, 33: 344–353.

Liu, Y., Sun, X. and Ouyang, A. 2010. Non-destructive measurements of soluble solid content of Navel orange fruit by visible-NIR spectrometric technique with PLS and PCA-BPNN. LWT –Food Sci.Technol. 43: 602–607.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronjé, P.J.R., Saeys, W. and Nicolaï, B. 2012a. NIR spectroscopy applications for internal and external quality analysis of citrus fruit – a review. Food and Bioprocess Technol. 5: 425–444.

Magwaza L.S., Opara, U.L., Terry L.A., Landahl. S., Cronjé, P.J., Nieuwoudt, H., Mouazene, A.M., Saeys, W. and Nicolaï, B.M. 2012b. Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. Postharvest Biol. Technol., 74:1-10.

McGlone, V.A., Fraser, D. G., Jordan, R. B. and Kunnemeyer, R. 2003. Internal quality assessment of mandarin fruit by vis–NIR spectroscopy. J. Near Infrared Spectrosc. 11: 323–332.

McGlone, V.A. and Kawano, S. 1998. Firmness, dry-matter and soluble solids assessment of postharvest kiwifruit by NIR spectroscopy. Postharvest Biol. Technol., 13: 131–141.

Merzlyak, M.N. and Chivkunova, O.B. 2000. Light-stress-induced pigment changes and evidence for anthocyanin photoprotection in apples. J. Photochem. Photobiol. B: Biol. 55: 155–163.

Nicolaï, B. M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K. I. and Lammertyn, J. 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46: 99-118.

Nicolaï, B. M., Bulens, I., De Baerdemaker, J., De Ketelaere, B., Hertog, M. L. A. T. M. Verboven, P. and Lamertyn, J. 2009. Non-destructive evaluation: detection of external and internal attributes frequently associated with quality and damage.Chapter 3 In D. Florkowiski (eds.), Postharvest Handling: A Systems Approach. Amsterdam: Academic Press, Elsevier. pp. 421–442.

Nordby, H.E. and McDonald, R.E. 1995. Variations in chilling injury and epicuticular wax composition of white grapefruit with canopy position and fruit development during the season. J. Agric. Food Chem. 43: 1828-1833.

Osborne, B. G. 2000. Near infrared spectroscopy in food analysis. Australia: BRI Australia Ltd. pp. 1–14.

Pasquini, C. 2003. Near Infrared Spectroscopy: Fundamentals, Practical Aspects and Analytical Applications. J. Braz. Chem. Soc. 14: 198-219.

Purvis, A.C. 1984. Importance of water loss in the chilling injury of grapefruit stored at low temperature. Scientia. Hort. 23: 261-267.

Purvis, A.C., Kawada, K. and Grierson, W. 1979. Relationship between mid-season resistance to chilling injury and reducing sugar level in grapefruit peel. HortScience14: 227-229.

Purvis, A.C. and Grierson, W. 1982. Accumulation of reducing sugar and resistance of grapefruit peel to chilling injury as related to winter temperatures. J. Am. Soc. Hort. Sci.107:139-142.

Kawano, S., Fujiwara, T., and Iwamoto, M. 1993. Non-destructive determination of sugar content in 'Satsuma' mandarins using NIRS transmittance. Journal of the Japanese Society for Horticultural Science, 62, 465–470.

Sevillano, L., Sanchez-Ballesta, M.T., Romojaroc, F. and Floresc, F.B. 2009. Mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. J. Sci. Food Agric. 89; 555–573.

Sites, J.W. and Reitz, H.J. 1949. The variation in individual 'Valencia' oranges from different locations of tree as a guide to sampling methods and spot picking for quality. Part1 Soluble solids in the juice. Proc. Am. Soc. Hortic. Sci. 54: 1-9.

Schaare, P. N. and Fraser, D. G. 2000. Comparison of reflectance and transmission modes of visible-near infrared spectroscopy for measuring internal properties of kiwifruit (*Actinidia chinensis*). Postharvest Biol. Technol. 20: 175–184.

Skoog, D. A., West, D. M., Holler, J. F. and Crouch, S. R. 2004. Fundamentals of Analytical Chemistry. Belmont, USA: The Lehigh Press, Inc.

Syvertsen, J.P. and Albrigo, L.G. 1980. Some effects of grapefruit tree canopy position on microclimate, water relations fruit yield and juice quality. J. Amer. Soc. Hort. Sci. 105: 454-459.

Williams, P. and Norris, K. H. 2001. Variable affecting near infrared spectroscopic analysis. In P. Williams, K. H. Norris (eds.), Near infrared technology in the agriculture and food industries. 2nd ed. The American Association of Cereal Chemists, St. Paul, MNL, pp. 171–185.

Williams, P.C. and Sobering, D.C., 1996. How do we do it: a brief summary of the methods we use in developing near infrared calibrations. In: Davies, A.M.C., Williams, P. (eds.), Near Infrared Spectroscopy: The future waves. Proc. 7th Interntl. Conf. Near Infrared Spectroscopy. Montreal, Chichester, UK, pp. 185–188.

Zheng, Y., He, S., Yi, S., Zhou, Z., Mao, S., Zhao, X. and Deng, L. 2010. Predicting oleocellosis sensitivity in citrus using Vis-NIR reflectance spectroscopy. Scientia Hort 125: 401–405.

Tables and Figures:

Table 1. Correlation between CI and physical parameters [mass, firmness, colour attributes (L, C, H)] of ‘Washington’ navel orange fruit measured before and after storage for 35 d at -0.6 °C.

Variable 1	Spearman	p-value
Mass before	-0.37	<0.01
Mass after	-0.39	<0.01
Firm before	0.03	0.62
Firm after	0.02	0.73
H before	-0.24	<0.01
L after	-0.14	0.04
H after	-0.33	<0.01
C after	-0.19	<0.01
H after	-0.21	<0.01
Diameter	-0.32	<0.01
Length	-0.34	<0.01
C_B diff	0.17	<0.01

Table 2. An overview of statistics from calibration (n = 48), validation (n = 32) and of models for individual quality parameters.

		Calibration				Validation		
Treatment	**parameter**	**r^2**	**RMSEE**	**RPD**	**Rank**	**r^2**	**RMSEP**	**RPD**
outside and inside	Mass before	62.46	25.20	1.63	6	45.35	31.30	1.36
	Mass after	33.73	32.00	1.23	3	27.13	35.50	1.17
	Firm before	13.18	20.40	1.07	1	27.49	18.10	1.17
	Firmness after	43.45	7.80	1.33	3	31.34	9.24	1.21
Large and small	Mass before	88.91	27.10	3.00	4	80.22	35.20	2.31
	Mass after	77.39	44.70	2.10	4	74.85	38.80	2.06
	Firm before	0.44	30.30	1.00	1	-5.89	30.60	0.98
	Firmness after	27.85	13.30	1.18	3	35.95	10.70	1.25
	C after	21.43	10.20	1.13	2	4.46	4.61	1.02
	H after	15.02	9.93	1.08	3	10.45	3.20	1.09
Green and orange	Mass before	38.04	36.10	1.27	1	26.23	31.20	1.17
	Mass after	41.05	34.80	1.30	2	1.94	35.10	1.01

Figure 1. Example of rind colour difference of 'Washington' navel orange fruit i.e. orange vs. green/ orange.

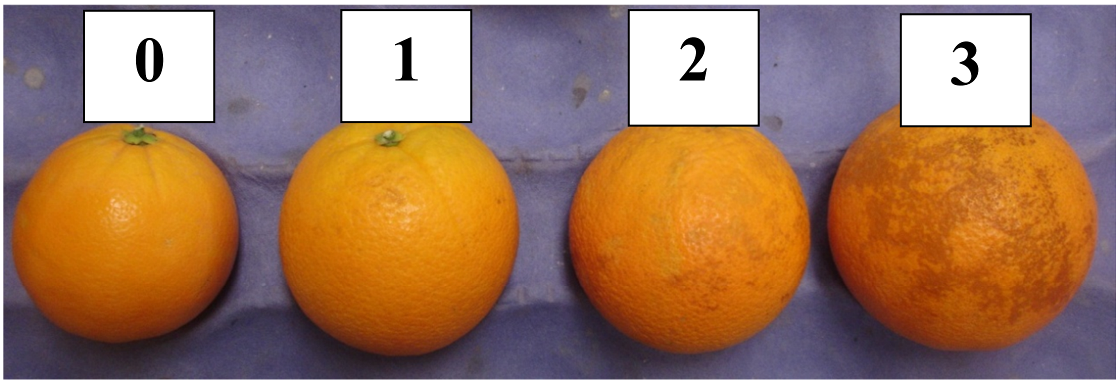


Figure. 2 Chilling injury (scalding) scoring of navel orange according to severity.

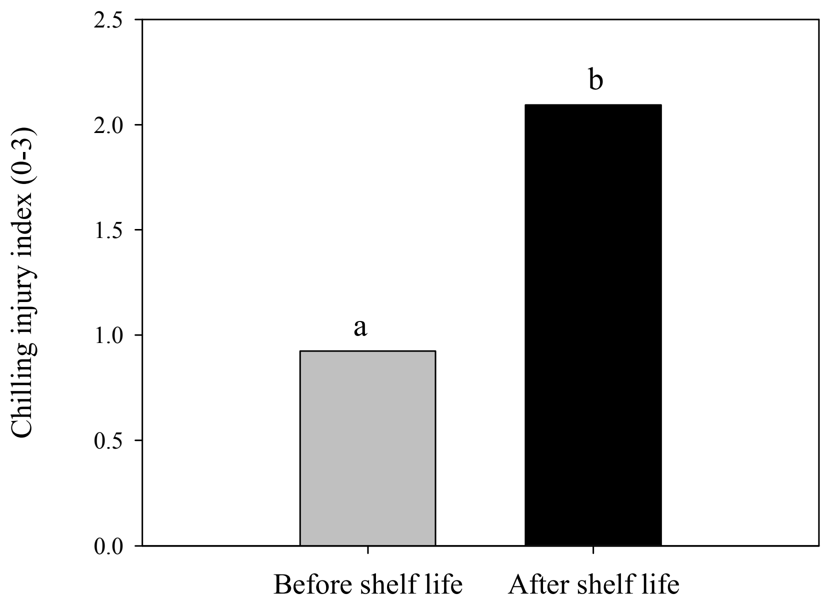


Figure 3. Chilling injury index (0-3) between 'Washington' navel orange fruit stored at -0.6 °C for 35 d determine before and after a week of shelf life (20°C) ($p \leq 0.05$).

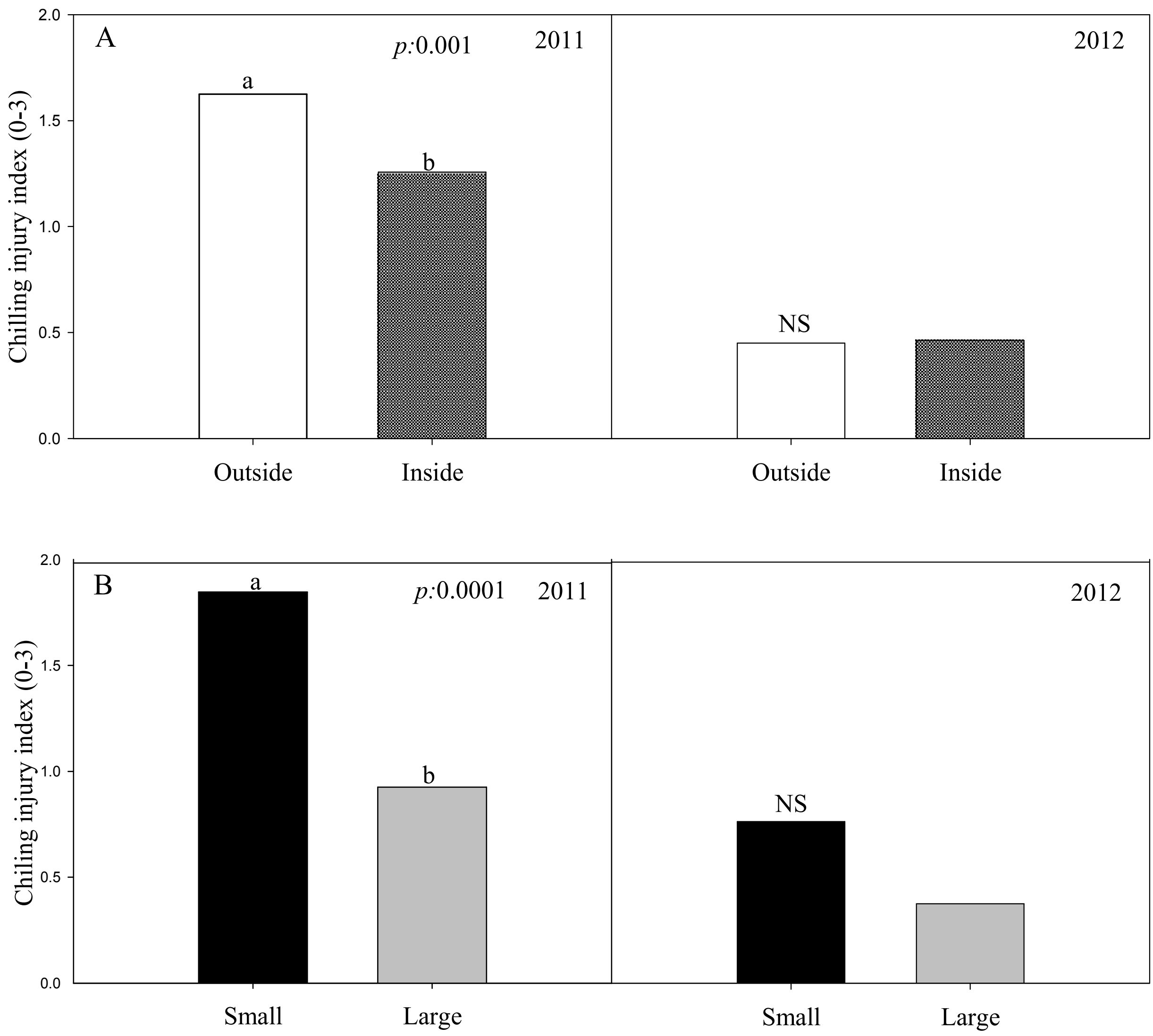


Figure 4. Chilling injury index (0-3) between 'Washington' navel orange fruit classify according to position (A) and fruit size (B) large (±82mm) and small (±62mm) in diameter. The fruit was stored at -0.6 °C for 35 d and 7 d of shelf life ($p \leq 0.05$).

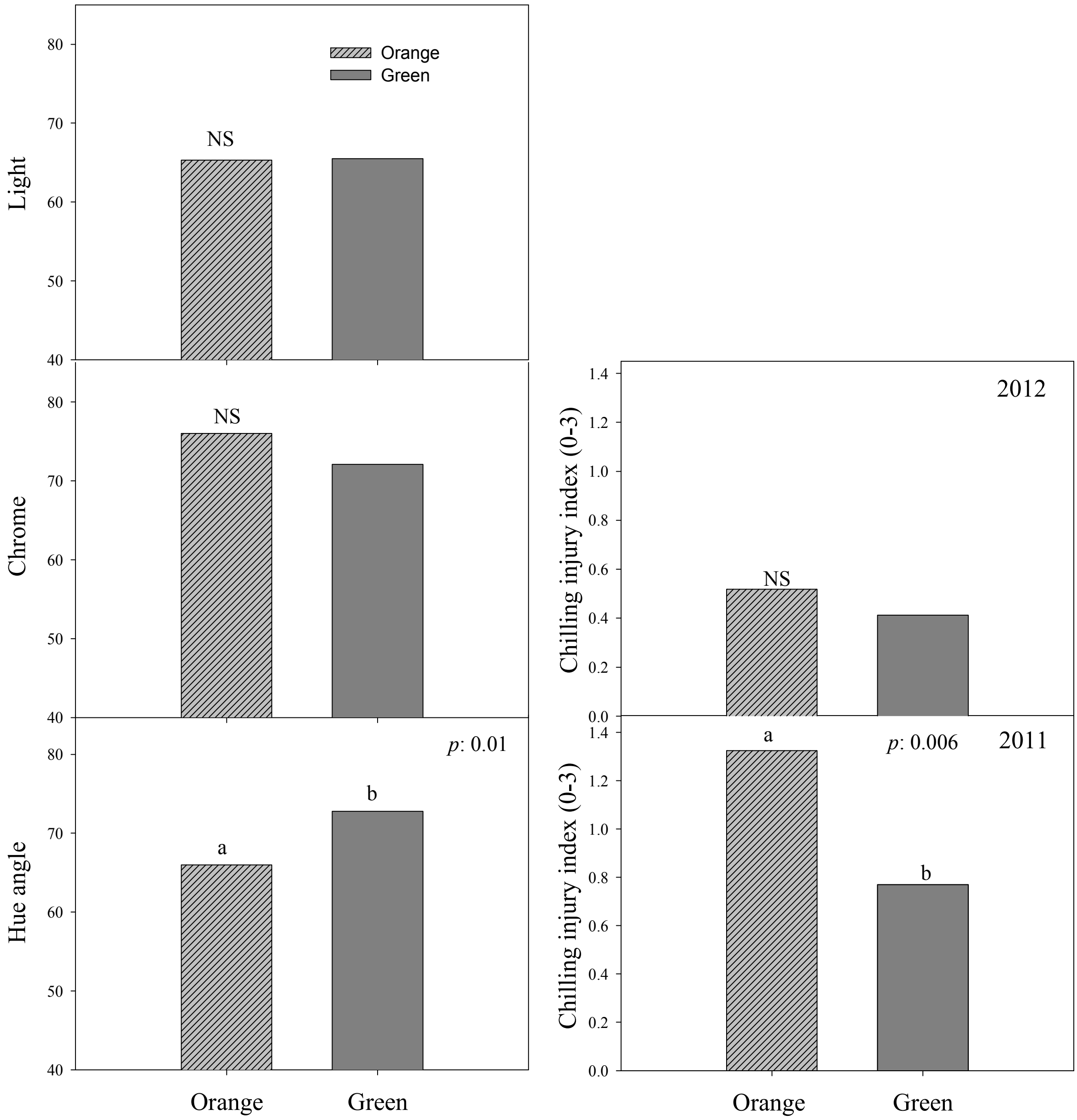


Figure 5. Colour attributes (L*, C*, H*) for 2011 and Chilling injury index (0-3) in 2011 and 2012 of green and orange colour 'Washington' navel orange fruit stored at -0.6 °C for 35 d.

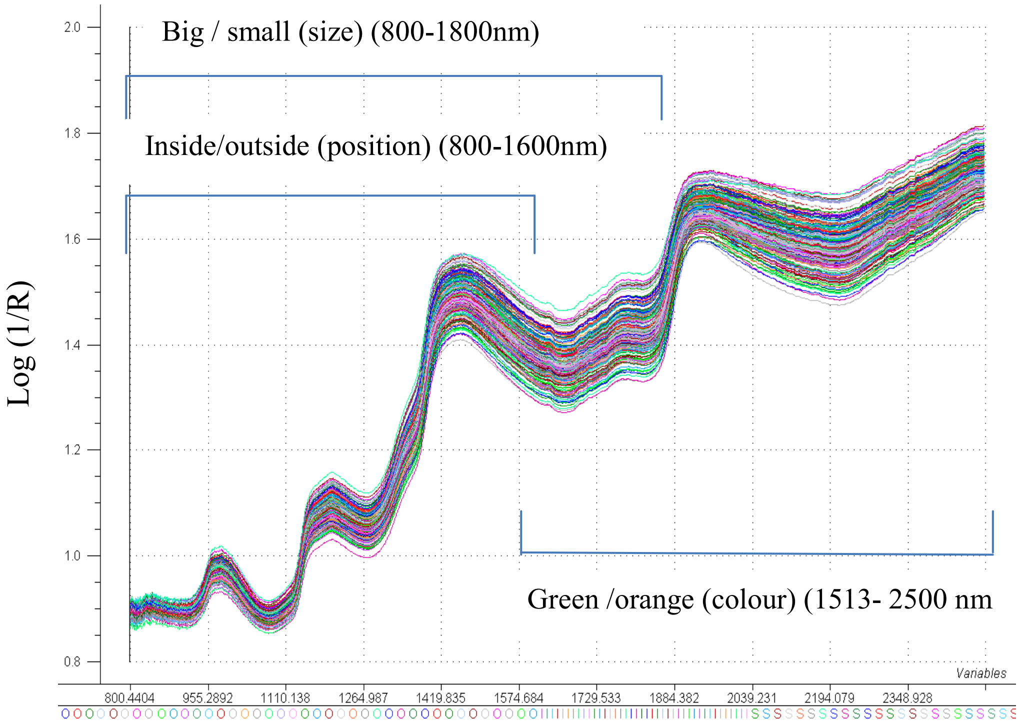


Figure 6. Absorbance spectra (log(1/R) of 'Washington' navel orange fruit in the wavelength 800nm -2500nm. R=reflectance

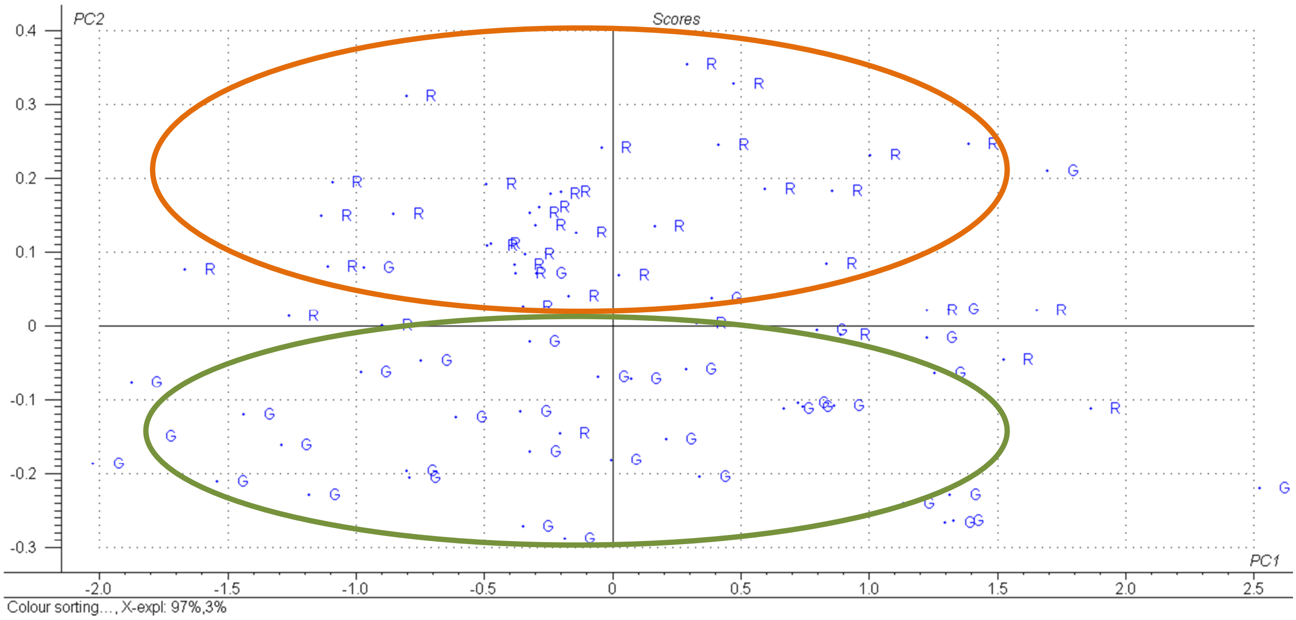


Figure 7. PCA plot for the two PC factors showing spectral ability to sort based on colour. 'Washington' navel orange fruit were stored for 35 days at temperature of -0.6°C. G represents green fruit and R represents orange fruit.

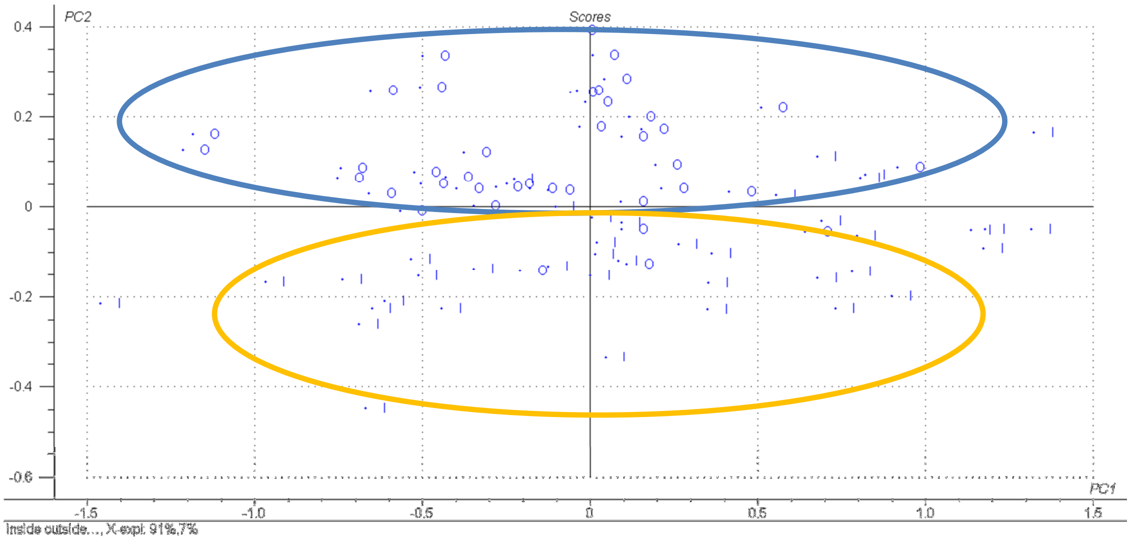


Figure 8. PCA plot for the two PC factors showing spectral ability to sort based on their origin within the tree canopy. 'Washington' navel orange fruit were stored for 35 days at temperature of -0.6 °C. I represents fruit from inside and O repersents fruit from outside.

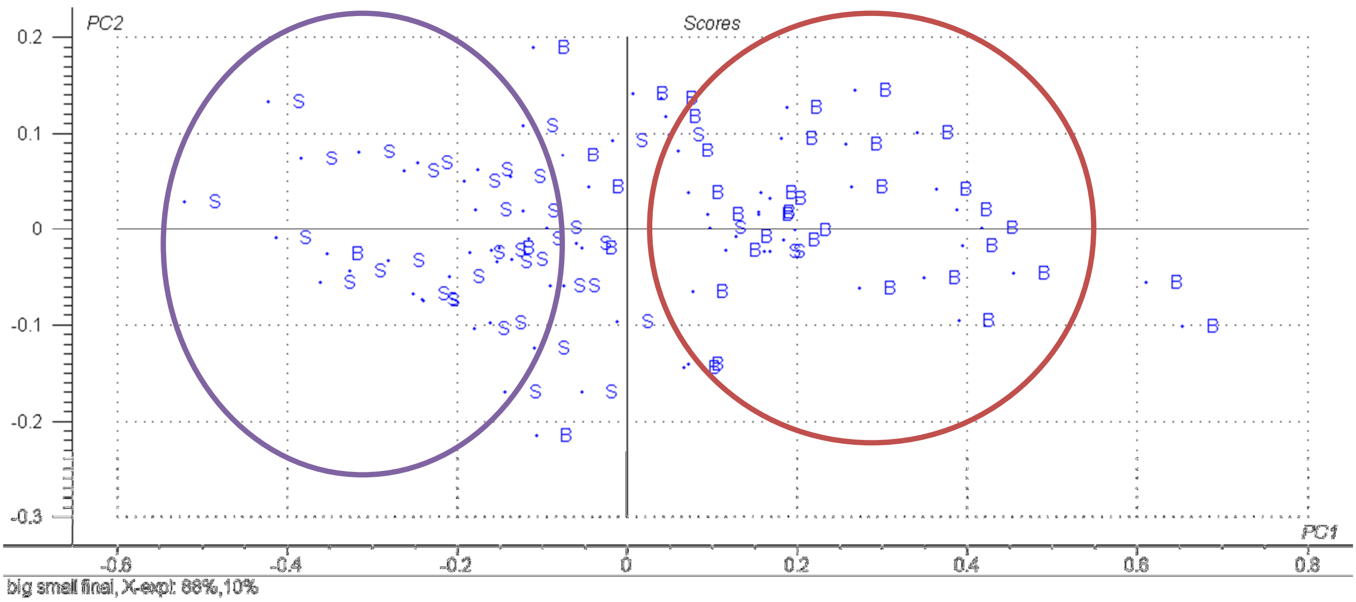


Figure 9. PCA plot for the two PC factors showing spectral ability to sort based on their size. 'Washington' navel orange fruit were stored for 35 days at temperature of -0.6°C. B represents large fruit (±80mm) from inside and S repersents small fruit (±60mm).

PAPER 3: POSTHARVEST APPLICATION OF THIABENDAZOLE REDUCES CHILLING INJURY OF NAVEL ORANGE FRUIT

Abstract

Previous studies indicated that the use of thiabendazole (TBZ) is effective in reducing the incidence of chilling injury (CI) in certain Citrus cultivars. The aim of this study was to determine the effect of different postharvest fungicides dips of the same chemical family, benzimidazoles, on the CI susceptibility of 'Washington' navel oranges (*Citrus sinensis* L. Osb.) in warm and cold water. A dose response of the most effective fungicide was done. In addition the use of a semi-commercial packline was used to apply waxes and drenches, with or without thiabendazole, to determine the effect on CI incidence. Different fungicides at commercial rate, Benomyl® ($2g.L^{-1}$), Benazid® ($2mL.L^{-1}$), Tecto® ($2mL.L^{-1}$) and ICA-TBZ®, ($4mL.L^{-1}$) were applied to 'Washington' navel oranges two minutes in either cold water (10 °C) or warm water (45 °C). The concentration of Tecto® tested as the commercial rate ($2mL.L^{-1}$) as well as a half and quarter of this concentration. 'Washington' navel and 'Autumn Gold' navels were also drenched with water and TBZ as well as carnauba wax incorporated with or without Tecto®. Thereafter the fruit was stored at a temperature of -0.6 °C for 35 days, followed by a shelf life (20 °C for 7 days), prior to scoring of CI incidence and severity. The results of the application of different fungicides in dip indicated that the Tecto® dip treatments were effective in reducing the incidence and severity of CI and were more effective when used in warm water than in cold water. Higher concentration i.e. the commercial recommended rate and half of the commercial recommended rate was effective in reducing the incidence of CI. Wax application was most effective in reducing the incidence of chilling injury; however, the additional application of TBZ in the wax further reduced the incidence of CI.

Introduction

The symptoms of chilling injury (CI) in citrus fruit can be described with different terms i.e. scalding, rind pitting and watery breakdown (Reuther et al., 1989). CI entails the collapse of the individual oil gland or tissue around the gland in the citrus rind which causes pits in the tissue, which in turn cause surface browning and result in large, darken lesions of the rind (Lindhout et al, 2007). CI in citrus fruit is known to be influenced by preharvest factors, i.e. cultivar and canopy position, but also postharvest treatment, i.e. time and temperature exposure below a certain threshold for a cultivar. However, it has been shown that application with postharvest fungicides thiabendazole (TBZ) reduces the incidence of CI in 'Tarocco' oranges (Schirra et al., 1998; Schira and Mulas, 1995) and 'Star Ruby' grapefruit (Schirra et al., 2000). Schirra et al. (2000) reported that a high concentration of 1200 $mg.L^{-1}$ TBZ in a dip for 3 min at room temperature, didn`t alleviate the incidence of CI. In contrast 200 $mg.L^{-1}$ in hot water at 50 °C dip for 3 min proved to be effective in reducing incidence of CI. Imazalil (IMZ) used to control *Pennicillium* spp. in citrus, with a different mode of action, was applied at 200 $mg.L^{-1}$ (50 °C) but no more beneficial effects were seen compared to only hot water dip application (Schirra et al., 2000). TBZ is a systematic benzimidazole fungicide used to control fruit and vegetable diseases such as mould, blight and stain, but in the past two decades there was an increased fungicide resistance towards benzimidazoles. Even though the effect of TBZ has been identified ±20 years ago the mode of action has not been elucidated (Holmes and Eckert, 1999)

Wax coatings are applied in the citrus pack houses to maintain fruit quality, prevent undesirable quality changes, i.e. water loss, and it is a useful carrier of fungicides (Hall, 1981; Mannheim and Soffer, 1996). Different coatings are used in citriculture i.e. polyethylene, carnauba, shellac and resin. Polyethylene is a synthetic wax coating which is manufactured from polymerizing ethylene gas obtained from petroleum gas (Petracek et al., 1998), whereas Carnauba wax is a protective coating extracted from the leaves of *Copernica cerifera,* a Brazilian palm tree. Shellac is a natural product gained from the refinement of raw lac, a resinous secretion of tiny insect called *Laccifer lacca,* which is a parasite on certain trees and originates from India and Thailand (Hagenmaier, 1998; Kaplan, 1986; Dou, 2004). Wax formulations change constantly and new formulations are used with new surfactants and emulsifiers to improve the water loss and decrease build up of CO_2. Hagenmaier (2000) reported that different type of waxes were applied such as carnauba and polyethylene synthetic to maintain fruit quality, reduced fruit weight loss and shrinkage. Furthermore Dou (2004) reported that with 'Marsh' grapefruit the application of wax coating reduces the incidence of CI.

The composition of the applied wax could negatively affect the fruit physiology processes and may prevent the gaseous exchange of CO_2 and O_2 over the artificial barrier, which could lead to the formation of off-flavours as a result of anaerobic respiration. As reported by Hagenmaier (2002) and Hagenmaier and Baker (1994), the coatings on mandarin hybrids which contain shellac or resin has a low O_2 permeability and consequently results in off-flavours producing ethanol and acetaldehyde (Petracek et al., 1998).

The first objective of this study was to determine the effect of different postharvest fungicides of the benzimidazole chemical family i.e. Carbendazim, (Benazid®), thiabendazole (TBZ) (Tecto® and ICA-TBZ®) as well as benomyl (Benomyl®) on the CI susceptibility of navel orange fruit and to determine the efficacy between applying these fungicides in warm or cold water. The second aim was to determine the effectiveness of TBZ in the semi commercial packline, where TBZ is applied in the waxes or/ and drenches. It is hypothesised that TBZ does not reduce the incidence of CI in navel orange fruit.

Materials and Methods

'Washington' navel orange fruit was harvested on 25 July 2011 at Brakfontein Citrusdal (32°.51'S 18°99' E) which was planted in 1982 on 'Rough' lemon (*Citrus jambhiri* Lush) rootstock in an east to the west direction. The tree density was 5.7 m by 3 m and the harvest load of the trees was 40 ton/ha in 2011. For this experiment 600 'Washington' navel orange fruit of equal size and colour was harvested and divided into 20 fruit for each treatment with three replicates. Different fungicides, benomyl (Benomyl®; $2g.L^{-1}$), Carbendazim (Benazid®; $2mL.L^{-1}$) and thiabendazole (Tecto®; $2mL.L^{-1}$) and ICA-TBZ®; $4mL.L^{-1}$) were the treatments. These different treatments were applied to the fruit for 2 min in either cold water (10 °C) or warm water (45 °C). The controls used were 'Washington' navel oranges dipped in warm (45 °C) water and cold (10 °C) water for 2 minutes. Thereafter the fruit was stored for 35 d at -0.6 °C. The fruits were scored for CI according to following index directly after storage and after a week of shelf life (20 °C for 7 d) (Fig.1).

$$\text{Chilling injury index (0-3)} = \sum \frac{[\text{Chilling injury (scale 0-3)} \times \text{no. of fruit in each class}]}{\text{Total number of fruit in replicates}}$$

After the results of the previous experiment were available, 'Cambria' and 'Autumn Gold' navel orange fruit (both late maturing cultivars) were harvested from Tien Riviere (32°.81'S, 19.08E) in Citrusdal on 14 Augustus 2011. For this experiment different concentrations of the TBZ (Tecto®) were tested viz. the commercial recommended rate ($2mL.L^{-1}$) as well as dilutions of a half and quarter of this concentration. These different treatments were applied to the fruit for two minutes in either cold water (10 °C) or warm water (45 °C). Thereafter, the fruit was stored at -0.6 °C for 35 d, kept at shelf life (20 °C for 7 d), prior to scoring of CI incidence and severity.

The 2012 experiment consisted of applying TBZ as a water dip (warm vs. cold) and in a drench as well as in the wax. The experiment was repeated with 'Washington' navel followed by 'Autumn Gold' navel orange fruit. 'Washington' navel (900) orange fruit was harvested from WST-Karingmelksvlei (32° 71'S 19.05E) in Citrusdal on 20 July 2012, divided into eight treatments with three replicates of 30 fruit per replicate while the rest of the fruit was used in the packline trial between the different treatments as pusher fruit to separate between treatments. 'Autumn Gold' navel orange fruit (700) were harvested from Rosedale in Robertson on 20 August 2012. For both sets of fruit the rind colour and size were within a narrow range to reduce variability.

The eight treatments consist of dipping, drenching and waxing treatments. In the dip treatments the fruit were dipped for 1 min in 1000 ppm TBZ solution and in water for the control, these treatments were applied either in cold water (10 °C) or hot water (35 °C) and the fruit were allowed to air dry. See Table 1 for detail on treatments.

The drench consists of pipes with openings, a water flow pump and a container with solution. The water or solution was pumped through the pipes out of openings onto the crate with fruit (Fig. 2). Fruit were exposed to the drench for 45 sec using water (control) and a 1000ppm TBZ solution where after the fruit were air dried. For the wax and drench treatments four different treatments were tested, i.e. first the application of only water in the drench and a clean wax, secondly drench with TBZ and clean wax, thirdly water in the drench and application of a TBZ wax, and lastly drench with TBZ and wax with TBZ.

The application of the wax was done with a custom-built experimental packline (Dormas, Johannesburg, South Africa) which is similar to packlines at commercial pack houses. This packline consist of four different units, an elevator which feed fruit into the line, spray–on recycling washing system over eight brushes, a commercial coating applicator (JBT Foodtech, Brackenfell, South Africa) and an air drying tunnel. The drying tunnel uses very high volume air at a slow speed to dry the fruit. The coating applicator was calibrated using one pulsating nozzle (0.5s on, 2s off) at 22 $mL.min^{-1}$ (3 bar). The fruit moves across the packline against a set speed since the packline is speed controlled and the coatings units has brush sweep paddles which moves the fruit (Njombolwana, 2011). The wax used was the natural based 18% solids consisting of carnauba-shellac based formulations (875 High Shine, John Bean Technologies, Brackenfell, South Africa). The fruit were treated with clean wax with no addition as well as wax with TBZ at a concentration of 4000 $\mu g.mL^{-1}$. The TBZ and wax was stirred throughout the trial with a magnetic stirrer. The fruit were exposed to the coating applicator for 20 s which affect a coating load of 1.2 $L.ton^{-1}$ of fruit.

After the application of the different treatments for the 'Washington' navel orange fruit, six fruit of each replicate were used to determine maximum residue level (MRL) and then six fruit were divided into the flavedo, albedo and pulp tissue to determine the TBZ content in each. The rest of the fruit and the 'Autumn Gold' navel fruit was stored at a temperature of -0.6 °C for 40 d, kept at shelf life (20 °C for 7 d), prior to scoring of CI incidence and severity.

The fruit was prepared for residue only in the following manner; two of the three replicates of the various treatments were used for TBZ residues for the 'Washington' navel orange fruit. From each treatment combination six fruit were frozen (-20 °C) after being weighed until further preparation. Fruit was defrosted, and by using a fruit blender the fruit was ground to a fine pulp (Salton Elite, Amalgamated Appliance Holdings Limited, Reuven, South Africa). For the determination of TBZ distribution in the fruit, part of the flavedo, albedo and pulp of three oranges of each treatment were separated. The samples were frozen in liquid nitrogen and stored at a temperature of -80 °C. Samples were submitted for TBZ residue analyses by an accredited analytical laboratory (Hearshaw and Kinnes Analytical Laboratory, Westlake, Cape Town, South Africa). Extraction of the samples was done by using acetonitrile and matrix solid phase dispersion extraction. The analysis of the extraction was done using a liquid chromatography mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA) (Njombolwana, 2011).

Statistical analysis

Data on CI and residues was analysed by the one way Anova and two way Anova test using Statistical analysis system (SAS v 6.12; SAS institute, Cary, UC, USA). Analysis witthout two-way Anova indicates that the interaction between temperature and the fungicide was not significant. Each treatment was compared with the other using Fischer least significance differences with one way Anova, which is a test to determine the difference between the treatments. The p-values illustrate the significance differences in the figures presented. If *p*-value is smaller than 0.05 there is a significant difference between treatments.

Results

CI of 'Washington' navel oranges significantly differ between fruit applied with Tecto in warm water and Tecto in cold water (Fig. 3). Using various fungicides in warm water was more effective in reducing CI compared to applying the fungicide in cold water.

In the dip experiment done in 2011 where four different chemicals of the benzimidazole family were each applied as dip to 'Washington' navel orange fruit, Tecto® significantly differed with the control and Benomyl; however, no significant differences were obtained from Benazid or ICA-TBZ (Fig, 4 and 5). The application of Benomyl shows a high incidence of CI at the calyx of the fruit (Fig. 5). Application of the four fungicides in cold water resulted in no significant differences in CI index, although Tecto® had the lowest incidence of CI compared to all the other fungicides used in cold water (Fig. 4).

A dose response to Tecto® was evident in the CI of 'Autumn Gold' navel oranges applied with dilutions in warm water. At the lowest concentration, a quarter of the recommended rate Tecto® ($2mL.L^{-1}$), there was a 52 % and 36% higher incidence of CI in comparison to the ½ and full concentration respectively (Fig. 6). Less of a dose response was evident in the 'Cambria' navel orange fruit (Fig.7).

During the second season of experiments (2012), application of TBZ in the drench, dip or wax resulted in reduction of CI of 'Washington' and 'Autumn Gold' navel orange fruit. Even though 'Autumn Gold' navel orange fruit dipped in warm water (35 °C) for 1 min without TBZ reduced the incidence of CI significantly compared to the cold water (control), the dip application of warm water with TBZ was more effective in reducing the incidence of CI (Table 1). However there wasn`t a significant difference in the application of TBZ in warm or cold water, the application of fungicide in cold water numerically has the highest incidence of CI.

All treatments which received wax (with or without TBZ) reduced CI in both cultivars and a 70% reduction was obtained compared to warm water dip treatments (Table 1). However when the drench and wax treatments (with or without wax) were analysed separately, for 'Autumn Gold' navel orange fruit the application of TBZ in the wax significantly decreases the incidence of CI further (Table 2). Warm water dip of 'Washington' and 'Autumn Gold' navel orange fruit respectively, resulted in 84% and 95% higher CI index compared to fruit drenched in TBZ and applied with wax incorporated with TBZ (Table 1). The residue levels of TBZ measured from the 'Washington' navel orange fruit were high in all the treatments i.e. TBZ dip, drench or wax (Table1). The residue of fruit dipped in warm or cold water TBZ, or where TBZ was applied in the drench and the wax was significantly higher compared to the treatments where TBZ was only applied in the drench or in the wax. The TBZ residues in the different tissue of the fruit i.e. flavedo, albedo, pulp follow the same pattern at lower concentrations in the following order flavedo>albedo>pulp (Table 3). The residues were not significantly different for fruit dipped in warm water or to cold water TBZ.

Discussion

TBZ applied as a dip in 2011 and 2012 on the three different navel orange cultivars 'Washington', 'Autumn Gold' and 'Cambria', did reduce the incidence of CI significantly when applied in warm water. Similarly, Schirra et al. (1998) reported that the application of

TBZ reduce the incidence of CI in 'Tarocco' oranges with concentration of 200ppm in warm water (50 °C) and 1200 ppm in cold water. No significant differences were found between using ICA-TBZ®, Benazid® and Tecto®; however, Tecto® was consistently the most effective in reducing the incidence of CI. Furthermore the application of this fungicide in hot water was more effective to reduce CI which could be due to the better uptake of the TBZ in the fruit rind. Increased efficacy of TBZ to reduce CI in combination with hot water was demonstrated previously in citrus fruit (McDonald et al., 1991; Schirra and Mulas, 1995). Schirra et al. (1996) also indicated that the enhanced activity of heated chemicals could be related to the higher deposition of the active ingredient on the fruit. Schirra and D'hallewin, (1997) explained that hot water dips induce a redistribution of the epicuticular wax and a melting of the waxy layer, which could aid in TBZ uptake into the rind.

By applying TBZ in a warm water dip (1 min, 35 °C) a reduction in CI was seen compared to only warm water dip. In contrast Lindhout, (2007) reported that dipping 'Lane Late' navel orange fruit in 50 °C for 3 min reduced the incidence of CI, but adding TBZ (1000 ppm) did nott reduce the incidence any further. The dip application with TBZ (Tecto®) at the higher temperature 45 °C for 2 min in 2011 seemed to be 56% more effective in reducing the incidence of CI compared to the dip treatment for 1 min at 35 °C for 'Washington' navel. This high temperature dipping is however, commercially difficult because of the significant amount of heat transfer when fruit are processed in packing runs and the cost for electricity to heat the water to 45 °C.

There was not a significant difference in the TBZ residue applied in a warm or cold dip, even though there was a significant difference in CI. This could indicate that the warm water, known to reduce CI (Schirra and D'hallewin, 1997) could to a large extend be responsible for the CI reduction. Some of the physiological effects of heat treatment are the accumulation of heat shock proteins, other molecular chaperones and the induction of other defence pathways (Lafuente et al., 1991). Molecular chaperones are proteins which bind to the folded protein and will help to promote the correct folding of the specific substrate. Heat shock proteins accumulate in response to sudden high temperatures (Lafuente et al., 1991). Therefore the reduction in CI by warm water TBZ could be the result of a synergistic effect of warm water induced protective process as well as a currently unknown mechanism of TBZ; however, this hypothesis need further testing. The maximum residue level which is measured for the whole fruit is 5-6 $mg.L^{-1}$ depending on the market (van Zyl, 2011), was not exceeded where the highest value was 2.95 $mg.L^{-1}$ for applying TBZ in the wax and in the drench (Table 1).

Even though the mode of action of TBZ is not known, the effect of TBZ may be associated with a decrease rate of rind senescence as shown with the reduction of rind colour development (Schiffman-Nadel et al., 1975). Benzimidazole treatments have also been associated with a decrease in senescence of wheat leaves (Mishra and Waygood, 1968), which is involved in the delay of protein degradation and net protein synthesis which is revealed through ratios of soluble and insoluble nitrogen fractions (Samborski et al., 1958, Lindhout, 2007). An additional explanation offered by Schiffman-Nadel et al. (1975) is that latent fungal infections develop in the cold storage with loss of fruit resistance also in combination with development of CI. Therefore it can be due to the inhibition of fungal infection that CI incidence is less with the application of TBZ.

The use of different dilutions of TBZ (Tecto®) applied as postharvest dips in warm water indicated that a dosage threshold for effective uptake and protection exists i.e. a quarter (1/4) of the commercial recommended rate wasn`t effective in reducing the incidence of CI in 'Autumn Gold' however in 'Cambria' there were no concentration effects . The commercial recommended rate ($2mL.L^{-1}$) and the application of the half of the commercial recommended rate was effective in reducing the incidence of CI for 'Autumn Gold navel oranges which correspond to the results by Schiffman-Nadel et al., (1972).

The application of wax was most effective in reducing the incidence of CI. Citrus fruit consist of a layer of epidermal wax and the cuticle, but this cuticle can form microscopic cracks (Cohen et al., 1994) and become damaged during the postharvest handling which allows moisture loss. Wax application is used to seal the fruit to water loss during export and cold storage. It proposes that the waxing of fruit creates more a physical barrier through preventing moisture loss (Lindhout, 2007). Lindhout, (2007) reported that the application of wax was most effective to reduce CI compared to other treatments and the additive effect of wax with other treatments i.e. heat treatments (3 min 50 °C), the dip of fruit in TBZ and the application of methyl jasmonate. For 'Autumn Gold' navel orange fruit, fruit applied with only wax had a significant higher incidence of chilling injury compared to fruit applied with

TBZ in the wax. The application of TBZ in the wax has the effect of both TBZ and wax which reduce the incidence of chilling injury.

The average residue levels in the flavedo, albedo and the pulp of the fruit indicate that 96% of the TBZ residue is located in the flavedo of the orange fruit. The highest residue level in the albedo and pulp was with fruit applied with warm and cold dips followed by the application of TBZ in the drench and in the wax. In addition when TBZ is only applied in the drench and not in the wax there is lower residue in the flavedo compared to the application of TBZ in the wax.

The application of TBZ in dip had a higher residue level compared to the application of wax with TBZ even though the CI incidence of the fruit dip in TBZ was significant higher compared to the fruit applied with wax. This could indicate that wax application could to a large extend be responsible for the CI reduction.

To conclude, the application of TBZ was the most effective in reducing the incidence of CI compared to the other fungicides in the family of the benzimidazoles. In addition TBZ has a higher efficacy when applied in warm water dip of 35ºC or 45 ºC at 1 min or 2 min, respectively. The specific mechanism of TBZ is still unknown but the positive association of TBZ and hot water could be in the up regulation of stress defence mechanism and enhancement of fruit resistance. The synergistic effect of the warm water protection process and the TBZ mechanism result in reduction in CI. The application of postharvest wax was more effective in reducing the incidence of CI compared to the TBZ dip treatments. However, the application of TBZ with wax was overall the most effective in reducing the incidence of CI.

References

Cohen, E., Shapiro, B., Shalom, Y. and Klein, J.D. 1994. Water loss: a non-destructive indicator of enhanced cell membrane permeability of chilling injured citrus fruit. J. Amer. Soc. Hort. Sci. 119: 983-986.

Dou, H. 2004. Effect of coating application on CI of grapefruit cultivars. Postharvest Biol. Technol. 39: 558-561.

Hagenmaier, R.D. 1998. Wax microemulsion formulations used as fruit on coatings. Proc. F. S. Hort. Soc. 111: 251-255.

Hagenmaier, R.D. 2000. Evaluation of a polyethylene-candelilla coating for 'Valencia' oranges. Postharvest Biol. Technol. 19: 147-154.

Hagenmaier, R.D. 2002. The flavor of mandarin hybrids with different coatings. Postharvest Biol. and Technol. 24: 79-87.

Hagenmaier, R.D. and Baker, R.A. 1994. Internal gases, ethanol content and gloss of citrus fruit coated with polyethylene wax, carnauba wax, shellac or resin at different application levels. Proc. F. S Hort. Soc. 107: 261-265.

Hall, D. 1981. Innovation in citrus waxing - An overview. Proc. FI. Hort. Soc. 94: 258-263.

Holmes, G.J. and Eckert, J.W. 1999. Sensitivity of *Penicillium digitatum* and *P. italicum* to Postharvest citrus fungicides in California. Postharvest Pathology and Mycotoxins. 89: 716-721.

Kaplan, H.J. 1986. Washing, waxing and color-adding. Chapter 2 In: Fresh Citrus Fruit. W.F. Wardowski, S. Nagy and W. Grierson, (eds.) Van Nostrand Reinhold Company, Florida, USA. pp. 379-395

Lafuente, M.T., Belver, A., Guye, M.G. and Saltveit, M.E. 1991. Effect of temperature conditioning on chilling injury of cucumber cotyledons. Possible role of abscisic acid and heat shock proteins. Plant Physiol. 95:443-449.

Lindhout, K. 2007. Physiology of chilling related postharvest rind breakdown of Navel oranges (*Citrus Sinensis* (L.) Osbeck). PhD dissertation, La Trobe University, Bundoora, Australia.

Mannheim, C. and Soffer, T. 1996. Permeability of different wax coatings and their effect on citrus fruit quality. Food Chemicals 44: 919-923.

McDonald, R. E., Miller, W. R., McCollum, T. G. and Brown, G. E. 1991. TBZ and imazalil applied at 53ºC reduce chilling injury and decay of grapefruits. HortScience 26:397-399.

Mishra, D. and Waygood, E.R. 1968. Effect of benzimidazole and kinetin on the nicotinamide nucleotide content of senescing wheat leaves. Can. Biochem. 46:167-178.

Njombolwana, N. 2011. Imazalil and wax coating application in citrus packhouses to control green mould and preserve fruit quality. MSc dissertation, Stellenbosch University, Stellenbosch, South Africa.

Petracek, P.D., Dou, H. and Pao, S. 1998. The influence of applied waxes on postharvest physiological behaviour and pitting of grapefruit. Postharvest Biol. Technol. 14: 99-106.

Reuther, W., Calavan E.C. and Carman, G.E. 1989. The citrus industry. Postharvest disorders and diseases. University of California, Division of Agricultural Sciences, Berkley, USA.

Samborski, D.J., Forsyth, F.R. and Person, C. 1958. Metabolic changes in detached wheat leaves floated on benzimidazole and the effect of these changes on rust reaction. Can. Bot. 36: 591-601.

Schiffman-Nadel, M., Chalutz, E., Waks, J. and Dagan, M. 1975. Reduction of chilling injury in grapefruit by TBZ and benomyl during longterm storage. Amer. Soc. Hort.Sci.100: 270-272.

Schirra, M., Cabras, P., Angioni, A. and Melis, M. 1996. Residue level of imazalil fungicide in lemons following prestorage dip treatment at 20 and 50 °C. J. Agric. Food Chem. 44:265-286.

Schirra, M., Cabras, P., D'allewin, G., Angioni, A. and Garau, V.L. 1998. Seasonal susceptibility to chilling injury of 'Tarocco' oranges as affected by hot water and thiabendazole postharvest dip treatments. J. Agric. Food Chem. 46: 1177–1180

Schirra, M. and D'hallewin, G. 1997. Storage performance of 'Fortune' mandarin following hot water dips. Postharvest Biol. Technol. 10: 229-237.

Schirra, M., D'hallewin, G., Cabras, P., Azngioni, A., Ben-Yehoshua, S. and Lurie, S. 2000. Chilling injury and residue uptake in cold-stored 'Star Ruby' grapefruit following thiabendazole and imazalil dip treatments at 20 and 50 ºC. Postharvest Biol. Technol. 20:91–98.

Schirra, M. and Mulas, M. 1995. Improving storability of 'Tarocco' oranges by postharvest hot-dip fungicide treatment. Postharvest Biol. Technol. 6:129-138.

Van Zyl, K. 2011. The control of fungal ,viral and bacterial diseases in plants. AVCASA, Halfway house, South Africa. pp 24-25.

Tables and Figures:

Table 1. Chilling injury index of ‘Washington’ and ‘Autumn Gold’ navel orange fruit as well as the TBZ residue loading application of TBZ (Tecto®) in the different treatments done in 2012. TBZ was applied at 1000 ppm in all dip and drench treatments and as 4000 ppm in the wax. The fruit were stored for 40 d at -0.6 °C plus shelf life (20 °C for 7 days). Residues were determined on fresh fruit weight basis ($P \leq 0.05$).

	‘Washington’		**‘Autumn Gold’**
	CI index	TBZ Residue (mg/kg FW)	CI index
Cold water dip (15 °C)	1.09a	0.00c	1.28a
Warm water dip(35 °C)	0.81a	0.00c	1.03b
Cold water TBZ dip	0.80ab	2.63a	0.88bc
Warm water TBZ dip	0.42bc	2.82a	0.75c
Water drench + wax	0.25c	0.00c	0.17d
TBZ drench + wax	0.18c	0.97b	0.08d
Water drench + TBZ wax	0.13c	0.70b	0.05d
TBZ drench +T BZ wax	0.13c	2.95a	0.03d
p-value	0.0002	0.0001	0.0001

*means with a different letter differ significantly at the 5% level (LSD)

Table 2. Chilling injury index of 'Washington' and 'Autumn Gold' navel orange fruit as well as the TBZ residue loading application of Tecto® in the different wax treatments done in 2012. TBZ was applied at 1000 ppm in all dip and drench treatments and as 4000 ppm in the wax. The fruit were stored for 40 d at -0.6 °C plus shelf life (20 °C for 7 d).Residue were determined on fresh fruit weight basis.

	'Washington'		**'Autumn Gold'**
	CI index	TBZ Residue (mg/kg FW)	CI index
Water drench + wax	0.25c	0.00c	0.17a
TBZ drench + wax	0.18c	0.97b	0.08ab
Water drench + TBZ wax	0.13c	0.70bc	0.05b
TBZ drench + TBZ wax	0.13c	2.95a	0.03b
p-value	0.247	0.023	0.045

*means with a different letter differ significantly at the 5% level (LSD)

Table 3. TBZ (Tecto®) residue loading in the flavedo, albedo and the pulp of 'Washington' navel orange fruit after various postharvest treatments. TBZ was applied at 1000 ppm in all dip and drench treatments and as 4000 ppm in the wax The fruit were stored for 40 d at -0.6 °C plus shelf life (20 °C for 7 d).

	TBZ residue (mg/kg FW)		
	Flavedo	Albedo	Pulp
Cold water dip (15 °C)	0.00c	0.00c	0.00c
Warm water dip(35 °C)	0.00c	0.00c	0.00c
Cold water TBZ dip	7.29a	0.18a	0.06ab
Warm water TBZ dip	7.59a	0.25a	0.07a
Water drench + wax	0.00c	0.00c	0.00c
TBZ drench + wax	2.84b	0.05b	0.03b
Water drench +TBZ wax	3.41b	0.05bc	0.04b
TBZ drench +TBZ wax	6.95a	0.14b	0.07a
p-value	0.0002	0.0002	0.0004

*means with a different letter differ significantly at the 5% level (LSD)

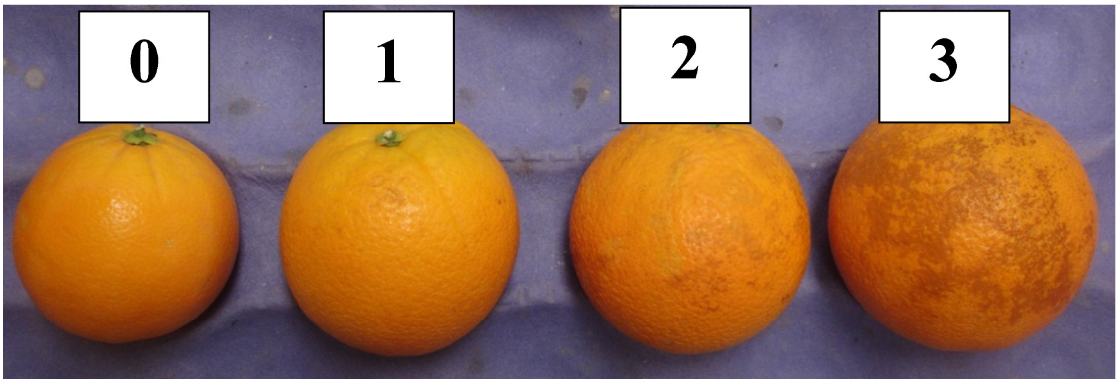


Figure 1. Chilling injury scoring of navel oranges (0 = zero, 3= severe)

Figure 2. Photo of the drench application used for 45 seconds on navel orange fruit.

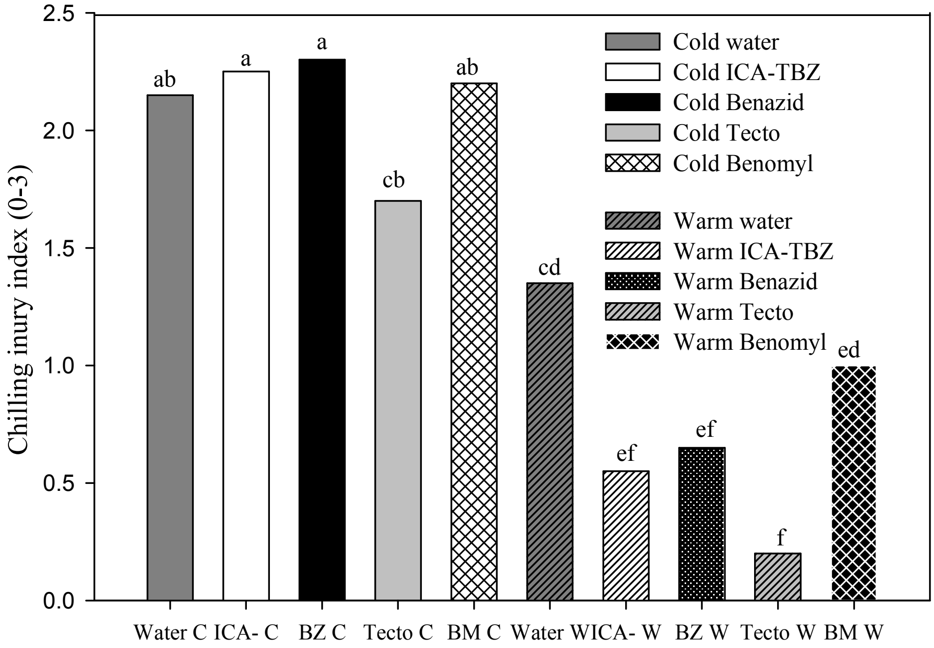


Figure 3. Difference in the chilling injury index (0-3) of 'Washington' navel oranges dipped with cold water 10 °C and warm water 45 °C water, with different fungicides benomyl (Benomyl®; 2g.L^{-1}), Carbendazim (Benazid®; 2mL.L^{-1}) and thiabendazole (Tecto®; 2mL.L^{-1}and ICA-TBZ®; 4mL.L^{-1}) for 2 minutes and stored for 35 d at -0.6 °C and kept at shelf life (20 °C for 7 d) done in 2011.

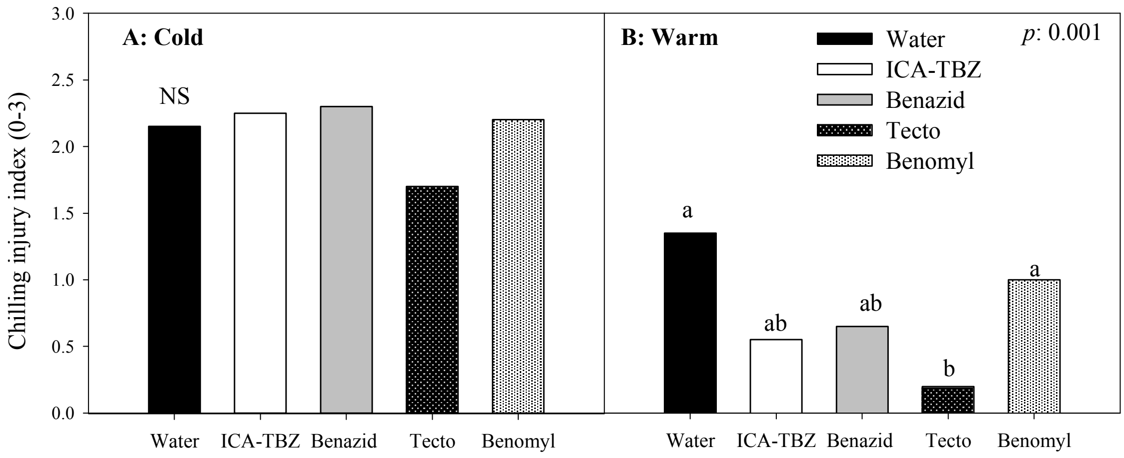


Figure 4. Difference in the chilling injury index (0-3) of 'Washington' navel orange fruit treated with different fungicides i.e. benomyl (Benomyl®; 2g.L^{-1}), Carbendazim (Benazid®; 2mL.L^{-1} and thiabendazole (Tecto®; 2mL.L^{-1} and ICA-TBZ®; 4mL.L^{-1}) for 2 minutes in cold water 10 °C (A) and warm water 45 °C (B) prior to storage at -0.6 °C for 35 d plus shelf life (20 °C for 7 d) ($p \leq 0.01$) done in 2011.

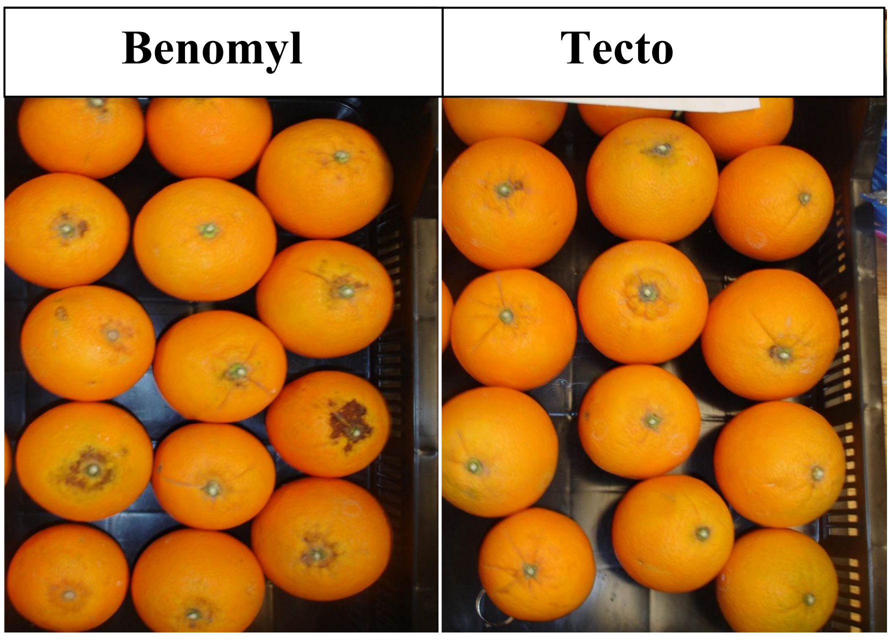


Figure 5. Example of the chilling injury incidence of ' 'Washington' navel dipped in Benomyl® and Tecto® for 2 min at 45 °C and stored for 35 d at -0.6 °C plus shelf life (20 °C for 7d) from 2011.

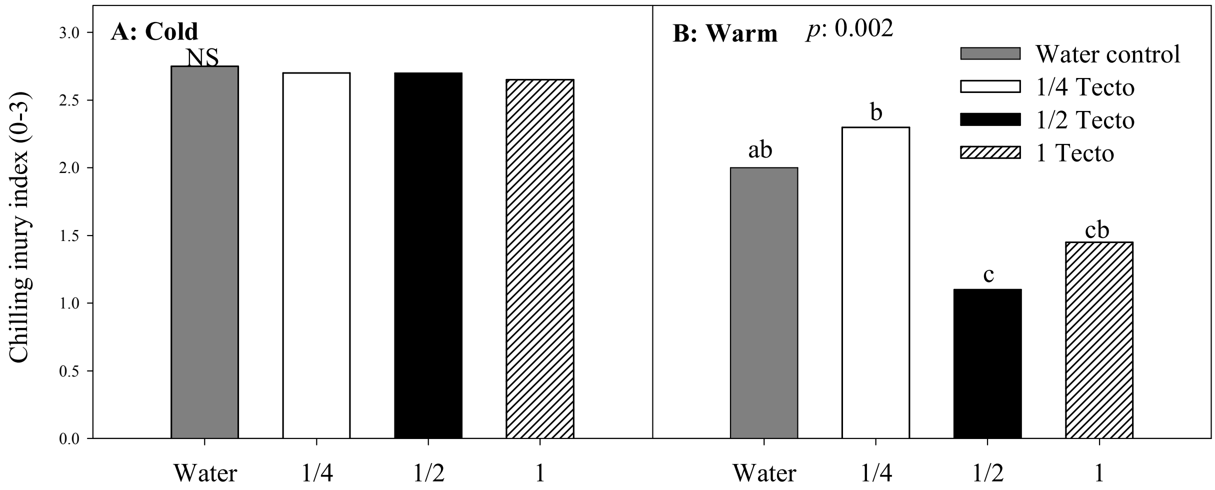


Figure 6. Difference in the chilling injury index of 'Autumn Gold' navel orange fruit treated with three concentrations of TBZ (Tecto®); full 2mL.L^{-1}, half 1mL.L^{-1} and quarter 0.5 mL.L^{-1}, for 2 minutes in cold water (A) and warm water 45 °C (B) and stored for 35 d at -0.6 °C plus shelf life (20 °C for 7d) done in 2011 ($p \leq 0.01$).

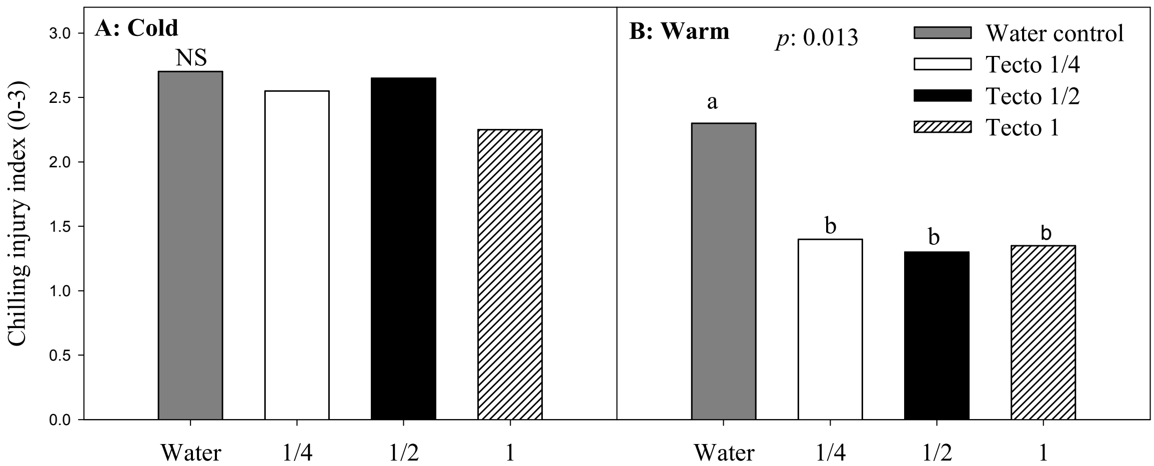


Figure 7. Difference in the chilling injury index of 'Cambria' navel orange fruit treated with three concentrations of TBZ (Tecto®); full 2mL.L^{-1}, half 1mL.L^{-1}and quarter 0.5 mL.L^{-1}, for 2 minutes in cold water (A) and warm water 45 °C (B) and stored for 35 d at -0.6 °C plus shelf life (20 °C for 7d) ($p \leq 0.01$) done in 2011.

GENERAL DISCUSSION AND CONCLUSION

Citrus fruit from South Africa are exported to countries such as the USA, Korea, Thailand and China with a mandatory cold sterilization treatments were fruit must be exposed to -0.6 °C for 24 days to kill all insect larvae of false codling moth and fruit fly in the fruit. However, the cold temperatures can result in CI which negatively affects the external quality of the fruit and can result in dramatic financial losses. The first objective of the study was to investigate the effect of different pre- and postharvest factors affecting the CI incidence of navel orange fruit. In this study it was shown that cultivar, harvest date and site significantly influence the susceptibility to CI incidence of navel orange fruit. ‘Washington’ navel orange fruit was found to be more susceptible to CI compared to the ‘Navelina’, and therefore cultivar choice for specific steri-markets is an important part of a strategy to reduce the incidence of CI and to ensure postharvest quality of navel orange fruit.

Incidence in CI differ between the different areas in the Citrusdal valley and at the top of the valley (Tharakama),the coldest part with highest rainfall, both cultivars had higher incidence of CI compared to lower down in the valley i.e. Hexrivier and Ouwerf where it is warmer and dryer. It therefore could be construed that the lower preharvest temperature could negatively affect the postharvest incidence of CI. In addition harvest date or maturity can affect the incidence of CI as immature and over mature fruit are generally more susceptible to CI compared to the fruit harvested at the optimum commercial harvest date.

It was hypothesised that there will be an increase in the malondialdehyde as the fruit were stored longer; however, malondialdehyde content (lipid peroxidation) did not show any specific trend in relation to CI. However the fruit that is harvested late vs eraly show some difference with the fruit harvested late had a higher average MDA concentration. There is the possibility that there is no trend or that other compounds in citrus rind interfered with the measurements. For future research, the lipid peroxidation could be measured with high performance liquid chromatographer (HPLC) to enhance accuracy. Measurement of ethylene production during storage showed a spike in the ethylene production for ‘Washington’ navel orange fruit stored for 21 or 35 days with an increase in chilling injury on day 35. However, commercially stored fruit also had chilling injury similar to early harvested fruit but with no spike in ethylene. However, 'Navelina', which was harvested later, did not develop CI but did have an increase in ethylene. It seems therefore unclear whether chilling injury can be noted by a spike in ethylene before symptom development.

Canopy position, fruit size and rind colour do affect the incidence of CI. Fruit from outside has a higher incidence of CI compared to fruit from inside of the canopy. Due to the high light intensities in, Citrusdal (the fruit growing area in this study), the fruit exposed to the sunlight, outside the canopy, could result in the fruit developing latent sunburn symptoms thereby increasing fruit susceptibility to CI, as seen in 'Star Ruby' grapefruit. Furthermore smaller fruit are more prone to CI compared to larger fruit and it is thought that larger fruit are generally a better sink, which would result in a higher carbohydrate accumulation in the flavedo and reduce CI susceptibility.

The NIR analysis was successful, after using PCA plots, to distinguish between fruit from different canopy positions and fruit sizes. However, the reliable prediction of colour parameters and firmness were difficult to obtain using integrated sphere NIR measurements from a wavelength between 800-2500nm, however according to literature it is possible to predict the rind colour using wavelength of 450-2500nm which includes the visible spectrum. The biochemical changes of the rind such as carotenoid and lipid peroxidation could therefore be included in future studies to possibly be determined with this non-destructive tool in order to develop a more robust prediction model for CI.

Various postharvest treatments such as fungicide and wax application investigated in this study reduced the incidence of CI. The application of TBZ on 'Washington', 'Autumn Gold' and 'Cambria'in 2011in warm water (45 ºC) reduced the incidence of CI more than the application in cold water. The residues of the TBZ applied in cold or warm water did not differ significantly even though CI incidence was reduced. Therefore the reduction in CI by warm water TBZ could be the result of a synergistic effect of warm water induced protective process as well as a current unknown mechanism of TBZ; however, this hypothesis needs further testing.

Approximately 96% of the TBZ residues were in the flavedo of the fruit rind and only a small portion in the albedo and pulp of the fruit. In a screening trial various commercial fungicides from the benzimidazoles family i.e. Benomyl®, Benazid®, Tecto®, ICA-TBZ® were tested for efficacy in reducing CI. However, the TBZ treatment of Tecto®, Benazid and ®, ICA-TBZ® was overall the most effective in reducing the incidence of CI, Tecto® had numerically the highest efficacy. A dose response of TBZ fungicide as dip application was evident in 'Autumn Gold' navel fruit and a too low concentration, ¼ the commercial recommended concentration, was not effective in reducing the incidence of CI. It is therefore advisable that TBZ application must be higher than half of the commercial recommended concentration. The application of postharvest wax was more effective in reducing the incidence of CI compared to the TBZ dip treatments. The wax layer covers the fruit and protects the fruit against moisture loss, however, the addition of TBZ in the wax was overall the most effective in reducing the incidence of CI.

For future prospects, it can be useful to determine a specific biochemical characteristic such as a change of the carotenoids, in the fruit rind which could be related to rind maturity or condition in order to determine optimum harvest date to decrease CI. Further research that focus on identifying non-destructive methods to assist in the sorting of the fruit according to rind quality are needed. Research on the use of NIR in postharvest technology in identifying fruit disorders offer the best opportunity to date and it is proposed that the NIR could be used to assay for example carotenoid content and possibly lipid peroxidation in the flavedo in order to develop a prediction model for CI susceptibility. To reduce the incidence of CI more research could be done about the mode of action of TBZ and how it affects the cellular structures and metabolism which is associated with chilling damage. In addition more research could be done to increase the effectiveness of wax application as well as identifying the components in the different waxes (polyethylene, shellac, bee wax and carnauba) which effect chilling injury. In addition, the interaction of wax with different concentrations of TBZ, should also be optimised to increase the efficacy against the incidence of chilling injury.

To conclude, cultivar and site differences have to be considered prior to orchard planting to avoid the high incidence of CI in exported fruit. Export of 'Navelina' to cold sterilization market could be more suitable because the fruit were less susceptible to CI compared to 'Washington' navel orange. In commercial handling particular attention should be given to 'Washington' navel orange fruit because of its high susceptibility to CI. Fruit have to be harvested at commercial harvest date and not when immature or over mature, however, the ideal will be to determine the optimum rind maturity or condition to reduce the incidence of chilling injury. To reduce the incidence of CI on the packing line, fruit has to be sorted according to size and only bigger (±75-82mm) should be packed for export. Furthermore, fruit with sunburn or other lesions should not be packed for export to these cold sterilization markets. Wax application as well as TBZ in the wax (4000ppm) is advisable to reduce the incidence of CI.