Influence of rind water content on mandarin *citrus* fruit quality

by

Jéanine Joubert

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Supervisor: Dr. P.J.R. Cronjé
Dept. of Horticultural Science
Stellenbosch University
South Africa

Co-supervisor: Dr. E.W. Hoffman
Dept. of Horticultural Science
Stellenbosch University
South Africa

Co-supervisor: Prof. L. Zacarías
Extraordinary professor: Dept. of Horticultural Science
Stellenbosch University
South Africa

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DECLARATION

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Date: March 2016

Jéanine Joubert

SUMMARY

‘Nules Clementine’ and ‘Nadorcott’ mandarin are commercially important *Citrus* cultivars in South Africa. Both cultivars are prone to develop rind breakdown and pitting which are considered non-chilling related postharvest physiological rind disorders. The progressive and erratic nature of these rind disorders result in high financial losses. The incidence of a rind disorder is thought to be associated firstly with an increased susceptibility, as influenced by pre-harvest aspects; and secondly with a trigger in the postharvest environment. A study was conducted over two seasons a study to determine the effect of late nitrogen (stage II and after summer flush) application, pre-harvest water stress and postharvest handling were conducted. The rind quality of ‘Nules Clementine’ and ‘Nadorcott’ mandarin fruit harvested from Citrusdal and Riebeeck Kasteel was evaluated.

Soil applications of nitrogen at 20 kg·ha^{-1} and 40 kg·ha^{-1} were done on 21 January and 26 March 2014/2015, respectively. This was in addition to the standard 300 kg·ha^{-1} nitrogen provided by the producer. During 2015 a 1% urea foliar application was sprayed on 26 March. During the post-harvest period all fruit were dehydrated at 25 °C and 60 to 80% RH (0.7 to 1.1 kPa vapour pressure deficit) for two days, followed by rehydration at 100% RH for one day. Subsequently fruit were stored at either -0.6 °C or 4 °C for a 30-day period. There were no significant differences in fruit colour or size between the different nitrogen treatments. No increase in rind disorders and no negative impacts on internal fruit quality were noted.

To determine the impact of pre-harvest water stress, the soil below the tree was covered with plastic sheets three weeks prior to harvest to exclude rainfall or irrigation. The effect of postharvest stress was established by dehydrating and rehydrating fruit at 0.7-1.1 kPa vapour pressure deficit for different periods after harvest. Wax was applied on day 5 and thereafter fruit was stored at 4 °C for 30 days. The results indicated that pre-harvest water stress did not have a detrimental effect on fruit susceptibility to disorders. By early wax application, however, a decrease in moisture loss was recorded, coinciding with lower incidences of rind disorders.

The final part of the study was aimed at determining if postharvest handling lending to water loss, as induced by high vapour pressure, could increase disorders. From the results of the trials it can be concluded that exposing fruit to dehydration increased rind disorder susceptibility. Dehydration prior to wax application on day 5 also increased pitting and rind breakdown, whereas an early wax application, 2 to 3 days after harvest, reduced incidence.

This study serves as a step to resolve the impact of factors predisposing the citrus fruit rind to progressive postharvest disorders.

OPSOMMING

'Nules Clementine’ en ‘Nadorcott' mandaryne is kommersieël belangrike *Citrus* kultivars in Suid-Afrika. Beide kultivars is geneig tot skilafbraak en gepokte skil wat as na-oes fisiologiese skildefekte beskou word en is onafhanklik van koueskade tydens opberging. Die progressiewe- en wisselvallige aard van hierdie skildefekte het groot finansiële verliese tot gevolg. Vermoedelik hou die voorkoms van 'n skildefek eerstens verband met ‘n verhoogde vatbaarheid soos beïnvloed word deur voor-oes aspekte; en tweedens met 'n sneller vanuit die na-oes omgewing. Die invloed van ‘n laat stikstof toediening (fase II en na die somer groei), voor-oes waterstres en na-oes hantering op skildefekte is oor twee seisoene bepaal. 'Nules Clementine’ en ‘Nadorcott' mandaryn vrugskilgehalte, geoes vanaf Citrusdal en Riebeeck Kasteel, is geëvalueer.

Toediening van korrelkunsmis (stikstof) teen 20 kg·ha^{-1} en 40 kg·ha^{-1} is gedoen op 21 Januarie en 26 Maart 2014/2015, respektiewelik. Dié toedienings was bykomend tot die produsent se standaardbehandeling van 300 kg·ha^{-1} stikstof. ’n Blaartoediening van 1% ureum is addisioneel op 26 Maart 2015 aangewend. Alle vrugte was na-oes gedehidreer teen 25 °C en 60 tot 80% RH (0.7 tot 1.1 kPa dampdrukverskil) vir twee dae, gevolg deur ‘n een dag rehidrasie periode van 100% RH. Vrugte is vervolgens opgeberg by óf -0.6 °C óf 4 °C vir 30 dae. Geen beduidende verskil in kleur en grootte van die vrugte tussen die verskillende stikstof behandelings of toename in skildefekte of negatiewe effek op interne vrugkwaliteit was waargeneem nie.

Die impak van voor-oes waterstres op na-oes skilgehalte is bepaal deur grond onder die eksperimentele bome drie weke voor oes met plastiekseile te bedek om sodoende die invloed van reënval en besproeiing uit te skakel. Die effek van na-oes stres was geëvalueer in vrugte wat gedehidreer en rehidreer was teen 0.7 tot 1.1 kPa dampdrukverskil. Die na-oes waks behandeling was op dag 5 toegedien, waarna vrugte opgeberg was by 4 °C vir 30 dae. Resultate dui daarop dat voor-oes waterstres geen negatiewe impak op die vrugte se vatbaarheid vir skildefekte gehad het nie. ‘n Afname in vogverlies is wel gemeet met die wakstoediening wat ooreenstem met 'n laer voorkoms van skildefekte.

Die finale gedeelte van die studie was daarop gemik om te bepaal of na-oes waterverlies, soos geïnduseer deur ‘n hoë dampdruk verskil, die voorkoms van skildefekte kan induseer. Resultate dui aan dat die blootstelling van vrugte aan dehidrasie wel die vatbaarheid vir skildefekte verhoog het. Dehidrasie voor ‘n wakstoediening op dag 5 het ook verhoogde skilafbraak en gepokte skil tot gevolg gehad, terwyl 'n vroeë waks, 2 tot 3 dae na oes, die voorkoms verlaag het. Hierdie studie dien as ‘n skakel om die impak van faktore wat skil-vatbaarheid ten opsigte van fisiologiese defekte verhoog verder te ontrafel en dus sodoende aan te spreek.

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The referencing and formatting style in this thesis is written according to the requirements, in general, of the *Postharvest Biology and Technology* journal. This thesis presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

GENERAL INTRODUCTION

South African citrus production: an export orientated industry

Citrus fruit are grown commercially in more than 50 countries worldwide (Ladaniya, 2008). The South African citrus industry comprising of 64 510 ha in 2014, is the 10th largest producer of citrus fruit internationally (Edmonds, 2015). More than 70% of South Africa's total citrus fruit are exported, making this industry the second largest exporter of fresh citrus worldwide, with export volumes currently at 1.4 million tons. The contribution of the South African citrus industry to the world economy is significant and was estimated in 2013 at more than R9.3 billion (Potelwa et al., 2014). This export-oriented industry is represented by approximately 1300 large scale producers exclusively focussed on export, with another 2200 smaller producers that are together responsible for the 31 million citrus trees cultivated throughout Southern Africa. This dynamic industry provides employment for over 100,000 skilled and unskilled workers throughout various sectors of the value chain, including harvesting, handling, transportation, storage and marketing operations.

Within South African citriculture the recent focus on planting of new cultivars leans heavily in the favour of the Clementine/Mandarin types, due to the ease of removing the rind and a highly desired eating quality. In the current market there is a special emphasis on Late Mandarins which share the same general harvesting window than 'Valencias' oranges in South Africa. Approximately 3040 ha of Mandarins were established by 2013 and approximately 10 million cartons were exported during 2014 (Edmonds, 2014). In 2013 the total Late Mandarin plantings in the Southern Hemisphere was estimated to be at 2032 ha, producing over two million cartons of fruit (Edmonds, 2014). This figure is predicted to expand to 4000 ha in 2017 and could potentially threaten South Africa's 600,000 tonnes of annual citrus exports to Europe - mainly consisting of oranges and lemons – at an estimated value of some 1 billion euros ($1.3 billion) (Edmonds, 2014).

Importance of the rind to attain high fruit quality

The commercial value of a citrus fruit is related to its external quality (Khalid et al., 2012), with emphasis on especially the colour, where orange not yellow is preferred along with the obvious lack of any lesions, due to physical or physiological breakdown (Magwaza et al., 2013). Therefore it is evident that the rind of a citrus fruit plays a critical part to determine the value of fruit.

Rind quality is known to be negatively influenced by various factors including: irrigation, fertilization, degreening protocols, pack house treatments and the cold chain management. The citrus rind is a particularly complex structure as citrus fruit is a special type of berry, known as a hesperidium, with a characteristic, leathery rind. It has two morphologically distinct regions, the pericarp, commonly known as the peel or the rind, and the endocarp, which represents the edible, juicy pulp. The outer layer of the rind is referred to as the flavedo, whilst the inner white, spongy tissue is known as the albedo. The citrus fruit primarily loses water from the rind and very seldom from the pulp (Alquezar et al., 2010), primarily due to the non-vascular connectivity of the juice vesicles. The water content of the rind is therefore directly related to any water loss from the fruit (Gonzalez-Aguilar et al., 1997). The rind is physiologically independent of the pulp and has the same leaf-like physiological processes such as stomatal control and photosynthesis. Also of interest is that the rind contains oil which can be a potential phytotoxic substance within the rind if released from the oil glands.

Impact of physiological rind orders

An increased drive towards higher production plus the long and complex export postharvest cold chain within South Africa and during transport to diverse world markets have led to an increase in the incidence of physiological disorders of the citrus rind such as rind breakdown, pitting and staining in addition to higher susceptibility to decay (Dodd et al., 2010). The Perishable Products Export Control Board (PPECB) reported in 2014 that soft citrus, which includes mandarins, had by far the most rejections at 2.46% in comparison to oranges where a rejection rate of 1.77% was reported for the 2015 season (Julius, 2015). For soft citrus, rind pitting is one of the top three reasons for rejections and was considered the main cause of rejection of approximately 85 500 cartons.

The specific mechanisms of the various physiological rind disorders are to a large extent unknown. It is hypothesized that the development of the disorders can be separated by two distinct phases: firstly fruit are rendered susceptible within a particular pre-harvest environment; secondly, a trigger within the postharvest chain is required to cause the expression of this disposition. It is often difficult to classify physiological disorders, particular where the rind is concerned. It is however important to understand that disorders are almost always associated with a particularly symptomology as the fruit is subjected to a set of specific environmental conditions. Rind staining can for example be described as the collapse of sub-epidermal tissue, with epidermis intact, whereas rind pitting is linked to the dramatic collapse of epidermal and sub-epidermal tissue, leading to round brown spots (chocolate spot) (Cronjé, 2007).

Effect of water relations on rind disorder development

In fruit, water diffuses readily from the internal high water content towards the much drier atmosphere. Fruit temperature and ambient environmental conditions are the primary factors to drive the vapour pressure deficit (VPD) value, mostly in favour of water loss, which is recorded as weight loss.

Data published by Spanish and Floridian researchers on non-chilling postharvest pitting and staining of grapefruit and 'Navel' orange clearly implicate the importance of rind water content in the development of these disorders. (Alférez, et al., 2003; Alférez, et al., 2005). Rind staining/pitting in 'Navelate' and 'Navelina' oranges was reported to be aggravated by the transferance of dehydrated fruits directly to conditions of high relative humidity (RH). In these studies the dehydration of the rind during low RH conditions, followed by a re-hydration with exposure to high RH conditions, resulted in turgor-stress in the transition cellular zone between the flavedo and albedo. This stress was suggested to cause cellular collapse followed by the visible staining or pitting lesions (Alquezar et al., 2010). In addition to the development of rind disorders, altered water relations in flavedo and albedo was also considered responsible for an early and transitory increase in the respiration rate and ethylene production (Alferéz et al., 2003).

Regrettably, little is known about the impact of postharvest practices on the occurrence of physiological disorders under local conditions. For instance, the extent to which citrus fruit rind loses water in the period directly after picking, but before packing, is unknown for citrus cultivars in South Africa.

Role of cultural practices on rind disorder development

Fruit development and quality are both associated with either positional effects on the tree and/or the environment. The canopy position and mineral nutrient allocation to sinks could lead to a predisposed susceptibility in a fruit which would then respond to environmental changes or extremes (temperature or relative humidity) (Ferguson et al., 1999), leading to the development of a disorder.

The principal mineral nutrients affecting rind pigments composition and concentration are N, P and K, although N is generally considered to have the greatest effect (Reitz and Embleton, 1986). High nitrogen is commonly associated in fruit with shorter shelf life and less favourable fruit colour. During flowering and fruit set of 'Washington' navels, the presence of high to very high levels of nitrogen has been associated with puffy and green fruit (Davies and Albrigo, 1994). However, it is uncertain at what phenological stage a late nitrogen application may still impact negatively on fruit quality. For example, a late nitrogen application, just before colour break, could possibly be allocated to the roots, instead of the above-ground component. If so, when additional nitrogen isavailable to be sourced for the accumulation of root reserves, instead of being channeled into vegetative flushing, flower development will be promoted in the next season. This hypothesis is based upon the results reported by Huff (1983; 1984) which concluded that the accumulation of sugars in the epicarp (citrus rind) is a major factor that regulates plastid metamorphosis (chloroplasts to chromoplasts), in conjunction with the abundance of nitrogen. Nevertheless, nitrogen flux does not play a major factor in determining plastid form during seasonal changes.

Most research on water relations in citriculture has focused on promoting yield during the pre-harvest stage. However, very seldom has the link of water stress been made with postharvest quality. For instance, no studies could be traced where pre-harvest water stresses are directly linked to disorder development, incidence and severity. It has been hypothesised that pre-harvest factors, in particular water stress in the month before harvest as well as a nitrogen application during stage II of fruit development, could increase fruit sensitivity to postharvest physiological disorders such as pitting, staining andrind breakdown (RBD). In addition postharvest water loss could induce rind disorders in sensitive cultivars and/or fruit that are predisposed to this disorder due to a cultural practise.

Study aim and objectives

The primary focus of this research was to determine the impact of water stress, induced both at a pre-harvest and postharvest level, on rind water content and fruit rind quality of two mandarin cultivars. Two widely planted cultivars namely ‘Nules Clementine’ and ‘Nadorcott’ were selected for the study as they have two distinct commercial harvest windows and are well-known to develop postharvest rind disorders.

The objectives of the study included:

- To review existing literature on the various factors affecting the water balance of citrus fruit, as influenced by the pre- to the postharvest environment, and its impact on fruit quality
- To determine if late N application, following the summer flush, may negatively affect the rind condition
- To investigate if pre-harvest water stress affects fruit susceptibility to postharvest disorders
- To evaluate if postharvest handling can induce fruit to develop a rind disorder

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LITERATURE REVIEW

The water balance in *citrus* fruit: controlling factors and impact on quality

1. Introduction

1.1. Economic importance

Cultivated *Citrus spp*. are believed to be native to tropical and subtropical regions of south-east Asia, probably north-eastern India, Burma and southern China, where they have been produced since remote times (Carr, 2012). During the 2014/2015 season the world fresh citrus production was estimated at 37,293 million tons (Edmonds, 2015). Oranges represented 64% of the total (= 64 510 ha) South African citrus production, lemons and limes combined 10%, and grapefruit (with pomelo) 14% (Edmonds, 2015). The contribution of the South African citrus industry to the world economy through exports was over R 9.3billion in 2013 (Potelwa et al., 2014).

The need for the South African citrus industry to deliver a range of cultivars to a discerning export market led to the industry actively diversifying its cultivar base. A major shift in plantings to incorporate new cultivars occurred during the 1990s with the development of mandarin orchards in both the Western and Eastern Cape and to a lesser extent in the northern production regions (Dodd et al., 2010). In citriculture, the market trend continues to show interest in new cultivars, especially with respect to clementine/mandarin, with special emphasis on late mandarins that generally have a corresponding harvest time with that of Valencia oranges.

In 2013, 5181 ha of mandarins were already established in South Africa, producing ± 10 million (15 kg) export-quality cartons during the 2014 season, equal to 1.5 million tons of fruit (Edmonds, 2014). Total late mandarin (harvest time predominantly from July to August) plantings in 2013 in the Southern Hemisphere were estimated to be approximately 2032 ha, producing over two million cartons of fruit or 300 000 tons (Edmonds, 2013). This cultivated area is predicted to expand to 4000 ha by as early as 2017. The expansion of hectares of mandarin plantings in South Africa could, however, threaten the current 600 000 tonnes of mainly oranges and lemons – estimated at a value of some 1 billion euros (US$1.3 billion) – which are mainly exported to Europe (Edmonds, 2013).

The ever-increasing pressure in the oversupplied market has compelled timely investments in research capacity to reduce losses in the marketplace. A critical agent to determine the value of citrus fruit is external quality, namely colour and rind condition. An increased drive towards higher production along with the long and complex export postharvest cold chain to different world markets has led to an increase in the incidence of physiological disorders in citrus rind. These disorders include rind breakdown, chilling injury, pitting, and staining; all may lead to higher susceptibility to decay. It has also been hypothesised that excessive water loss from the rind of citrus fruit aggravates these physiological disorders; however this concept has not been tested in mandarin citrus types.

Statistics by the Perishable Product Export Control Board (PPECB) indicated that soft citrus, which includes mandarins, had by far the most rejections (2.46%) of all the cultivars (vs oranges, 1.77%) during the 2015 season (Julius, 2015). Of all the mandarin fruit inspected, 85 369 cartons were rejected owing to the postharvest disorder, rind pitting. For soft citrus, rind pitting is amongst the top three reasons for rejection.

In this study, the influence of rind water content on specifically mandarin citrus fruit quality will be investigated. The literature study will thus review the various factors affecting the water balance of citrus fruit, as influenced from the pre- to the postharvest environment, and its impact on fruit quality.

1.2. Incidence and importance of rind disorders within the South African context

The market value of citrus fruit, especially for fresh consumption, is highly dependent on external appearance (Khalid et al., 2012). Fruit should therefore not only be free of physical blemishes, but also physiological disorders (Magwaza et al., 2013). The rind susceptibility to disorders is determined by the rind quality of citrus fruit, which consists of the sum of many physical and chemical components, including primary and secondary metabolites (Magwaza et al., 2013). The introduction of the mandarin cultivars to the South African citrus industry, for example, ‘Nules Clementine’ (*Citrus reticulata*) and ‘Satsuma’ (*C. unshiu*) led to the increased occurrence of postharvest rind disorders such as zebra skin, rind breakdown and oleocellosis in the market (Dodd et al., 2010). Other rind disorders such as peteca spot on lemons, and pitting in grapefruit and oranges also contribute to serious losses (Cronjé, 2007).

Since the mid-1990s, a continuing effort has been made to identify problem areas and implement new production and postharvest practices aimed at the reduction of rind disorders (Dodd et al., 2010). One aspect highlighted was the occurrence of postharvest rind pitting or staining of ‘Valencia’ orange and mandarin fruit in South Africa, which continues to result in serious losses during export. Unfortunately, very little is known about the impact of postharvest practices on the occurrence of physiological disorders in these citrus cultivars under southern African conditions. In 2010 Alquezar et al. reiterated what Spanish researchers (Zaragoza and Alonso; Almela) had concluded in 1975 and 2000, respectively: “The occurrence of these rind disorders is erratic and unpredictable, showing high variability from year to year, among orchards and even among fruit of a given tree.” However, research published by Spanish and Floridian researchers on non-chilling postharvest pitting and staining of grapefruit and ‘Navel’ orange (Alférez et al., 2003, 2005), provides clear evidence of the importance of rind water content (Cronjé et al., 2011b). In these studies the dehydration of the rind, when exposed to low relative humidity (RH) conditions, followed by a re-hydration at high RH conditions, can result in turgor stress in the transition cellular zone between the flavedo and albedo. With shrunken cytoplasm and the tonoplast disrupted (Alquezar et al., 2010), this stress is thought to cause cellular collapse, followed by a visible staining or forming of pitting lesions.

The extent to which the citrus rind loses water in the period after picking but before packing is unknown for citrus cultivars in South Africa. In fruit, the movement of water is more often than not out of the fruit towards the drier atmosphere, owing to its high internal water content. In orange and mandarin, a water loss of 5–6% could result in some changes in appearance and firmness of the fruit, which could be detrimental to its marketability (Ladaniya, 2008).

For fresh produce, the temperature of the fruit and ambient environmental conditions (wet and dry bulb temperature and RH) are the primary factors influencing the vapour pressure deficit (VPD) value and eventual water loss (determined as weight loss). In citrus fruit, being a hesperidium berry with a leathery rind, water is primarily lost from the rind and very seldom from the pulp (Alquezar et al., 2010). In mature citrus fruit there are also no functional vascular connections between the pulp and the rind tissue (Ben-Yehoshua, 1987). The water content of the rind is therefore directly related to any water loss from the fruit (Gonzalez-Aguilar et al., 1997).

In 2001 trials by Agustí et al., the fruit of ‘Navelate’ orange on the tree had initial symptoms of rind breakdown which commenced in days of low temperature and high RH, following a period of high temperature, low RH and high evapotranspiration (Alquezar et al., 2010). Subsequently studies by Alférez et al. (2003) as elaborated in 2004 by Alférez and Burns, indicated that during postharvest handling of fruit from several *Citrus* species and varieties, such as ‘Navelate’ and ‘Navelina’ oranges, ‘Marsh’ grapefruit and ‘Fallglo’ tangerine, rind pitting was induced and/or aggravated by transferring fruit from low to high RH (Alquezar et al., 2010). Nevertheless, morphological changes in the rind and their potential relation to the alterations in water status as induced by moderate dehydration of citrus fruit during postharvest handling are mostly unknown (Alquezar et al., 2010).

In a preliminary study on ‘Nadorcott’ mandarin (*Citrus reticulata* Blanco), it was reported that postharvest physiological rind disorders (pitting and staining) were influenced by type of rootstock (known to influence water supply to fruit) as well as postharvest handling practices (low vs high RH) (Cronjé, 2013). The results indicated a significantly higher susceptibility of fruit from rough lemon rootstocks compared with fruit from 'Carrizo' citrange. The postharvest dehydration prior to wax application induced significantly higher levels of rind disorders compared with fruit waxed within 24 hours after harvest. The data concurs with findings on different citrus rind disorders where a dramatic water loss, due to high vapour pressure deficit (VPD) resulted in an inadequate adjustment of the water status of the rind, leading to cellular collapse and tissue damage.

It is hypothesised that rough lemon rootstocks produce fruit with rinds which have in general a lower ability to prevent water loss and therefore have a higher probability of resulting in rind disorder development (Cronjé, 2013). In addition, certain postharvest handling practices could aggravate the incidence of rind disorders. Therefore, known and implementable postharvest practices such as removal of field heat and reduction of fruit VPD could possibly decrease citrus postharvest rind disorders.

2. Water loss from horticultural products

2.1. Fruit anatomy and physiology

Plant growth, crop agricultural productivity, and quality are adversely affected by both biotic and abiotic stress factors such as water availability and loss. However, in spite of the relevance of water stress on fruit quality, an understanding of how these factors impact on citrus fruits is still limited (Romero et al., 2012).

Plant cuticles contain cuticular waxes that play an important role at the interface between primary plant tissues and the atmosphere (Jetter et al*.,* 2000). The very high structural and compositional complexity of plant cuticles (Veraverbeke, Lammertyn et al., 2001, Veraverbeke, Van Bruaene et al. 2001) necessitates the parallel transport to occur through natural surface pores such as stomata, lenticels, or cracks in the cuticles (Veraverbeke et al., 2003a). However, stomata control most of the bulk water vapour and gas transport for transpiration, respiration and photosynthesis (Veraverbeke et al*.,* 2003a). These stomata are normally open. When a loss of turgor in the surrounding guard cells or simple hydraulic relationships in the subsidiary epidermal cells occurs, stomata will close (Veraverbeke et al., 2003a) – typically associated with conditions of water vapour deficit, darkness, or enhanced CO_2 concentrations (Assmann and Gershenson, 1991; Kerstiens, 1996). Equipped with an unique morphology and the ability to fill its antechamber with wax (Jeffree et al*.,* 1971), stomata can prevent excessive water loss without completely impeding the gaseous exchange of CO_2 and O_2, which is essential for metabolic reactions (Veraverbeke et al., 2003a).

Compared to leaves, stomata on most fruit (Veraverbeke et al., 2003a) are closed, inactive, covered with wax or simply fewer per unif surface area (Araus et al*.,* 1991; Blanke et al*.,* 1994; Veraverbeke, Van Bruaene et al. 2001). The contribution of stomata to water loss during storage of various horticultural products is therefore considered to be small. Regarding water loss, non-controlled openings such as lenticels and surface cracks are much more influential in commodities like apples (Veraverbeke et al*.,* 2003a).

If an imbalance between wax production and growth of apple fruit emerges, an interconnected network on the fruit surface may develop, resulting in the formation of cracks (Roy et al*.,* 1994; Veraverberke et al., 2003a). Even though they might disappear during storage and shelf life, these cracks, together with lenticels, are considered to significantly increase the cuticular permeance of apples (Veraverbeke, Van Bruaene et al. 2001). Citrus fruit, however, does not have any lenticels.

Diurnal shrinkage and expansion because of hydration changes have been reported for many different species and for various plant parts (Chaney and Kozlowski, 1971). At night, when stomata are closed and transpiration rate is low, differences in water potential between various organs and tissues of the plant are small. However, during the day internal water deficits typically develop, as absorption of water by roots usually lags behind transportation (Kramer, 1937). Under such conditions, redistribution of water within the plant may occur (Chaney and Kozlowski, 1971). In1964 Kozlowski stated that “internal movement of water from fruits to leaves during the day is common and occurs in a variety of fruit, including lemons, oranges, walnuts, cherries (as cited by Kaufmann et al., 1970b), cotton bolls, avocados, pears and apples”. Alternatively, fruit shrinkage during the day, due to the reverse flow of water to other parts of the tree, has also been reported. This phenomenon is more closely related to leaf water potential than to the vapour pressure deficit of the air (Tromp, 1984).

In the early 1970s it was already well known to researchers that growth of various fruits does not occur continuously throughout the day and night, but shows a diurnal growth rhythm. In 1984 Tromp further elaborated by adding that fruits expand during the night, whereas during the day – especially on dry sunny days– actual shrinkage of fruits has been observed due to water loss. Chaney and Kozlowski (1971) and Klepper (1968) have supplied evidence in cherry and pear, respectively, that reverse flow of water is an important factor in fruit shrinkage. In a comparison of the moisture content of excised fruit-bearing and de-fruited branches of orange trees over a period of days, Chaney and Kozlowski (1971) found higher values for branches bearing fruits. In a series of experiments with potted apple trees, cultivar 'Cox's Orange Pippin', Tromp (1984) concluded that fruits may lose water directly by evaporation or indirectly by redistribution to other tissues of the plant, that is, to the leaves.

Mangos consist of more than 80% water (Lakshminarayana et al*.,* 1970). During fruit growth, the accumulation of water and dry matter in the various compartments greatly determines the fruit size (Léchaudel and Joas, 2007). To accumulate water and dry matter at different rates, the skin, flesh and stone of mango fruit have specific compositions, and their appearance is dependent on environmental conditions (Léchaudel et al*.,* 2002). Variations in fruit volume – enlargement of fruit cells through water accumulation (Léchaudel and Joas, 2007) – can occur with changes in the balance between incoming fluxes from the phloem and xylem, and outgoing fluxes such as those driven by transpiration (Ho et al., 1987).

The shelf life of mangos can be viewed in the context of dry matter content (Léchaudel and Joas, 2007), which is directly affected by the water (Ho et al., 1987) and carbohydrate (Medlicott and Thompson, 1985) fluxes at the fruit level during its growth. Thus the changing carbon availability is influenced by both the dry mass and water mass of the three (skin, pulp and stone) main compartments of the fruit (Léchaudel et al., 2002). Léchaudel et al. (2007) used a model (based on water relations) to analyse changes in elastic and plastic components of mango fruit growth. Léchaudel and Joas (2007) concluded that shrinkage was sensitive to the surface conductance of the fruit skin, the elastic modulus and the hydraulic conductivity of fruit.

Large fruit have a high leaf-to-fruit ratio, but the higher surface-to-volume ratio of small fruit cannot be ascribed to a certain leaf-to-fruit ratio, it is simply a physical phenomenon. For smaller apple fruit this unfavourable leaf-to-fruit ratio can result in an increase in water loss per unit of fruit mass via transpiration, and in calcium accumulation as supplied by the xylem (Wilkinson, 1968). Leaves in close proximity to small fruit have shown the highest leaf diffusive conductance. Xylem sap generally flows from fruit to tree during the day, but from tree to fruit during the night (Lang and Volz, 1998). Thus, the fruits may lose larger amounts of water during the day to compensate for the higher transpiration rate of their surrounding leaves (Léchaudel and Joas, 2007). At night, larger amounts of water should come into fruits via the xylem to compensate for these water losses during the day (Lang and Volz, 1998). The additional influx of sap also provided the associated additional imports of calcium into the fruit (Léchaudel and Joas, 2007).

2.2. Pre-harvest

Environmental conditions such as temperature and humidity are critical in controlling the rate of water movement. Horticultural products are prone to weight loss caused by moisture loss due to the evaporation of water from products based on the vapour pressure difference (VPD) between in the product and the surrounding air (Scheer, 1994). The VPD, which is considered a strong indicator of the driving force behind water movement, is determined by firstly, the temperature of the product; secondly, the difference in temperature between the product and air; and thirdly, the relative humidity of the air (Scheer, 1994).

Romero et al. (2012) revisited the metabolic actions during dehydration of grape berries following harvest (Grimplet et al., 2007; Zamboni et al., 2010) and that of berries from water-stressed vines (Deluc et al., 2009) and concluded that dehydration may have a profound effect on the expression of genes associated with the biosynthesis of relevant compounds that ultimately impact on berry flavour and fruit quality.

2.3. Storage conditions

To achieve continuous sales and market value of fruit, the use of cold storage facilities to ensure quality is a necessity. However, especially for the inland South African production regions, the long-term shipping and storage of their fruit isvery complex and challenging, as the harvested products are living entities subject to carbohydrate and water losses through their storage period. From the moment of harvest the product is subject to weight loss as a result of respiration and water loss by evaporation. Respiration rate in turn is largely proportional to the storage temperature. In addition, a higher product temperature further implicates higher carbon hydration and its associated water losses, necessitating the correct use of cold storage.

Decrease in weight of apples, caused by moisture loss during long-term storage, not only implies quality concerns [surface shrivels, wax structure, such as greasiness or glossiness, changes (Wills et al*.,* 1998; Maguire et al., 1999; Veraverbeke, Lammertyn et al*.*, 2001; Veraverbeke, Van Bruaene et al., 2001)], but implies direct economic loss consequences (Veraverbeke et al*.,* 2003b). Alternatively Veraverbeke et al. (2003b) also stated a positive outcome as described by Hatfield and Knee (1988), where water loss may also result in enhanced cell cohesion and higher compressibility of the cells, which in turn can cause lower development or a reduced perception of mealiness and fewer bruises.

Moisture loss can be controlled by altering the fruit surface through the application of artificial wax coatings. This can be achieved by spraying or dipping fruit following the removal of the natural wax through citrus pack lines, during heat treatments, or by implementing proper control of storage conditions (Veraverbeke et al., 2003b). Taking the factors that determine the effectiveness of the coating (thickness, concentration and type) (Amarante et al*.,* 2001a, 2001b) into consideration, optimal coatings are regarded as reducing water loss, but without altering the gas exchange properties and internal fruit atmosphere and should improve skin finish (Veraverbeke et al., 2003b).

Control of storage conditions to avoid water loss involves the critical compromise of relative humidity, higher than the water activity of the fruit (Veraverbeke et al*.,* 2003b), but without promoting fungal decay (Wills et al., 1998). Additionally, maintaining a low temperature difference between product and environment will limit or reduce the vapour pressure deficit (Veraverbeke et al*.,* 2003b). An expensive but effective alternative to consider is individual packaging of produce in film, boxes or wrappers (Wills et al., 1998).

Any mechanical injury of the fruit rind during harvest, grading, sorting and storing of the produce may lead to greater weight loss and result in a lowering of the resistance of the fresh produce to pathogens. Therefore, an integrated approach that links both pre- and postharvest is essential to improve final fruit quality traits such as size, colour, taste, nutritional value and flavour, as these impacts on the various aspects of fruit quality (Léchaudel and Joas, 2007).

3. Water loss from *Citrus* fruit

3.1. Rind anatomy and water relations

Growth and development of a citrus fruit follow a typical sigmoid curve that can be divided into three phases (Bain, 1958). Iglesias et al. (2007) and Carr (2012) described the initial phase (Phase I) following anthesis as a two-month period of cell division and slow growth; during the second phase (Phase II – a four-to-six-month period of rapid growth), the cells enlarge and accumulate water; finally, the maturation period (a non-climacteric process in citrus) of slower fruit growth and external (colour change) and internal (a decline in acidity and an increase in sugars) ripening processes occurs. Mehouachi et al*.* (1995) described developing fruitlets as utilising sinks during Phase I, which then change to storage sinks during Phase II. Deviations from this typical growing pattern can be caused by physical forces as a result of varying cell growth rate in the different regions of the rind (Scott and Baker, 1947).

The citrus fruit rind is an isolated structure that has various anatomically and physiologically separated tissue types, and is therefore most appropriate to use as a model system for studying the combined effects of water relations, carbohydrate translocation and their interactions together with the role of tissue strength during fruit growth (Kaufmann, 1970b). Relatively few studies on citrus have been done that specifically focus on the important role of the anatomical structure of the rind within rind disorders.

The *Citrus* fruit is a special type of berry, referred to as a hesperidium (Scott and Baker, 1947; Iglesias et al., 2007; Carr, 2012). Two morphologically distinct regions are recognised within a hesperidium, namely, the pericarp, commonly recognised as the tough, leathery rind, and the endocarp (Fig. 1). The pericarp is further divided into the exocarp or colourful outer rind, also referred to as the flavedo, and the mesocarp or white inner rind, the albedo, containing the oil glands (Fig. 2; Fig. 3). The mesocarp tissue contains a vascular network (Kaufmann, 1970b). Oil glands are abundant near the surface of the fruit, as similarly observed in the leaf (Scott and Baker, 1947). The fleshy interior of the endocarp or pulp, which is peculiar to the citrus fruit, is composed of separate segments (carpels) of edible fluid-filled (juice) vesicles that are actually specialised hair cells (Fig. 1). The juice vesicles contain sink cells (Scott and Baker, 1947), and in citrus fruit, these juice sacs are isolated from any vascular bundles (Iglesias et al., 2007).

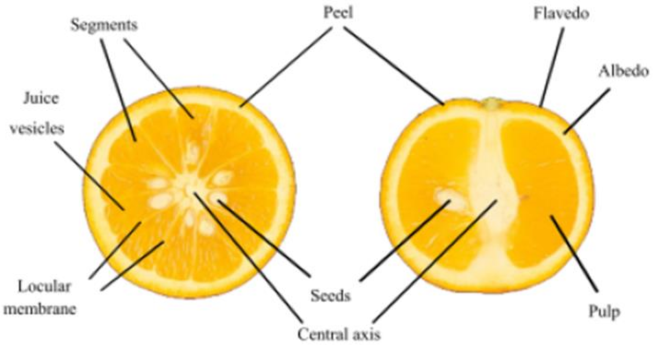

Fig.1. A photograph showing a cross- (left) and longitudinal section (right) through a *citrus* fruit with its various morphological regions (Iglesias et al., 2007).

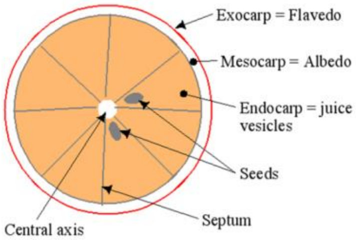

Fig. 2. A diagrammatic representation of a cross-section through a *citrus* fruit showing the distinct locations of the exo-, meso- and endocarp.

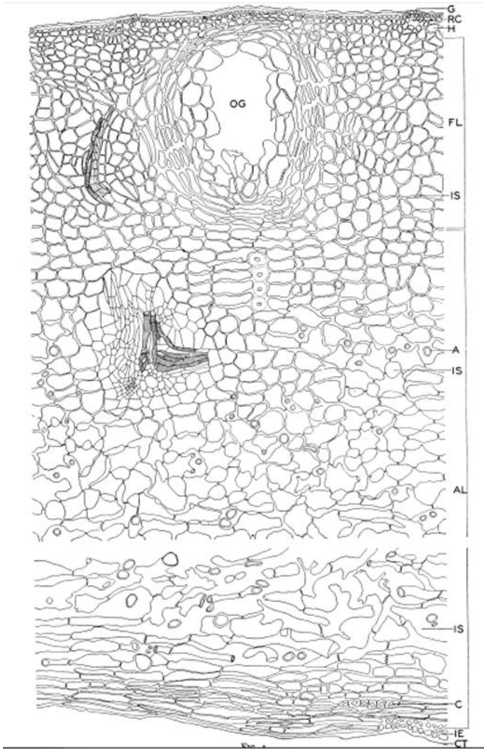

Fig. 3. A transection of the rind of a mature *citrus* fruit at 8mm of diameter, from outer to inner epidermis: G, guard cell; RC, respiratory chamber; H, hypodermis; FL, flavedo; A, arm of albedo cell; IS, intercellular space; AL, albedo; C, calcium oxalate crystal; IE, inner epidermis; CT, cutin; OG, oil gland (Scott and Baker, 1947).

Epidermal cells in the flavedo are polygonal and isodiametric in shape, with no intercellular spaces (Agustí et al., 2001). Located immediately below the epidermis are hypodermal parenchymatous cells which surround the oil glands (Fig. 3). These cells are spherical to slightly oval in profile, thin walled, highly vacuolated, and they increase gradually in size deeper into the rind, with small intercellular spaces (Agustí et al., 2001). In contrast, the albedo cells in white display an irregular shape and have meristematic cells with long intracellular air spaces.

Between citrus species, there are large differences in pericarp (rind) anatomies. For instance, mandarin fruit have a much thinner albedo compared with that of other citrus fruit. This might be an underlying reason for the different observed symptomatic development of various physiological rind disorders (Cronjé et al., 2011b), although the relationship has not been tested.

Carbohydrates, contributing 30–50% of rind dry weight (Liu et al., 2012) along with non-volatile organic acids, are the predominant primary metabolites in the citrus rind (Magwaza et al., 2013), while carotenoids and phenolic compounds constitute the major secondary compounds (Iglesias et al., 2007). Oil glands, specialised oil cavities sequestered with characteristic essential oils, add to the citrus rind distinctiveness (Iglesias et al., 2007).

Kaufmann (1970b) described the water and carbohydrate movement through a citrus fruit, based on anatomical evidence: water follows the vascular bundles in the mesocarp, diffuses through the exocarp, and evaporates on the surface (Kaufmann, 1970b). Carbohydrates from the source (leaves) utilise the vascular network for translocation into the mesocarp. These metabolites ultimately reach the juice vesicles first by crossing the endocarp, underlying the mesocarp, and then pass through the vesicle stalk into the vesicle. The vesicles, located within the fruit, are not involved in the water transport pathway during fruit transpiration as they are located at the end of the carbohydrate translocation pathway, known as the non-vascular pathway, which exists between the vesicles and the phloem or the xylem in the pericarp (Kaufmann, 1970a). Based upon these anatomical considerations, it can be hypothesised that factors affecting transpiration or re-translocation will have little effect on the water relations of the vesicles and will only occur through osmosis. This hypothesis is supported by the work of Rokach (1953), which suggested that diurnal changes in water content of the fruit as mediated by transpiration and translocation result from changes of water content of the pericarp (rind) rather than that of the anatomically isolated vesicles of the pulp (Kaufmann, 1970b).

Citrus leaf and fruit cuticles have a thin film of epicuticular waxes on the surface of their cutin matrix (Baker et al., 1975). These waxes are effective barriers to gas permeation. Most of the water vapour and other gases are understood to move in detached citrus fruit, by flowing freely throughout the spongy parenchyma of the mesocarp (albedo) and the central axis, diffusing through the exocarp and the coloured portion of the rind (flavedo), and by evaporating on the surface (Kaufmann, 1970a). In this system, the flavedo portion of the rind seems to be the primary site of resistance (Ben-Yehoshua, 1969).

Researchers such as Burg and Kosson (1983) reported that water transport occurs, presumably as a liquid phase; Schönherr (1976), along with Schönherr and Schimdt (1979), suggested that evaporation at the outer cuticular surface creates a difference in water potential and draws liquid water by mass flow from the epidermal cells, through cuticular membrane to the air/water interface. These findings all suggested that mass transport of water and fixed gases occurs by different mechanisms in fruit (Ben-Yehoshua et al., 1985) and concluded that stomata of harvested citrus fruits are essentially closed, but that ethylene, O_2 and CO_2 may still diffuse mainly through residual stomatal openings, while water moves preferentially through a liquid aqueous phase in the cuticle. Cuticular transpiration as a mechanism was proposed for fruit, rather than stomatal transpiration. In a study on ‘Fortune’ mandarin fruits, low temperature induced a breakdown of the more external cellular strata of the rind which then modified the physiological function of the cuticle, and led to a dramatically increased water permeability (Vercher et al., 1994).

Moon et al. (2003) observed that the wax density on the rind, and thus also the coverage of stomata, increased with maturity, in both water-stressed and control fruit. Of interest, however, is that these authors concluded that water stress in ‘Okitsu wase Satsuma’ mandarins decreased the proportion of photosynthates meant for wax production on fruit surfaces, resulting in these cuticles being more water permeable and probably more susceptible.

Léchaudal and Joas (2007) investigated postharvest behaviour in relation to pre-harvest factors and reported that another source of water loss is the increase in cuticle cracking (as described by Moreshet et al., 1999). Cohen et al*.* (1994) reported significant water loss in citrus fruit associated with the development of microscopic cracking of rind tissue when the fruit was moved from two to 20 °C.

3.2. Physiological interaction within the tree

3.2.1. Fruit

Citrus fruit, unlike climacteric crops, have a greater potential to maintain quality postharvest or can provide the producer with the option of leaving fruit for some time to mature on the tree (Grierson and Ben-Yehoshua, 1986). By having the choice to delay the harvest period to some extent, flexibility exists to optimise market opportunities, as well as to accommodate changes in the weather around harvest or to maximise available labour. However extending the harvest period also carries significant risks. Over-extending the post-bloom storage period on the tree can severely limit the potential for postharvest cold storage and reduce the subsequent year’s yield (Grierson and Ben-Yehoshua, 1986). Pre-harvest factors that influence the storage potential and postharvest performance of citrus fruits include: rootstock and scion selection, tree condition, cultural practices (sanitation: pruning, removing decayed and fallen fruit), the prevailing weatherat harvest and orchard treatments such as fertilisation, irrigation, soil composition, and crop protection programme (Grierson and Ben-Yehoshua, 1986).

Citrus is well known to be extremely sensitive to moisture stress during flowering and fruit set, thus any water shortage during this time is likely to adversely affect yield (Hutton et al., 2007). Leaves and fruit normally do not fall during the period of water stress; however citrus has an uncharacteristic behaviour regarding leaf and fruit abscission induced by water stress (Iglesias et al., 2007), where these organs abscise after rehydration (Addicott, 1982). A relationship between fruit size and water loss was established in early research (Haas, 1927). Haas described that transpiration rate is greater in smaller fruits such as oranges, which have a larger surface area per unit weight or volume. However, this finding was later found not be applicable to grapefruit (McCornack, 1975).

3.2.2. Rootstock

The choice of *Citrus* rootstocks can significantly affect tree size and crop load, together with fruit size and quality (Castle et al., 1993) and is therefore important during planting of a citrus orchard (Romero et al., 2006). For example, the root system of 'Cleopatra' was found to be more effective at water uptake, mainly because of a higher root density or because of its deeper and more densely branched root system compared to 'Carrizo' (Davies and Albrigo, 1994). This finding was later confirmed by Romero et al. (2006) where 'Cleopatra' was reported to retain a better tree water status than 'Carrizo', especially during water-stress periods. Rootstock characteristics, like root distribution, water- and nutrient-uptake efficiencies, vessel element anatomy and root hydraulic conductivity, are genetically determined and all have an effect on plant–water relations (Romero et al., 2006). They have therefore been associated with differential fruit development and sugar accumulation of citrus fruit (Albrigo, 1977; Barry et al., 2004; Koshita and Takahara, 2004).

3.2.3. Water relations

Healthy water relations in *Citrus* is of prime importance as a reduction in growth will occur under conditions of critical, low levels of humidity. In response to high VPD (such as low RH and high temperature), plants reduce the excessive water loss from their leaves by preventing light from reaching the leaf surface.

At night, when stomata are closed and transpiration rate is low, differences in water potential between various organs and tissues of the plant are relatively small. During the day, however, absorption of water by roots usually lags behind transpiration (Kramer, 1937) and internal water deficits develop. To compensate for these internal water deficits, the internal movement of water from fruits to leaves during the day (Kaufmann, 1970b) is common and has been reported in a variety of fruits, including lemons, oranges, walnuts, cherries, avocados, pears and apples (Kozlowski, 1964).

For most plants, growth tends to improve at high RH; however excessive humidity can also encourage some unfavourable growth attributes. Low VPD causes low transpiration that limits the transport of minerals, affecting particularly calcium, as its transport is reliant on the transpiration stream through the xylem. If VPD is very low (95–100% RH), so that plants are unable to transpire any water into the atmosphere, coupled with a wet root zone, a build-up of root pressure is experienced, which then typically results in guttation.

Leaves remain fresh much longer on detached fruit-bearing branches than detached non-fruit bearing branches. Rokach (1953) showed that water is drawn by the leaves primarily from the rind and not from the pulp. In branches bearing ripe fruit, a very large difference in osmotic values was established; however the osmotic value of the fruit pulp remained unchanged by leaf suction, strongly suggesting that the pulp does not participate in the water transfer. Recordings of fruit contraction and those of changes in water content, osmotic values and, to a certain degree, of water saturation deficits, showed that a citrus fruit has a reduced fresh-weight transpiration compared with that of a leaf, mainly owing to lower surface-volume ratio. The transpiration pull from leaves has been shown to readily transport water from a fruit on a defoliated main branch to a leafy lateral. No measurable transfer of water from the fruit was found to take place before the fruit attained a size comparable to that of a large olive (Rokach, 1953).

3.2.4. Microclimatic conditions

Finally, among the many environmental factors that affect citrus rind quality, the microclimatic conditions created by the canopy position (Magwaza et al., 2013) occupy a predominant role. They are thought to exert a very strong influence on the biochemical composition of rind and whole fruit and thus play a role in the fruit's sensitivity towards physiological rind disorders (Cronjé et al., 2011a, 2011b, 2013). For instance, after assessing 'Navelate' orange trees studied in Spain (northen hemisphere), for the incidence of rind breakdown, Agustí et al. (2001) concluded that fruit exposed to the north-western face of the tree is more prone to develop rind breakdown, with the highest proportion of fruit with symptoms found in the outer portion of the N-W exposed canopy (Magwaza et al., 2012). In addition, the disorder has been reported to be more prevalent at the exposed face of the fruit, irrespective of its location on the tree (Magwaza et al., 2014).

3.3. Environmental conditions

The impact of climatic and micro-climatic conditions on fruit enlargement can be directly attributable to environmental influences on plant water relations and/or to temperature effects (Kaufmann, 1970b).

The three main factors most likely to affect water stress in citrus trees foliage are soil water availability, soil temperature and VPD of the atmosphere. In a study by Elfving and Kaufmann (1972), where data were collected at night, it was reported that as soil water became increasing limited, night-time leaf water potential decreased. The effects of VPD on leaf water potential and tree stem water potential indicated that a high VPD during the day resulted in more water loss from the leaves due to transpiration, which resulted in lower water potential and eventually stomatal closure. At night the recovery of stem and leaf water potential is accomplished under conditions of sufficient soil moisture.

In a review in 2007, Iglesias et al. noted that the additional abiotic factors affecting citrus fruiting are dependent upon the soil composition, quality and quantity of water and the risk of low temperatures. Contributing negative soil characteristics that may affect *Citrus* fruit quality include factors such as excess calcium, high pH, and mineral imbalances. Iglesias et al. (2007) concluded that for citrus, salinity, iron chlorosis, flooding or freezing were the most common adverse environmental conditions.

Water stress evoked by conditions of high temperatures together with dry winds induces tree dehydration (Iglesias et al., 2007), even in the presence of soilmoisture. Although water stress was recognised as a strong inducing factor of flowering, most other physiological parameters were negatively affected by water stress (Iglesias et al., 2007), including characteristic leaf injuries and abscission typically resulting from water deficit conditions (Tudela and Primo-Millo, 1992). As the soil-water balance is known to directly influence fruit quality, it is recommended that citrus fruit should not be picked when the trees are wet or within a week of severe drought stress, or after drought having been relieved by rain or irrigation (Grierson, 1965; Grierson, 1983).

3.4. Sugar accumulation

3.4.1. Sugar accumulation in the pulp

The concentration and ratio of sugars and organic acids in the pulp determine the characteristic taste of citrus fruit. Fruit sugar content depends on imports of photosynthetic products and is therefore influenced by the rate of fruit photosynthesis and respiration (Huang et al., 1992). It is well known that water stress due to low soil water availability increases sugar content in fruit (Kramer and Boyer, 1995). Similarly, water stress has been reported to result in an increase in acidity in the central portion or mid-section of fruit, whereas it may lead to a reduction in the outer portions (Moon et al., 2003).

In studies conducted to enhance sugar accumulation in ‘Satsuma’ mandarin (*Citrus unshiu* Marc.) pulp, water stress induced by non porous mulching (e.g. Tyvec) not only increased concentrations of sucrose, glucose, and fructose respectively, but also elevated the absolute sugar content (monosaccharide accumulation) in the fruit. This was achieved mainly through the process of osmoregulation, while the acidity in the fruit juice decreased owing to high respiration (Yakushiji et al., 1996).

Meyer and Boyer (1981) first reported water stress as triggering the physiological function of osmoregulation. Many plants that need to tolerate water stress are largely dependent on their capacity for osmoregulation to maintain cell turgor through the accumulation of solutes (Morgan, 1984). The mechanisms to maintain cell turgor involve sufficient solute accumulation in cells which decrease the osmotic potential of cells when water potential of cells declines, owing to low water potential of the water source (water stress), faster than the decrease in water potential so that water can be re-absorbed by cells (osmo-regulated) without decreasing in cell volume (Yakushiji et al., 1996).

3.4.2. Sugar accumulation in the rind

Cronjé et al. (2011a, 2011b, 2013) and Magwaza et al. (2012, 2013) observed differences in rind quality on fruit harvested from different canopy positions. Magwaza et al. (2013) concluded that the results of the various studies indicated fruit position, and therefore exposure to high (outside) or low (inside) light levels in the canopy, to affect the rind content of sugars during fruit development (Cronjé et al., 2011b). In addition it was recorded that the flavedo from fruit borne on the outside of the canopy had a significantly higher sugar content than fruit borne inside the canopy.

Water-stressed citrus trees tend to yield smaller fruit, although higher sugar levels were found to accumulate in these fruit. The presence of stomata on the pericarp surfaces of citrus fruit may allow for dehydration to occur owing to transpiration under water stress conditions, which in turn will result in sugar accumulation as water is lost from rind cells (Yakushiji et al., 1996).

The quality of fruit is frequently evaluated by measuring soluble solids content (SSC) with a refractometer. The SSC of ‘Satsuma’ mandarin fruit is known to consist mainly of sugars (sucrose, fructose, and glucose) and citric acid (Daito and Sato, 1985). The total sugar content of water-stressed fruit was recorded to be significantly higher than in well-watered fruit (Yakushiji et al., 1996), indicating dehydration could not solely explain the sugar accumulation in fruit under water stress. Sugar accumulation in ‘Satsuma’ mandarin fruit could not be ascribed to dehydration under water stress, but rather to sugars accumulated by active osmoregulation in response to water stress (Yakushiji et al., 1996).

4. Postharvest aspects affecting water loss

4.1. Handling pick to packhouse

During postharvest handling, several processes can reduce storage and shelf life as well as fruit quality, such as decay, weight loss and shrinkage, over-maturity resulting in off-flavours, and undesirable colour changes and softening (Grierson and Ben-Yehoshua, 1986). Citrus fruits can be classified as non-climacteric fruit as their respiration declines gradually after harvest. Optimum maturity and flavour are achieved on the tree and should be able to be retained for a long period after harvest, owing to a low inherent respiration rate.

Anatomically, the fruits of most citrus cultivars are well suited to long postharvest life (30–90d), if undamaged and picked at their prime maturity (Grierson and Ben-Yehoshua, 1986). Successful postharvest shipment and storage of citrus thus depends largely on minimising losses from fungal decay, shrinkage and softening, together with control of any development of physiological disorders.

In general, postharvest storage problems are often associated with the time of harvest within the season. Early in the season, citrus fruit is more sensitive to oleocellosis and other harvest-induced blemishes, but more resistant to decay (Grierson and Ben-Yehoshua, 1986). Over-mature fruit in the later season is experienced to be more susceptible to decay and disorders. Thus, climate and varietal characteristics should be considered when developing handling protocols for the successful storage of mandarin-type varieties (Grierson and Ben-Yehoshua, 1986).

The importance of harvesting practices is emphasised by Grierson and Ben-Yehoshua (1986): “The single most critical factor in fruit storage is careful harvesting”. Poor storage potential is often associated with fruit that has been physically abused during harvesting. Such fruit should neither be shipped as fresh fruit, nor be stored, because of the high risk of decay. Care should also be taken to follow best harvest practices, for example, fruit should be clipped rather than yanked off, as the rind can tear when fruit is pulled off during harvesting.

Decays such as mould, and to a lesser degree, sour rot, are known to be directly related to various types and severities of rind injury (Smoot and Melvin, 1961; Smoot et al., 1971). Even rind injuries not identified at the grading belt can act as avenues of infection (Smoot and Melvin, 1965). As discussed earlier, the citrus rind is a complex structure, as it has similar processes to that of a leaf, such as stomatal control and photosynthesis, while containing oil glands that can release a potential phytotoxic substance within the rind. A further complication is that the rind is physiologically independent from the pulp.

During handling and storage, various postharvest physiological rind disorders manifest by a multitude of symptoms on fresh citrus fruit (Magwaza et al., 2013). In earlier studies Chalutz et al. (1973) had suggested that for warm days in low-humidity areas (especially towards the end of the season), precooling of fruit would help maintain firmness and increase resistance to decay during and even after storage. The erratic incidence of these physiological rind disorders may be related to the environmental conditions at harvest (Alquezar et al., 2010). Evidence obtained by Agustí et al. (2001) and Alférez et al. (2005) indicated that fruit exposed to moderate dehydration in the field are much prone to develop rind injury after storage at high RH, similar to fruit dehydrated postharvest.

The rate of water loss postharvest is directly related to fruit temperature. Time from harvest to market for South African citrus fruit can vary from four to eight weeks, which emphasises the need for effective postharvest measures such as cold storage to maintain high fruit quality and prevent decay. The citrus fruit rind plays a critical part in determining fruit value. Pre-harvest irrigation and fertilisation can influence rind development and stability along with postharvest practices such as degreening, packhouse operations and various aspects of cold chain management (Grierson and Ben-Yehoshua, 1986).

4.2. Postharvest treatments

Some cultivars could be less or more tolerant to prevailing environmental conditions such as low RH, but the basic handling practices in essence remain similar.

The handling sequence of citrus in South Africa (Fig. 4) can be described by a representation of the packhouse process flow (Lesar, 2008). Fruit received from the orchard must be identified by means of a production unit code, PUC, and orchard number. Bins appointed for degreening (to be marked with the fruit colour on arrival), are drenched to remove field heat, kill fungal spores and leave fungicide residues on the fruit for protection during degreening. Proper drying of fruit after drenching is crucial. During degreening fruit is exposed to ethylene gas at a specific concentration and for a certain period to break down the chlorophyll pigments. The packhouse process commences after degreening or directly from the orchard, when fruit is either wet or dry dumped. Wet dump (dumped into water) is regarded as the gentlest way of handling the fruit. Alternatively, fruit can be dry dumped onto a conveyor belt. Clean fruit is pre-sorted (by means of mechanical sizer and sorters) to eliminate any fruit that may not meet the packing standards, such as fruit which is badly infected or destined for the juicing factory. After pre-sorting, the fruit is subjected to a fungicide bath (dip treatment), to apply postharvest protection through adequate fungicide residue loading. In the next phase, fruit must be air dried properly before waxing. Wax containing added fungicides prevents moisture loss and maintains fruit quality during export. After wax is applied, drying commences through a drying tunnel. Skilled sorters grade fruit into various grades and classes namely, export fruit with minimum blemish; export fruit with some blemishes; and fruit suited to the domestic market. Fruit destined for the local market is packed by a separate pack line. The sizer classifies the fruit mechanically into a count (according to pre-determined size specifications) and optically (grade colour, shape and blemishes). Mechanical fruit labelling is provided according to market requirements.

Export fruit is packed in the required cartons. Individually wrapped fruit can be a prerequisite. Cartons are labelled, indicating the variety, grade, size, packing date, packline number, production unit code, and packhouse code. Cartons are weighed to conform to the minimum weight requirements. Palletising of cartons is done according to the exact specification of the client and includes the pallet base, four protective corner pieces and the number of cartons, as specified by shipping requirements. Each pallet is provided with a unique barcode identification that includes, amongst others, the destination, date packed and packing type, and is linked to the production unit code as presented on each carton, to ensure full traceability of every packed carton on every pallet.

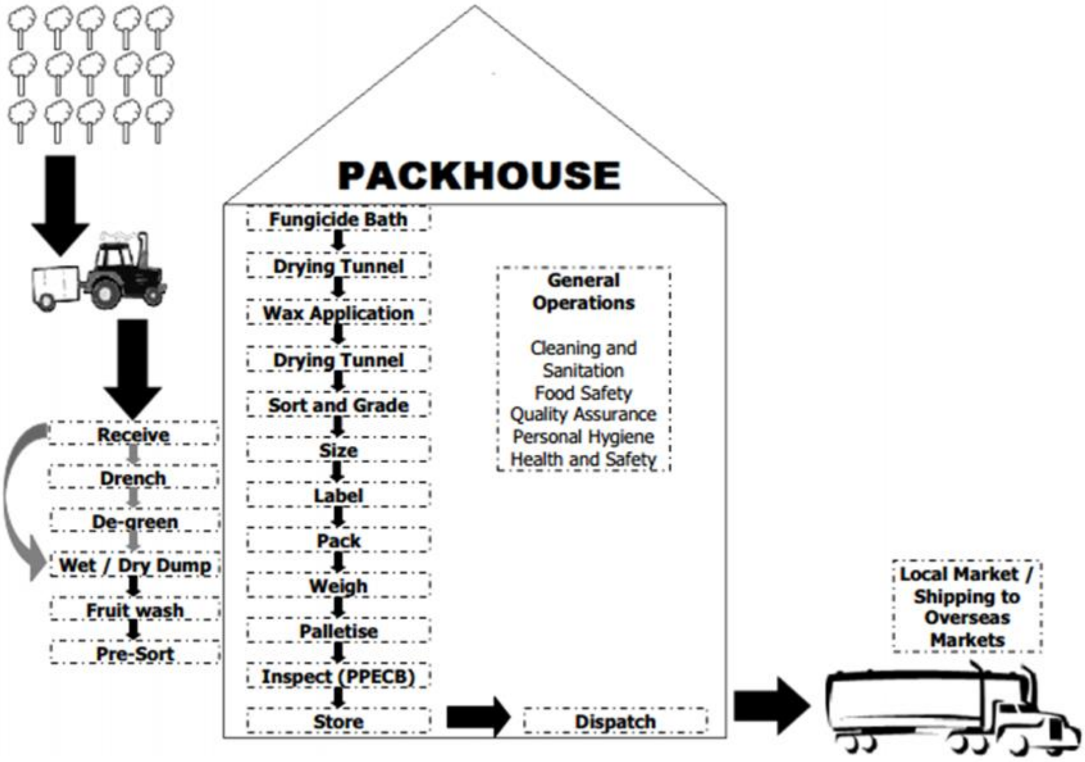

Fig. 4. Diagrammatic representation of a typical handling sequence of citrus fruit in South Africa (Lesar, 2008).

4.2.1. Drench

Treatment with 2,4-dichloro phenoxy acetic acid (2,4-D) and fungicide before degreening is needed to maintain greenness of the pedicel (Ladaniya, 2008). After degreening, the fruit is waxed or stored for later waxing, depending on the requirement. It was once common practice in most citrus areas to cure fruit before shipping or storing (Grierson and Ben-Yehoshua, 1986), but more recent evidence suggests that curing involving drying is detrimental to oranges.

4.2.2. Wash

Washing through a line of brushes may remove the natural wax coating from the fruit surface, thus increasing the water loss from the flavedo (Alferéz et al., 2010). The slow loss of moisture directly from orange fruit is also evident in commercial handling practices. In contrast, waxed fruit has an improved appearance and extended shelf life, probably due to less water loss and reduced shrinkage.

4.2.3. Wax

The natural wax coating of citrus fruits is deposited in lightly attached irregular platelets and can largely be removed by washing, most effectively so, in an alkaline fungicidal treatment. The risk exists that in any storage facility other than that with ultra-high humidity, the rind of non-waxed, but washed citrus fruit tends to dry to a thin, hard, unsightly shell, thereby making the fruit unmarketable. It is therefore necessary to replace the missing natural wax with an artificial coating to reduce weight loss by up to 60%, inhibit shrinkage, and provide fruit with a shiny gloss (Grierson and Ben-Yehoshua, 1986).

It is standard practice to coat citrus fruit with waxes. Hagenmaier and Baker (1993) concluded that wax coverage results in reduced weight loss of fruit and an elevated internal CO_2 concentration, due to a reduced gas exchange between fruit and the atmosphere. Commercial waxing reduce transpiration of the fruit as it results in a new surface layer over the fruit which has many pits and cracks for water transport, but it specifically restricts gas exchange (by plugging the stomatal pores) so effectively off-flavours may result (Ben-Yehoshua et al., 1985).

4.2.4. Drying tunnels

A drying tunnel can damage fruit, resulting in burn or major fruit moisture loss, if it is not operated correctly. Due to the various climatic conditions under which South African citrus are produced, each packhouse has to establish the optimal drying conditions for their climate. The recommended temperatures for soft citrus is 40 to 48°C maximum, whereas oranges, grapefruits and lemons, can be set up anything from 56 to 58°C maximum (Lesar, 2008).

4.3. Impact of shipping and cold storage on moisture loss of citrus

Weight loss during cold storage is a significant factor in the postharvest deterioration of citrus fruit. A major process leading to this weight loss is transpiration (Grierson and Ben-Yehoshua, 1986). Ben-Yoshua (1969) reported that not only does transpiration cause desiccation, shrivelling, accelerated softening, and loss of the attractive appearance of fruit, but the resultant water stress also accelerates senescence.

Grierson (1969) noted that the loss of water vapour from citrus fruits is a passive process, with the main driving force provided by the vapour pressure gradient between the rind and the air surrounding the fruit. The study further concluded that the relative humidity

(RH), considered alone, can be meaningless, as effects on the fruit should be evaluated in terms of VPD.

4.3.1. Precooling

Rapid cooling of fruit before packing, from field temperature to 10 °C, decreased weight loss by about 50% and also somewhat decreased the incidence of decay that developed during storage, without causing injury to the fruit (Chalutz et al., 1973).

4.3.2. Shipment

Shipping temperatures for different *Citrus* species from various production regions have been determined and are implemented (Lutz et al., 1968). Shipping temperature has a considerable effect on eventual rind colour of *Citrus* fruit and is one of the conclusive factors that can influence rind colour in the fruit production and supply chain (Van Wyk et al., 2009).

The predominant shipping mode in the 1980s was specialised reefer vessels that handled some 80% of the export volume (Dodd et al., 2010). In 1998, only 13% of citrus fruit was exported in containers, but this figure is currently closer to 50%. Comparing the export mode of deciduous fruits over the same time frame, the changeover to containers has moved from 32% to 70% to date (Dodd et al., 2010). The volume of container usage is likely to further increase owing to a decrease in the bulk vessel fleet and the convenience of loading fruit at the packhouse, along with the benefits of introducing and maintaining the cold chain during road transport.

All citrus fruit exported from South Africa to the United States of America (USA) and China currently has to be subjected to a mandatory 22-day exposure to a temperature regime of -0.6 °C following a pre-cooling period at the same temperature to comply with cold sterilisation. This compulsory low-temperature cold treatment is commonly referred to as a cold-steri protocol (Hordijk et al., 2013), as these protocols are mandatory to kill insect larvae of false codling moth (FCM) and those of the Mediterranean fruit fly in fruit destined for these markets (White and Elson-Harris, 1992; EPPO, 2007). The increased focus of South African producers and exporters on exporting various citrus cultivars to China has resulted in an emphasis on all harvest, packhouse and postharvest handling practices that could alleviate incidences of chilling injury (CI).

Horticultural industries can suffer severe economic lossesdue to fruit moisture loss and the onset of rind disorders in citrus (Henriod, 2006) which occur during refrigerated storage and transport (Table 1). To maintain high fruit quality throughout commercial supply chains, the application of proper cooling and storage practices is regarded as fundamental (Henriod, 2006). Ben-Yehoshua et al. (2001) and Porat et al. (2004) reported fruit to have a higher degree of fruit quality (rind firmness and tissue turgidity) at its final destination when moisture loss was reduced during the postharvest period under conditions of low temperature and reduced air velocities. This enhanced postharvest quality is evidently a result of high RH conditions that decrease fruit transpiration or sub-cuticle evaporation (Henriod, 2006). Moreover, the link between high RH conditions (that reduce fruit moisture loss) and decreased incidence of rind disorders such as chilling injury (Pantastico et al., 1968; Fujisawa et al., 2001; Porat et al., 2004), stem-end breakdown and button tissue collapse (Ben-Yehoshua et al., 2001; Porat et al*.,* 2004) have also been reported (Henriod, 2006).

Table 1. Typical storage conditions for citrus fruits (Grierson and Ben-Yehoshua, 1986).

		Storage conditions			
Type of citrus fruit	**Temperature (° C)**	**Relative humidity (%)**	**Estimated storage life (weeks)**	**Chilling injury symptoms**	**Typical storage diseases**
Orange, Calif. & Arizona	4.4–6.7	85–90	6	Rind staining and pitting	Stem-end rot, *Penicillium* moulds, stem-end rind breakdown
Grapefruit, Midswason	12.8	90–95	7	Severe peel pitting, oil gland darkening	Stem-end rot
Tangerines, Temples	3.3–4.4	85–90	2	Off-flavours, rind staining,	Stem-end rot
Coorg mandarins	6–7	85–90	8	shrivelling	*Penicillium* moulds
Japanese satsumas	1–2	85–90	16	Rind puffing	*Penicillium* moulds
Limes	10.0	90–95	4	Severe peel pitting	Yellowing, peel collapse
Lemons (Green)	14.4–15.5	85–90	20	Peel pitting (peteca), membranous staining, albedo browning	*Penicillium* moulds, stem-end rot, sour rot
Lemons (Yellow)	3.3–4.4	85–90	4	Peel pitting (peteca), membranous staining, albedo browning	*Penicillium* moulds, stem-end rot, sour rot

5. Influence of water loss on fruit quality

5.1. Physiological disorders associated with rind water balance

Physiological disorders in this respect are defined as conditions such as rind breakdown, rind staining, or rind pitting that affect rind quality of fruit from several citrus varieties, including oranges, grapefruit and mandarins at non-chilling temperatures, without compromising internal quality, but negatively affecting fresh market value (Alquezar et al., 2010).

Certain cultivars are more prone to specific postharvest physiological disorders than others. Zaragosa and Alonso (1975) stated that under certain conditions, 'Navelate' sweet orange is highly sensitive to rind breakdown, which can cause rind stain during ripening. Rind breakdown of this cultivar has also been observed during postharvest storage (Alférez and Zacarias, 2001). Postharvest rind pitting at non-chilling temperatures is a severe disorder that affects fruit from several citrus cultivars worldwide and results in significant economic losses (Alférez et al., 2010). Sudden changes from low to high RH during postharvest storage, induced rind pitting in 'Navelina' and 'Navelate' oranges in Valencia, Spain (Alférez et al., 2003) and 'Marsh' grapefruit (Alférez and Burns, 2004) in Florida, USA, aggrevated these disorders. Even the exact cause of this physiological disorder is still unknown, research clearly identified altered water relations in the fruit rind as a major inducer of rind pitting, regardless of the geographical origin of the samples (Alférez et al., 2010).

Many rind blemishes and physiological disorders of citrus fruit exhibit similarities regarding visual symptoms and morphological appearance; hence accurate diagnosis and differentiation is difficult (Alquezar et al., 2010). Effective sources are, however, available to facilitate identification (Cronjé, 2007).

5.2. Physiological reaction to water loss: proposed mechanism(s)

5.2.1. Biochemical change in flavedo cells

Fruit colour, flavour, firmness, and juice content are all subject to changes during storage, depending on cultivar, storage conditions (e.g., temperature or RH), packhouse treatments (e.g., degreening), and even orchard conditions. During storage of oranges, softening and subsequent deformation of the rind are accompanied by a range of metabolic changes. These changes include the concomitant increase in soluble pectins and pectinates at the expense of insoluble protopectin (Sinclair and Jolliffe 1958); an increase in malonic, succinic and malic acids, as well as an increase in electrical conductivity (Sasson and Monselise, 1977); and a decrease in uremic acid as the main component of pectins together with an increase in relative amounts of cellulose and neutral sugars, especially galactose, arabinose and rhamnose (Ben-Gad et al., 1981).

Alférez et al. (2005) suggested that the water status of the rind could be a major determinant of rind pitting incidence when the postharvest handling of fruit has been delayed during the first three days after harvest, especially under conditions of low RH. When fruit is again transferred to high RH, VPD is suddenly reduced and water potential recovers faster in the outer fruit layer (flavedo) than in the subtending albedo cells (Alférez et al., 2003). Elaborating further, Alférez et al. (2005) hypothesised that one explanation for this effect may be that at short durations of low RH storage, dehydrated flavedo can draw water from albedo cells, which then become water deficient. The increased water demand of the albedo and the resulting suction force may subsequently cause collapse of internal flavedo and external albedo cell layers, owing to their reduced ability to rehydrate (Alferéz et al., 2003; Alferéz and Burns, 2004). Oil glands in affected areas can become compressed and rupture, causing browning of tissue in advanced stages. If dehydration continues for prolonged periods, albedo cell layers may collapse and lose their cellular contents, before rehydration treatments commence (Alférez et al., 2005).

The extent to which the citrus rind is prone to losing water in the period after picking, but before packing, is unknown under South African conditions. In the case of fruit, the movement of water is more often than not out of the fruit, owing to its high water content towards the drier atmosphere (Fig. 5). The temperature of the fruit and ambient environmental conditions such as wet and dry bulb temperature and RH are the factors that influence the VPD value and eventual water loss (determined as weight loss).

Mechanisms of cellular collapse underlying rind breakdown disorder (RBD) are proposed to be the dehydration at low RH (45%) and subsequent rehydration at high RH (95%), which leads to water potential differences and tension in the flavedo-albedo interface (Cronjé et al., 2011b). Studies by Alquezar et al. (2010) confirmed the flavedo to be the rind tissue most sensitive to water stress, and that the albedo is most likely to maintain a functional water status at the expense of flavedo.

In ‘Washington’ Navel and ‘Navelina’ oranges, the elevated activities of a range of cell wall enzymes (pectinesterase, exo-polygalacturonase, endo-polygalacturonase, and endo-1,4-ß-D-glucanase) in the albedo and flavedo of creased compared with healthy fruit at harvest appear to be associated with enhanced loss of pectin. This loss is accompanied by starch accumulation in the cell walls of the albedo, together with the consequent reduction in the hardness, stiffness and tensile force of the rind, possibly leading to cell wall loosening and crack formation (Saleem et al., 2014). This confirmed that postharvest water loss could be an important factor in inducing rind disorders (Magwaza et al., 2013).

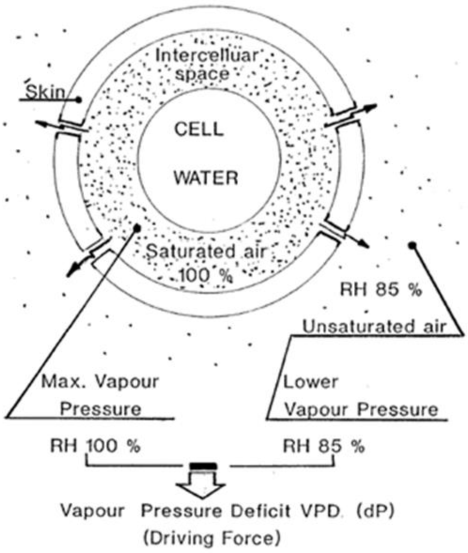

Fig. 5. Possible driving forces and mechanisms of water loss from a fresh produce product (Scheer, 1994).

5.2.2. Genetic response within rind to changes in water balance

Genetics during development

Fruit development is a highly regulated process and involves changes in the expression of a large number of genes (Gillaspy et al., 1993). The ‘Satsuma’ mandarin, however, differs from the orange in that its rind adhesion to the juice sac membrane loosens during development and allows for easy peeling, which is an agronomically important trait (Kita et al., 2000). Morphological and anatomical analyses showed different changes in shape and pectin substances during rind development and maturation of ‘Satsuma’ mandarin, compared with oranges (Kita et al., 2000).

Northern blot analyses to characterise the expression of candidate genes during citrus rind development revealed four types of expression patterns in the albedo (Kita et al., 2000). Taking the rind developments of ‘Satsuma’ mandarin into consideration, Type-I and Type-II genes can be related to the changes in the rind which occur during Stage II and Stage III; also, Type-III and partly Type-II genes may play important roles during Stage I, whereas Type-IV genes may be necessary for normal fruit development owing to their ubiquitous expressions (Kita et al., 2000). Thus, Type-I, Type-II and Type-III genes, such as endo-xyloglucan transferase, expansin, extensin, glycine-rich protein and pectin-acetyl-esterase homologues, may contribute to rind development, resulting in the formation of large intercellular spaces in the albedo (Kita et al., 2000).

Accumulation of sugars, poly-alcohols, amino acids, amines, and abscisic acid (ABA) in response to water stress as a survival method has been reported in several horticultural crops (Seki et al., 2007). These metabolites function as osmolytes, antioxidants, scavengers, and/or signalling molecules that assist plants to tolerate abiotic stresses; changes in their homeostasis are thought to be associated with maintenance of the structure and function of cellular component networks (Alférez et al., 2008).

In water-stressed plants, the removal of water from the cytoplasm to the extracellular space may cause a reduction in the cytosolic and vacuolar volumes. This change may occur along with an alteration in reactive oxygen species homeostasis, which may lead to an accumulation of toxic substances along with the production of signal transduction molecules (Miller et al., 2010) and finally result in damaged cellular tissues (Alférez et al., 2008). The phytohormone abscisic acid (ABA), is the primary signal to water stress, and affects many agronomic traits such as the loss of fruit quality in many citrus cultivars that develop rind damage in response to dehydration (Romero et al., 2012). The close relationship between ABA and dehydration of the rind is well illustrated in 'Pinalate' oranges, a wild-type 'Navelate' which develops distinctive yellow-coloured fruits due to a partial blockage of the carotenoid biosynthetic pathway, causing a fruit-specific ABA deficiency (Rodrigo et al., 2003). Moreover, harvested 'Pinalate' fruits show higher dehydration and a much greater susceptibility than their parental fruits to developing rind depressions, which in advanced stages become bronze and necrotic (Alférez et al., 2005; Sala et al., 2005). Gene ontology analysis indicated the higher ability of 'Navelate' fruits (in respect of mutant 'Pinalate' fruit) to induce a molecular dehydration responsiveness which required ABA-dependent and -independent signals to water stress (under conditions of 12 °C and 70–75% relative humidity), allowing a reduction in their susceptibility to developing non-chilling related rind disorders (Romero et al. 2012).

Romero et al. (2012) concluded that these responses to water deprivation include active di- andtri-valent inorganic cation transport, which fit into the classical plant responses to water deficit and osmotic adjustment as ascribed by Shinozaki et al. (1998) and elaborated by Ramanjulu and Bartels (2002).

The involvement of ABA-independent genes (Riera et al., 2005) in response to dehydration in plants and the occurrence of alternative dehydration responsive pathway(s) to minimise water loss in plants under ABA deficiency have also been reported (Wilkinson and Davies, 2010). Therefore, and considering that the number of stomata per unit surface area in fruits of both cultivars ('Navelate' and 'Pinalate') is similar (Alférez and Zacarias, unpublished data), the above results indicate a higher ability of 'Navelate' fruits to synthesise ABA, which controls stomata closure to reduce dehydration, and also to modulate ABA-related genes important for cell homeostasis and viability for the reduction of rind-damaged fruits (Romero et al., 2012).

In support of the above-mentioned results, Sala et al. (2005) suggested that the presence of metal transporters might play a role in the tolerance of citrus fruit to dehydration by modulating an ABA-responsive pathway of ‘Navelate’ fruit to developing NCPP. Puig et al. (2007) elaborated that an excess of metals may lead to the disruption of cellular processes and finally to cell death. On the contrary, the prevention of such harmful effects requires the participation of metal-binding proteins and transporters. Thus, it can be concluded that elevated levels in the expression levels of iron and copper transporters detected in the dehydration-protected wild-type fruit suggest that the impaired ability of the ABA-deficient mutant to regulate metal homeostasis could be relevant for its higher susceptibility to dehydration and NCPP (Romero et al., 2012).

5.3. Pathological response to postharvest water loss

Control of RH and temperature is important in reducing the weight loss of citrus fruit that leads to shrinkage, softening, and susceptibility to decay. Proper control of RH should include the prevention of water condensation on the fruit surface (Grierson, 1969), since the presence of free water at relatively high temperatures provides an ideal medium for the development of decay organisms (Eckert, 1978).

Waks et al. (1979) recommended that fruit be kept at high humidity and cool temperatures before packing to decrease loss of postharvest quality and market value. Grierson and Ben-Yehoshua (1986) further elaborated on the research of Waks et al. (1979) and concluded that drying conditions (low RH, ambient temperature) at the packhouse can accelerate weight loss and increase the incidence of stem-end rot during subsequent storage. Temperatures such as 28–29 °C and very high RH (95%) were also found to improve healing of minor wounds in the flavedo through lignification (Grierson and Ben-Yehoshua, 1986). During the lignification process a two- to threefold increase in phenolic compounds occurs, catalysed by the enzyme phenylalanine ammonia-lyase (PAL) (Ismail and Brown, 1979). Lesions of pathological origin normally develop quickly and increase in size, whereas physiological disorders normally do not expand dramatically in surface area.

McCollum (1989) found that chilled squash fruit had a greater weight-loss rate after transfer to 15 °C than non-chilled fruit. In citrus fruit this loss can also be associated with the development of microscopic cracking in the otherwise apparently healthy rind tissue. Schiffmann-Nadel et al. (1980) proposed the existence of such cracks as an underlying cause of the development of mould in affected citrus fruit. Cohen et al. (1988) revealed these cracks by dipping fruit in malachite green solution. The cuticle and outer epidermal cells of fruit that had been stored at 2 °C, and which appeared macroscopically intact, were exposed by the colourant to be extensively injured. Such fruit has a greater susceptibility to infection by *Penicillium digitatum*, a typical opportunistic wound fungus. Cohen et al. (1994) investigated whether water loss could serve as a reliable non-destructive indicator of chilling injury (CI). Their results indicated that the water-loss rate increased significantly after removing the fruit from cold storage (2 °C) and holding at 20 °C (ambient conditions), with large cracks around the stomata revealed through scanning electron microscopy (SEM). Cohen et al. (1994) thus concluded that such cracks occur after exposure to chilling temperatures and are most likely sites for fungal infection.

Rootstocks have been reported to affect tree growth and fruit rind characteristics that could in turn affect stem-end rind breakdown (SERB) and decay susceptibility (Agustí, 1999). For example, fruit with a thinner rind may lose water more readily leading to SERB. Tree growth characteristics that result in more canopy dead wood may lead to greater infection from anthracnose (*Colletotrichum gloeosporiodes*) and organisms causing stem-end rot such as *Diplodia natalensis* and *Phomopsis citri* (Brown and Miller, 1999).

In southern California, water spot within 'Washington' navel oranges is of increasing concern to the citrus industry and is characterised by a local swelling and waterlogging of the rind in the maturing fruit (Scott and Baker, 1947). The progress of this disease appears to be dependent on weather conditions, with injury occurring during cold rainy periods in winter. Scott and Baker (1947) described that the surface of the rind becomes raised and more or less translucent, and a consequence when fluid replaces the air normally present in the respiratory chamber beneath the guard cells, and in the intercellular spaces. If rain continues, the initial swelling may spread, and in a matter of hours or days, the epidermis may crack in all directions. Should rain cease, the cracked epidermis will heal by normal cicatrisation. The rind at maturity will be scarred and shrivelled, but the fruit will be otherwise undamaged. On the other hand, if wet weather persists, the ever-present spores of mould such as *Penicillium* and *Cladosporium* may enter the cracks, germinate, and spread to rot the entire fruit within 48 hours (Scott and Baker, 1947).

6. Conclusion

The success of the South African citrus industry is based on three aspects: firstly, quick market feedback to the relevant role players on issues relating to quality; secondly, research in focus areas that could establish sustainable protocols; and thirdly, the transfer of technology to producers and other stakeholders of the industry.

Environmental factors are one of the main sources of variation in fruit quality. These pre-harvest factors affect both fruit growth during its development by changing the accumulation of water and dry matter, including biochemical and mineral compounds, and fruit behaviour during its storage. Water and carbon balance of fruit are important to fruit quality (Ho et al., 1987). Having knowledge of and then being able to control changes in fruit quality in response to environmental conditions may be essential to adopting cultural practices that will provide high-quality fruits and assist in defining optimal postharvest procedures, taking fruit production conditions into account. Continued research will ensure a better understanding of the various factors that add to the complexity of exporting citrus, such as cultivar differences (mandarins vs oranges), production areas (winter vs summer rainfall) and shipping temperatures (traditional vs cold sterilisation) (Dodd et al., 2010).

Fruit-water balance is determined by the entry of water via xylem and phloem (Pate et al., 1977; Pate et al., 1978), accumulation in fruit, and losses due to either fruit transpiration (Syvertsen and Albrigo, 1980) and/or xylem backflow to transpiring leaves or other sinks within the plant (Elfving and Kaufmann, 1972). Variations in rind water status are determinants of rind pitting induction and its development in sensitive citrus fruit (Alférez and Burns, 2004).

Changes in postharvest RH, and consequently in fruit rind water status, are important factors inducing rind pitting in citrus fruit (Alférez et al., 2003; Alquezar et al., 2010). Fruit development also appears to affect the rate of water loss from the fruit surface owing to factors such as surface:volume ratio, cuticle thickness (Syvertsen and Albrigo, 1980), stomatal frequency (Blanke and Lenz, 1989), and stomatal responsiveness (Blanke and Leyhe, 1987). Therefore these and other studies suggest that alterations in rind water status, which can be modified by RH, can play an important role in the expression of some rind disorders.

Most research on water relations in citriculture have focused on increased yield during the pre-harvest stages or on the effect of long-term water stress for increased sugars, without making the inevitable link to postharvest quality. Furthermore, the influence of pre-harvest water stress on the fruit rind and its impact when exposed to the various postharvest stresses such as dehydration and packaging is unknown and will be the primary focus of the research reported in this study.

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PAPER 1

Influence of late nitrogen soil and urea foliar spray applications on mandarin (*Citrus reticulata* blanco) fruit quality

ABSTRACT

The level of nitrogen (N) application is known to have a greater influence on tree growth, yield, quality and appearance of citrus fruit than any other single mineral element, as it is most often deficient in soils. Adequate availability of N during the critical stages of fruit initiation and development is important to support optimal yield of high quality citrus fruit. The objective of this study was to determine the influence of late soil applied nitrogen on mandarins when applied during the phase II and III period of fruit growth and whether such application could negatively impact on rind colour and susceptibility to rind disorders. In addition the nutrient content of the rind and foliage were recorded. The experiment focused on two widely planted mandarin cultivars, in South Africa, namely ‘Nules Clementine’ and ‘Nadorcott’ mandarin, because of their two distinct respective commercial harvest dates (May and June/August) and susceptibility to postharvest rind disorders. In general across areas, season, or cultivars studied, no significant difference in fruit colour or size was recorded. Furthermore, no consistent difference was seen after fruit were stored at either -0.6 °C or 4 °C among the N treatments. From this study, the expected decreased rind colour due to increased nitrogen application was not consistently observed. In this study incidence of pitting was recorded, but was generally at too low levels for all treatments to be included in the data analysis and therefore could not be correlated with N treatments. Thus it was not possible to link rind mineral content pre-harvest and the eventual incidence of rind pitting as recorded during subsequent storage with additional N applications.

Keywords: Citrus; Colour; ‘Nadorcott’; ‘Nules Clementine’; Nutrients; Pre-harvest factors

1. Introduction

The South African citrus industry identified research to resolve challenges relating to market access such as false codling moth (FCM), citrus black spot (CBS), as well as rind disorder as the most important factors which will determine the future sustainability of this industry. Therefore studies addressing rind disorders have received a high priority on the horticultural research agenda. Literature and industry feedback have identified various factors contributing either towards increased susceptibility or those triggering higher rind disorders incidence viz. nutritional imbalance, sudden weather changes such as rainy periods followed by cold wind and postharvest handling protocols (Ritenour et al., 2003).

Central to production of high internal and external citrus fruit quality is an understanding of soil and nutrient requirements. Nitrogen (N) is a key component of enzymes, vitamins, chlorophyll and other cell constituents and therefore one of the most important macro-nutrients required in leaves, flowers and fruit to collectively ensure regular high citrus crop yields (Iglesias et al., 2007). An optimum level of N fertilisation is especially critical to ensure the best possible tree growth, yield, quality and appearance (Appendix 3) of the citrus fruit compared to any other single mineral nutrient, as it is mostly this nutrient that is often deficient in soils. In citrus trees adequate supply of N is needed to promote rapid growth and development of young non-bearing trees as well as to promote flower development. Therefore it is a standard practice in citriculture to adjust or augment N levels, either through foliar or soil applications.

The principal mineral nutrients affecting rind pigment composition and concentration are N, P and K, although N has the greatest effect (Reitz and Embleton, 1986) where high N levels in the leaves and rind are known to be a strong inhibitor of rind colour development (Iglesias et al., 2001). According to Koo and Reese (1977) shoot development in a warm environment leads to increased vegetative and reproductive growth and the resulting translocation of N to physiological sinks which cause a reduction of rind N content. Ritenour et al. (2003) reported that lower N levels in the citrus tree resulted in more orange-coloured fruit, however the N content reported could be sub-optimal for maximum yield at 2.5 to 2.7% leaf N (Appendix 2). This research elaborated on findings from a study by Coggins et al. (1981) which reported that high N-nutrition under warm and humid environmental conditions favourable for plant growth, increased the degree of regreening of late maturing oranges. Excessive applications of N can have several adverse effects in addition to reduced colour development and may include increased rind thickness and toughness, a decreased sugar:acid ratio, and increased susceptibility of new foliage to winter frost damage (Appendix 3). Excessive foliar applied N can cause leaf burn. Valencia orange fruit from trees exhibiting a high foliar N content were more susceptible to rind staining than fruit from trees with a normal or subnormal content (Reuther et al., 1989).

Adequate availability of N during the critical stages of fruit initiation and development is important to support optimal yields of good quality citrus fruits. Leaf tissue analysis (Appendix 2) is a valuable indicator of soil and tree nutritional status, particularly with respect to mobile nutrients such as N and K, and for micronutrients such as copper, iron, manganese, and zinc (Obreza et al., 1992) to develop fertilizer regimes for improved yield and quality (Mills and Benton-Jones, 1996). The nutritional values measured in the leaf tissue in a given situation can be compared with the critical range of concentration norms that are established for that particular crop based on years of experimentation (Bennett, 1993; Mills and Benton-Jones, 1996). Typically, the fruit yield increased with an increase in leaf N concentration to an optimal level. Further increase in leaf N concentration leads to a decrease in fruit yield due to the effect of luxurious consumption of N (Alva et al., 2006). In citrus, emphasis is often placed to achieve optimum pre-harvest quality of the fruit – sugar content, size, and fruit colour – however, little focus is still being placed on parameters that are considered indicators for good storage potential of the fruit (Kruger et al., 2003). This is ironically an extremely important aspect for South African citrus producers who are heavily dependent on exports.

Development of physiological disorders during postharvest ripening and storage of fruit depends on a range of pre-harvest factors (Ferguson et al., 1999). The factors affecting fruit development are mostly associated with either positional effects on the tree or the environment. The canopy position and mineral nutrient allocation to sinks could lead to a predisposed susceptibility in a fruit which would then respond to environmental changes or extremes (temperature or relative humidity) (Ferguson et al., 1999), leading to the development of a disorder. In ‘Navel’ orange the cause of the non-chilling rind physiological disorder known as rind stain or rind breakdown has been related to nutritional imbalances, drought and rainy periods in alternation (Zaragoza and Alonso, 1975) as well as cold periods (Klotz et al., 1966). Variation in the incidence and intensity of ‘Navel’ orange rind stain may occur from season to season, amongst orchards and as well as between trees and individual fruit. Rind breakdown has been reported to be as high as 50 % affected at harvest in 'Navelate' orange produced in Spain (Agustí et al., 2002).

During the last ten years a shift in production has occurred in the South African Citrus industry in favour of later maturing (July to August in southern hemisphere), high- yielding and value mandarin cultivars. However, these cultivars have not been adequately researched to determine the impact of basic horticultural practises such as nutrition and crop management on fruit quality and storability. In addition, these cultivars have shown to be susceptible to postharvest rind disorders (Cronjé, 2013). It is being hypothesised that incorrect N fertilisation could result in an increased sensitivity of mandarin fruit to postharvest physiological rind disorders. The objective of this study was to ascertain whether additional N, applied later than the current practice, during the phase II and III fruit growth periods, could negatively impact on rind colour and susceptibility to rind disorders of ‘Nules Clementine’ and ‘Nadorcott’ mandarin.

2. Materials and methods

2.1. Experimental site and plant material

Two geographically different sites for each mandarin cultivar, ‘Nules Clementine’ and ‘Nadorcott’ were selected to include fruit from different climatic conditions (Table 1). For ‘Nules Clementine’ mandarin used the first season, the experiment was conducted in two commercial orchards budded on ‘Carrizo citrange’ {[*Poncirus trifoliate* (L.) Raf.] X [*Citrus sinensis* (L.) Osb.]} located in Citrusdal (Brakfontein) and Riebeeck Kasteel (Wynkeldershoek) in the Western Cape Province, South Africa, respectively. In Citrusdal (32°.25’ S 18°99’ E) trees were planted in 1991 at a spacing of 4.5 x 2.5 m. In Riebeeck Kasteel (33°.40’ S 18°.84’ E), approximately 110 km from Citrusdal, trees were planted in 1993 at a tree spacing of 4.5 x 2.0 m. Commercial harvest maturities (Appendix 1) differed between 7 to 14 days for the two respective experimental orchards. In the second season, the experiment on ‘Nules Clementine’ was only conducted at the Riebeeck Kasteel experimental site.

For ‘Nadorcott’ mandarin, during the first season, the experiment was conducted in two commercial orchards budded on ‘Carrizo citrange’ {[*P. trifoliate* (L.) Raf.] X [*C. sinensis* (L.) Osb.]} at the same sites as described for the ‘Nules Clementine’ mandarin above. The Citrusdal orchard was planted in 2008 at a spacing of 4.5 x 2.5 m, whilst the Riebeeck Kasteel orchard was planted in 2007 at a spacing of 5.0 x 2.4 m. The commercial harvest date for ‘Nadorcott’ differed between 7 to 14 days for the respective sites. For the second season, the experiment on ‘Nadorcott’ was only repeated in Riebeeck Kasteel.

2.2. Treatments

All experimental sites and cultivars received the same soil nitrogen applications at 20 $kg{\cdot}ha^{-1}$ (90 g limestone ammonium nitrate per tree [LAN; 28% N]) and 40 $kg{\cdot}ha^{-1}$ (180 g limestone ammonium nitrate per tree [LAN; 28% N]), respectively, in addition to the standard commercial recommended N fertigation regime of approximately 300 $kg{\cdot}ha^{-1}$ (Table 2). The first and second application was made on 21 January and 26 March 2014, respectively, according to a complete randomised block design on single tree replications (n=10), with one buffer tree between treated trees. These additional N application treatments were repeated during the 2015 season in the same orchard, but on previously untreated trees. During the 2015 season an additional foliar application of 1% urea was sprayed on 26 March to ten single tree replicates (Table 2).

The likeliness of the development of postharvest pitting was increased by subjecting all fruit, harvested at commercial maturity, to a dehydration protocol which was followed by a rehydration water stress as described in Alférez et al. (2003). The dehydration postharvest stress involved dehydrating the fruit at 25 °C and 60 to 80%. RH to induce high vapour pressure deficit (VPD) (0.7 to 1.1 kPa) for two days, followed by rehydrating in a 100% RH environment for one day by placing fruit in plastic bags, lined with wet paper. Temperature and %RH were monitored by means of loggers (Tinytag View 2 TV-4500 Gemini Data Loggers (UK) Ltd. Temperature/Relative Humidity Logger [-25 to +50 °C/0 to 100% RH]) to confirm and ensure constant treatment conditions. On the fifth day following harvest and immediately prior to cold storage the fruit were waxed in a simulated commercial pack line with natural based wax (18% solids) consisting of carnauba-shellac based formulations (875 High Shine, John Bean Technologies, Brackenfell, South Africa). No fungicide or plant growth regulators were included in the postharvest treatments.

2.3. Data collection

From each tree 20 fruit per treatment were harvested at commercial harvest maturity and transferred to the Department of Horticultural Science at the University of Stellenbosch, where the fruit was washed with chlorine (0.147 $mg.mL^{-1}$) and left to air dry. Ten fruit per replicate (n=10), based on uniformity of size and colour and lack of visual rind injuries, were selected for cold storage, either at -0.6 °C (n=5) or 4 °C (n=5) respectively. The remaining 10 fruit per replicate were used for immediate external and internal quality analysis.

Visual rind colour was assessed using a No. 36 CRI colour chart for mandarins [Citrus Research International (CRI), 2004; Appendix A]. In addition 'Nules Clementine' mandarin rind colour was measured using a Minolta chroma meter (Model CR200; Minolta Camera Osaka, Japan) and the data were expressed as hue angle (H) where a higher hue angle value represents a more green colour whereas a lower hue value would represent a more orange rind colour (0 red to purple; 90º yellow and 150º green). Colour measurements were taken at two opposite sides of the fruit to include lighter and darker rind colour of fruit whereafter an average value was used. No colorimeter data were recorded for the 'Nadorcott' mandarin as no visual difference was detected, mainly due to the high rind colour development. The fruit diameter was measured using an electronic calliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan). Fruit weight was determined prior to and after cold storage using an Elec checking scale NBK-30 (Model NWH 10422, UWE South Africa) in order to calculate the total percentage (%) weight loss during storage.

Internal quality was determined by cutting fruit in half on the equatorial line, whereafter the flesh was juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth to remove any solid particles. Juice percentage was calculated by dividing the weight of the juice by the total weight of the fruit. °Brix content of the juice was determined by using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan) and titratable acidity (TA) was determined by titrating 20 ml of juice against 0.1562 N sodium hydroxide. Phenolphthalein was used as indicator and titration was complete when the liquid turned pink in colour. Acid was expressed as citric acid content. The °Brix:TA ratio was determined by dividing the °Brix values by the TA values. Cold stored fruit were evaluated for external quality after the cold storage period, where external and internal quality was assessed, after a seven day shelf life period. The incidence of rind disorders was determined by visually inspecting fruit. A scoring rind disorder incidence on a rating scale from 0 (no disorders noted) to 3 (severe disorder incidence) based on the extent and intensity of the symptom was used [Fig. 1.F]. The rating values were used to calculate the rind disorder index (RDI) according to the following formula:

$$\text{RDI} = \sum \frac{[\text{Rind disorder scale } (0-3) \text{ x number of fruit within each category}]}{\text{Total number of fruit in replicate } (\text{n}=10)}$$

2.4. Mineral nutrient analysis

Prior to N-application as well as at harvest, the fresh rind (containing the flavedo and albedo), pulp and leaves of ten replicates of all treatments were sent to a commercial analytical laboratory for mineral analysis (Bemlab (Pty) Ltd., Strand, South Africa) according to the following protocol. A volume of 50 ml in solution was analysed on the Nitric / Hydrochloric total Acid digestion, ICP–OES (Inductively Coupled Plasma–Optical Emission spectrometer) (Varian PRX–OEX, Varian, Inc. Corporate, Palo Alto, CA, USA) against suitable standards. For the nitrogen analysis, 0.15 g of the sample was combusted (Total combustion method) at 850 °C and analysed on a LECO Nitrogen analyser by thermal conductivity (LECO FP528 Nitrogen analyser, LECO cooperation, St. Joseph, MI, USA) [W.A.G. Kotzé, Bemlab (Pty), Ltd., Strand, South Africa; personal communication] (Cronje et. al., 2011a). The results of the mineral analysis of the leaves are expressed as a percentage (%), whereas the rinds and pulp are expressed as mg.100g^{-1} fresh weight, later to be converted to a percentage (%) value. All macro and microelements (including % water) were analysed, but only the N, P, K, Ca and Mg data are presented.

2.5. Scanning Electron Microscopy (SEM)

In order to identify the concentration as well as locality of nutrients in the rind, samples at harvest were prepared for scanning electron microscopy (SEM) studies. Thin, uniform rind pieces (approximately 0.125 cm^3) were cut with a razor blade and fixed in 1:1 ($^{v}/_{v}$) solution of 2.5% glutaraldehyde and 2.5% formaldehyde for 1 to 2 hours at room temperature. The samples were then rinsed three times with distilled water. The material was then dehydrated in a graded (50%, 60%, 70%, 80%, 90%, 100%) ethanol series for 2 h. The material was thereafter washed with acetone, before being placed in a critical point drier in liquid carbon dioxide.

Imaging of the samples was taken using a Leo® 1430VP Scanning Electron Microscope at the Central Analytical Facilities, Stellenbosch University, South Africa. Prior to imaging, the samples were mounted on SEM stubs with double sided carbon tape. The mounted material was coated with a thin layer of gold in a Polaron E-6100 sputter coater. This was done in order to increase the electrical conductivity of the sample surface. SEM was performed on a LEO 1450VP scanning electron microscope (SEM), at an acceleration voltage of <30 kV. The Scanning Electron (SE) image detects the surface structure of inspected material. Beam conditions during surface analysis were 7 kV and approximately 1.5 nA, with a spot size of 150.

BSE-EDS analysis was accomplished through mass spectrometry using a Zeiss EVO® MA15 Scanning Electron Microscope. The system is designed to perform high-resolution imaging concurrently with quantitative analysis, with errors ranging from ± 0.6 to 0.01 weight % on the major elements using EDS. Quantitative analysis of phase compositions of the SEM samples and their backscatter images require 15 μm thickness of carbon coating, a flat and polished surface. Samples were identified with backscattered electron images, and phase compositions were quantified by EDS analysis using an Oxford Instruments® X-Max 20 mm^2 detector and Oxford INCA software. Beam conditions during the quantitative analyses were 20 kV and approximately 1.0 A, with a working distance of 8.5 mm and a specimen beam current of - 20.00 nA. For the mineral analyses counting time was 10 s live-time. Internal Astimex Scientific mineral standards were used for standardization and verification of the analyses. Pure Co were used periodically to correct for detector drift.

2.6. Statistical analysis

Data were analysed with each nitrogen and time combination as a treatment. Cultivars or areas were not statistically compared. Statistical analyses of fruit physico-chemical properties were carried out using the statistical software package *SAS Enterpise Guide* (*SAS EG* v.5.1; SAS Institute, Cary, VC, USA). Ranked data were subjected to one-way ANOVA analysis, where possible interaction could be detected. Means of treatments were separated by Fisher's least significant difference (LSD; $p = 0.05$). A p-value <0.05 is interpreted as a significant difference between treatments.

3. Results and discussion

3.1. Size and colour

3.1.1. 'Nules Clementine' mandarin

A significant difference in fruit size (diameter) between the different pre-harvest N-treatments was detected for fruit from Citrusdal during the 2014 season at harvest, but no distinct trend could be described. The higher application rate during the late application date significantly increased fruit size compared to the control, but also to the higher application rate applied in January, along with the lower application rate, applied in March (Table 3).

At harvest there was no significant difference in colour chart value or Hue° between the treatments. This serves as a good indication that there was uniformity in colour during picking of the fruit. Likewise, after storing fruit at 4 °C for 30 days, no significant difference in colour, irrespective of method, between different nitrogen treatments was measured. However, the colour improved dramatically during cold storage at 4 °C compared to that at harvest. Colour in general improved with storage (Van Wyk et al., 2009), but compared to fruit stored at 4 °C, the fruit stored at -0.6 °C had far less rind colour change, both according to the colour charts and Hue° measurement. Furthermore, in fruit stored at -0.6 °C indicated that the control displayed a better colour compared to the different nitrogen treatments. Still, fruit that was obtained from the March (late) application of N at the higher rate did not differ significantly compared to that of fruit from the control or earlier N-applications (Table 3).

In 2014, at harvest in Riebeeck Kasteel, no significant difference in fruit size between treatments was recorded. However, by comparison to the Citrusdal fruit diameters of the same season (Table 3), the late N applications resulted in a reduced fruit size (Table 4).

No significant difference between the control and the fruit which received the various additional pre-harvest N-treatments could be detected in both colour determinations that were performed at harvest. The colour after storage improved compared to fruit examined at harvest and more so at the higher storage temperature of 4 °C (Table 4). According to the colour chart and the hue° measured, fruit stored at -0.6 °C showed no significant difference between different N-treatments. On the other hand, fruit that was stored at 4 °C and rated on the colour chart showed notable differences between nitrogen treatments (Table 4). The greatest difference occured between early January application at 20 kg·ha^{-1} N and the late March application at 40 kg·ha^{-1} N, with the latter treatment favouring less colour development. Of interest is that no significant difference was observed between control fruit and fruit from trees receiving the later, higher nitrogen application rate (Table 4).

In 2015 at Riebeeck Kasteel the measurements taken at harvest showed a significant difference in size of fruit (diameter) between the different pre-harvest N treatments, but again no distinct pattern emerged. The later additional application of 40 kg·ha^{-1} resulted in improved fruit size compared to the control and the early, lower N application at 20 kg·ha^{-1} N (Table 4).

At harvest, significant differences in rind colour were detected between the different N treatments (Table 4). The additional 1% urea in March and 40 kg·ha^{-1} January N applications improved colour, and differed significantly from the fruit obtained from the late, 20 kg·ha^{-1} N treatment. Nevertheless, no consistent pattern could be established that would correlate or predict a decrease in rind colour with an increase in N application, irrespective of application rate or timing. After cold storage at -0.6 °C and 4 °C no significant difference developed between fruit from the different N-treatments in the colour chart or hue° determination value (Table 4).

3.1.2. 'Nadorcott' mandarin

For 'Nadorcott' mandarin in Citrusdal, during the 2014 season significant differences in fruit between treatments were measured, but again without a distinct trend (Table 5). A significant reduction in colour was observed at harvest in fruit from the March 40 kg·ha^{-1} N application, compared to control fruit and that of other N treatments. Fruit stored at -0.6 °C also displayed significant differences between different nitrogen applications, but under these postharvest conditions, fruit obtained from the additional January at 40 kg·ha^{-1} application displayed a significant reduction in colour development. The fruit stored at 4 °C showed even more dramatic improved colour development compared to fruit stored at -0.6 °C, but the significant difference between treatments that was observed at harvest was lost after cold storage. Hue° values were not measured on 'Nadorcott' mandarin as no visible colour difference could be detected (Table 5).

In Riebeeck Kasteel for the 2014 season there was a significant difference in fruit size where the January, higher application rate together with the lower March N-applications resulted in smaller fruit compared to the control and the additional January application at the lower rate (Table 6). At harvest there was no significant difference in colour within treatments or in comparison to the control. Likewise fruit stored at -0.6 °C did not show any significant difference between treatments and/or the control. However, storing at 4 °C resulted to significant differences between N-treatments. The additional application of 20 kg·ha^{-1} in March reduced colour development significantly compared to the control (Table 6). As mentioned above, colorimetry was not performed with 'Nadorcott' mandarins as no visible colour differences between treatments could be detected.

For 'Nadorcott' mandarin produced in the 2015 season in Riebeeck Kasteel a significant difference in size of fruit, as noted in the previous season, was detected between respective additional pre-harvest nitrogen treatments and the control. Fruit which was obtained from trees which received 40 kg·ha^{-1} N in January or March was significantly smaller than fruit which received the lower application rate of 20 kg·ha^{-1} in March. However, fruit from trees subjected to the additional, late urea applications, did not differ in size from fruit of the control, or earlier N applications (Table 6). No colour differences, irrespective of quantification method (colour chart or Hue°) could be detected at harvest or following long-term cold storage (Table 6).

3.2. Internal quality

3.2.1. 'Nules Clementine' mandarin

At harvest, the internal quality of fruit cultivated in Citrusdal during the 2014 season did not differ between treatments with respect to juice content (%), % titratable acidity (TA) or °Brix (Table 7). Even after cold storage no significant differences emerged between treatments and the control. An increase in °Brix and °Brix:TA ratio compared to harvested fruit was, however, noted after storage of fruit at both temperatures (Table 7).

Fruit harvested from the Riebeeck Kasteel experimental site during 2014 were not significantly different among the various nitrogen treatments or with respect to the control, regarding any of the internal fruit quality parameters (Table 8). These results remain valid after fruit had been stored at 4 °C for 30 days. However fruit that were stored at -0.6 °C for 30 days resulted in significant differences observed in %TA and °Brix among the different N-treatments. The higher application rate of 40 kg·ha^{-1} N application in March resulted in both lower TA and °Brix values compared to the more desirable internal quality obtained with the additional application of 20 kg·ha^{-1} N, during the same application time (Table 8).

In the following season of 2015 in Riebeeck Kasteel the internal quality indices showed significant differences between the different nitrogen applications at harvest (Table 8). The % juice content was the highest in fruit harvested from trees exposed to the additional 40kg·ha^{-1} N application in January and from the 20 kg·ha^{-1} N application in March. For %TA at harvest, treatments and the control were comparable, except for fruit from the January 20 kg·ha^{-1} N application, which were significantly higher in %TA than fruit from the higher application rate of 40 kg·ha^{-1} N, applied during the same period (Table 8). °Brix and °Brix:TA ratioat harvest differed in fruit from the various N treatments, but were not significantly different from the control for both parameters.

A general trend was observed where %TA decreased and the °Brix increased when fruit was stored at -0.6 °C for 30 days compared to values obtained at harvest (Table 8). The trend was most evident for fruit from the treatment which involved the additional application of 20 kg·ha^{-1} N in March.

Fruit stored at 4 °C for 30 days did not differ significantly among the various N-treatments with respect %TA (Table 8). Differences in °Brix for fruit associated with the various N treatments were recorded at harvest and following cold storage at both temperatures. However, none of these values differed significantly from that of control fruit (Table 8).

A significant difference in °Brix:TA ratio was observed for fruit at harvest between fruit from the respective N treatments. However, as applicable in °Brix discussed above, these values did not differ significantly from that of the control fruit. Also, the difference observed at harvest for °Brix:TA was not retained after the storage of fruit at both temperatures (Table 8).

3.2.2. 'Nadorcott' mandarin

The internal quality of 'Nadorcott' harvested in Citrusdal in 2014 was not significantly affected by treatments for the juice content (%), TA (%), °Brix, or ratio thereof at harvest or after cold storage (Table 9).

Fruit cultivated in Riebeeck Kasteel, similar to that described for Citrusdal in the 2014 season, did not display any significant difference in the internal quality between the N-treatments and that of the control fruit (Table 10). The only exception was a significant difference in °Brix values between the various N-treatments, after the fruit were stored at -0.6 °C for 30 days. However, values for the control fruit with this treatment did not differ significantly from stored fruit associated with any of the additional N applications.

In 2015 for the Riebeeck Kasteel experimental site, there were no significant differences in the juice content (%), TA (%) at harvest for the various N-treatments (Table 10). A significant difference in the °Brix at harvest was detected where fruit from the additional application 1% urea in March was recorded to be being significantly lower in °Brix than that of fruit from the control and all other N treatments.

Following cold storage at both -0.6 °C and 4 °C a decrease in TA (%) was observed in comparison to fruit freshly harvested (Table 10). After storage at -0.6 °C the significant difference in °Brix at harvest described above was not evident anymore. However, °Brix differed significantly for fruit stored at 4 °C and also resulted in the °Brix:TA ratio be significantly higher following long-term cold storage at 4 °C (Table 10).

3.3. Mineral analysis

3.3.1. 'Nules Clementine' mandarin

The macro-nutrients N, K, Ca and Mg as recorded at harvest in the leaf, pulp or rind, respectively, from treated trees or fruit from Citrusdal during the 2014 season did not differ significantly between treatments or from the control (Table 11). In leaves, %Ca and %Mg was, in general, higher among the various N-treatments compared to that of the base value that was established prior to treatment, whereas %N and %K maintained relatively similar values (Table 11). Mineral content values of the pulp, in particular the %N, decreased from that recorded prior to treatment to lower values at harvest. By contrast, an increase in the % N, K and Ca, but not in %Mg of the rind of fruit subjected to additional N treatments was observed at harvest, compared to the base pre-treatment value.

For experimental trees during 2014, located at Riebeeck Kasteel, significant differences in % K and Mg were recorded with leaf analysis for each of the respective elements compared to control fruit. However, this observation was not repeated in 2015 where no significant difference among the various N-treatments or with the control was recorded for any of the minerals studied (Table 12). Fruit pulp mineral content did not differ significantly in 2014 among the various N-treatments (Table 12). An apparent decrease in nutrients, however, was detected from the base values measured prior to N treatment compared to those recorded at harvest, both in the control and N treatments (Table 12).

In 2015, for mineral analysis done on material obtained from Riebeeck Kasteel, a somewhat different pattern was recorded for leaf and pulp samples where all the minerals studied were detected at lower levels prior to treatment than was later recorded following N treatment or in the control. The % Ca, as was measured in the pulp, showed significant differences between the various N-treatments.

Mineral analysis of the rind contents of fruit from Riebeeck Kasteel showed significant differences for % N and K in 2014 as well as for % N, Ca and Mg, respectively, in 2015 (Table 12). For both these seasons, fruit from trees subjected to the March N application at a rate of 20 $kg{\cdot}ha^{-1}$ N indicated significantly higher %N rind values, compared to that of control fruit. Similarly, in 2014, fruit that was harvested from this particular treatment also indicated significantly higher %K than was recorded in control fruit, but in 2015 only % Ca and Mg differed significantly among the N treatments as well as the control. During this season, notably lower initial base rind values for all minerals studied were recorded compared to values reported at harvest for the fruit from the treatments and control (Table 12).

3.3.2. 'Nadorcott' mandarin

In 2014 no significant difference was detected among the various treatments or control for any of the minerals recorded in the leaf, pulp or rind analysis for 'Nadorcott' mandarin harvested in Citrusdal (Table 13).

For both the 2014 and 2015 season, no significant differences were detected for any of the minerals analysed either in the leaves or fruit from Riebeeck Kasteel (Table 14), with the exception where %Ca differed significantly between treatments in the leaves and pulp for 2015. In this season, the N treatment applied in late in March at 40 kg·ha^{-1} resulting in a lower Ca% in both the leaves and pulp compared to the control (Table 14). The pulp %N in 2015 was also significantly affected where all late N applications resulted in lower N-content compared to the control (Table 14). A similar trend, although not significant, was observed in 2014, with a reduction in %N in the pulp for N-treated fruit compared to control fruit. Regarding the %Ca in the pulp in 2015, the late additional applications N and urea, recorded significantly lower to values obtained from rind associated with the additional 20 kg·ha^{-1} Napplication in January (Table 14). Rind mineral content did not differ significantly for either 2014 or 2105 for any of the minerals investigated (Table 14).

3.4. Scanning electron microscopy (SEM)

Mineral content as located and analysed by SEM in the different rind layers of 'Nadorcott' mandarin recorded significant differences in the albedo between the various N treatments and control, as well as in fruit that was diagnosed with either pitting or rind breakdown disorder (RBD), for all minerals analyzed (% K, Ca and Mg). In addition, %Mg in both the epidermis and flavedo was also detected to be significantly different between the respective treatments (Table 15), but with no significant differences detected for % K and Ca located in either the epidermis or the flavedo of the various categories of fruit inspected. %K, %Ca and %Mg in the 3 sections of the rind (epidermis, flavedo and albedo) all increased with fruit either receiving the additional N-applications or containing rind disorder lesions, compared to the control. Notably %K was the highest in die flavedo and albedo in fruit receiving the additional 40 kg·ha^{-1} N soil application in March (prior to harvest), but however did only differ significantly in the albedo compared to the control. These values are dramatically higher than those measured in fruit rind with lesions of pitting and RBD. Again the fruit which received additional 40 kg·ha^{-1} N soil application of N in March indicated higher values of %Ca in the albedo compared to values measured in fruit with pitting and RBD lesions. These values, however, only differ significantly compared to the control. The %Mg for RBD differed significantly in the epidermis and albedo compared to the other treatments, being significantly higher. In the flavedo the %Mg differed significantly between fruit containing RBD lesions and the other treatments. The fruit with lesions (RBD and pitting) were, however, significantly higher in %Mg compared to the control and additional 40kg·ha^{-1} N soil application of LAN in January (Table 15).

Low magnification images of the cuticle, epidermis, flavedo and albedo layers of ‘Nadorcott’ mandarin fruit rind, following storage at -0.6 °C for 30 days, projected the rind structure as intact with well-compacted cells, showing no signs of cell disruption or collapse in either epidermal or flavedo cells (Fig. 2A). Fruit that was harvested from trees which received the additional 40 $kg{\cdot}ha^{-1}$ N soil application prior to harvest showed no cellular damage occurring within the rind sections, but provided images of a healthy and gradual transition from densely packed flavedo cells to loosely congregated albedo cells. Similar observations were also witnessed and described by Alquezar et al. (2010), who concluded that the storage of fruit under high relative humidity (RH) had little effect on the morphology of the flavedo tissue. Alquezar et al. (2010) reported that cells from the epidermis and flavedo remained almost intact during storage for more than 30 days, whilst no signs of cell disruption or collapse were observed. Rind breakdown lesions manifest in ‘Nadorcott’ mandarin fruit as clearly collapsed regions with the flavedo and albedo (Fig. 2B). A similar condition was reported by Alquezar et al. (2010) on ‘Navelate’ oranges which were stored postharvest at various relative humidities, which ranged from 45% to as high as 95% RH. By day 3 albedo cells formed a zone of compressed tissue at the transition between albedo and flavedo and after prolonged storage, the albedo appeared twisted. This observation appeared also similar to that of initial symptoms noted during ‘Navelate’ rind breakdown which manifested in the transitional zone of the albedo-flavedo (Agustí et al., 2001). Our studies also reported collapsed oil glands of ‘Nadorcott’ mandarin fruit which developed pitting lesions after 30 days of storage at -0.6 °C (Fig. 2C). Alférez et al. (2003) stated that occasionally with these rind disorders the underlying oil glands remain intact, or at least during the initial stages of the disorder (Agustí et al., 2001), before further collapsing in the flavedo-albedo intercellular zone, in-conjunction with the collapsed oil gland (Fig. 2D). SEM images done by Agustí et al. (2010) confirmed this by showing more accurately the ‘Navelate’ rind breakdown to initiate in the deeper layers of flavedo cells and the external layers of albedo cells. The affected cells appear twisted and compressed, forming a layer of collapsed cells between healthy intact cells of the flavedo and albedo. Leakage of essential oils from oil glands in the flavedo proceeds to oxidize the albedo and then finally the epidermis, where oxidized tissue characteristically is detected as dark-brown coloured areas.

3.5. Rind disorders

Very low incidences of any rind disorders such as rind pitting or chilling injury as induced at -0.6 °C, in both cultivars and in both experimental areas were recorded, therefore no data areshown. Even after storage of fruit from eitherof the experimental sites for 30 days (at -0.6 °C or 4 °C), very low incidences of rind disorders prevailed, thus data are not presented.

3.6. Fruit colour as affected by additional N application

Fruit size and colour are critical factors in determining fruit quality and the profitability of citrus marketing. Colour change is of particular economic importance since the external colour of citrus fruits is a critical quality parameter for the fresh market (Alos et al., 2006). The colour break in citrus rind results from the differentiation of chloroplasts to chromoplasts, a process that in citrus is influenced by environmental conditions (in particular temperature), nutrient availability, and phyto-hormones (ethylene and gibberellins) (Alos et al., 2006). The effects of nutrients and hormones are typically assessed and studied by manipulations of external chemical applications (Alos et al., 2006).

Nutrient imbalances have also evidently been connected to rind disorders. Numerous studies have been done on fruit colour and rind disorders, linking the effect of inadequate fertilization. Changes in rind pigments are mostly affected by the principal mineral nutrients N, P and K, although N has the greatest effect (Reitz and Embleton, 1986). The role of other macronutrients has also been implied as Cronjé et al. (2011a) reported that the outer tree canopy fruit which exhibits typically a more orange colour may contain higher levels of Mg and Ca, and is less prone to rind disorders than the pale fruit, located inside the tree canopy. Rind blemishes often develop in susceptible fruit to such a degree as to render the fruit unmarketable, although solely for cosmetic reasons (Ritenour et al., 2013).

GA (Cooper and Peynado, 1958) and cytokinins (Eilati et al., 1969) are strongly associated with juvenility, along with the maintenance of the vegetative phase and chlorophyll synthesis, thus often resulting in delayed fruit maturation (Cooper and Peynado, 1958; Eilati et al., 1969). Gibberellins favour regreening of *Citrus* under environmental conditions favourable for vegetative growth, viz. high temperatures, sufficient soil moisture, high light and N levels (Cooper and Peynado, 1958). Under higher soil temperatures (>12.8 °C), root activity is stimulated and promotes the uptake and translocation of N-containing compounds and production of phyto-hormones (which are antagonistic to chlorophyll degradation and carotenoid biosynthesis) by the roots (Young and Erickson, 1961). At low soil temperatures (<12.8 °C) there is cessation of root growth, leading to a reduction in the uptake of N-containing compounds and thus the inhibition of hormones associated with juvenility and chlorophyll production.

In this study, no clear link could be established between additional applications of N and fruit size. The results varied from area to area, year to year and between cultivars. In some cases the different N-treatments increased fruit size, whilst the opposite outcome was also achieved. Still, it is of importance to consider that smaller-sized fruit have a greater tendency to develop stem-end rind breakdown (Reuther et al., 1989) and thus should rather be eliminated for packing and export purposes.

Nitrogenous fertilisation is one of the modifiable factors that most influences rind colour of *Citrus* (Sala et al., 1992). Early studies on citrus reported high N applications, at approximately 1.1 kg per tree per year, to delay fruit maturity, delay colour break and reduce colour development at harvest as well as promote regreening (Reuther and Smith, 1952; Reitz and Koo, 1960; Coggins et al., 1981). In this study, investigating the effect of additional, later N-applications on fruit rind colour of mandarins produced contradictory results to that of the general beliefthat nitrogen has a strong inhibitory effect on rind colour change (Iglesias, 2001). In general across areas, seasons, or cultivars studied, no significant difference in fruit colour was recorded compared to the control, although significant differences were detected among different N treatments. Furthermore, no significant difference was consistently detected after fruit were stored at either -0.6 °C or 4 °C among the various N treatments. However, in general an improved colour was obtained after storage at 4 °C. The colour of the paler fruit also improved with storage, although it remained inferior (higher hue angle) to that of fruit which display the desired orange colour at harvest. The fruit which recorded more in the orange spectrum and were stored at −0.6 °C developed a better rind colour (lower hue angle) after storage compared to the paler fruit. From our studies, the expected pattern of increased nitrogen concentration and decreased rind colour was not consistently observed. However, the additional, late application of higher nitrogen rates of 40 $kg{\cdot}ha^{-1}$ in March may indicate some detrimental effects with respect to rind colour, although this contraindicates some positive effects that wereobserved that can be obtained by an additional late nitrogen application, but at the lower application rate of 20 $kg{\cdot}ha^{-1}$. An optimum N concentration, later during the season, which supports maximum production and supports good colour development, can thus not be recommended unconditionally.

3.7. Internal fruit quality as affected by additional N application

Fresh citrus characteristics such as taste and juiciness are well-known to be influenced by mineral nutrition. It is a general belief that increasing doses of the inorganic N adversely affects fruit shelf life. Increased N supplied to ‘Valencia’ orange trees has been recorded to increase soluble solids, acid and juice percentage, along with promoting the percentage of green fruit, whilst reducing individual fruit weight and the percentage of packable fruit. Reese and Koo (1975) reported that incidences of decay during storage were negatively correlated with nitrogen rates whereas Grierson and Hatton (1977) concluded the effects of fertilizer treatments, particularly on storage quality, to be inconsistent. In the sweet orange cv. Mosambi, higher doses at 800-1200 g.tree^{-1} N reduced storage decay and accelerated fruit weight loss compared to fruit from trees subjected to lower or no applications of nitrogen (Govind and Prasad, 1981). Despite inconsistency in literature, it is generally recognized that high nitrogen application, as a rule, decreases storage potential of fruit (Ladaniya, 2008). However, contradictory to views held by Ladaniya (2008), our results indicated the internal fruit quality of mandarin, at harvest, for both cultivars, seasons and areas did not differ significantly when additional N was applied either as LAN or urea, early or late in the season, and at two different rates. In addition, mostly if not always, internal fruit quality of control fruit that were borne on trees not subjected to additional N treatments, were similar to that of fruit from trees that received a late N application.

3.8. Leaf and fruit mineral analysis as affected by additional N application

The mineral content of plant parts, in particular leaves, is widely used to identify nutrient deficiencies, excesses or imbalances within a crop (Story and Treeby, 2000; Kruger et al., 2003; Alva et al., 2006; Appendix 2). For example, creasing, a physiological disorder that develops predominantly on over-mature citrus fruit on the tree has been, among other factors, also associated with nutritional imbalances, particularly K, Ca and Mg nutrition (Grierson, 1983; Story and Treeby, 2000).

In this study, as also reported by Kruger et al. (2003), mineral nutrients were found not to be uniformly distributed between structural parts of the fruit. Our results indicated minerals in general, including % N, to be higher in the rind compared to the pulp. Story and Treeby (2000) stated that both xylem and phloem transport, determine the distribution of mineral nutrients to fruit parts, and not only xylem flow as was initially understood by Ferguson and Watkins (1992). Thus, the much higher concentrations of Ca in the rind compared to that of the pulp reported in this study could possibly be attributed to the low mobility of Ca in phloem (Marschner, 1995). Ca in particular has been shown to have a significant effect on the development of postharvest disorders (Ferguson et al., 1999; Saure, 2005). Story and Treeby (2000) concluded that fruit from mature trees, with a history of frequent and severe outbreaks of the rind disorder albedo breakdown (crease) had higher albedo K/Ca and Mg/Ca ratios during stage I of fruit development, compared to unaffected fruit from young trees.

Late season fluctuations in mobile nutrient content, such as N, were found to have an important effect on the development of rind pitting in ‘Valencia’ orange (Kruger et al., 2006). Extra N application to ‘Marsh’ grapefruit and late N application to ‘Valencia’ orange resulted in higher rind pitting after storage (Kruger et al., 2005). Similarly, fruit from trees exposed to high nitrogen levels, as indicated by leaf analysis, was found to be more susceptible to rind staining than fruit from trees with normal or subnormal levels of nitrogen (Reuther et al., 1989). In this study the incidence of pitting was recorded, but levels were generally too low for all treatments to be included in the data analysis and therefore could not be correlated with N levels of leaves or fruit.

Other rind disorders which may be morphologically similar to rind breakdown of ‘Nules Clementine’ mandarin have also been associated with nutritional imbalances. Zaragoza et al. (1996) demonstrated that the application of $Ca(NO_3)_2$ to ‘Fortune’ mandarin fruit at colour-break significantly reduced rind pitting incidence at harvest. Superficial rind pitting of ‘Shamouti’ orange was also reduced with the application of a K-spray fertilizer (Tamim et al., 2001).

From our study it was not possible to link rind mineral content prevalent pre-harvest and the eventual incidence of rind pitting as recorded during subsequent storage with additional N applications. Information on the seasonal changes in nutrient concentrations of whole fruit and structural parts of the fruit remains useful in providing a more in-depth understanding of the nutrient requirements of the tree and the effect thereof on yield and fruit quality. Nutrient management needs to be done through soil and foliar applications as the need arises.

The susceptibility of a particular crop and not other crops is apparently associated with weather, fertilization, irrigation and other cultural practices, but the interrelationships are unclear (Reuther et al., 1989). Agustí et al. (2001) assessed trees for the incidence of rind breakdown. Results from this study clearly illustrate the randomness and variable incidence of this disorder and suggested variations in climatic conditions during the growing season and within each particular orchard to be causal to the susceptibility of fruit to rind disorders. Cronjé et al. (2011b) further elaborated that the differences in levels of RBD incidence between seasons is an indication that these disorders cannot be ascribed to one single, specific factor. It is therefore likely that the sensitivity of the fruit to rind disorders is determined during pre-harvest fruit rind development (where exposure to nutrient imbalances or water stress can occur) and that a “trigger/signal” during the postharvest handling period (such as the transfer from low to high RH) initiates the onset of symptom development (Cronjé et al., 2011b). To further complicate the condition, no single factor is thought to act alone as a “trigger/signal”, rather a combination of factors is hypothesized to induce the response leading to non-chilling related physiological rind disorder symptoms. Although pre-harvest weather and cultural practices influence the fruit’s inherent susceptibility, postharvest conditions generally determine incidence and severity (Reuther et al., 1989). However, the visual manifestations of symptoms of physiological rind disorders differ between cultivars, climatic regions, and with postharvest handling protocols, along with the time of symptom development and type of disorder developing (Cronjé et al., 2011b). An understanding of the role of pre-harvest induction in the predisposition of *Citrus* to postharvest disorders is critical to develop orchard practices that will be successful to significantly reduce risk for rind disorders (Ferguson et al., 1999).

4. Conclusion

The late application of nitrogen had no negative effect on the fruit rind colour, contrary to expectations. The late N application, following the summer flush, can thus be considered as an option to increase tree N content, as rind condition was not negatively affected. However, results from this study should be regarded as preliminary, and further research is required to address relevant questions on the possible impact that a late N application may have on flower and fruit set in the subsequent season. In addition, more studies on the influence of N-status on the citrus rind and disorders are needed, particularly with regard to the impact of the position of the fruit in the canopy on rind disorders, also with special emphasis on the effect of sunburn. Comparisons between different cultivars is of prime importance as this aspect is being debated in the industry, where the general feeling is that not all citrus have the same inherent sensitivity towards rind disorders. As new norms for different nutrient concentrations are being developed, later applications of nutrients and especially that of nitrogen, can be proposed as part of an optimum mineral composition, especially formulated to improve rind strength without compromising quality.

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Table 1

Demographic and cultivation information of ‘Nules Clementine’ and ‘Nadorcott’ mandarin on Carrizo citrange rootstock at two experimental sites (Citrusdal and Riebeeck Kasteel, South Africa) as was used in 2014 and 2015. A north-south row orientation and the use of drip irrigation was consistent throughout the trials.

Location	Farm and climatic data	Mandarin cultivar	Plant density (m)	Year planted	Yield (t.ha^{-1})
Citrusdal	Brakfontein	'Nules Clementine'	4.50 x 2.50	1991	50
	(32°.25' S, 18°99'E)	'Nadorcott'	4.50 x 2.50	2008	60
	Annual Rainfall: 334 mm Temp: max/min: 25.3 °C /10.9 °C				
Riebeeck Kasteel	Wynkeldershoek	'Nules Clementine'	4.50 x 2.00	1993	40
	(33°.40’ S 18°.84’ E)	'Nadorcott'	5.00 x 2.40	2007	60
	Annual Rainfall: 403 mm Temp: max/min: 19.2 °C /12.0 °C				

Table 2

Summary of the demographic and cultivation information, indicating the harvest dates and rates of various soil/foliar nitrogen applications of ‘Nules Clementine’ and ‘Nadorcott’ mandarin at two experimental sites (Citrusdal and Riebeeck Kasteel, South Africa) as was used in 2014 and 2015. The number of trees per treatment (n=10) was consistent throughout the trials.

Area	Cultivar	Harvest date	Nitrogen application (N* kg·ha^{-1}) or % Urea				
			21 January		26 March		
2014							
Citrusdal	‘Nules Clementine’	6 May	20	40	20	40	---[z]
Riebeeck Kasteel	‘Nules Clementine’	29 May	20	40	20	40	---
Citrusdal	‘Nadorcott’	21 July	20	40	20	40	---
Riebeeck Kasteel	‘Nadorcott’	28 July	20	40	20	40	---
2015							
Riebeeck Kasteel	‘Nules Clementine’	18 May	20	40	20	40	1%
Riebeeck Kasteel	‘Nadorcott’	13 July	20	40	20	40	1%

* N-source: LAN (Limestone ammonium nitrate).

[z] Measurements not performed.

Table 3

External fruit quality (n=10) of 'Nules Clementine' mandarin cultivated at Citrusdal, quantified as diameter and colour (scoring by chart and hue°), at harvest and after cold storage at -0.6 °C or 4 °C for 30 days, following a range of additional soil nitrogen (LAN - limestone ammonium nitrate) treatments, applied either early (21 January) or late (26 March) during 2014.

Nitrogen treatment [N(kg.ha^{-1})]	External fruit quality						
	Diameter (mm)	Colour Chart (1-8)[w]			Hue°		
	Harvest	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C
Control	58.76bc[y]	6.50[ns]	3.50ab	1.70[ns]	109.58[ns]	86.24b	87.95[ns]
20 kg.ha^{-1} Early	61.46ab	6.40	4.10ab	1.90	110.00	88.44ab	89.55
40 kg.ha^{-1} Early	59.22bc	6.30	3.20b	1.50	109.98	89.48ab	89.17
20 kg.ha^{-1} Late	56.78c	6.00	4.50a	1.90	110.84	91.51a	91.39
40 kg.ha^{-1} Late	64.07a	6.30	4.30a	1.70	110.15	88.58ab	90.52
p-value	*0.0004*	*0.3957*	*0.0034*	*0.4134*	*0.7841*	*0.0226*	*0.3795*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

[w] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 4

External fruit quality (n=10) of 'Nules Clementine' mandarin cultivated at Riebeeck Kasteel, quantified as diameter and colour (scoring by chart and hue°), at harvest and after cold storage at -0.6 °C or 4 °C for 30 days, following a range of additional soil nitrogen (LAN - limestone ammonium nitrate) treatments as well as foliar urea (%) treatments, applied either early (21 January) or late (26 March) during 2014 and 2015.

	External fruit quality						
Nitrogen treatment [N (kg.ha^{-1}) or Urea (%)]	Diameter (mm)	Colour Chart (1-8)[w]			Hue°		
	Harvest	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C
2014							
Control	73.12[ns]	3.2[ns]	1.90[ns]	1.50ab[y]	77.81[ns]	68.88[ns]	70.76[ns]
20 kg.ha^{-1} Early	58.95	3.75	2.0	1.10b	78.59	71.51	69.46
40 kg.ha^{-1} Early	59.33	3.50	2.2	1.30ab	79.02	70.22	71.05
20 kg.ha^{-1} Late	54.99	3.40	1.7	1.30ab	76.51	68.17	71.15
40 kg.ha^{-1} Late	54.49	3.50	2.3	1.90a	78.81	71.09	71.48
p-value	*0.2015*	*0.8419*	*0.2588*	*0.0342*	*0.7302*	*0.9329*	*0.1071*
2015							
Control	60.92b	2.70ab	1.00[ns]	1.00[ns]	77.25b	73.44[ns]	72.55[ns]
20 kg.ha^{-1} Early	62.08b	2.80ab	1.00	1.00	78.06b	74.69	72.13
40 kg.ha^{-1} Early	66.70a	2.10b	1.00	1.00	77.16b	73.20	72.60
20 kg.ha^{-1} Late	63.60ab	3.10a	1.00	1.00	78.30b	74.20	73.61
40 kg.ha^{-1} Late	66.70a	2.60ab	1.00	1.00	83.30a	78.56	76.88
1% Urea Late	63.02ab	2.00b	1.00	1.00	80.42ab	76.35	74.37
p-value	*0.0146*	*0.0057*	---[x]	---	*0.0313*	*0.0976*	*0.0636*

[ns] No significant differences.
[y] Means with a different letter within a column differ significantly at the 5% level (LSD).
[x] P-value is non-significant.
[w] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 5

External fruit quality (n=10) of ‘Nadorcott’ mandarin cultivated at Citrusdal, quantified as diameter and colour (scoring by chart and hue°), at harvest and after cold storage at -0.6 °C or 4 °C for 30 days, following a range of additional soil nitrogen (LAN - limestone ammonium nitrate) treatments, applied either early (21 January) or late (26 March) during 2014.

Nitrogen treatment [N (kg.ha^{-1})]	External quality			
	Diameter (mm)	Colour Chart (1-8)[w]		
	Harvest	Harvest	-0.6 °C	4 °C
Control	67.34ab[y]	1.40b	1.20b	1.20[ns]
20 kg.ha^{-1} Early	63.66ab	1.50ab	1.70ab	1.60
40 kg.ha^{-1} Early	62.18b	2.10ab	2.10a	1.50
20 kg.ha^{-1} Late	68.89a	1.40b	1.20b	1.10
40 kg.ha^{-1} Late	67.75a	2.40a	1.70ab	1.00
p-value	*0.0019*	*0.0084*	*0.0068*	*0.0785*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

[w] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 6

External fruit quality (n=10) of ‘Nadorcott' mandarin cultivated at Riebeeck Kasteel, quantified as diameter and colour (scoring by chart and hue°), at harvest and after cold storage at -0.6 °C or 4 °C for 30 days, following a range of additional soil nitrogen (LAN - limestone ammonium nitrate) treatments as well as foliar urea (%) treatments, applied either early (21 January) or late (26 March) during 2014 and 2015.

	External quality						
Nitrogen treatment [N ($kg.ha^{-1}$) or Urea (%)]	Diameter (mm)	Colour Chart (1-8)[w]			Hue°		
	Harvest	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C
2014							
Control	73.02a[y]	1.20[ns]	1.00[ns]	1.00b	---[z]	---	---
20 $kg.ha^{-1}$ Early	73.45a	1.50	1.20	1.10ab	---	---	---
40 $kg.ha^{-1}$ Early	67.35b	1.90	1.10	1.00b	---	---	---
20 $kg.ha^{-1}$ Late	65.59b	1.80	1.70	1.50a	---	---	---
40 $kg.ha^{-1}$ Late	68.26ab	1.00	1.40	1.30ab	---	---	---
p-value	*0.0002*	*0.0567*	*0.1050*	*0.0090*	---	---	---
2015							
Control	66.64ab	1.40[ns]	1.00[ns]	1.00[ns]	55.20[ns]	55.94[ns]	55.53[ns]
20 $kg.ha^{-1}$ Early	67.80ab	1.50	1.00	1.00	55.35	55.82	55.81
40 $kg.ha^{-1}$ Early	65.34b	1.60	1.00	1.00	55.98	56.13	56.09
20 $kg.ha^{-1}$ Late	69.16a	1.40	1.00	1.00	54.99	55.63	55.43
40 $kg.ha^{-1}$ Late	65.39b	1.80	1.00	1.00	57.29	57.39	56.92
1% Urea Late	68.68ab	2.10	1.00	1.00	56.04	56.43	56.16
p-value	*0.0029*	*0.2980*	---[x]	---[x]	*0.1670*	*0.7338*	*0.6214*

[ns] No significant differences.
[z] Measurements not performed.
[y] Means with a different letter within a column differ significantly at the 5% level (LSD).
[x] P-value is non-significant.
[w] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 7

Internal fruit quality of 'Nules Clementine' mandarin from Citrusdal, quantified as juice content, Titratable acidity (TA), °Brix and °Brix:TA, both at harvest and following long term cold storage at -0.6 °C and 4 °C for 30 days, following additional soil (LAN - limestone ammonium nitrate) nitrogen (kg·ha^{-1}) treatments (n=10), applied either early in the season on 21 January or later on 26 March 2014 respectively.

Nitrogen treatment [N (kg.ha^{-1})]	Internal fruit quality									
	Juice content (%)	Titratable acidity (TA) (%)			°Brix			°Brix:TA		
	Harvest	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C
Control	58.82^{ns}	1.02^{ns}	1.04^{ns}	1.03^{ns}	9.43^{ns}	11.17^{ns}	11.24^{ns}	9.37^{ns}	10.83^{ns}	11.05^{ns}
20 kg.ha^{-1} Early	60.23	1.04	0.99	1.04	9.74	11.16	11.23	9.40	11.30	10.85
40 kg.ha^{-1} Early	59.65	1.04	1.04	1.05	9.55	11.36	11.28	9.29	11.08	10.77
20 kg.ha^{-1} Late	60.39	1.07	1.04	1.04	8.78	11.06	10.84	8.23	10.72	10.47
40 kg.ha^{-1} Late	59.38	1.02	1.01	1.05	9.32	10.38	10.73	9.19	10.30	10.20
p-value	*0.8776*	*0.6369*	*0.7392*	*0.8727*	*0.0506*	*0.0586*	*0.4814*	*0.0664*	*0.3291*	*0.4245*

^{ns} No significant differences.

Table 8

Internal fruit quality of 'Nules Clementine' mandarin from Riebeeck Kasteel, quantified as juice content, Titratable acidity (TA), °Brix and °Brix:TA, both at harvest and following long term cold storage at -0.6 °C and 4 °C for 30 days, following additional soil (LAN - limestone ammonium nitrate) nitrogen (kg·ha^{-1}) and foliar urea (%) treatments (n=10), applied either early in the season on 21 January or later on 26 March 2014 and 2015 respectively.

Nitrogen treatment [N (kg.ha^{-1}) or Urea (%)]	Internal quality									
	Juice content (%)	Titratable acidity (TA) (%)			°Brix			°Brix:TA		
	Harvest	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C
2014										
Control	57.44[ns]	0.95[ns]	0.83ab[y]	0.88[ns]	12.41[ns]	13.06ab	13.07[ns]	13.07[ns]	15.91[ns]	14.91[ns]
20 kg·ha^{-1} Early	56.75	1.00	0.87ab	0.91	12.18	12.98ab	13.31	12.18	14.99	14.64
40 kg·ha^{-1} Early	51.89	1.04	0.83ab	0.86	12.53	12.69b	12.71	12.22	15.48	14.89
20 kg·ha^{-1} Late	57.63	1.14	1.07a	1.08	13.33	14.32a	14.15	12.25	14.15	13.73
40 kg·ha^{-1} Late	55.94	0.95	0.82b	0.86	11.98	12.51b	12.85	12.87	15.48	15.07
p-value	*0.5057*	*0.2463*	*0.0202*	*0.0630*	*0.0921*	*0.0049*	*0.0720*	*0.5246*	*0.3857*	*0.3774*
2015										
Control	54.52b	0.96ab	0.92ab	0.90[ns]	12.34ab	13.53abc	13.86ab	12.98ab	14.90[ns]	15.48[ns]
20 kg·ha^{-1} Early	53.44b	1.05a	0.93ab	0.94	12.89a	14.20a	14.09a	12.30b	15.52	15.34
40 kg·ha^{-1} Early	59.38a	0.89b	0.90ab	0.89	12.75a	13.15bc	13.71ab	14.29a	14.67	15.6
20 kg·ha^{-1} Late	60.08a	0.99ab	1.00a	0.99	11.88a	13.83ab	13.96ab	12.13b	13.97	14.16
40 kg·ha^{-1} Late	51.99b	0.98ab	0.86b	0.87	12.32ab	12.88c	13.00b	12.67b	15.07	14.88
1% Urea Late	54.52b	1.01ab	0.97ab	0.98	12.79a	13.36abc	13.56ab	12.85b	13.94	13.94
p-value	*<.0001*	*0.0223*	*0.0338*	*0.0646*	*0.0038*	*0.0009*	*0.0192*	*0.0002*	*0.1257*	*0.1272*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 9

Internal fruit quality of ‘Nadorcott' mandarin from Citrusdal, quantified as juice content, Titratable acidity (TA), °Brix and °Brix:TA, both at harvest and following long term cold storage at -0.6 °C and 4 °C for 30 days, following additional soil (LAN - limestone ammonium nitrate) nitrogen (kg·ha^{-1}) treatments (n=10), applied either early in the season on 21 January or later on 26 March 2014 respectively.

Nitrogen treatment [N (kg.ha^{-1})]	Internal fruit quality									
	Juice content (%)	Titratable acidity (TA) (%)			°Brix			°Brix:TA		
	Harvest	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C
Control	56.82^{ns}	1.23^{ns}	0.99^{ns}	1.00^{ns}	11.29^{ns}	11.62^{ns}	11.54^{ns}	9.22^{ns}	11.92^{ns}	11.65^{ns}
20 kg·ha^{-1} Early	55.41	1.09	0.91	0.90	11.30	11.72	11.68	10.46	12.92	13.08
40 kg·ha^{-1} Early	56.17	1.13	0.91	0.95	10.83	11.11	11.48	9.68	12.29	12.11
20 kg·ha^{-1} Late	58.45	1.18	0.97	1.72	11.01	11.40	11.46	9.42	11.86	11.48
40 kg·ha^{-1} Late	58.10	1.15	0.93	0.87	10.80	11.22	11.40	9.57	12.25	13.36
p-value	*0.6573*	*0.1743*	*0.4270*	*0.4320*	*0.1550*	*0.2209*	*0.8647*	*0.0894*	*0.4123*	*0.1452*

^{ns} No significant differences.

Table 10

Internal fruit quality of ‘Nadorcott' mandarin from Riebeeck Kasteel, quantified as juice content, Titratable acidity (TA), °Brix and °Brix:TA, both at harvest and following long term cold storage at -0.6 °C and 4 °C for 30 days, following additional soil (LAN - limestone ammonium nitrate) nitrogen (kg·ha^{-1}) and foliar urea (%) treatments (n=10), applied either early in the season on 21 January or later on 26 March 2014 and 2015 respectively.

Nitrogen treatment [N (kg.ha^{-1}) or Urea (%)]	Internal fruit quality									
	Juice content (%)	Titratable acidity (TA) (%)			°Brix			°Brix:TA		
	Harvest	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C	Harvest	-0.6 °C	4 °C
2014										
Control	61.78[ns]	1.06[ns]	0.79[ns]	0.78[ns]	10.87[ns]	11.16ab[y]	11.30[ns]	10.31[ns]	14.40[ns]	14.59[ns]
20 kg·ha^{-1} Early	60.03	1.00	0.80	0.79	11.17	11.76a	11.46	11.20	14.84	14.64
40 kg·ha^{-1} Early	62.78	1.02	0.80	0.74	10.61	11.24ab	10.96	10.50	14.28	15.00
20 kg·ha^{-1} Late	61.41	1.01	0.71	0.74	10.64	10.74b	10.97	10.63	15.24	15.13
40 kg·ha^{-1} Late	61.68	1.07	0.78	0.79	10.75	11.20ab	11.39	10.09	14.58	14.68
p-value	*0.1783*	*0.5622*	*0.3162*	*0.5851*	*0.2379*	*0.0216*	*0.0613*	*0.1760*	*0.6425*	*0.9307*
2015										
Control	58.10[ns]	1.31[ns]	1.11ab	0.98ab	12.21a	12.51[ns]	12.75a	9.42[ns]	11.32[ns]	13.23ab
20 kg·ha^{-1} Early	57.07	1.34	1.21a	1.18a	12.04a	12.59	12.72a	9.06	10.57	10.93b
40 kg·ha^{-1} Early	57.51	1.25	1.09ab	1.09ab	12.08a	12.38	12.15ab	9.86	11.50	11.52ab
20 kg·ha^{-1} Late	58.55	1.23	1.10ab	0.96ab	11.87a	12.50	12.76a	9.79	11.46	13.48a
40 kg·ha^{-1} Late	54.08	1.29	1.10ab	1.07ab	11.81a	12.48	12.38ab	9.30	11.65	11.94ab
1% Urea Late	57.73	1.11	0.95b	0.90b	10.62b	11.79	11.72b	9.64	12.67	13.28ab
p-value	*0.0571*	*0.059*	*0.0352*	*0.0072*	*0.0012*	*0.3605*	*0.0313*	*0.6029*	*0.115*	*0.0071*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 11

The macronutrient content of 'Nules Clementine' mandarin expressed as % N, K, Ca, and Mg of leaf, pulp and rind respectively, as observed in fruit from Citrusdal, sampled (n=10) prior to any additional N application and at harvest, following the application of a range of soil (LAN - limestone ammonium nitrate) nitrogen treatments (kg·ha^{-1}), either on 21 January (early application) or 26 March (late application) 2014. The average values of the mineral nutrients the day prior to the first applications are supplied as a reference and not used in the statistical analysis.

Treatment	%N	%K	%Ca	%Mg
Leaf analysis				
Prior to treatment	2.57	1.59	2.02	0.36
Control	2.57^{ns}	1.38^{ns}	2.53^{ns}	0.48^{ns}
20 kg·ha^{-1} Early	2.62	1.62	2.53	0.49
40 kg·ha^{-1} Early	2.60	1.56	2.42	0.37
20 kg·ha^{-1} Late	2.51	1.77	2.73	0.43
40 kg·ha^{-1} Late	2.67	1.82	2.42	0.44
p-value	*0.6128*	*0.5016*	*0.8351*	*0.1307*
Pulp analysis				
Prior to treatment	2.61	2.00	0.52	0.20
Control	1.91^{ns}	1.68^{ns}	0.29^{ns}	0.13^{ns}
20 kg·ha^{-1} Early	1.82	1.61	0.26	0.12
40 kg·ha^{-1} Early	1.75	1.61	0.28	0.12
20 kg·ha^{-1} Late	2.05	1.91	0.26	0.13
40 kg·ha^{-1} Late	1.61	1.60	0.29	0.12
p-value	*0.0504*	*0.1054*	*0.9583*	*0.3001*
Rind analysis				
Prior to treatment	2.26	2.00	0.75	0.20
Control	3.40^{ns}	2.61^{ns}	1.23^{ns}	0.26^{ns}
20 kg·ha^{-1} Early	3.26	2.72	1.24	0.22
40 kg·ha^{-1} Early	3.18	2.55	1.23	0.23
20 kg·ha^{-1} Late	3.53	2.73	1.08	0.25
40 kg·ha^{-1} Late	3.16	2.72	1.27	0.28
p-value	*0.701*	*0.9156*	*0.7549*	*0.6518*

^{ns} No significant differences.

Table 12

The macronutrient content of 'Nules Clementine' mandarin expressed as % N, K, Ca, and Mg of leaf, pulp and rind respectively, as observed in fruit from Riebeeck Kasteel, sampled (n=10) prior to any additional N application and at harvest, following the application of a range of soil (LAN - limestone ammonium nitrate) and foliar urea (1%) nitrogen treatments (kg·ha^{-1}), either on 21 January (early application) or 26 March (late application) 2014 and 2015. The average values of the mineral nutrients the day prior to the first applications are supplied as a reference and not used in the statistical analysis.

Treatment	%N	%K	%Ca	%Mg	%N	%K	%Ca	%Mg
	2014				**2015**			
Leaf analysis								
Prior to treatment	2.32	1.10	3.36	0.54	2.17	1.12	2.98	0.43
Control	2.42[ns]	0.80b[y]	4.06[ns]	0.64a	2.37[ns]	1.32[ns]	3.06[ns]	0.47[ns]
20 kg·ha^{-1} Early	2.60	1.16ab	3.84	0.60ab	2.23	1.34	3.05	0.49
40 kg·ha^{-1} Early	2.58	1.37a	3.52	0.47c	2.19	1.55	3.01	0.44
20 kg·ha^{-1} Late	2.33	1.08ab	3.73	0.49bc	2.29	1.42	3.24	0.46
40 kg·ha^{-1} Late	2.66	1.54a	3.35	0.56abc	2.37	1.36	3.11	0.47
1% Urea Late	---[z]	---	---	---	2.35	1.39	2.87	0.44
p-value	*0.0988*	*0.0017*	*0.3148*	*0.0277*	*0.5039*	*0.8903*	*0.8802*	*0.4317*
Pulp analysis								
Prior to treatment	1.92	1.75	0.53	0.20	0.33	0.33	0.10	0.03
Control	1.25[ns]	1.47[ns]	0.21[ns]	0.11[ns]	1.40[ns]	1.81[ns]	0.22ab	0.11[ns]
20 kg·ha^{-1} Early	1.33	1.42	0.18	0.10	1.51	1.69	0.28a	0.11
40 kg·ha^{-1} Early	1.28	1.07	0.17	0.08	1.82	1.73	0.24ab	0.11
20 kg·ha^{-1} Late	1.61	1.61	0.19	0.11	1.66	1.70	0.17b	0.11
40 kg·ha^{-1} Late	1.13	1.36	0.20	0.10	1.88	1.84	0.18ab	0.10
1 % Urea Late	---	---	---	---	2.00	1.76	0.18ab	0.10
p-value	*0.3627*	*0.0849*	*0.8413*	*0.1166*	*0.0814*	*0.4151*	*0.0139*	*0.0477*
Rind analysis								
Prior to treatment	1.62	1.25	0.80	0.28	0.41	0.43	0.24	0.08
Control	1.86bc	2.29b	0.97[ns]	0.19[ns]	1.96b	2.77[ns]	1.17a	0.25ab
20 kg·ha^{-1} Early	2.03abc	2.16b	1.01	0.22	2.36ab	2.51	1.07ab	0.27a
40 kg·ha^{-1} Early	2.13ab	2.18b	1.08	0.25	2.15b	2.42	1.06ab	0.22ab
20 kg·ha^{-1} Late	2.29a	3.02a	0.86	0.18	2.77a	2.78	0.83ab	0.20ab
40 kg·ha^{-1} Late	1.72c	2.09b	0.98	0.20	2.23ab	2.44	0.80ab	0.17ab
1% Urea Late	---	---	---	---	1.98b	2.62	0.63b	0.15b
p-value	*0.0509*	*0.0505*	*0.2156*	*0.1602*	*0.0024*	*0.3514*	*0.0113*	*0.0279*

[ns] No significant differences.

[z] Measurements not performed.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 13

The macronutrient content of 'Nadorcott' mandarin expressed as % N, K, Ca, and Mg of leaf, pulp and rind respectively, as observed in fruit from Citrusdal, sampled (n=10) prior to any additional N application and at harvest, following the application of a range of soil (LAN - limestone ammonium nitrate) nitrogen treatments (kg·ha^{-1}), either on 21 January (early application) or 26 March (late application) 2014. The average values of the mineral nutrients the day prior to the first applications are supplied as a reference and not used in the statistical analysis.

Treatment	%N	%K	%Ca	%Mg
Leaf analysis				
Prior to treatment	3.17	1.10	2.72	0.57
Control	2.63^{ns}	0.81^{ns}	3.19^{ns}	0.55^{ns}
20 kg·ha^{-1} Early	2.91	0.87	3.37	0.54
40 kg·ha^{-1} Early	2.55	0.96	3.41	0.55
20 kg·ha^{-1} Late	3.07	1.15	3.10	0.46
40 kg·ha^{-1} Late	2.77	0.85	3.24	0.51
p-value	*0.1431*	*0.1848*	*0.8902*	*0.1311*
Pulp analysis				
Prior to treatment	2.81	2.00	0.85	0.20
Control	1.58^{ns}	1.50^{ns}	0.26^{ns}	0.12^{ns}
20 kg·ha^{-1} Early	1.67	1.61	0.28	0.13
40 kg·ha^{-1} Early	1.70	1.61	0.27	0.13
20 kg·ha^{-1} Late	1.83	1.64	0.23	0.12
40 kg·ha^{-1} Late	1.73	1.58	0.30	0.13
p-value	*0.2511*	*0.4662*	*0.8337*	*0.706*
Rind analysis				
Prior to treatment	2.31	2.50	1.70	0.45
Control	2.68^{ns}	2.53^{ns}	0.91^{ns}	0.21^{ns}
20 kg·ha^{-1} Early	2.37	2.08	0.95	0.25
40 kg·ha^{-1} Early	2.50	2.14	0.75	0.18
20 kg·ha^{-1} Late	2.54	2.41	0.76	0.20
40 kg·ha^{-1} Late	2.38	2.00	0.97	0.24
p-value	*0.5551*	*0.1122*	*0.4256*	*0.4745*

^{ns} No significant differences.

Table 14

The macronutrient content of ‘Nadorcott’ mandarin expressed as % N, K, Ca, and Mg of leaf, pulp and rind respectively, as observed in fruit from Riebeeck Kasteel, sampled (n=10) prior to any additional N application and at harvest, following the application of a range of soil (LAN - limestone ammonium nitrate) and foliar urea (1%) nitrogen treatments (kg·ha^{-1}), either on 21 January (early application) or 26 March (late application) 2014 and 2015. The average values of the mineral nutrients the day prior to the first applications are supplied as a reference and not used in the statistical analysis.

Treatment	%N	%K	%Ca	%Mg	%N	%K	%Ca	%Mg
	2014				**2015**			
Leaf analysis								
Prior to treatment	2.35	0.81	2.54	0.51	1.93	0.88	2.41	0.62
Control	2.59[ns]	0.80[ns]	3.07[ns]	0.54[ns]	2.70[ns]	0.79[ns]	3.58a[y]	0.62[ns]
20 kg·ha^{-1} Early	2.62	0.69	3.36	0.53	2.72	0.81	3.33a	0.64
40 kg·ha^{-1} Early	2.70	0.64	3.42	0.49	2.58	0.78	3.46a	0.60
20 kg·ha^{-1} Late	2.78	0.77	3.21	0.55	2.70	0.88	3.09ab	0.61
40 kg·ha^{-1} Late	2.41	0.52	3.89	0.61	2.78	0.98	2.59b	0.57
1% Urea Late	---[z]	---	---	---	2.64	0.75	3.34a	0.67
p-value	*0.2760*	*0.2050*	*0.4174*	*0.2218*	*0.3203*	*0.1280*	*0.0025*	*0.1205*
Pulp analysis								
Prior to treatment	2.16	2.00	0.75	0.33	0.24	0.28	0.08	0.03
Control	1.68[ns]	1.39[ns]	0.24[ns]	0.13[ns]	2.35a[z]	1.55[ns]	0.42ab	0.15[ns]
20 kg·ha^{-1} Early	1.52	1.45	0.24	0.12	1.73b	1.62	0.49a	0.15
40 kg·ha^{-1} Early	1.50	1.39	0.24	0.13	1.56b	1.59	0.39ab	0.15
20 kg·ha^{-1} Late	1.45	1.31	0.21	0.13	1.62b	1.55	0.37b	0.14
40 kg·ha^{-1} Late	1.40	1.13	0.19	0.12	1.45b	1.50	0.36b	0.14
1% Urea Late	---	---	---	---	1.51b	1.53	0.36b	0.14
p-value	*0.2634*	*0.4465*	*0.6866*	*0.8512*	*<0.0001*	*0.5860*	*0.0090*	*0.4869*
Rind analysis								
Prior to treatment	1.43	1.00	0.80	0.28	0.31	0.31	0.25	0.12
Control	1.70[ns]	1.81[ns]	0.85[ns]	0.19[ns]	1.77[ns]	1.65[ns]	1.39[ns]	0.35[ns]
20 kg·ha^{-1} Early	1.80	1.83	0.87	0.17	1.92	1.83	1.29	0.33
40 kg·ha^{-1} Early	1.78	1.73	0.83	0.17	2.02	1.67	1.13	0.34
20 kg·ha^{-1} Late	1.77	1.61	0.84	0.20	1.91	1.78	1.10	0.30
40 kg·ha^{-1} Late	1.78	1.73	0.83	0.17	1.87	1.72	0.94	0.26
1% Urea Late	---	---	---	---	1.95	1.60	1.13	0.29
p-value	*0.8539*	*0.6322*	*0.9763*	*0.7795*	*0.3635*	*0.6726*	*0.1387*	*0.2743*

[ns] No significant differences.
[z] Measurements not performed.
[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 15

Mineral content (% K, Ca and Mg) analysed within the rind epidermis, -flavedo, and -albedo of 'Nadorcott' mandarin, cultivated in Riebeeck Kasteel during the 2014 season. Treatments (n=10) included fruit from trees that received 40kg·ha^{-1} additional (LAN - limestone ammonium nitrate) soil application of nitrogen, either on 21 January (Early) or 26 March 2014 (Late). Fruit that was diagnosed with pitting and rind breakdown disorder (RBD) lesions, irrespective of treatments, were also included for analysis. % Nitrogen data was not shown due to almost undetectable low levels.

Treatment	%K			%Ca			%Mg		
	Rind sections analysed			Rind sections analysed			Rind sections analysed		
	Epidermis	Flavedo	Albedo	Epidermis	Flavedo	Albedo	Epidermis	Flavedo	Albedo
Control	0.23^{ns}	0.20^{ns}	0.18b^{y}	1.98^{ns}	0.46^{ns}	0.39b	0.03c	0.03c	0.03c
40 kg·ha^{-1} Early	2.31	0.73	0.42b	5.41	1.25	0.76ab	0.10c	0.06c	0.08bc
40 kg·ha^{-1} Late	1.35	2.14	1.28a	5.18	2.59	1.76a	0.28b	0.12bc	0.12b
Pitting fruit	1.15	0.42	0.42b	5.58	1.68	1.59a	0.26b	0.19ab	0.13b
RBD fruit	0.32	0.38	0.37b	3.98	1.69	1.69a	0.47a	0.25a	0.24a
p-value	*0.1060*	*0.0724*	*<.0001*	*0.4216*	*0.1708*	*0.0002*	*<.0001*	*<.0001*	*<.0001*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

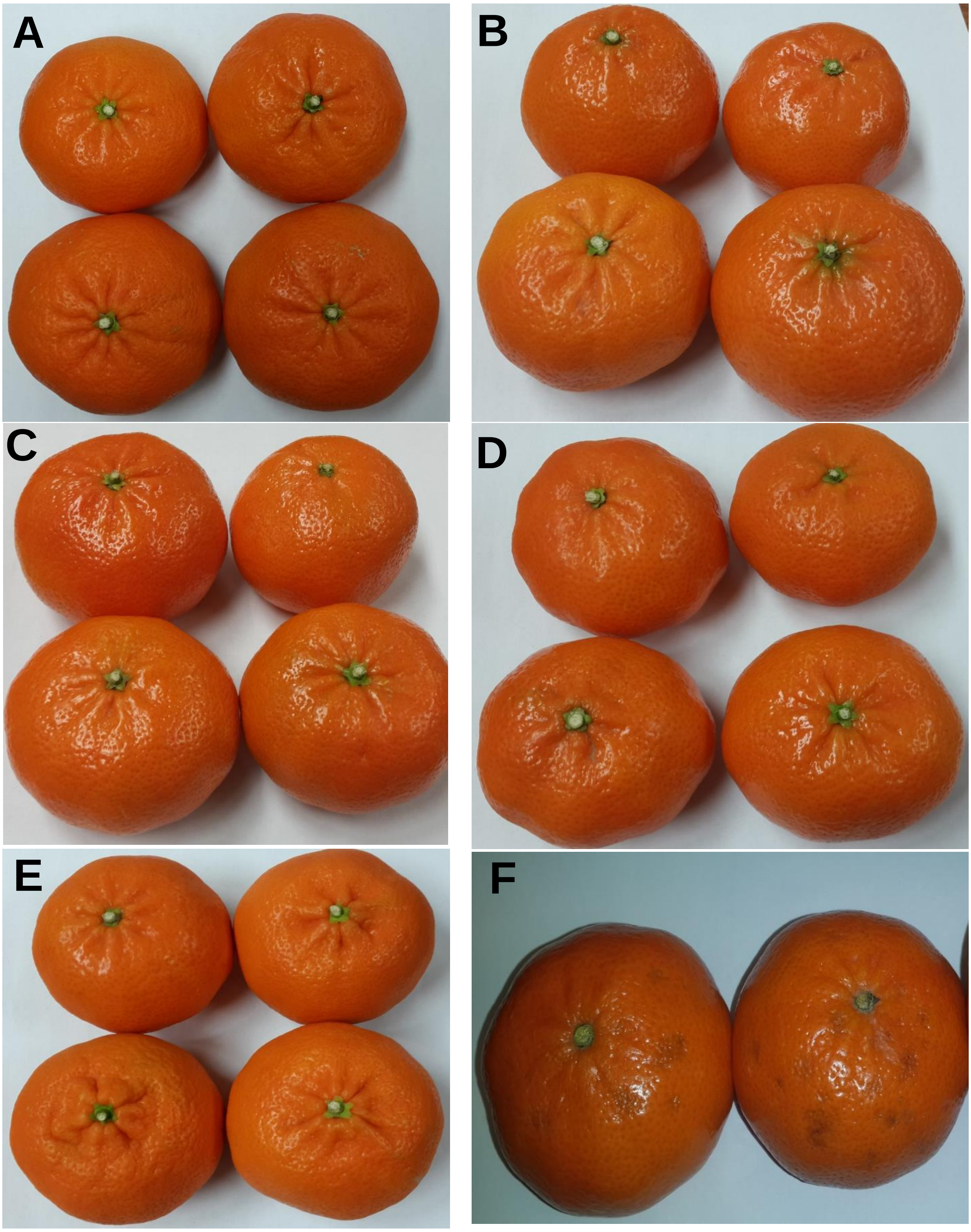

Fig. 1. Photographic evidence of rind colour and pitting lesions observed in 'Nadorcott 'mandarin fruit at harvest as produced in Riebeeck Kasteel in the 2014 season, following the application (Early- 21 Jan. 2014; Late- 26 March 2014) of various additional soil (LAN) N treatments prior to harvest. **A,** Control; **B,** 20 kg·ha^{-1} Early; **C,** 40 kg·ha^{-1} Early; **D,** 20kg·ha^{-1} Late; **E,** 40kg·ha^{-1} Late; **F,** Pitting lesions.

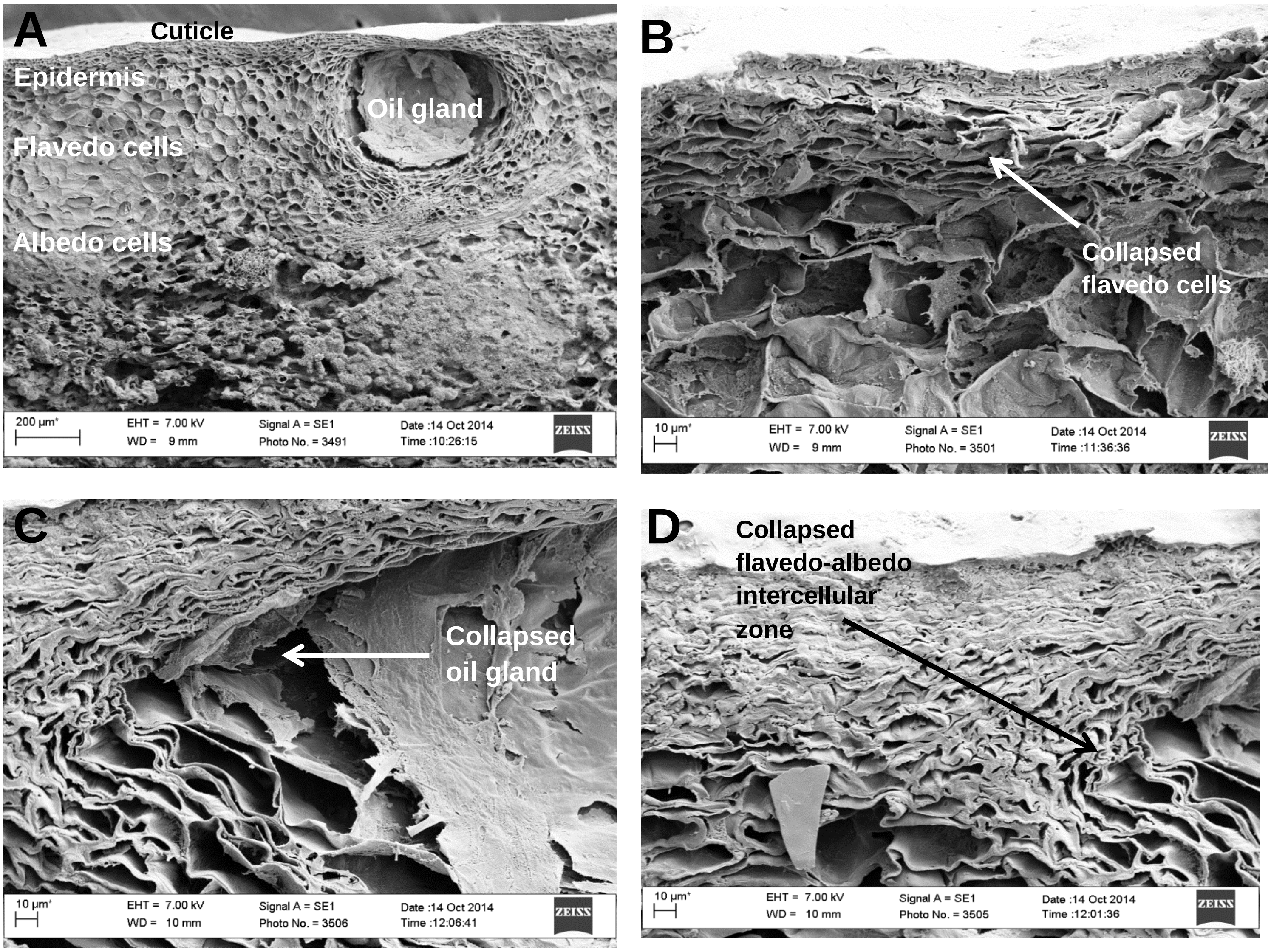

Fig. 2. Scanning electron microscopy (SEM) comparative cross sections of different cellular sections of ‘Nadorcott’ mandarin fruit rind after storage at -0.6 °C for 30 days, showing either no rind disorder lesions (A) or where it manifested with distinct rind disorder lesions (B-D). Fruit, produced in Riebeeck Kasteel in 2014, were subjected to the additional application (Early- 21 Jan. 2014; Late- 26 March 2014) of various soil N (LAN) treatments prior to harvest. **A.** No cellular damaged occurred within the rind sections of fruit which received a late N soil application at a rate of 40kg.ha^{-1}. The gradual transition from the densely packed flavedo cells to the loosely congregated albedo cells is visible; **B.** Rind breakdown lesions, with collapsed flavedo and albedo sections evident; **C.** A collapsed oil gland which developed into a pitting lesion after cold storage; **D.** A collapsed flavedo-albedo intercellular zone, in conjunction with a disintegrated oil gland.

Appendix 1

Minimum requirements of mandarins for export (Department of Agriculture and forestry [DAFF] 2015).

Variety	Juice content (%)	°Brix	Titratable acid (TA) (%)	°Brix:Acid
‘Nules Clementine'	48%	9.0+	0.8 - 1.5	8.0:1
‘Nadorcotts'	48%	9.0+	0.8 - 1.5	8.0:1

Appendix 2

Guidelines for interpretation of orange tree leaf N (%) analysis based on 4-to 6-month-old spring flush leaves from non-fruiting twigs (Adapted from Zekri and Obreza, 2013).

	Deficient	Low	Optimum	High	Excessive
% N	<2.2	2.2-2.4	2.5-2.7	2.8-3.0	>3.0

Appendix 3

Effects of N on citrus fruit quality (Adapted from Zekri and Obreza, 2013).

Variable	Rating
Juice Quality	
Juice content	+
Soluble Solids (SS)	+
Acid	+
SS/A Ratio	-
Solids/Box	+
Solids/Acre	+
External Fruit Quality	
Size	-
Weight	-
Green Fruit	+
Peel Thickness	+
* Increase (+), Decrease (-)	

PAPER 2

The effect of pre-harvest water stress on mandarin (*Citrus reticulata* blanco) fruit rind susceptibility to postharvest physiological disorders

ABSTRACT

Pre-harvest factors may predispose fruit to subsequent disorder development. Most research to date relating to water relations and irrigation in citriculture has focused on increasing yield and improving internal quality during the pre-harvest stage. This study provides a first account within a South African context, on the role of pre-harvest water stress on postharvest rind condition, especially as pertaining to disorder incidence. The aim of this study was to determine whether pre-harvest water stress could play a role in enhancing fruit susceptibility to postharvest physiological disorders. In addition, we aimed to determine if the postharvest handling of stressed fruit will increase pitting. 'Nules Clementine' and 'Nadorcott' mandarins harvested from Citrusdal and Riebeeck Kasteel, respectively, were studied during the 2014/2015 season. Results from this study concluded that pre-harvest water stress seemed to have little or no effect on fruit susceptibility to postharvest physiological disorders. It was, however, observed that an early postharvest wax application may significantly decrease moisture loss, coinciding with lower incidences of rind disorders. Underlying differences between cultivars was also elicited. An understanding of pre-harvest factors that may predispose fruit to rind disorders supports the modification of existing production protocols that may affect fruit development beneficially for improved storage quality. In addition, methods can be developed for accurate prediction of disorder risk development.

Keywords: 'Nadorcott'; 'Nules Clementine'; Relative humidity; Rootstocks; Soil moisture

1. Introduction

Citrus, which is considered internationally to be the most economically important fruit crop, is widely grown in both developed and developing countries (Iglesias et al., 2007). As citrus also constitutes one of the main sources of vitamin C, an increasing demand for "high quality fresh citrus" is driven by recommendations from the World Health Organization (Iglesias et al., 2007). In view of these distinct beneficial traits associated with citrus fruit, improvement of external fruit quality is a major research priority as the market value of citrus fruit, especially for fresh consumption, is highly dependent on appearance (Khalid et al., 2012). Fruits should therefore not only be free of physical blemishes, but also of any physiological rind disorders (Magwaza et al., 2013). These rind disorders effectively reduce external fruit quality and therefore decrease the commercial value of the fruit.

Very few disorders in fruit, for instance storage disorders due to carbon dioxide injury, manifest completely independently of pre-harvest factors (Ferguson et al., 1999). Pre-harvest factors that influence the storage potential and postharvest performance of citrus fruit include: rootstock and scion selection, tree condition, cultural practices like fertilisation, irrigation and crop load management, in addition to the weather prevailing during fruit growth as well as at harvest (Grierson and Ben-Yehoshua, 1986). Of these factors, water balance has been well researched for its positive impact on fruit growth, but has generally been neglected from a postharvest fruit quality perspective.

Water deficit has long been recognized as a strong signal for flowering in citrus, but beside this beneficial effect, most other physiological parameters are known to be negatively affected by water stress, with leaf injury and abscission, along with reduced fruit growth and poor fruit quality as typical consequences (Tudela and Primo-Millo, 1992). High temperatures together with dry winds may also produce similar effects to those promoted by water stress, even in the presence of sufficient soil moisture (Iglesias et al., 2007). Exposure to these types of conditions induces tree dehydration, and even though leaves and fruit do not fall during the period of water stress (Iglesias et al., 2007), this might happen rapidly after re-hydration (Addicott, 1982). The atypical composition of a citrus fruit, consisting of two physiologically separate entities, namely the rind and pulp, add to the complexity regarding the reaction of the fruit to water stress.

The citrus rind reacts daily to water demand from leaves and the environment (Kaufmann, 1970). During the day water flow is directly from the rind to the leaves, resulting in diameter loss, only to be restored during the night. In the case of fruit, the movement of water is more often than not, out of the fruit, with its high water content in relation to the much drier atmosphere as the driving force. The temperature of the fruit and ambient environmental conditions, as described by wet and dry bulb temperatures and % relative humidity (%RH), are the primary factors to influence the vapour pressure deficit (VPD) and the eventual water loss, as determined by weight loss. Rokach (1953) concluded that the water content of young ‘Navel’ orange fruit which was reduced by 25-30% during hot and dry weather, as was observed by Coit and Hodgson (1919), appeared to be solely due to leaf suction.

The incidence of ‘Fortune’ mandarin rind pitting is recorded to vary from year to year and may even vary among fruits within a given tree (Vercher et al., 1994). The causal factors and their mechanisms of action are, however, not known. Almela et al. (1992) suggested that the combination of prevalent strong and cold winds, together with low temperature and low relative humidity might be involved in the development of pitting. Changes in the physiological properties of the cuticle and membranes are thought to modify the water balance of rind areas which will develop pitting (Vercher et al., 1994). Excessive loss of water is associated with the “crushed” appearance typically observed in both epidermal and hypodermal cells of ‘Fortune’ mandarin fruits displaying severe symptoms of rind pitting (Vercher et al., 1994).

Research on non-chilling postharvest pitting and staining of grapefruit and ‘Navel’ orange provided evidence on the importance and involvement of rind water content (Alférez et al., 2003; 2005). In these studies the dehydration of the rind as would occur when subjected to low RH conditions, followed by a re-hydration at high RH conditions, can result in turgor-stress in the transition cellular zone between the flavedo and albedo. This stress is thought to cause cellular collapse, where oil glands can become compressed and rupture, leading to the characteristic brown/dark pitting or staining lesions.

Prevailing weather during fruit development can exert an interaction which will impact on the occurrence of some rind disorders. For instance, thinner-skinned fruit such as grapefruit and ‘Valencia’ oranges fruit from humid growing environments were found to be more prone to stem-end rind breakdown (SERB) than thicker-skinned fruit grown in arid environments (Grierson, 1965). Both pre-harvest and postharvest factors such as VPD as well as cold storage temperature- and duration may affect SERB development (Ritenour et al., 2004). In addition, the choice of rootstocks can significantly affect tree size and crop load, along with fruit size and quality (Castle et al., 1993), as well as have an impact on the postharvest performance of fruit from a specific growing location (Agustí, 1999; Ritenour et al., 2004).

The effect of the rootstock on tree water relationships and fruit peduncle development were shown to significantly affect the incidence and severity of rind breakdown of ‘Navelate’ orange budded on Carrizo citrange (Agustí et al., 2001), Cleopatra mandarin and sour orange (Romero et al., 2006). Similarly, a preliminary study which reported on postharvest physiological rind disorders (pitting and staining) in ‘Nadorcott’ mandarin (*Citrus reticulata* Blanco) established a link between particular rootstocks, as well as to specific postharvest handling practices which involved substantial variations in RH (Cronjé, 2013), to the development of rind disorders. Results indicated a significantly higher susceptibility in fruit from rough lemon rootstocks compared to fruit obtained from Carrizo citrange. The data concur with findings on other citrus rind disorders where significant water loss, due to high VPD, resulted in an inadequate adjustment of the water status of the rind, leading to cellular collapse and tissue damage (Cronje, 2013). Fruit harvested from trees grafted on rough lemon rootstocks are thought have a reduced ability to control water loss and are therefore more susceptible to rind disorder development (Cronje, 2013).

The aim of this study was to investigate a possible link between pre-harvest water stress and postharvest rind condition, especially disorder incidence, on two widely planted mandarin cultivars in South Africa, namely 'Nules Clementine' and 'Nadorcott'. These cultivars have distinct commercial harvest dates, but are both prone to the development of postharvest rind disorders. The first objective was to determine whether pre-harvest moisture stress, timed at approximately three weeks before harvest, would negatively affect rind quality. The second objective was to evaluate whether specific post-harvest treatments, documented to be causal for other citrus cultivars, could lead to increased loss of moisture under high vapour pressure defect (VPD) conditions and subsequently result in increased rind disorders.

2. Materials and methods

2.1. Experimental site and plant material

Two geographically different sites for each mandarin cultivar, 'Nules Clementine' and 'Nadorcott' were selected to include fruit from different climatic conditions (Table 1). For 'Nules Clementine' mandarin used the first season, the experiment was conducted in two commercial orchards budded on 'Carrizo citrange' {[*Poncirus trifoliate* (L.) Raf.] X [*Citrus sinensis* (L.) Osb.]} located in Citrusdal (Brakfontein) and Riebeeck Kasteel (Wynkeldershoek) in the Western Cape Province, South Africa. In Citrusdal (32°.25' S 18°99' E) trees were planted in 1991 at a spacing of 4.5 x 2.5 m. In Riebeeck Kasteel (33°.40' S 18°.84' E), approximately 110 km from Citrusdal, trees were planted in 1993 at a tree density of 4.5 x 2.0 m. Commercial harvest maturities (Appendix 1) differed between 7 to 14 days for the two respective experimental orchards. The second season, the experiment on 'Nules Clementine' was only conducted at the Citrusdal experimental site.

For 'Nadorcott' mandarin, during the first season, the experiment was conducted in two commercial orchards budded on 'Carrizo citrange' {[*P. trifoliate* (L.) Raf.] X [*C. sinensis* (L.) Osb.]} at the same sites as described for the 'Nules Clementine' mandarin above. The Citrusdal orchard was planted in 2008 at a spacing of 4.5 x 2.5 m, whilst the Riebeeck Kasteel orchard was planted in 2007 at a spacing of 5.0 x 2.4 m. The commercial harvest date for 'Nadorcott' differed between 7 to 14 days for the respective sites. For the second season, the experiment on 'Nadorcott' was repeated at both farms.

2.2. Pre-harvest treatment

The two cultivars from the two different experimental sites received the same pre- and postharvest treatments. Treatment consisted either of a water-stressed treatment, where trees received no irrigation or rain water, compared to the control which received the commercial irrigation schedule in addition to its natural exposure to above-ground rain water and run-off. Access to any above-ground rain water availability for water-stressed trees was eliminated by covering the soil at the base of a row of approximately 15 trees, whilst irrigation was disabled by placing the pipes on top of the plastic sheeting. Both interventions were made starting roughly three weeks prior to harvest. Trees which occupied the ten middle positions within the experimental covered row were labelled as water-stressed trees, whilst trees from an opposite row were used as the control. A complete randomised block design could not be followed due to the requirement for a continuous plastic sheet to deliver the required water-stressed treatment to experimental trees. As the severity of excluding water availability to bearing trees in a commercial orchard was unknown, along with the possible associated financial losses, the use of the exclusion plastic strip was limited to only 15 trees per orchard. However, the uniformity of the trees allowed for the ten innermost trees within the row to serve as appropriate replicates.

Soil samples (n=10) were obtained prior to laying plastic sheets from untreated, uncovered soil and again at harvest from both covered and open soil to determine effectiveness of depleting the soil water content. Wet soil weight was measured immediately after sampling and dry soil weight was measured after 47 h at 70 °C, from which the percentage soil water was determined (Barry et al., 2004). Soil from both experimental sites and different orchards, were classified as a clay-loam.

In order to determine the effectiveness of water removal on the tree water balance, stem water potential was measured at harvest with a pressure bomb (PMS 600 pressure chamber - PMS Instruments, Albany, Ore., USA). 60 mature, healthy leaves were bagged with black polyethylene envelopes covered with aluminium foil, three to two hours prior to measuring stem water potential (Ψ_{stem}). This allowed for the equilibration of the plant water status in experimental leaves with that of the whole-tree plant water status, thereby providing a realistic measurement of the tree water status at the time of measurement. Three measurements per replicate tree (n=10) of stressed and non-stressed (control) trees were obtained.

Only fruit from the pre-harvest water stressed trees were used for detailed measurements after the postharvest treatments. This was done to exclude a more complex factorial design and specifically to record the effect of pre-harvest water stress. A comparison between the rind disorder index of non-stressed and stressed fruit (Table 13) has however been included in the tables and can be the focus of future studies.

2.3. Postharvest treatment

From each experimental (water-stressed) tree unit a total of 45 fruit were harvested at the respective commercial harvest dates and transferred to the Department of Horticultural Science at the University of Stellenbosch, where the fruit was washed with chlorine (0.147 mg.mL^{-1}) and left to air dry. Ten fruit from each replicate were randomly allocated to three postharvest treatments, A, B, and C (Fig. 1). All treatments were applied for five days after harvest, whereafter the fruit was placed in storage at 4°C for 30 days. Treatment A which served as the control was kept at room temperature (20 °C ± 1 °C) for the entire five days following harvest, before being subjected to cold storage. The likelihood of the development of postharvest pitting was increased by subjecting selected treatments of fruit to a dehydration protocol, which was followed by a rehydration water stress as described in Alférez et al. (2003). Treatment B and C consisted of a postharvest stress whereby the fruit was dehydrated at a constant temperature of 25 °C and 60 to 80% RH to create conditions of high (0.7 to 1.1 kPa) vapour pressure deficit (VPD) for a two day period (Fig. 1). Dehydration was followed by rehydration within a 100 % RH environment for 24h, by placing fruit in sealed plastic bags, each containing a standard sheet of water-saturated paper towel. Micro-environments were monitored throughout the experimental procedure by means of temperature- and RH loggers (Tinytag View 2 TV-4500 Gemini Data Loggers (UK) Ltd. Temperature/Relative Humidity Logger [-25 to +50°C/0 to 100% RH]). On day 5 following harvest treatments A and B were waxed with a carnauba-shellac based wax formulation containing 18% solids,, as used commercially (875 High Shine, John Bean Technologies, Brackenfell, South Africa). For treatment C the fruit did not received any postharvest wax application on day 5.

2.3. Postharvest assessment

2.3.1. External and internal quality

Ten fruit per treatment and replicate (n=10), based on uniformity of size and colour and lack of visual rind injuries, were selected for cold storage, 4 °C for 30 days. The remaining 10 fruit per replicate were used for immediate external and internal quality analysis.

Visual rind colour was assessed using a No. 36 CRI colour chart for mandarins [Citrus Research International (CRI), 2004; Appendix A]. In addition 'Nules Clementine' mandarin rind colour was measured using a Minolta chroma meter (Model CR200; Minolta Camera Osaka, Japan) and the data were expressed as hue angle (H) where a higher hue angle value represents a greener colour whereas a lower hue value would represent a more orange rind colour (0° red to purple; 90º yellow and 150º green). Colour measurements were taken at two opposite sides of the fruit to include lighter and darker rind colour of fruit whereafter an average value was used. No colorimeter data were recorded for the 'Nadorcott' mandarin as no visual difference was detected, mainly due to the high rind colour development. The fruit diameter was measured using an electronic caliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan). To calculate moisture loss, fruit weight (g) of 10 fruit per replicate was determined at each step in the postharvest handling (Fig. 1) using an electric scale (Elec checking scale NBK- 30 (Model NWH 10422, UWE South Africa). Internal quality was determined by cutting fruit in half on the equatorial line, whereafter the flesh was juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth to remove any solid particles. Juice percentage was calculated by dividing the weight of the juice by the total weight of the fruit. °Brixof the juice were determined by using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan) and titratable acidity (TA) was determined by titrating 20 ml of juice against 0.1 N sodium hydroxide. Phenolphthalein was used as indicator and titration was complete when the liquid turned pink in colour. Acid was expressed as citric acid content. The °Brix:TA ratio was determined by dividing the °Brixvalues by the TA values. Cold stored fruit were evaluated for external quality following the cold storage period, where external and internal quality was assessed, after a seven day shelf life period.

2.3.2. Rind water potential

Rind water potential *(Ψ_p)* was measured destructively using a PSYPRO Water Potential System (PSYPRO-CR7, Wescor®, Inc., Logan, UT USA), equipped with eight Model C-52-SF sample chambers. The psychrometric output was calibrated as a function of the water potential in the chamber by using filter paper disks (7 x 1.25mm) saturated with a 0.55 M NaCl solution (-25 bars) for each sample chamber.

Two hours prior to measurements fruit were placed in a climatic controlled environment of 17-18 °C to ensure a uniform temperature between the rind and equipment. Discs, containing both the flavedo and albedo, were removed with a 10mm cork borer from the fruit rind and sealed in a 9.5 x 2.5 mm sample chamber. The rind disks were left sealed within the chamber to equilibrate for 20 min before the readings were recorded. Two readings per fruit were obtained at the various stages during the postharvest handling protocol (Fig. 1).

2.3.3. Determination of incidence of rind disorders

A postharvest rind disorder index (RDI) for fruit was calculated at 15 and 30 d of storage at 4°C as well as after 7d of shelf life (20 °C). The RDI represents the severity of the incidence as well as the percentage of rind disorder incidence. In order to calculate the RDI, a rating scale from 0 (no disorders noted) to 3 (severe disorder incidence), based on extent and intensity of the symptom, was used (Appendix 3). The rind disorder index (RDI) was then calculated according to the following formula:

$$\text{RDI} = \sum \frac{[\text{Rind disorder scale } (0-3) \text{ x number of fruit within each category}]}{\text{Total number of fruit in replicate } (\text{N}=10)}$$

2.4. Statistical analysis

The trial consisted of 10 single tree replications (n=10) assigned to each preharvest treatment. Data from each orchard were analysed separately using analysis of variance from the statistical software package, *SAS Enterpise Guide* (*SAS EG* v.5.1; SAS Institute, Cary, VC, USA). The pre-harvest stress was considered in combination with its particular postharvest treatment, at a single storage temperature of 4 °C. Cultivars or areas were not statistically compared. Means of treatments were separated by Fisher's least significant difference (LSD; p = 0.05). A p-value <0.05 is interpreted as a significant difference between treatments.

3. Results and discussion

3.1. Soil moisture

3.1.1. 'Nules Clementine' mandarin

The % moisture content recorded in soils from the 'Nules Clementine' mandarin orchard as obtained during the 2014 season in Citrusdal provides evidence of the efficacy of the plastic sheets to exclude soil water as only 2% moisture at harvest was measured, compared to 14% in uncovered soil from the control treatment (Table 2). However, this result could not be repeated for the 2015 season as almost no difference in soil moisture could be measured for this orchard between plastic covered soil and that from the control (Table 2). The inability of the plastic water exclusion sheets to induce soil moisture differences in 2015 may be linked to the occurrence of heavy rains which resulted in saturated soil conditions, immediately prior to the fitting of the water exclusion sheets. The soils then retained this high moisture content over the three-week period to harvest. During the 2014 season soil samples which were obtained from the 'Nules Clementine' mandarin orchard in Riebeeck

Kasteel provided similar evidence than that observed in soil from Citrusdal where the presence of plastic sheets reduced the % soil moisture by almost 60% (Table 2).

3.1.2. 'Nadorcott' mandarin

A major difference in % soil moisture from the 'Nadorcott' mandarin orchard in Citrusdal for both seasons was recorded between the covered and the open, control soils (Table 2). In 2014, the difference in % soil moisture as was observed in covered and open soils at harvest from the 'Nadorcott' mandarin orchard in Riebeeck Kasteel (3% vs 22%) were fairly consistent with that recorded for soils from the 'Nadorcott' mandarin orchard in Citrusdal (7% vs 24%). However, as was observed for the 'Nules Clementine' described above for the 2015 season in Citrusdal, the plastic sheets appeared fairly ineffective in loweringthe % moisture content of the covered soils of the 'Nadorcott' mandarin orchards, particularly that of the Riebeeck Kasteel area (Table 2). Again, a heavy downpour (Appendix 2) experienced during the period of sheet coverage may have contributed to the ineffectiveness of the sheets to induce a reduction in % soil moisture.

In addition to the effect of the rainfall, also with respect to harvest time of the respective cultivars and orchards, differences in soil types such as the lower water retention ability of sandy soils compared to that of soils with a higher clay content, will also play a role in % soil moisture content and the subsequent pre-harvest water stress of trees.

3.2. Stem water potential (Ψ_{stem})

3.2.1. 'Nules Clementine' mandarin

Significantly lower stem water potential values were recorded for stressed trees from Citrusdal, both the 2014 and 2015 seasons, although values obtained for the latter season were less marked than that measured in 2014 (Table 3). Trees monitored in 2014 from Riebeeck Kasteel, similar to that of Citrusdal, also showed a significant difference in stem water potential values as was expressed in the leaves of pre-harvest water stressed and non-stressed trees (Table 3). These differences appeared to be in general less distinct compared to that of 'Nules Clementines' recorded during the 2014 season in Citrusdal.

3.2.2. 'Nadorcott' mandarin

As for 'Nules Clementine' mandarin, a significant difference in stem water potential at harvest between treatments in both Citrusdal and Riebeeck Kasteel orchards, for both seasons, was observed (Table 3). Apparently, the presence of the exclusion sheets affected the tree water balance even though its efficacy was not always reflected in a difference in % soil moisture content between respective treatments.

3.3. External and internal fruit quality

3.3.1. External fruit quality of 'Nules Clementine' and 'Nadorcott' mandarin

During the 2014 season 'Nules Clementine' fruit harvested in Citrusdal were either waxed five days after a dehydration-rehydration stress treatment (treatment B) or received no wax, following the same stress treatment (treatment C) (Fig.1). After storage for 30 days at 4 °C, a significant improvement in rind colour, expressed as colour chart and hue°, was detected compared to values recorded at harvest, in particular that of non-waxed fruit (Table 4). Non-waxed fruit (treatment C) also showed a superior colour (colour chart and hue°) to that of waxed fruit (treatment B).

During the 2015 season, an additional postharvest treatment was imposed on 'Nules Clementine' fruit from Citrusdal, where fruit were kept at ambient temperature and waxed five days postharvest (control, treatment A) in order to better distinguish the effect of the dehydration-rehydration protocol on the pre-harvest stressed fruit. In this season, however, no significant differences between postharvest treated fruit following storage were observed (Table 4). Hue° values were therefore not measured. Fruit colour did, however, improve with storage compared to that recorded at harvest. Slightly bigger fruit were recorded during the 2015 season in Citrusdal than during the 2014 season (data not shown).

For 'Nules Clementine' harvested from Riebeeck Kasteel during 2014, no significant difference in colour was detected between treatments following storage at 4°, however fruit colour did improve compared to that observed at harvest (Table 5).

'Nadorcott' mandarin harvested in Citrusdal and Riebeeck Kasteel in 2014 showed no significant difference in rind colour among the different post-harvest treatments, following the 30-day cold storage period (Table 6). Due to the lack of distinguishable visual colour differences at harvest, no hue° measurements were recorded for either season.

3.3.2 Internal fruit quality of 'Nules Clementine' and 'Nadorcott' mandarin

The internal quality attributes of °Brix, total acidity and % juice were measured in both 2014 and 2015, after cold storage of pre-harvest stressed fruit. However, no significant differences between postharvest treatments were detected (data not shown). All internal quality indices achieved pre-requisite export standards [Appendix 1: (DAFF, 2015)].

3.4. Weight loss and rind disorder index

3.4.1. 'Nules Clementine' mandarin

During the 2014 season fruit harvested from Citrusdal showed no significant difference in % weight loss per day, total % weight loss or rind disorder index (RDI) between the waxed and non-waxed postharvest stress treatments throughout the postharvest chain (Table 7). In addition, the incidence of rind disorder was too low to be scored effectively.

During the 2015 season significant differences emerged among the different postharvest treatments within aspects of the postharvest chain (Table 7). From harvest to the end of dehydration stress (T1), postharvest stressed fruit (treatment B and C) had significantly higher % weight loss compared to the control fruit (treatment A), effectively illustrating the impact of postharvest dehydration. For the period from end of dehydration until end of rehydration (T2), the period immediately prior to waxing, a significant difference in % weight loss per day was again recorded for postharvest stressed fruit compared to the control (Table 7). For this period where treatments B and C received a rehydration opportunity, only a low % weight loss (moisture loss) resulted, whereas control fruit which remained at ambient temperature displayed a much higher % weight loss per day. In the period from day one to 15 days of cold storage (T3), again significant differences in % weight loss between treatments were recorded (Table 7). The lower % weight loss in the control and waxed, postharvest stressed fruit compared to unwaxed, postharvest stressed fruit provides evidence for the positive effect of wax prior to storage. For the latter half of the cold storage period (T4), however, no significant differences between the different treatments were detected (Table 7). This result suggests the ongoing positive effect attributed to an earlier wax application. In the period which included the termination of storage to the conclusion of shelf-life (T5) a significant difference between treatments was again recorded and provides clear evidence of the benefits of wax applications. Fruit which received the earliest wax application, without any postharvest stress showed similar moisture loss to fruit which were postharvest stressed, but waxed. However, the waxed fruit, irrespective of any postharvest stress intervention, had significantly lower % weight loss compared to fruit which received no wax application (Table 7).

For the total % weight loss over the entire trial period, from harvest until the end of shelf-life, a trend (significant at the 95 % confidence interval) emerged where non-waxed, stressed fruit (Treatment C) displayed the highest moisture loss, followed by waxed, stressed fruit, with the control, waxed fruit exhibiting the lowest % weight loss. No difference in rind disorder incidence was detected among treatments, either following cold storage or shelf life evaluation (Table 7).

Fruit harvested during the 2014 season in Riebeeck Kasteel produced comparable results to those observed in Citrusdal for 2015 regarding moisture loss (Table 8). As the postharvest protocol was similar for both treatments from harvest to end of the rehydration period (T1-2), no significant difference was observed. However, following waxing, from cold storage to day 7 of shelf life (T3-5), significant differences emerged where non-waxed fruit consistently showed a higher % moisture loss (Table 8). The positive effect of wax on preventing moisture loss, however, was not evident when % total weight loss was calculated. In addition, a low incidence of rind disorders was recorded, with no significant difference among treatments (Table 8).

3.4.2. 'Nadorcott' mandarin

Fruit harvested during the 2014 season in Citrusdal was significantly affected by treatment during the period from harvest to dehydration (T1-2; Table 9). As to be expected, control fruit which were at ambient temperature for 5 days prior to waxing, had significantly lower % moisture loss compared to the dehydrated fruit. As with the 'Nules Clementine' of Citrusdal in 2015, % moisture loss recorded during the period of rehydration, reflected the treatments, where control fruit not being exposed to rehydration and left at ambient temperature, recorded the highest % moisture loss (Table 9). Following waxing, in the period where cold storage commenced up to day 15 of cold storage (T3), the non-waxed, postharvest stressed fruit (treatment C) recorded significantly higher % moisture loss than the control, waxed fruit (treatment A), but had comparable moisture loss to waxed, stressed fruit (Table 9). During 16 to 30 days of cold storage (T4), the % weight loss between treatment B and C differed significantly, but neither differed significantly from the control. During the 7 day shelf life period (T5) the % weight loss with treatment C differed significantly compared to the control, but not so compared to treatment B. For total % weight loss, there were significant differences among all treatments. When considered at the 95 % confidence interval, the highest incidence of rind disorders was observed with postharvest stressed, non-waxed fruit immediately following cold storage (Table 9), although this trend was not as evident following the shelf life evaluation period. These results could possibly be ascribed to the higher % moisture loss that was evident when % total weight loss was calculated for the entire postharvest period.

Results on % moisture loss for the 2015 season were mostly insignificant, except for the period prior to waxing and during the first phase of cold storage where results were inconclusive, with no real pattern emerging (Table 9).

During 2014 in 'Nadorcott' mandarin from Riebeeck Kasteel, results proved mostly to be non-significant between treatments, except for the first phase up to the end of the dehydration period where stressed, non-waxed fruit (treatment C) showed the highest moisture loss (Table 10). Of interest is that for these fruit, a significantly higher incidence of rind pitting was also recorded both following cold storage and after the completion of shelf life evaluation (Table 10). Results from this season could not be repeated for 2015 as no significant trend, either in the first postharvest interval (T1), or in any the rind disorder indices, could be detected (Table 10).

3.5. Rind water potential (Ψ_p) of 'Nadorcott' mandarin

Water potential readings of the fruit rind were recorded at various time intervals to determine the effect of the pre-harvest and postharvest applied water stress on the water balance. No significant results were obtained in pre-harvest stressed fruit rind during any of the postharvest stages between any of the treatments from fruit harvested from both the Citrusdal and Riebeeck Kasteel area during the 2015 season (Table 11; Table 12).

3.6. Rind disorder index of 'Nules Clementine' and 'Nadorcott' mandarin

No clear pattern emerged when the rind disorder index of control fruit was compared with that of stressed fruit for the different cultivars within a particular production year in the respective areas (Table 13). However when the rind disorder indices for 'Nadorcott' from Citrusdal were compared between production years, a distinct difference was recorded, whereas the results for 'Nadorcott' mandarin rind from Riebeeck Kasteel stayed unchanged between 2014 and 2015. This phenomenon illustrates the erratic incidence of these disorders.

3.7. Fruit rind quality

The quality of citrus rind is determined by the development of the cellular structure during fruit growth. Quality is defined by the visual appearance of the fruit as well as their ability to withstand postharvest stress and a low incidence of rind disorders. Pre-harvest factors which may predispose fruit to subsequent disorder development are primarily linked to the position of the fruit on the tree, the characteristics of the fruiting branch and unit, crop load, the availability of mineral and carbohydrate nutrition to the developing fruit, water relations, and ambient environmental conditions (Cronje 2013; Cronje et al., 2011). An understanding of these factors permits modification of fruit development to optimize storage quality and a possible reduction of the risk of disorder incidence (Magwaza et al., 2014). Feedback from South African technical experts and producers and exporters along with preliminary studies indicated that an interrupted or low irrigation for crop, followed by a lengthy post-harvest chain (time between picking, waxing and cooling) often leads to an increased rate of rind disorders. This coincides with international research which also identified water loss from the rind as causal to many types of defects (Alférez et al., 2010; 2005).

In terms of general postharvest product quality, the pre-harvest impact is implicit in the use of maturity indices for optimizing harvest times (Ferguson et al., 1999). Susceptibility to rind pitting also appears to be related to the ripening stage of fruit at maturity, which in turn is dependent on the environmental conditions of low temperatures before cold storage. The interaction between temperature and duration of cold storage and the environmental conditions during ripening appear to be the main causal factors for rind pitting of 'Fortune' mandarin fruits, and have been suggested for other *Citrus* species and cultivars (Grierson, 1986). Low temperatures modify the pattern of wax secretions from the epidermal cells of 'Fortune' fruits, which apparently containing lower amounts of waxes, proved to be more susceptible to pitting (Vercher et al., 1994).

Citrus species may differ distinctly in pericarp (rind) anatomy (Cronje et al., 2011). For instance, mandarin fruit have a much thinner albedo compared with that of other citrus fruit. This might be an underlying reason for the different observed symptomatic development of various physiological rind disorders (Cronjé et al., 2011), although the relationship has not been established.

3.8. Pre- and postharvest water stress and rind disorder development

The three main factors known to affect water stress in citrus trees foliage are soil water availability, soil temperature and vapour pressure deficit (VPD) of the atmosphere. In a study by Elfving and Kaufmann (1972), where data were collected at night, it was reported that as soil water became increasingly limited, night-time leaf water potential decreased. The effects of VPD on leaf water potential and tree stem water potential indicated that a high VPD during the day resulted in more water loss from the leaves due to transpiration, which resulted in lower water potential and eventually stomatal closure. At night recovery of stem and leaf water potential is accomplished under conditions of sufficient soil moisture.

Haas (1927) established a relationship between fruit size and water loss where the transpiration rate was found to be greater in smaller fruits such as oranges as compared to grapefruit, which have a larger surface area per unit weight or volume. This association was, however, found not to be applicable to grapefruit in a study by McCornack (1975). Continued water loss from the fruit rind during storage is thought to result in SERB development (Ritenour et al., 2004). In this study cumulative SERB development after storage in 2002 was significantly lower than in 2001 on fruit harvested from the same trees. This finding thus points to possible pre-harvest factors such as changes in temperature or rainfall that might be different between the respective seasons, and which could have affected the susceptibility of fruit to SERB. Grierson (1965) cited work by Stearns (1942) where a decline in SERB of ‘Valencia’ oranges was reported if harvest was delayed during dry weather, until sufficient irrigation could be provided. SERB is thus considered to become more severe when trees are water stressed during the growing season.

In trials done on ‘Nadorcott’ mandarin by Cronjé (2013) it was observed that the postharvest dehydration prior to wax application induced significantly higher levels of rind disorders compared to fruit that were waxed within 24 hours after harvest. It is standard practice to coat citrus fruit with waxes, as the natural wax coating of citrus fruits is largely removed by washing during postharvest packing. It is therefore necessary to replace the lost natural wax with an artificial coating to reduce weight loss by up to 60%, inhibit shrinkage and provide fruit with a shiny gloss (Grierson and Ben-Yehoshua, 1986), thus generally improving the marketability of the fruit. Citrus fruit are therefore routinely waxed during postharvest handling to improve appearance and extend shelf-life by reducing water loss and shrinkage (Alférez et al., 2010).

3.9. Pre- and postharvest fruit water stress and internal fruit quality

The concentration and ratio of sugars and organic acids in the pulp determine the characteristic taste of citrus fruit. For fresh fruit consumption smaller fruit size and the slight reduction in sugar:acid ratio is undesirable (Hutton et al., 2007). During ripening, in general there is a decline in titratable acidity and an increase in sugars, usually expressed as total soluble solids (Iglesias et al., 2007). Fruit sugar content depends on imports of photosynthetic products and is therefore influenced by the rate of fruit photosynthesis and respiration (Huang et al., 1992).

It is well known that water stress due to limited soil water availability increases sugar content in fruit (Yakushiji et al., 1996; Kramer and Boyer, 1995). Water stress induced by extending irrigation intervals also altered fruit quality by increasing juice acidity and total soluble solids levels (Hutton et al., 2007), with a consequent decrease in sugar:acid ratios. This would be viewed as a negative result by both the fresh market and processing sectors of the citrus industry, despite the fact that higher total soluble solid levels were recorded due to extended irrigation intervals.

In studies conducted to enhance sugar accumulation in ‘Satsuma’ mandarin (*Citrus unshiu* Marc.), Yakushiji et al. (1996) recorded the total sugar content of water-stressed fruit to be significantly higher than that of fruit borne on well-watered trees. This sugar accumulation could not be ascribed to dehydration under water stress, but rather to active osmoregulation in response to water stress. Rootstocks have also been associated with differential fruit development and sugar accumulation of citrus fruit (Albrigo, 1977; Barry et al., 2004; Koshita and Takahara, 2004).

3.10. Rind anatomy and the development of rind disorders

Cronjé (2013) concluded that within the 'Nadorcott' fruit the effect of pre-harvest drought was not as noticeable as in ‘Navel’ oranges. Similarly, this study showed different responses for the same pre-and post-harvest stresses when ‘Nules Clementine’ and ‘Nadorcott’ mandarin were compared. Our data support the general industry debate and impression that not all citrus have the same sensitivity to rind disorders.

The thick albedo of ‘Navel’ oranges is thought to play a major role in the moisture balance in the rind, also as it drastically differs from that of the mandarin types. However, when waxing was delayed for seven days it had a negative impact on the rind of both citrus groups (Cronjé, 2013).

4. Conclusion

Our results highlight that all implications of tree and fruit water balance in fruit quality and specific disorders are not fully understood. It is also clear that this study should to be extended to more cultivars, as the responses between cultivars, within area and between areas (e.g. Lowveld), may vary significantly. Methodology for detailed studies of plant water balance now exist and make it possible to define more clearly the role of plant moisture in fruit growth and development and ultimately fruit quality.

The rind is thought to experience a predisposing action due to a stress during fruit development. Subsequently, the rind responds negatively to a postharvest stress, resulting in the physiological breakdown of the rind tissue and visible symptoms. It is thus clear that postharvest protocols based on this hypothesis should be designed to accelerate postharvest practices, from transport to packhouse, through to waxing and cold storage, especially for soft citrus. Also specific protocols may be required to reduce the impact of these rind disorders for not only the different cultivars but also for different areas.

Future research should aim to identify pre-harvest factors which could predispose fruit to postharvest disorders, in order to change orchard practices to reduce risks. An understanding of these factors would allow for the predicting of potential incidences, which would alert packhouses and exporters to implement appropriate, additional postharvest practices, as required. Prediction can be based either on significant relationships between pre-harvest factors and postharvest disorder incidence, or on premature expression of the disorder by artificial induction. The former has been the most successful. As a slight reduction in the disorder incidences and severity will have a significant commercial impact, continued research into this important area is warranted for the South African citrus industry.

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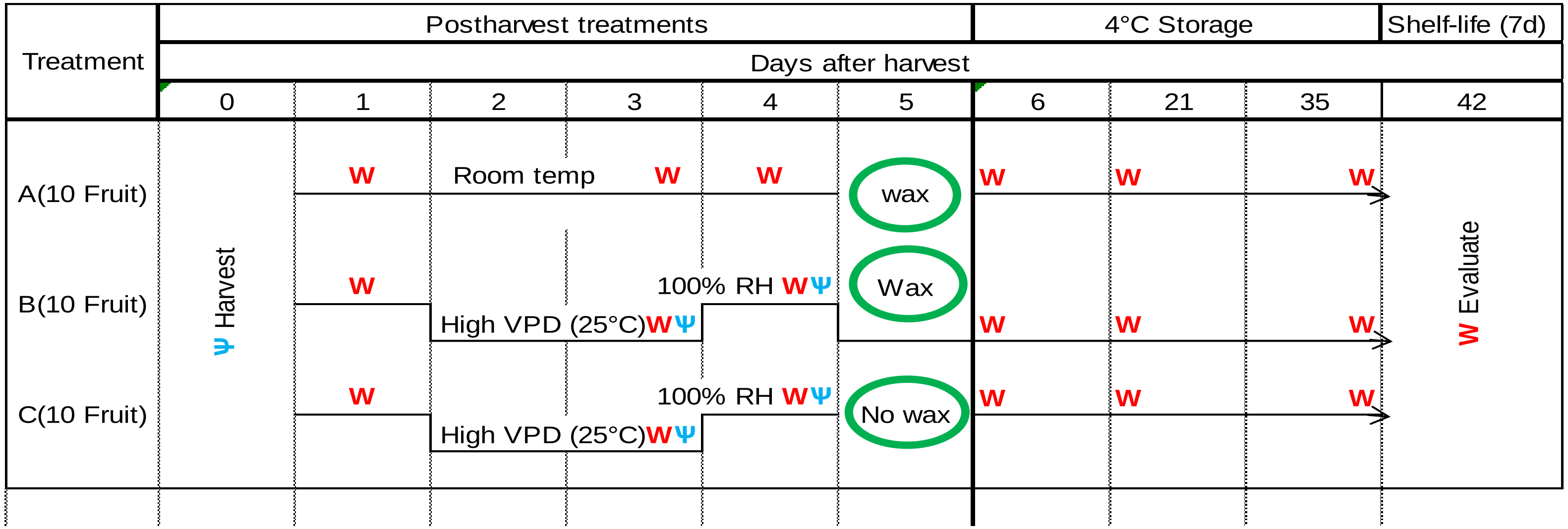

Fig. 1. A diagrammatic flow chart depicting the timeline and experimental conditions of the application of various postharvest treatments (n=10) to water-stressed fruit on 'Nules Clementine' and 'Nadorcott' mandarin as harvested from Citrusdal and Riebeeck Kasteel in 2014 and 2015. Fruit weight (**W**) and rind water potential (**ψ**) determination, as well as waxing and evaluation for rind disorder indices are indicated as was performed over a 42 day postharvest period, of which fruit were stored at 4 °C for 30 days. Treatment B and C consisted of a postharvest stress whereby the fruit was dehydrating at a constant temperature of 25 °C and 60-80% RH to create conditions of high (0.7 to 1.1 kPa) vapour pressure deficit (VPD) for a two day period. Dehydration was followed by rehydration within a 100 % RH environment for 24h. Treatment B was waxed on day 5. Treatment C received no wax application. Treatment A was kept at room temperature (20 °C ± 1 °C), and waxed on day 5.

Table 1

Demographic and cultivation information of ‘Nules Clementine’ and ‘Nadorcott’ mandarin on Carrizo citrange rootstock at two experimental sites (Citrusdal and Riebeeck Kasteel, South Africa) as was used in 2014 and 2015. A north-south row orientation and the use of drip irrigation was consistent throughout the trials.

Location	Farm and climatic data	Mandarin cultivar	Plant density (m)	Year planted	Yield (t.ha^{-1})
Citrusdal	Brakfontein (32°.25' S, 18°99'E)	'Nules Clementine'	4.50 x 2.50	1991	50
	Annual Rainfall: 334 mm Temp: max/min: 25.3 °C /10.9 °C	'Nadorcott'	4.50 x 2.50	2008	60
Riebeeck Kasteel	Wynkeldershoek (33°.40’ S 18°.84’ E)	'Nules Clementine'	4.50 x 2.00	1993	40
	Annual Rainfall: 403 mm Temp: max/min: 19.2 °C /12.0 °C	‘Nadorcott'	5.00 x 2.40	2007	60

Table 2

The efficacy of an exclusion plastic sheet and the elimination of irrigation to reduce % soil moisture for the purpose of inducing pre-harvest water stress, over the three-week period prior to harvest, in 'Nules Clementine' and ‘Nadorcott’ mandarin orchards, as based in Citrusdal and Riebeeck Kasteel respectively (2014/2015).

	% Soil moisture	
2014	Control, open soil	Covered soil
‘Nules Clementine’ – Citrusdal	14.00 ± 0.75[z]	2.00 ± 0.48
‘Nules Clementine’ – Riebeeck Kasteel	12.00 ± 0.49	7.00 ± 0.44
‘Nadorcott’ – Citrusdal	24.00 ± 0.77	7.00 ± 0.67
‘Nadorcott’ – Riebeeck Kasteel	22.00 ± 0.55	3.00 ± 0.35
2015		
‘Nules Clementine’ – Citrusdal	8.00 ± 0.40	8.00 ± 0.49
‘Nadorcott’ – Citrusdal	11.00 ± 0.86	5.00 ± 0.52
‘Nadorcott’ – Riebeeck Kasteel	8.00 ± 0.34	8.00 ± 0.65

[z] Values are the mean ± S.D. of measurements.

Table 3

Stem water potential (mPa) as recorded by means of a pressure bomb from pre-harvest water stressed trees (n=10) where soil was covered with plastic sheets three week prior to harvest compared to non-stressed, control trees of 'Nules Clementine' and 'Nadorcott mandarin, Citrusdal and Riebeeck Kasteel (2014 and 2015).

2014	Stem water potential (Ψ_{stem}- MPa)		*p-value*
	Control, open soil	Covered soil	
'Nules Clementine' – Citrusdal	-11.84b[y]	-17.15a	*<.0001*
'Nules Clementine' – Riebeeck Kasteel	-7.33b	-11.03a	*<.0001*
'Nadorcott' – Citrusdal	-9.25b	-11.33a	*0.0023*
'Nadorcott' – Riebeeck Kasteel	-9.83b	-12.02a	*0.0085*
2015			
'Nules Clementine' – Citrusdal	-8.50b	-13.95a	*<.0001*
'Nadorcott' – Citrusdal	-9.25b	-11.43a	*0.0007*
'Nadorcott' – Riebeeck Kasteel	-9.83b	-11.88a	*0.0183*

[y] Means with a different letter within a row differ significantly at the 5% level (LSD).

Table 4

The external fruit quality (n=10) of pre-harvest stressed fruit of 'Nules Clementine' mandarin, cultivated at Citrusdal during 2014/2015, following a range of additional postharvest stress treatments. The treatments entailed control fruit to be waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. Fruit quality was quantified as colour (scoring by chart and hue°) at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	External fruit quality			
	Colour Chart (1-8)[v]		Hue°	
	Harvest	4 °C	Harvest	4 °C
2014				
B	6.30 ± 0.46[z]	4.0a[y]	109.28 ± 7.39	91.30a
C	6.30 ± 0.46	2.3b	109.28 ± 7.39	85.12b
p-value	*1.0000*	*0.0021*	*1.00*	*<.0001*
2015				
A	3.83 ± 0.58	1.50[ns]	---[x]	---
B	3.83 ± 0.58	1.50	---	---
C	3.83 ± 0.58	1.50	---	---
p-value	*1.0000*	*1.0000*	---	---

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

[x] Measurement not performed.

[v] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 5

The external fruit quality (n=10) of pre-harvest stressed fruit of 'Nules Clementine' mandarin, cultivated at Riebeeck Kasteel during 2014, following a range of additional postharvest stress treatments. The treatments entailed fruit to be first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. Fruit quality was quantified as colour (scoring by chart and hue°) at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	External fruit quality			
	Colour Chart (1-8)[v]		Hue°	
	Harvest	4 °C	Harvest	4 °C
B	3.30 ± 1.00[z]	1.80[ns]	75.12 ± 7.08	67.16[ns]
C	3.30 ± 1.00	1.40	75.12 ± 7.08	67.52
p-value	*1.0000*	*0.1387*	*1.0000*	*0.3801*

[ns] No significant differences.
[z] Values are the mean ± S.D. of measurements.
[v] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 6

The external fruit quality (n=10) of pre-harvest stressed fruit of ‘Nadorcott' mandarin, cultivated at Citrusdal and Riebeeck Kasteel during 2014/2015, following a range of additional postharvest stress treatments. The treatments entailed control fruit to be waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. Fruit quality was quantified as colour (scoring by chart) at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	External fruit quality			
	Citrusdal Colour Chart (1-8)[v]		Riebeeck Kasteel Colour Chart (1-8)[v]	
	Harvest	4 °C	Harvest	4 °C
2014				
A	1.80 ± 0.74[z]	1.30[ns]	1.00 ± 0.00	1.00[ns]
B	1.80 ± 0.74	1.40	1.00 ± 0.00	1.00
C	1.80 ± 0.74	1.60	1.00 ± 0.00	1.00
p-value	*1.0000*	*0.5678*	*1.0000*	---[w]
2015				
A	1.10 ± 0.30	1.00[ns]	1.20 ± 0.40	1.00[ns]
B	1.10 ± 0.30	1.00	1.20 ± 0.40	1.00
C	1.10 ± 0.30	1.00	1.20 ± 0.40	1.00
p-value	*1.0000*	---[w]	*1.0000*	---[w]

[ns] No significant differences.
[z] Values are the mean ± S.D. of measurements.
[w] P-value is non-significant.
[v] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 7

Moisture loss, expressed either as % weight loss per day or % total weight loss, following postharvest stress treatments of pre-harvest stressed 'Nules Clementine' mandarin from Citrusdal, harvested in 2014/2015. The treatments entailed control fruit to be waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. Percentage (%) weight loss was determined at various time intervals (T1-5) within the postharvest chain, which included a 30 day cold storage period at 4 ˚C, followed by shelf life evaluation for 7 days at ambient temperature. Where relevant, wax was applied between T2 and T3. The incidence of rind disorders was expressed as an index value (RDI) based on a 0-3 rating scale.

Postharvest treatment	% Weight loss per day					%Total weight loss (harvest to day 7 of shelf life)	Rind disorder index (0-3)	
	Harvest to dehydrate (T1)	Dehydrate to rehydrate (T2)	Day 0-15 of cold storage (T3)	Day 16-30 of cold storage (T4)	Day 0-7 of shelf life (T5)		Post storage (4 °C)	Day 7 of shelf life (ambient temperature)
2014								
B	3.03[ns]	0.46[ns]	0.14[ns]	0.04[ns]	0.53[ns]	12.80[ns]	0.00[ns]	0.00[ns]
C	2.85	0.73	0.14	0.05	0.57	11.79	0.00	0.00
p-value	*0.2768*	*0.3348*	*0.8262*	*0.2104*	*0.7390*	*0.4350*	---[w]	---
2015								
A	1.28b[y]	1.22a	0.08ab	0.15[ns]	0.13b	6.74[ns]	0.16[ns]	0.17[ns]
B	3.61a	0.33b	0.07b	0.22	0.15b	9.05	0.08	0.09
C	4.11a	0.21b	0.10a	0.21	0.28a	10.44	0.24	0.30
p-value	*<.0001*	*<.0001*	*0.0155*	*0.8037*	*0.0019*	*0.0775*	*0.2928*	*0.1323*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

[w] P-value is non-significant.

Table 8

Moisture loss, expressed either as % weight loss per day or % total weight loss, following postharvest stress treatments of pre-harvest stressed 'Nules Clementine' mandarin from Riebeeck Kasteel, harvested in 2014. The treatments entailed fruit to be first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. Percentage (%) weight loss was determined was recorded at various time intervals (T1-5) within the postharvest chain, which included a 30 day cold storage period at 4 ˚C, followed by shelf life evaluation for 7 days at ambient temperature. Where relevant, wax was applied between T2 and T3. The incidence of rind disorders was expressed as an index value (RDI) based on a 0-3 rating scale.

Postharvest treatment	% Weight loss per day					%Total weight loss (harvest to day 7 of shelf life)	Rind disorder index (0-3)	
	Harvest to dehydrate (T1)	Dehydrate to rehydrate (T2)	Day 0-15 of cold storage (T3)	Day 16-30 of cold storage (T4)	Day 0-7 of shelf life (T5)		Post storage (4 °C)	Day 7 of shelf life (ambient temperature)
B	1.68^{ns}	0.29^{ns}	0.07b^{y}	0.07b	0.31b	8.18^{ns}	0.20^{ns}	0.25^{ns}
C	1.65	0.60	0.10a	0.13a	0.60a	8.99	0.05	0.10
p-value	*0.8834*	*0.0926*	*0.0001*	*0.0001*	*<.0001*	*0.1292*	*0.0792*	*0.0933*

^{ns} No significant differences.

^{y} Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 9

Moisture loss, expressed either as % weight loss per day or % total weight loss, following postharvest stress treatments of pre-harvest stressed ‘Nadorcott’ mandarin from Citrusdal, harvested in 2014/2015. The treatments entailed control fruit to be waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. Percentage (%) weight loss was determined was recorded at various time intervals (T1-5) within the postharvest chain, which included a 30 day cold storage period at 4 ˚C, followed by shelf life evaluation for 7 days at ambient temperature. Where relevant, wax was applied between T2 and T3. The incidence of rind disorders were expressed as an index value (RDI) based on a 0-3 rating scale.

	% Weight loss per day					%Total weight loss (harvest to day 7 of shelf life)	Rind disorder index (0-3)	
Postharvest treatment	Harvest to dehydrate (T1)	Dehydrate to rehydrate (T2)	Day 0-15 of cold storage (T3)	Day 16-30 of cold storage (T4)	Day 0-7 of shelf life (T5)		Post storage (4 °C)	Day 7 of shelf life (ambient temperature)
2014								
A	$0.66c^{y}$	0.56a	0.07b	0.08ab	0.33a	4.74c	0.03^{ns}	0.04^{ns}
B	1.74b	0.26b	0.09ab	0.06b	0.27ab	7.15b	0.00	0.12
C	2.22a	0.10b	0.12a	0.10a	0.21b	9.73a	0.07	0.21
p-value	*<.0001*	*0.0004*	*0.0029*	*0.0386*	*0.0155*	*<.0001*	*0.0832*	*0.1140*
2015								
A	1.77ab	0.81a	0.12^{ns}	0.11a	0.46^{ns}	8.87^{ns}	0.24^{ns}	0.27^{ns}
B	2.00a	0.09b	0.07	0.07b	0.48	7.41	0.18	0.19
C	1.52b	0.07b	0.09	0.09b	0.50	7.54	0.24	0.24
p-value	*0.0218*	*<.0001*	*0.5320*	*0.0002*	*0.9586*	*0.4390*	*0.8694*	*0.8377*

^{ns} No significant differences.

^{y} Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 10

Moisture loss, expressed either as % weight loss per day or % total weight loss, following postharvest stress treatments of pre-harvest stressed 'Nadorcott' mandarin from Riebeeck Kasteel, harvested in 2014/2015. The treatments entailed control fruit to be waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. Percentage (%) weight loss was determined was recorded at various time intervals (T1-5) within the postharvest chain, which included a 30 day cold storage period at 4 ˚C, followed by shelf life evaluation for 7 days at ambient temperature. Where relevant, wax was applied between T2 and T3. The incidence of rind disorders was expressed as an index value (RDI) based on a 0-3 rating scale.

Postharvest treatment	% Weight loss per day					%Total weight loss (harvest to day 7 of shelf life)	Rind disorder index (0-3)	
	Harvest to dehydrate (T1)	Dehydrate to rehydrate (T2)	Day 0-15 of cold storage (T3)	Day 16-30 of cold storage (T4)	Day 0-7 of shelf life (T5)		Post storage (4 °C)	Day 7 of shelf life (ambient temperature)
2014								
A	0.29b[y]	0.24[ns]	0.13[ns]	0.04[ns]	0.41[ns]	3.59[ns]	0.02b	0.07b
B	0.34b	0.04	0.10	0.06	0.29	3.32	0.02b	0.04b
C	0.46a	0.08	0.09	0.14	0.41	4.88	0.22a	0.22a
p-value	*0.0030*	*1.0290*	*0.3548*	*0.0848*	*0.1401*	*0.1187*	*0.0005*	*0.0058*
2015								
A	1.88[ns]	0.89a	0.08b	0.08b	0.37[ns]	8.20[ns]	0.01[ns]	0.04[ns]
B	1.90	0.12b	0.10a	0.09a	0.45	7.87	0.03	0.06
C	1.71	0.08b	0.08b	0.08ab	0.40	6.84	0.02	0.06
p-value	*0.4622*	*<.0001*	*0.0011*	*0.0199*	*0.6620*	*0.3725*	*0.7770*	*0.8570*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 11

Fruit rind water potential of pre-harvest stressed ‘Nadorcott’ mandarin harvested from Citrusdal in 2015 taken at various intervals during the postharvest chain. The treatments entailed control fruit to be waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. The rind water potential was measured by means of a Psypro water potential system.

Treatment	Rind water potential (Ψ_p)				
	After harvest (-MPa)	After dehydration (-MPa)	After rehydration (-MPa)	After 30 days cold storage at 4 ˚C (-MPa)	Day 7 of shelf life at ambient temperatures (-MPa)
A	-3.32 ± 0.27^{z}	-2.75^{ns}	-2.33^{ns}	-2.28^{ns}	-3.72^{ns}
B	-3.32 ± 0.27	-3.10	-2.25	-2.24	-2.83
C	-3.32 ± 0.27	-2.50	-2.80	-2.64	-2.68
p-value	*1.0000*	*0.6363*	*0.2018*	*0.6214*	*0.7123*

^{ns} No significant differences.

^{z} Values are the mean ± S.D. of measurements.

Table 12

Fruit rind water potential of pre-harvest stressed ‘Nadorcott’ mandarin harvested from Riebeeck Kasteel in 2015 taken at various intervals during the postharvest chain. The treatments entailed control fruit to be waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (C), five days postharvest. The rind water potential was measured by means of a Psypro water potential system.

Treatment	Rind water potential (Ψ_p)				
	After harvest (-MPa)	After dehydration (-MPa)	After rehydration (-MPa)	After 30 days cold storage at 4 °C (-MPa)	Day 7 of shelf life at ambient temperatures (-MPa)
A	-3.01 ± 0.23	-2.43^{ns}	-2.32^{ns}	-2.64^{ns}	-3.17^{ns}
B	-3.01 ± 0.23	-2.51	-2.13	-2.90	-3.96
C	-3.01 ± 0.23	-3.10	-2.35	-2.67	-2.95
p-value	*1.0000*	*0.7727*	*0.9349*	*0.7900*	*0.6982*

^{ns} No significant differences.

^{z} Values are the mean ± S.D. of measurements.

Table 13

Rind disorder index comparisons between control, non-water stressed fruit and pre-harvest water stressed fruit 'Nules Clementine' and ‘Nadorcott’ mandarin as harvested from Citrusdal and Riebeeck Kasteel in 2014 and 2015. Postharvest, all fruit was kept at ambient temperature for 5 days, followed by wax treatment on day 5 and cold storage for 30 days at 4 °C.

Cultivar and area	Rind disorder index (0-3)	
	Control (Non- stressed fruit)	Pre-harvest stressed fruit
2014		
‘Nadorcott’ – Citrusdal	0.00 ± 0.00[z]	0.03 ± 0.05
‘Nadorcott’ - Riebeeck Kasteel	0.06 ± 0.10	0.02 ± 0.04
2015		
‘Nules Clementine’ – Citrusdal	0.22 ± 0.20	0.16 ± 0.20
‘Nadorcott’ – Citrusdal	0.21 ± 0.40	0.24 ± 0.20
‘Nadorcott’ - Riebeeck Kasteel	0.05 ± 0.10	0.01 ± 0.00

[z] Values are the mean ± S.D. of measurements.

Appendix 1

Minimum requirementstesting results of mandarins for export (Department of Agriculture and forestry [DAFF], 2015).

Variety	Juice content (%)	°Brix	Titracible acid (TA) (%)	°Brix:Acid
‘Nules Clementine'	48%	9.0+	0.8 - 1.5	8.0:1
‘Nadorcotts'	48%	9.0+	0.8 - 1.5	8.0:1

Appendix 2

Weather summary for Citrusdal and Riebeeck Kasteel, April to July 2014 and 2015. Rainfall is expressed as mm for the monthly total, whereas temperature is given as °C mean daily maximum and minimum, respectively [Western Cape Department of Agriculture, http://www.elsenburg.com/sites/default/files/agri-outlook/ (2015)].

Locality	Rainfall (mm) monthly total			Temperature (°C) mean daily max.			Temperature (°C) mean daily min.		
	2015	2014	LT*	2015	2014	LT	2015	2014	LT
April									
Citrusdal	0.00	14.20	25.60	29.60	30.20	29.40	10.80	10.70	10.40
Riebeeck Kasteel**	3.60	16.80	39.70	25.90	27.40	25.80	13.50	14.60	13.40
May									
Citrusdal	0.60	32.00	46.80	25.30	23.90	24.10	7.80	8.20	7.60
Riebeeck Kasteel	20.00	39.20	80.00	21.70	20.60	20.70	11.20	10.70	11.20
June									
Citrusdal	85.50	36.80	50.70	20.40	18.70	20.20	5.70	4.10	5.00
Riebeeck Kasteel	58.00	81.60	92.00	17.30	17.10	17.70	8.60	8.30	9.30
July									
Citrusdal	6.80	37.80	48.70	18.50	19.30	20.60	4.40	4.30	3.90
Riebeeck Kasteel	51.80	60.20	62.70	15.80	16.80	17.30	7.20	7.70	8.00

*LT = long term average

** Data for Riebeeck Kasteel is a representation of data measured from the Malmesbury (Langgewens) weather station

Appendix 3: Photographic evidence of pitting lesions observed in ‘Nadorcott ‘mandarin fruit at harvest as produced in Riebeeck Kasteel in the 2014 season.

PAPER 3

The influence of postharvest dehydration on mandarin (*Citrus reticulata* Blanco) fruit quality

ABSTRACT

Citrus fruit are prone to develop various types of physiological rind disorders which manifest as a multitude of symptoms during handling and storage. Limited knowledge of the physiological mechanism(s) underlying these disorders is available, thus the challenge in reducing physiological rind disorders in citrus is significant. Sudden changes from low to high relative humidity (RH) have been shown to aggravate postharvest-induced rind pitting in 'Navelina' and 'Navelate' oranges in Valencia, Spain, and 'Marsh' grapefruit in Florida, USA. Altered water relations in the fruit rind were identified as a major inducer of rind pitting, regardless of the geographical origin. The objective of this study was to confirm this proposed mechanism underlies rind disorders under South African conditions. Results suggest that exposing fruit to dehydration may indeed promote rind disorder susceptibility. Dehydration prior to wax application also increased disorders. A wax treatment not more than 2-3 days postharvest is recommended to reduce incidence of pitting/staining/rind breakdown.

Keywords: Dehydrate; 'Nadorcott'; 'Nules'; Shade nets; Relative humidity; Rind disorders

1. Introduction

Citrus fruits rank first in the world with respect to production (Ladaniya, 2008). However, this widely consumed fruit is prone to developing, various types of physiological rind disorders, which manifest as a multitude of symptoms during handling and storage (Alquezar et al. 2010; Magwaza et al., 2014). Most disorders appearing on the surface of various citrus fruits are related to the rupture of oil glands, resulting in the phytotoxic injury to tissues and subsequent water loss (Ladaniya, 2008). The incidence of fruit affected by these disorders can reach as high as 60% of the total production (Agustí et al., 2001). Physiological rind disorders include a wide range of conditions namely: chilling injury, peteca spot, rind breakdown, non-chilling rind/peel pitting and rind staining, puffiness, creasing, oleocellosis, stem end rind breakdown (SERB) and stylar end breakdown (Cronjé, 2007). Physiological disorders for the purpose of this study are defined as conditions that affect rind quality of fruit from several citrus varieties including oranges, grapefruit and mandarins, at non-chilling temperatures without compromising internal quality, but negatively affecting fresh market value (Alquezar et al., 2010).

There is a great challenge in reducing the occurrence of physiological rind disorders in citrus, due to the lack of knowledge in physiological processes resulting in these disorders. Typically physiological rind disorders such as rind breakdown of 'Nules Clementine' mandarins (Cronjé et al., 2011), rind breakdown of 'Navel' orange (Agustí et al., 2001) and non-chilling postharvest rind pitting of 'Marsh' grapefruit (Alférez and Burns 2004) characteristically do not manifest during harvest or packhouse grading, but only after a few weeks into the cold chain. As the highest incidence of rind disorder development mostly coincides with the time period required for commercial shipping, substantial financial losses are experienced. Another factor which makes the control of rind disorders extremely challenging is the erratic incidence of these physiological rind disorders between fruit within a tree, within orchards, between cultivars and also between seasons.

It is suspected that non-chilling related physiological rind disorders as well as chilling injury can be aggravated by prevailing temperatures during fruit development and at harvest. Therefore the unpredictability of rind disorder development has led researchers to link these conditions to environmental conditions at harvest (Alquezar et al., 2010). Grierson (1969) noted that the loss of water vapour from citrus fruits is a passive process, with the main driving force provided by the vapour pressure gradient between the rind and the immediate air surrounding the fruit. Agustí et al. (2001) and Alférez et al. (2005) indicated that fruit exposed to moderate dehydration in the field are much more prone to develop rind injury after storage at high relative humidity RH, similar to fruit which was dehydrated postharvest.

Zaragoza and Alonso (1975) reported that an increase in the sensitivity to rind breakdown and staining during ripening was noted in 'Navelate' sweet orange that was cultivated under conditions where nutritional imbalances, climatic changes such as drought vs rainy periods, varying temperature regimes, or high vapour pressure deficit (VPD) occurred. Rind breakdown of this cultivar has also been observed during postharvest storage (Alférez and Zacarias, 2001). Sudden changes from low to high relative humidity (RH) were shown to induce postharvest rind pitting in 'Navelina' and 'Navelate' oranges in Valencia, Spain (Alférez et al., 2003) and 'Marsh' grapefruit in Florida, USA (Alférez and Burns, 2004). Even though the exact cause or mechanism(s) on a cellular level of this physiological disorder is still largely unknown, research clearly identified altered water relations in the fruit rind as a major trigger for rind pitting, regardless of the geographical origin of fruit (Alférez et al., 2010).

It however remains difficult to classify these physiological disorders to a large degree of certainty, as many rind blemishes and physiological disorders of citrus fruit exhibit similar visual symptoms and morphological appearance (Alquezar et al., 2010). Although accurate diagnosis and differentiation proove to be difficult, it is important in order to link a particular disorder to specific symptomology which, in turn, will identify possible causes and allow for the development and implementation of strategies to reduce the impact. Effective sources are available to facilitate identification, but currently still require further research and development (Cronjé, 2007).

Lately there has been significant growth in plantings of mandarin cultivars, despite a lack of information of the impact of postharvest practises on the occurrence of physiological disorders under Southern African conditions. The aim of this study was therefore to determine whether the postharvest water loss that results from a change in the vapour pressure deficit would induce pitting incidence in two susceptible mandarin cultivars, 'Nules Clementine' and 'Nadorcott', from four different sites and where orchards were open-cultivated, or consisted of net-covered trees.

2. Materials and methods

2.1. Experimental sites and plant material

2.1.1. Citrusdal and Riebeeck Kasteel

Two geographically distinct sites were selected for each mandarin cultivar, 'Nules Clementine' and 'Nadorcott', to include fruit from different climatic conditions (Table 1). For the 'Nules Clementine' mandarin studied in 2014, the experiment was conducted in two commercial orchards budded on 'Carrizo citrange' {[*Poncirus trifoliate* (L.) Raf.] X [*Citrus sinensis* (L.) Osb.]} located in Citrusdal (Brakfontein) and Riebeeck Kasteel (Wynkeldershoek) in the Western Cape Province, South Africa. In Citrusdal (32°.25' S 18°99' E) trees were planted in 1991 at a spacing of 4.5 x 2.5 m. In Riebeeck Kasteel (33°.40' S 18°.84' E), approximately 110 km from Citrusdal, trees were planted in 1993 at a tree density of 4.5 x 2.0 m. Commercial harvest maturities (Appendix 1) differed between 7 to 14 days for the two respective experimental orchards.

For 'Nadorcott' mandarin, during 2014, the experiment was conducted in two commercial orchards budded on 'Carrizo citrange' {[*P. trifoliate* (L.) Raf.] X [*C. sinensis* (L.) Osb.]} at the same sites as described for the 'Nules Clementine' mandarin above. The Citrusdal orchard was planted in 2008 at a spacing of 4.5 x 2.5 m, whilst the Riebeeck Kasteel orchard was planted in 2007 at a spacing of 5.0 x 2.4 m. The commercial harvest date for 'Nadorcott' differed between 7 to 14 days for the respective sites. During 2015 the experiment was repeated on both farms using the same mandarin cultivars.

2.1.2. Patensie and Bonnievale

Additional to the sites in Citrusdal and Riebeeck Kasteel, two additional sites with 'Nadorrcott' mandarin was included as part of a screening trial. In both these sites a conventional, open orchard was compared with a net-covered orchard (20% shading rate using white nylon nets).

The first additional site was based at Patensie (33° 43' S 24° 46' E), Eastern Cape Province, South Africa. This site included two commercial orchards of 'Nadorcott' mandarin budded on 'Carrizo' citrange {[*Poncirus trifoliate* (L.) Raf.] x [*Citrus sinensis* (Osb.) *L*.]}. This orchard represented conventional, open cultivated trees, planted in 2005, at a spacing of 5.0 x 2.0 m, whilst the second orchard consisted of net covered trees, planted the same year, with identical tree spacing.

The second additional site included two commercial orchards of 'Nadorcott' mandarin budded on 'Carrizo' citrange {[*Poncirus trifoliate* (L.) Raf.] x [*Citrus sinensis* (Osb.) *L*.]} in Bonnievale (34° 02' S 20° 05' E), Western Cape Province, South Africa. The first Bonnievale orchard was open cultivated, whilst the second orchard consisted of net-covered trees. Both orchards were planted in 2007 at a spacing of 5.5 x 2.0 m.

2.2. Postharvest treatments

50 fruit were harvested from ten adjacent trees in the specified commercial orchards (Citrusdal, Riebeeck Kasteel, Patensie and Bonnievale) and transferred to the Department of Horticultural Science at Stellenbosch University. On arrival fruit were washed with chlorine (0.147 mg.mL^{-1}) and left to air dry. From the 40 fruit from each tree (replicate) ten fruit were randomly allocated to each of the four treatments (Fig.1). Each treatment was therefore replicated ten times (n=10). In addition, 10 fruit for each replicate were used for destructive internal quality analysis the following day and did not receive any postharvest treatments.

The possible induction of postharvest pitting was followed as described in Alférez et al. (2003) whereby all fruit, harvested at commercial maturity, were subjected to a dehydration protocol which was followed by a rehydration water stress. Postharvest treatments stretched over a five-day period, whereafter all fruit were placed in storage at 4°C for 30 days. Environmental conditions for all treatments were monitored by means of temperature and RH loggers (Tinytag View 2 TV-4500 Gemini Data Loggers (UK) Ltd. Temperature/Relative Humidity Logger [-25 to +50°C/0 to 100% RH]) to confirm constant treatment conditions (data not shown).

Treatment A which served as the control was kept at room temperature (20 °C ± 1 °C) prior to cold storage. A postharvest stress was applied to the fruit of treatment B by dehydrating the fruit at constant conditions of 25 °C and 60-80% RH, to create an environment conducive to induce high vapour pressure deficit (0.7-1.1 kPa). After two days of the dehydration protocol, fruit were exposed for one day to a 100 % RH environment by placing fruit in plastic bags lined with water saturated paper. On day 5 treatment A and B were waxed using a natural (18% solids), carnauba-shellac based formulation (875 High Shine, John Bean Technologies, Brackenfell, South Africa).

Fruit that comprised treatment C was also kept at room temperature (20 °C ± 1 °C) until a similar wax treatment on day 3 as was described for treatment B, where after the fruit remained at room temperature until cold storage. Fruit allocated to treatment D received a similar postharvest stress to that of treatment B, with the difference being the fruit of treatment D received no wax application prior to cold storage.

2.3. Postharvest assessment

2.3.1. External and internal quality

Ten fruit per replicate uniform in size and colour and lacking visual rind injuries (n=10) were kept in cold storage at 4 °C. An additional ten fruit per replicate of each treatment were used for immediate external and internal quality analysis. Visual rind colour was assessed using a No. 36 CRI colour chart for mandarins [Citrus Research International (CRI), 2004; Appendix A]. In addition 'Nules Clementine' mandarin rind colour was measured using a Minolta chroma meter (Model CR200; Minolta Camera Osaka, Japan). Chroma meter data were expressed as hue angle (H) where a higher hue angle value represents a greener colour, whilst a lower hue value would represent a more orange rind colour (0 red to purple; 90^o yellow and 150^o green). Colour measurements were recorded at two opposite sides of the fruit to include lighter and darker rind colour of fruit whereafter an average value was used. Colorimeter data were not recorded for 'Nadorcott' mandarin as no visual differences were detected, mainly due to the high rind colour development. The fruit diameter was measured using an electronic caliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan). Fruit weight was determined prior to and after cold storage using an electric checking scale NBK-30 (Model NWH 10422, UWE South Africa) in order to calculate the total percentage (%) weight loss during storage.

Internal quality was determined by cutting fruit in half on the equatorial line, whereafter the flesh was juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth to remove any solid particles. Juice percentage was calculated by dividing the weight of the juice by the total weight of the fruit. °Brix of the juice was determined by using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan) and titratable acidity (TA) was determined by titrating 20 ml of juice against 0.1 N sodium hydroxide. Phenolphthalein was used as indicator and titration was complete when the liquid turned pink in colour. Acid was expressed as citric acid content. The TSS:TA ratio was determined by dividing the TSS values by the TA values. Cold stored fruit were evaluated for external quality after the cold storage period, whereafter external and internal quality was accessed, following a seven day shelf life period.

2.3.2. Rind water potential

Rind water potential (Ψ_p) was measured destructively using a PSYPRO Water Potential System (PSYPRO-CR7, Wescor®, Inc., Logan, UT USA), equipped with eight Model C-52-SF sample chambers. The psychrometric output was calibrated as a function of the water potential in the chamber, using filter paper disks (7 x 1.25mm) saturated with a 0.55 M NaCl solution (-25 bars) for each sample chamber.

Two hours prior to measurements fruit were placed in a climatic controlled environment of 17-18 °C to ensure a uniform temperature between the rind and equipment. Discs, containing both the flavedo and albedo, were removed with a 10mm cork borer from the fruit rind and sealed in a 9.5 x 2.5 mm sample chamber. The rind disks were left sealed within the chamber to equilibrate for 20 min before the readings were recorded. Two readings per fruit were obtained at the various stages during in postharvest handling protocol (Fig. 1).

2.3.3. Determination of incidence of rind disorders

A postharvest rind disorder index (RDI) for fruit was calculated at 15 and 30 d of storage at 4°C as well as after 7d of shelf life (20 °C). The RDI represented the severity of the incidence as well as the percentage of rind disorder incidence. In order to calculate the RDI, a rating scale from 0 (no disorders noted) to 3 (severe disorder incidence), based on extent and intensity of the symptom, was used (Appendix 2). The rind disorder index (RDI) was then calculated according to the following formula:

$$\text{RDI} = \sum \frac{[\text{Rind disorder scale } (0-3) \text{ x number of fruit within each category}]}{\text{Total number of fruit in replicate } (\text{N}=10)}$$

2.4. Statistical analysis

The trial consisted of 10 single tree replications (n=10) for each treatment. Cultivars or areas were not statistically analysed and only data of trials in the same orchard were compared. Due to uniformity of the trees, 10 trees were statistically treated as replicates. Statistical analyses of data quantifying the fruit physico-chemical properties were carried out using the statistical software package *SAS Enterpise Guide* (*SAS EG* v.5.1; SAS Institute, Cary, VC, USA). Ranked data were subjected to analysis of variance (One-way ANOVA). Means of treatments were separated by Fisher's least significant difference (LSD; $p = 0.05$). A *p*-value <0.05 was interpreted as a significant difference between treatments.

3. Results and discussion

3.1. Colour

3.1.1. 'Nules Clementine' mandarin

During the 2014 season, fruit from Citrusdal recorded a significant difference among the various post-harvest treatments for colour as measured with the colour chart and hue° values after 30 days storage at 4 °C (Table 2). The colour chart identified the postharvest stressed, but non-waxed fruit (treatment D) to present the best colour, whereas the control fruit (treatment A) was recorded to have the best coloured fruit according to hue° values. However, in the following season (2015), no significant or visible differences were evident between treatments in rind colour of fruit from Citrusdal, following 30 days of cold storage at 4 °C (Table 2). Colorimetry was thus not performed on these fruit.

For fruit from Riebeeck Kasteel no significant differences were recorded between treatments after storage of 30 days during the 2014 season when using the colour chart (Table 3). However the hue° value indicated control fruit (treatment A) to have a significantly better colour, whereas postharvest stressed, but waxed fruit (treatment B) had significantly lower colour development than that of the control or postharvest stressed, but non-waxed fruit (Treatment D) (Table 3). During the 2015 season the colour of fruit improved for the various treatments from that recorded at harvest to that noted on cold-stored fruit. Postharvest stressed, but non-waxed fruit (Treatment D) resulted in significantly better coloured fruit in relation to the other treatments following cold storage (Table 3).

3.1.2. 'Nadorcott' mandarin

Rind colour did not differ significantly for 'Nadorcott' in either of the seasons or areas, except for fruit harvested in 2014 from Citrusdal (Table 4; Table 5). The latter case was however considered not to be of biological value or commercial concern due to the inconsistent pattern observed between treatments. From our colour results on the two mandarin cultivars, it is evident that in comparison to ‘Nules’, ‘Nadorcott’ mandarin, predominantly develop full rind colour pre-harvest, with no or little further developing during postharvest storage. The impact of storage temperature and duration on rind colour of ‘Nadorcott’ would thus be of less importance than would be expected for ‘Nules’ mandarin.

3.2. Internal quality of ‘Nules Clementine’ and ‘Nadorcott’ mandarin

The internal quality of ‘Nules Clementine’ fruit harvested from Citrusdal or Riebeeck Kasteel did not show any significant difference for the various postharvest treatments, for either of the two seasons (Table 6; Table 7). Similarly, no clear significant results were found for the internal quality of ‘Nadorcott’ fruit exposed to the various postharvest treatments, irrespective of season or production area (Table 8; Table 9).

3.3. Weight loss and rind disorders

3.3.1. ‘Nules Clementine’ mandarin

Fruit harvested in 2014 from Citrusdal showed significant differences in total weight loss between the treatments (Table 10) as postharvest stressed fruit, irrespective of a wax treatment or not, resulted in the highest % moisture loss over the evaluation period. The same observation was also applicable during the period from harvest until the end of dehydration (T1), but when treatments were again compared at the end of the seven-day shelf-life period (T5), only the stressed, non-waxed fruit (treatment D) recorded significantly higher % weight loss (Table 10). A possible trend where early waxed, control fruit (treatment C) had the lowest overall % weight loss is noted, although within the current analysis this value did not differ significantly from later waxed, control fruit (treatment A) (Table 10). Irrespective of the differences observed for moisture loss between the various treatments no significant difference, either directly after cold storage or following shelf life evaluation, emerged regarding rind disorder indices among the treatments (Table 10). It should, however, be noted that a progressive nature in rind disorder development was evident from the initial evaluation to that done at the termination of shelf-life.

Similar trends for % weight loss as was observed in 2014 was reported for fruit harvested during 2015, with significant differences only reported after the first interval (T1) and then again after the first 15 days of cold storage (T3) (Table 10). In the first incidence stressed, but non-waxed fruit (treatment D) was significantly affected compared to the other treatments (Table 10). For the 2015 season, no significant differences were recorded in total % weight loss or any of the rind disorder indices (Table 10). As with fruit harvested in 2014, a distinct aggravation in the non-chilling physiological rind disorder incidence can be seen with an increase during post storage shelf life.

In the data from fruit harvested in Riebeeck Kasteel during 2014 (Table 11), similarities emerged to trends observed in the Citrusdal data (Table 10), with differences in climatic and soil demographics. During the 2014 season at the end of the dehydration (T1) a significantly higher % moisture loss was observed with postharvest stressed fruit (treatments B and D) compared to the non-stressed fruit (A and C). This trend continued unexpectedly into the next interval, to the end of rehydration phase (T2). The positive effect of the wax application was not as evident from cold storage through to the end of the first seven days of shelf life (T3-T5) as was observed in fruit harvested from Citrusdal. Still, postharvest stressed fruit recorded the highest % total moisture loss over the entire monitoring period. Again, irrespective of the differences observed for moisture loss between the various treatments at points throughout the postharvest chain, no significant difference, either directly after cold storage or following shelf life evaluation, was reported in any of the rind disorders indices (Table 11).

During the 2015 season for fruit harvested from Riebeeck Kasteel, the results were fairly consistent with that obtained for the 2014 and 2015 seasons on fruit from Citrusdal (Table 10; Table 11). The results confirm the positive impact of earlier application of wax as was also reported above. The incidence of rind disorders were particularly low for fruit harvested in Riebeeck Kasteel during the 2015 season, and therefore no significant differences between treatments were detected, even though the postharvest stressed and non-waxed fruit (treatment D) were the only fruit which developed rind disorders following the cold storage period (Table 11).

3.3.2. 'Nadorcott' mandarin

Fruit harvested in Citrusdal during 2014 had significantly different in % weight loss between postharvest treatments as recorded from harvest to the end of the dehydration period (T1) (Table 12). Both waxed (treatment B) and non-waxed (treatment D), stressed fruit experienced significantly higher % moistureloss than the unstressed fruit (Treatment A and C) (Table 12). Stressed, but non-waxed fruit also experienced significantly higher moisture loss during the final 15 days of cold storage (T4). The incidence of rind disorders following the 7 day shelf life period were significantly higher in fruit which were exposed to dehydration/rehydration (treatments B and D) as compared to fruit which were only left at ambient and waxed (treatments A and C) (Table 12). Even though the rind order index did not reveal any significant differences between treatments immediately after cold storage, a progressive development in the severity and incidence of rind disorders, in particular for that of the stressed fruit (treatment B and D), led to a significantly higher incidence after shelf life. Waxing of stressed fruit (B) did not prevent development of rind disorders and did not alleviate the development of rind disorders (treatment D), also stressed but not waxed, had similar levels of disorders. Thus waxing did not reduce the rind disorders.

For the next season (2015) ‘Nadorcott’ fruit from Citrusdal showed a fairly inconsistent pattern in % moisture loss between treatments and also in comparison to data recorded for 2014 (Table 12). For the total % moisture loss stressed, non-waxed fruit (treatment D), had comparable % moisture loss to that of control fruit (treatment A) or stressed, waxed fruit (treatment B). Early waxed, non-stressed fruit (treatment C) displayed significantly lower % moisture loss than that of late waxed fruit (treatment A) or stressed, non-waxed fruit (treatment D) (Table 12). However, despite significant difference in % total moisture loss, rind disorder indices, both after storage and an extended period of shelf life were not significantly different in the 2015 season (Table 12).

The ‘Nadorcott’ fruit harvested in Riebeeck Kasteel during the 2014 season did not show any significant difference during the period from harvest to dehydration (T1) (Table 13). The significant effect of a wax application can be seen during time interval 4, at the end of the cold storage period as stressed fruit where wax has been omitted resulted in the highest % moisture loss (treatment D). This particular treatment also recorded significantly higher values in the rind disorder indices, both after cold storage and following the extended shelf life period (Table 13).

The 2015 fruit harvested from Riebeeck Kasteel had significant differences in % weight loss at all the intervals from exposure to dehydration, then with wax application and until the end of cold storage, but not during the seven day shelf life evaluation or in % total weight loss (Table 13). Regarding the rind disorder incidence, a significantly lower value was recorded in unstressed, early waxed fruit (treatment C) compared to stressed, waxed fruit (treatment B) no they do not differ significantly, or unstressed, later waxed fruit (treatment A), for both evaluation times (Table 13). These results indicate the potential negative effect of the dehydration/rehydration cycle on fruit quality, irrespective of the total moisture loss experienced throughout the trial period. It also highlighted the possible benefits of an early wax application during a normal postharvest cycle.

Fruit harvested from both open and shade net-covered orchards in Patensie in 2015 revealed that stressed, non-waxed fruit (treatment D) consistently had the highest % total moisture loss over the trial period (Table 14). This increase in moisture loss observed in treatment D however did not extend its impact to create any significant rind disorder incidence (Table 14). Fruit harvested from the shade-net covered orchards however did differ from fruit harvested from conventional, open orchards as the fruit from netted orchards which received the postharvest stress, but no wax application (treatment D) had comparable % moisture loss to the unstressed control fruit (treatment A), which received a later wax application. Again, despite significant differences in % total moisture loss between treatments, almost no rind disorder incidence was recorded at either of the evaluation opportunities (Table 14). These results outline the erratic nature of physiological rind disorders, as the production area of Patensie is well-known as an area with orchards heavily susceptible to rind breakdown and pitting.

For fruit harvested from the open-cultivated orchard in Bonnievale during 2015, no significant difference in % total weight loss was documented between the different postharvest treatments, with also no occurrence of rind disorders recorded (Table 15). However, for fruit harvested from the shade-net covered orchard, the postharvest stressed fruit which received no wax application (treatment D) had significantly higher % moisture loss than any of the other postharvest treated fruit (Table 15). Some incidence of rind disorders was recorded, but no significant difference was detected among the different postharvest treatments (Table 15). A study done by Chang et al. (2015) on the effects of preharvest shading and postharvest storage temperatures on the quality of ‘Ponkan’ mandarin, confirmed that the weight loss of fruit is mainly affected by storage temperatures, regardless of whether pre-harvest shading was used or not.

3.4. Rind water potential (Ψ_p)

For ‘Nadorcott’ fruit harvested from both Citrusdal and Riebeeck Kasteel during 2015, the rind water potential did not differ significantly between treatments (Table 16; Table 17). The only exception that was recorded was for fruit from Citrusdal following dehydration and directly after the cold storage period, from which inconsistent results were obtained (Table 16; Table 17).

3.5. Postharvest fruit water stress and rind disorder development

Grierson and Ben-Yehoshua (1986) reported that anatomically, the fruits of most citrus cultivars are well suited to an extended postharvest life (30–90d), provided the rind remains undamaged and fruit are picked at their optimum maturity. Postharvest handling impacts on moisture content. For instance, Chalutz et al. (1973) reported that rapid cooling of fruit before packing, from field temperature to 10 °C, decreased weight loss by about 50%, without causing injury to the fruit.

During the postharvest period – harvest, packing and cold storage- transpiration (moisture loss) and respiration are processes which contribute to the physiological deterioration of citrus fruits after harvest. Moisture loss results in a rapid deterioration in the appearance of the fruit. It is thus not surprising that previous studies have focused their attention on controlling transpiration and less so on respiration which is controlled by temperature management. As a result waxes, along with the implementation of high humidity during cold storage have successfully been introduced to reduce moisture loss from fresh citrus fruits during transit and storage. Additional benefits also derived from cold storage such as reduced respiratory activity of the fruit as well as reduced invasion and growth of fungal pathogens (Purvis, 1983). Citrus leaves and fruit cuticles are well-known to carry a thin film of epicuticular waxes on the surface of their cutin matrix (Baker et al., 1975). However, a washing protocol which involves a line of brushes is likely to remove the natural wax coating from the fruit surface, thus increasing the water loss from the flavedo (Alférez et al., 2010). Consequently, additional wax is applied during packhouse procedures. Waxed fruit has an improved appearance and extended shelf life, probably due to less water loss and reduced shrinkage.

In a preliminary trial by Cronjé (2013), the response of ‘Nadorcott’ fruit to harvest treatments could be divided into three main categories. The first group, identified as fruit which were waxed the day after harvest and kept for five days at room temperature before cooling, had the lowest incidence of rind disorders. In the next group which developed the second worst rind disorder indices, fruit was only waxed after seven days, whereas the third group which displayed the highest incidence of rind disorders never received any wax treatment. The fruit from this group also showed the highest total water loss. Our results concur with findings from Cronjé (2013) as more often than not fruit which were moved from a high vapour pressure difference to high RH% to induce a dehydration and rehydration effect, but which received no wax application, resulted in the highest % moisture loss, along with a higher pitting incidence (Table 10; Table 11).

The postharvest rate of water loss has been found to be directly related to fruit and ambient temperature. Shading is an effective method that can be used in the field to reduce heat, direct solar radiation, and create microclimate (Chang et al., 2015). Chang et al. (2015) demonstrated that white net shading could be used during the late pre-harvest period in orchards to reduce the occurrence of sunscald and granulation of ‘Ponkan’ mandarin fruit without affecting fruit maturity and quality at harvest. However, its postharvest quality and shelf-life may be directly related to storage conditions, such as temperature and humidity.

Horticultural industries can suffer severe financial losses due to fruit moisture loss, which, in citriculture, can further translate to rind disorder development (Henriod, 2006). Ben-Yehoshua et al. (2001) and Porat et al. (2004) reported fruit to display better fruit quality, especially as pertaining to rind firmness and tissue turgidity at its final destination when moisture loss was reduced during the postharvest period under conditions of low temperature and reduced air velocities. The link between high RH conditions (that reduce fruit moisture loss) and decreased incidence of rind disorders such as chilling injury (Pantastico et al., 1968; Fujisawa et al., 2001; Porat et al., 2004), stem-end breakdown and button tissue collapse (Ben-Yehoshua et al., 2001; Porat et al*.,* 2004) have also been reported (Henriod, 2006).

Mechanisms of cellular collapse underlying rind breakdown are proposed to be the dehydration at low RH (45%) and subsequent rehydration at high RH (95%), which leads to water potential differences and tension in the flavedo-albedo interface. Studies by Alquezar et al. (2010) confirmed the flavedo to be the rind tissue most sensitive to water stress and that the albedo is most likely to maintain a functional water status at the expense of the flavedo.

Interpreting the incidence of postharvest disorders in response to a particular treatment is often made complex by the differences and erratic incidences of a particular disorder between seasons. However, the widespread incidence of non-chilling disorders, such as rind pitting, staining and rind breakdown in various citrus production areas strongly suggest common factors central to the cause of these disorders. Although some cultivars have been recognized to be less or more tolerant to prevailing environmental conditions such as low RH, to date, the basic handling practices, irrespective of susceptibility to rind disorders, in essence remain identical. In reaction to the known factors that impact on rind disorders it is recommended that climatic and varietal characteristics should be considered when developing handling protocols for the successful harvesting and storage of mandarin-type varieties (Grierson and Ben-Yehoshua, 1986).

4. Conclusion

Results from this study suggest that exposing fruit to dehydrating conditions such high VPD could impact on % moisture loss and potentially also increase rind disorder susceptibility. Dehydration prior to wax application is likely to increases disorders, however application of wax two to three days after harvest may reduce incidences of pitting/staining/rind breakdown compared to waxing five days after harvest. Further research is, however, required to verify these results and also to evaluate the applicability of these findings to other cultivars. A better understanding of the optimal manipulation of pre-harvest shading, such as the optimal shading materials, and shading periods, is needed to ensure the best possible quality at harvest, as well as the best conditions during the postharvest period to maintain high quality of fruit. Therefore, in the future, more research is needed to explore the effects of shading on postharvest quality, especially in relation to rind disorder incidence. Prevous results for Spanish indicated that this is the case in ‘Navel’ oanrges however these results do not concured.

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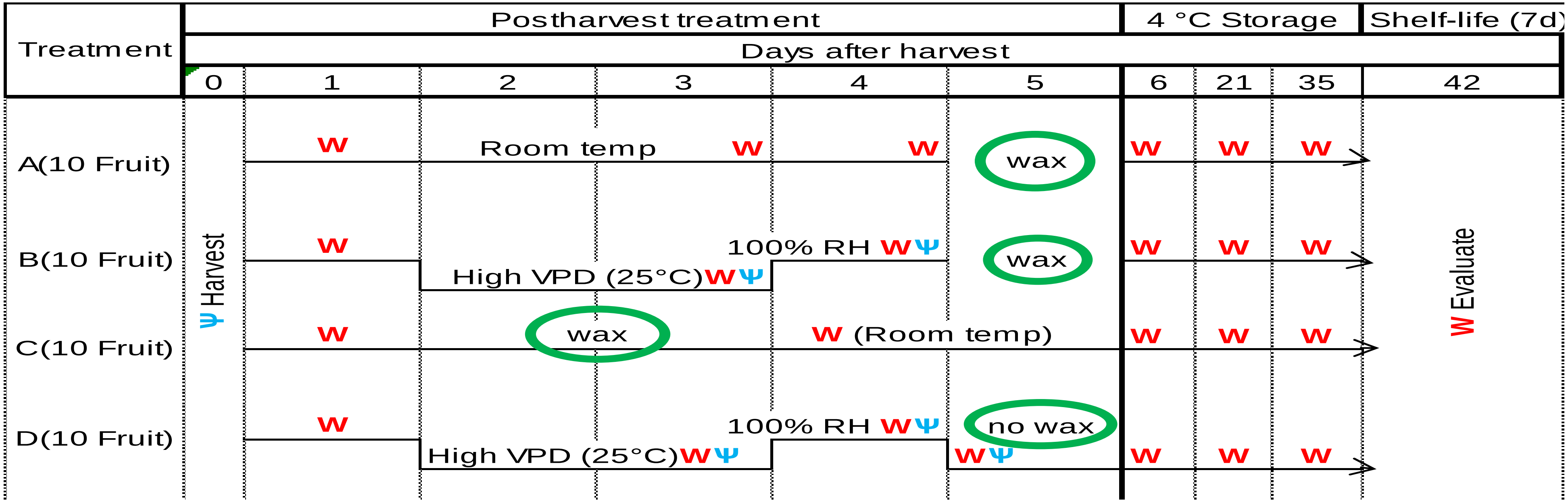

Fig. 1. A diagrammatic flow chart depicting the timeline and experimental conditions of the application of various postharvest treatments (n=10) on ‘Nules Clementine’ and ‘Nadorcott’ mandarin as harvested from Citrusdal and Riebeeck Kasteel in 2014 and 2015. Fruit weight (W) and rind water potential (ψ) determination, as well as waxing and evaluation for rind disorder indices are indicated as was performed over a 42 day postharvest period, of which fruit were stored at 4 °C for 30 days. Treatment B and D consisted of a postharvest stress whereby the fruit was dehydrated at a constant temperature of 25 °C and 60-80% RH to create conditions of high (0.7 to 1.1 kPa) vapour pressure deficit (VPD) for a two day period. Dehydration was followed by rehydration within a 100 % RH environment for 24h. Treatment B was waxed on day 5, whereas treatment D received no wax application. Treatment A and C was kept at room temperature (20 °C ± 1 °C), and waxed on day 5 or day 3, respectively.

Table 1

Demographic and cultivation information of ‘Nules Clementine’ and ‘Nadorcott’ mandarin on Carrizo citrange rootstock at two experimental sites (Citrusdal and Riebeeck Kasteel, South Africa) as was used in 2014 and 2015. A north-south row orientation and the use of drip irrigation was consistent throughout the trials.

Location	Farm and climatic data	Mandarin cultivar	Plant density (m)	Year planted	Yield (t.ha^{-1})
Citrusdal	Brakfontein (32°.25' S, 18°99'E)	'Nules Clementine'	4.50 x 2.50	1991	50
	Annual Rainfall: 334 mm	'Nadorcott'	4.50 x 2.50	2008	60
	Temp: max/min: 25.3 °C /10.9 °C				
Riebeeck Kasteel	Wynkeldershoek (33°.40’ S 18°.84’ E)	'Nules Clementine'	4.50 x 2.00	1993	40
	Annual Rainfall: 403 mm	'Nadorcott'	5.00 x 2.40	2007	60
	Temp: max/min: 19.2 °C /12.0 °C				

Table 2

The external fruit quality (n=10) of 'Nules Clementine' mandarin, cultivated at Citrusdal during 2014/2015, following a range of postharvest stress treatments. Fruit of treatment A and C was kept at room temperature (20 °C ± 1 °C), whereafter fruit were waxed on day 5 or day 3, respectively. Fruit of treatment B and D were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest. Fruit quality was quantified as colour (scoring by chart and hue°) at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	External fruit quality			
	Colour Chart (1-8)[v]		Hue°	
	Harvest	4 °C	Harvest	4 °C
2014				
A	6.10 ± 0.30[z]	3.90a[y]	110.78 ± 5.85	84.61c
B	6.10 ± 0.30	4.30a	110.78 ± 5.85	92.78a
C	6.10 ± 0.30	3.1ab	110.78 ± 5.85	87.44b
D	6.10 ± 0.30	2.30b	110.78 ± 5.85	87.09b
p-value	*1.0000*	*0.0002*	*1.0000*	*<.0001*
2015				
A	4.48 ± 0.55	1.60[ns]	---[x]	---
B	4.48 ± 0.55	1.90	---	---
C	4.48 ± 0.55	1.90	---	---
D	4.48 ± 0.55	1.60	---	---
p-value	*1.0000*	*0.3041*	---	---

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

[x] Measurement not performed.

[v] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 3

The external fruit quality (n=10) of 'Nules Clementine' mandarin, cultivated at Riebeeck Kasteel during 2014/2015, following a range of postharvest stress treatments. Fruit of treatment A and C was kept at room temperature (20 °C ± 1 °C), whereafter fruit were waxed on day 5 or day 3, respectively. Fruit of treatment B and D were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest. Fruit quality was quantified as colour (scoring by chart and hue°) at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	External fruit quality			
	Colour Chart (1-8)[v]		Hue°	
	Harvest	4 °C	Harvest	4 °C
2014				
A	3.40 ± 0.80[z]	2.20[ns]	84.07± 7.96	71.46c[y]
B	3.40 ± 0.80	2.20	84.07± 7.96	74.21a
C	3.40 ± 0.80	2.10	84.07± 7.96	72.76bc
D	3.40 ± 0.80	2.20	84.07± 7.96	72.82b
p-value	*1.0000*	*0.9721*	*1.0000*	*<.0001*
2015				
A	3.50 ± 0.50	2.00a	---[x]	---
B	3.50 ± 0.50	1.90a	---	---
C	3.50 ± 0.50	2.00a	---	---
D	3.50 ± 0.50	1.00b	---	---
p-value	*1.0000*	*<.0001*	---	---

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

[x] Measurement not perfomed.

[v] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 4

The external fruit quality (n=10) of ‘Nadorcott' mandarin, cultivated at Citrusdal during 2014/2015, following a range of postharvest stress treatments. Fruit of treatment A and C was kept at room temperature (20 °C ± 1 °C), whereafter fruit were waxed on day 5 or day 3, respectively. Fruit of treatment B and D were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest. Fruit quality was quantified as colour (scoring by chart and hue°) at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	External fruit quality	
	Colour Chart (1-8)[v]	
	Harvest	4 °C
2014		
A	1.20 ± 0.40[z]	1.00b[y]
B	1.20 ± 0.40	1.00b
C	1.20 ± 0.40	1.50a
D	1.20 ± 0.40	1.10b
p-value	*1.0000*	*0.0020*
2015		
A	1.00 ± 0.00	1.00[ns]
B	1.00 ± 0.00	1.00
C	1.00 ± 0.00	1.00
D	1.00 ± 0.00	1.00
p-value	*1.0000*	---[w]

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

[W] P-value is non-significant.

[v] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 5

The external fruit quality (n=10) of 'Nadorcott' mandarin, cultivated at Riebeeck Kasteel during 2014/2015, following a range of postharvest stress treatments. Fruit of treatment A and C was kept at room temperature (20 °C ± 1 °C), whereafter fruit were waxed on day 5 or day 3, respectively. Fruit of treatment B and D were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest. Fruit quality was quantified as colour (scoring by chart and hue°) at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	External fruit quality	
	Colour Chart (1-8)[v]	
	Harvest	4 °C
2014		
A	1.00 ± 0.00[z]	1.00[ns]
B	1.00 ± 0.00	1.00
C	1.00 ± 0.00	1.00
D	1.00 ± 0.00	1.00
p-value	*1.0000*	---[w]
2015		
A	1.10± 0.40	1.20[ns]
B	1.10± 0.40	1.20
C	1.10± 0.40	1.00
D	1.10±0.40	1.10
p-value	*1.0000*	*0.4994*

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[w] P-value non-significant.

[v] Colour chart for soft citrus (1: Orange, 8: Green) (Set no. 36, CRI, 2004).

Table 6

The internal fruit quality (n=10) of 'Nules Clementine' mandarin, cultivated at Citrusdal during 2014/2015, following a range of postharvest stress treatments. Fruit of treatment A and C was kept at room temperature (20 °C ± 1 °C), whereafter fruit were waxed on day 5 or day 3, respectively. Fruit of treatment B and D were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest. Internal fruit quality was quantified as % titratable acidity (TA), °Brix, °Brix:TA at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	Internal fruit quality					
	Titratable acidity (% TA)		°Brix		°Brix:TA	
	Harvest	4 °C	Harvest	4 °C	Harvest	4 °C
2014						
A	0.98 ± 0.05[z]	1.01[ns]	9.74 ± 0.35	11.11[ns]	9.94 ± 0.58	11.07[ns]
B	0.98 ± 0.05	0.99	9.74 ± 0.35	11.41	9.94 ± 0.58	11.58
C	0.98 ± 0.05	1.02	9.74 ± 0.35	11.10	9.94 ± 0.58	10.87
D	0.98 ± 0.05	1.05	9.74 ± 0.35	11.43	9.94 ± 0.58	10.93
p-value	*1.0000*	*0.1992*	*1.0000*	*0.2668*	*1.0000*	*0.0858*
2015						
Average	0.88 ± 0.35	---[x]	10.00 ± 0.40	---	11.36 ± 0.65	---

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[x] Measurement not performed.

Table 7

The internal fruit quality (n=10) of 'Nules Clementine' mandarin, cultivated at Riebeeck Kasteel during 2014/2015, following a range of postharvest stress treatments. Fruit of treatment A and C was kept at room temperature (20 °C ± 1 °C), whereafter fruit were waxed on day 5 or day 3, respectively. Fruit of treatment B and D were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest. Internal fruit quality was quantified as % titratable acidity (TA), °Brix, °Brix:TA at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	Internal fruit quality					
	Titratable acidity (% TA)		°Brix		°Brix:TA	
	Harvest	4 °C	Harvest	4 °C	Harvest	4 °C
2014						
A	0.94 ± 0.11[z]	0.80[ns]	11.63 ± 1.24	11.64[ns]	12.43 ± 1.14	14.78[ns]
B	0.94 ± 0.11	0.78	11.63 ± 1.24	11.48	12.43 ±1.14	14.79
C	0.94 ± 0.11	0.83	11.63 ± 1.24	11.63	12.43 ± 1.14	14.43
D	0.94 ± 0.11	0.81	11.63 ± 1.24	11.99	12.43 ± 1.14	15.17
p-value	*1.0000*	*0.9222*	*1.0000*	*0.4966*	*1.0000*	*0.8420*
2015						
Average	0.97 ± 0.08	---[x]	9.90 ± 1.40	---	10.24 ± 0.78	---

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[x] Measurement not performed.

Table 8

The internal fruit quality (n=10) of 'Nadorcott' mandarin, cultivated at Citrusdal during 2014/2015, following a range of postharvest stress treatments. Fruit of treatment A and C was kept at room temperature (20 °C ± 1 °C), whereafter fruit were waxed on day 5 or day 3, respectively. Fruit of treatment B and D were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest. Internal fruit quality was quantified as % titratable acidity (TA), °Brix, °Brix:TA at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	Internal fruit quality					
	Titratable acidity (% TA)		°Brix		°Brix:TA	
	Harvest	4 °C	Harvest	4 °C	Harvest	4 °C
2014						
A	0.97 ± 0.12[z]	0.81[ns]	11.22 ± 0.75	11.85[ns]	11.64 ± 1.29	14.69[ns]
B	0.97 ± 0.12	1.46	11.22 ± 0.75	12.40	11.64 ± 1.29	13.24
C	0.97 ± 0.12	0.82	11.22 ± 0.75	11.83	11.64 ± 1.29	14.57
D	0.97 ± 0.12	1.60	11.22 ± 0.75	11.83	11.64 ± 1.29	13.34
p-value	*1.0000*	*0.5556*	*1.0000*	*0.0449*	*1.0000*	*0.6257*
2015						
Average	1.37 ± 0.11	---[X]	13.30 ± 0.23	---	9.95 ± 0.87	---

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[x] Measurement not performed.

Table 9

The internal fruit quality (n=10) of ‘Nadorcott’ mandarin, cultivated at Riebeeck Kasteel during 2014/2015, following a range of postharvest stress treatments. Fruit of treatment A and C was kept at room temperature (20 °C ± 1 °C), whereafter fruit were waxed on day 5 or day 3, respectively. Fruit of treatment B and D were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest. Internal fruit quality was quantified as % titratable acidity (TA), °Brix, °Brix:TA at harvest and after cold storage at 4 °C for 30 days.

Postharvest treatment	Internal fruit quality					
	Titratable acidity (% TA)		°Brix		°Brix:TA	
	Harvest	4 °C	Harvest	4 °C	Harvest	4 °C
2014						
A	1.14 ± 0.15[z]	0.93[ns]	11.51 ± 0.56	12.07[ns]	10.25 ± 1.14	13.40[ns]
B	1.14 ± 0.15	0.96	11.51 ± 0.56	12.39	10.25 ± 1.14	13.22
C	1.14 ± 0.15	0.93	11.51 ± 0.56	12.07	10.25 ± 1.14	13.31
D	1.14 ± 0.15	0.95	11.51 ± 0.56	12.25	10.25 ± 1.14	13.17
p-value	*1.0000*	*0.9863*	*1.0000*	*0.6916*	*1.0000*	*0.9946*
2015						
Average	1.55 ± 0.06	---[X]	13.91 ± 0.54	---	9.01 ± 0.34	---

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

[x] Measurement not performed.

Table 10

Moisture loss, expressed either as % weight loss per day or % total weight loss, following postharvest stress treatments of ‘Nules Clementine’ mandarin from Citrusdal, harvested in 2014/2015. The treatments entailed control fruit waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest or fruit was kept at room temperature, waxed on day 3 and again left at room temperature (C). Percentage (%) weight loss was determined at various time intervals (T1-5) within the postharvest chain, which included a 30 day cold storage period at 4˚C, followed by shelf life evaluation after 7 days at ambient temperature. Where relevant, wax was applied between T2 and T3. The incidence of rind disorders was expressed as an index value (RDI) based on a 0-3 rating scale.

	% Weight loss per day					%Total weight loss (harvest to day 7 of shelf life)	Rind disorder index (0-3)	
Postharvest treatment	Harvest to dehydrate (T1)	Dehydrate to rehydrate (T2)	Day 0-15 of cold storage (T3)	Day 16-30 of cold storage (T4)	Day 0-7 of shelf life (T5)		Post storage (4 °C)	End of shelf life (ambient temperature) day 7
2014								
A	2.15b[y]	0.95[ns]	0.18[ns]	0.04[ns]	0.34b	10.02b	0.58[ns]	0.85[ns]
B	3.23a	0.44	0.19	0.06	0.39b	13.39a	0.54	0.69
C	1.87b	0.20	0.12	0.04	0.35b	7.86b	0.59	0.76
D	3.18a	0.43	0.25	0.06	0.59a	14.08a	0.62	0.75
p-value	*<.0001*	*0.9049*	*0.3536*	*0.0520*	*<.0001*	*<.0001*	*0.9744*	*0.9056*
2015								
A	0.99b	2.71[ns]	0.08b	0.16[ns]	2.39[ns]	9.24[ns]	0.22[ns]	0.51[ns]
B	3.24a	0.50	0.07b	0.12	2.64	8.87	0.24	0.38
C	1.47b	1.02	0.07b	0.14	2.85	8.25	0.22	0.36
D	3.30a	0.41	0.11a	0.14	2.71	9.78	0.30	0.50
p-value	*<.0001*	*0.1308*	*<.0001*	*0.8332*	*0.6104*	*0.6666*	*0.8086*	*0.6015*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 11

Moisture loss, expressed either as % weight loss per day or % total weight loss, following postharvest stress treatments of 'Nules Clementine' mandarin from Riebeeck Kasteel, harvested in 2014/2015. The treatments entailed control fruit waxed five days postharvest (A), whereas other fruit where first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest or fruit was kept at room temperature, waxed on day 3 and again left at room temperature (C). Percentage (%) weight loss at various time intervals (T1-5) within the postharvest chain, which included a 30 day cold storage period at 4˚C, followed by shelf life evaluation after 7 days at ambient temperature. Where relevant, wax was applied between T2 and T3. The incidence of rind disorders was expressed as an index value (RDI) based on a 0-3 rating scale.

	% Weight loss per day					%Total weight loss (harvest to end of day 7 shelf life)	Rind disorder index (0-3)	
Postharvest treatment	Harvest to dehydrate (T1)	Dehydrate to rehydrate (T2)	Day 0-15 of cold storage (T3)	Day 16-30 of cold storage (T4)	Day 0-7 of shelf life (T5)		Post storage (4 °C)	End of shelf life (ambient temperature) day 7
2014								
A	1.36b^{y}	0.40b	0.11^{ns}	0.08^{ns}	0.33^{ns}	7.28b	0.27^{ns}	0.33^{ns}
B	1.89a	0.96a	0.08	0.07	0.92	8.69a	0.35	0.49
C	0.97c	0.16b	0.14	0.18	0.96	5.52c	0.23	0.30
D	1.87a	0.77a	0.09	0.13	0.93	9.35a	0.24	0.32
p-value	*<.0001*	*<.0001*	*0.1861*	*0.497*	*0.1055*	*<.0001*	*0.6415*	*0.2211*
2015								
A	1.42b	1.13a	0.08^{ns}	0.11^{ns}	1.19ab	6.41^{ns}	0.00^{ns}	0.03^{ns}
B	2.86a	0.35b	0.07	0.06	1.14ab	6.12	0.00	0.02
C	1.28b	0.95a	0.08	0.14	0.48b	5.68	0.00	0.00
D	2.84a	0.35b	0.09	0.07	1.78a	7.10	0.02	0.07
p-value	*<.0001*	*<.0001*	*0.1001*	*0.4335*	*0.0063*	*0.4845*	*0.0992*	*0.4125*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 12

Moisture loss, expressed either as % weight loss per day or % total weight loss, following postharvest stress treatments of 'Nadorcott' mandarin from Citrusdal, harvested in 2014/2015. The treatments entailed control fruit waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest or fruit was kept at room temperature, waxed on day 3 and again left at room temperature (C). Percentage (%) weight loss was determined at various time intervals (T1-5) within the postharvest chain, which included a 30 day cold storage period at 4˚C, followed by shelf life evaluation after 7 days at ambient temperature. Where relevant, wax was applied between T2 and T3. The incidence of rind disorders was expressed as an index value (RDI) based on a 0-3 rating scale.

Postharvest treatment	% Weight loss per day					%Total weight loss (harvest to day 7 shelf life)	Rind disorder index (0-3)	
	Harvest to dehydrate (T1)	Dehydrate to rehydrate (T2)	Day 0-15 of cold storage (T3)	Day 16-30 of cold storage (T4)	Day 0-7 of shelf life (T5)		Post storage (4 °C)	End of shelf life (ambient temperature) day 7
2014								
A	0.62b[y]	0.36a	0.49[ns]	0.08bc	0.35[ns]	10.64[ns]	0.00[ns]	0.01b
B	2.25a	0.00b	0.05	0.06c	0.39	8.30	0.21	0.36a
C	0.80b	0.49a	0.07	0.10b	0.40	5.33	0.08	0.10b
D	2.17a	0.07b	0.12	0.13a	0.48	10.02	0.21	0.35a
p-value	*<.0001*	*0.0032*	*0.0797*	*<.0001*	*0.1307*	*0.2329*	*0.3428*	*0.0138*
2015								
A	1.78ab	0.88a	0.07b	0.12a	2.38b	7.61a	0.21[ns]	0.26[ns]
B	2.59a	0.09c	0.07b	0.08bc	2.17b	6.97ab	0.03	0.03
C	1.54b	0.60b	0.08a	0.09b	2.13b	6.67b	0.19	0.21
D	1.54b	0.08c	0.09a	0.09b	3.59a	7.72a	0.05	0.05
p-value	*0.0025*	*<.0001*	*0.0002*	*0.0049*	*<.0001*	*0.0214*	*0.2830*	*0.1503*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 13

Moisture loss, expressed either as % weight loss per day or % total weight loss, following postharvest stress treatments of ‘Nadorcott’ mandarin from Riebeeck Kasteel, harvested in 2014/2015. The treatments entailed control fruit waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest; or fruit was kept at room temperature, waxed on day 3 and again left at room temperature (C). Percentage (%) weight loss was determined at various time intervals (T1-5) within the postharvest chain, which included a 30 day cold storage period at 4˚C, followed by shelf life evaluation after 7 days at ambient temperature. Where relevant, wax was applied between T2 and T3. The incidence of rind disorders was expressed as an index value (RDI) based on a 0-3 rating scale.

	% Weight loss per day					%Total weight loss (harvest till day 7 shelf life)	Rind disorder index (0-3)	
Postharvest treatment	Harvest to dehydrate (T1)	Dehydrate to rehydrate (T2)	Day 0-15 of cold storage (T3)	Day 16-30 of cold storage (T4)	Day 0-7 of shelf life (T5)		Post storage (4 °C)	End of shelf life (ambient temperature) day 7
2014								
A	0.64^{ns}	$0.42a^{y}$	0.17^{ns}	0.06b	0.23^{ns}	5.65^{ns}	0.06b	0.07b
B	0.50	0.04b	0.15	0.05b	0.29	4.45	0.02b	0.03b
C	0.66	0.00b	0.15	0.06b	0.31	4.38	0.02b	0.04b
D	0.38	0.17b	0.12	0.13a	0.37	4.57	0.19a	0.19a
p-value	*0.4375*	*<.0001*	*0.9262*	*<.0001*	*0.0999*	*0.6356*	*0.0002*	*0.0007*
2015								
A	1.60^{ns}	0.89a	0.07b	0.07b	2.19^{ns}	6.62^{ns}	0.05b	0.05b
B	1.32	0.11b	0.08b	0.08ab	2.43	6.12	0.18a	0.18a
C	1.65	0.72a	0.07b	0.10a	2.16	6.94	0.06b	0.06b
D	1.55	0.11b	0.12a	0.09ab	2.40	6.91	0.10ab	0.10ab
p-value	*0.2170*	*<.0001*	*<.0001*	*0.0062*	*0.6422*	*0.1975*	*0.0386*	*0.0386*

^{ns} No significant differences.

^{y} Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 14

Moisture loss, expressed either as % total weight loss, following postharvest stress treatments of 'Nadorcott' mandarin from Patensie harvested in 2015 from open cultivated- and shaded-net orchards. The treatments entailed control fruit waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest; or fruit was kept at room temperature, waxed on day 3 and again left at room temperature (C). Percentage (%) weight loss was determined for the total postharvest chain, which included a 30 day cold storage period at 4˚C, followed by shelf life evaluation after 7 days at ambient temperature. The incidence of rind disorders were expressed as an index value (RDI) based on a 0-3 rating scale.

Postharvest treatment	%Total weight loss (harvest till day 7 shelf life)	Rind disorder index (0-3)	
		Post storage (4 °C)	End of shelf life (ambient temperature) day 7
Open-cultivated orchard			
A	7.10b[y]	0.00[ns]	0.00[ns]
B	5.80b	0.00	0.00
C	6.75b	0.00	0.00
D	9.18a	0.00	0.00
p-value	*0.0004*	---[w]	---
Netted orchard			
A	7.49ab	0.00[ns]	0.00[ns]
B	6.69b	0.00	0.01
C	6.53b	0.00	0.01
D	8.93a	0.00	0.03
p-value	*0.0054*	---	---

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

[w] P-value is non-significant.

Table 15

Moisture loss, expressed either as % total weight loss, following postharvest stress treatments of ‘Nadorcott’ mandarin from Bonnievale harvested in 2015 from open cultivated- and shaded-net orchards. The treatments entailed control fruit waxed five days postharvest (A), whereas other fruit where first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest; or fruit was kept at room temperature, waxed on day 3 and again left at room temperature (C). Percentage (%) weight loss was determined for the total postharvest chain, which included a 30 day cold storage period at 4˚C, followed by shelf life evaluation after 7 days at ambient temperature. The incidence of rind disorders were expressed as an index value (RDI) based on a 0-3 rating scale.

Postharvest treatment	%Total weight loss (harvest till day 7 shelf life)	Rind disorder index (0-3)	
		Post storage (4 °C)	End of shelf life (ambient temperature) day 7
Open Orchard			
A	7.13^{ns}	0.01^{ns}	0.01^{ns}
B	5.70	0.00	0.00
C	4.95	0.03	0.03
D	6.42	0.02	0.03
p-value	*0.7599*	*0.6409*	*0.6077*
Net Orchard			
A	4.77b^{y}	0.08^{ns}	0.08^{ns}
B	5.03b	0.00	0.00
C	4.25b	0.04	0.04
D	7.67a	0.03	0.03
p-value	*<.0001*	*0.4929*	*0.4929*

[ns] No significant differences.

[y] Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 16

Fruit rind water potential of ‘Nadorcott’ mandarin harvested from Citrusdal in 2015, as recorded at various intervals during the postharvest chain, after being exposed to different postharvest treatments. The treatments entailed control fruit waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest or fruit was kept at room temperature, waxed on day 3 and again left at room temperature (C). The rind water potential was measured by means of a Psypro water potential system.

Treatment	Rind water potential (Ψ_p)				
	After harvest (-MPa)	After dehydration (-MPa)	After rehydration (-MPa)	After 30 days cold storage at 4 ˚C (-MPa)	After 7 days shelf life at ambient temperature (-MPa)
A	-2.75 ± 0.20^z	-2.94a^y	-2.82^{ns}	-3.41b	-3.29^{ns}
B	-2.75 ± 0.20	-3.78b	-2.51	-3.16b	-3.17
C	-2.75 ± 0.20	-2.74a	-2.26	-2.40a	-3.29
D	-2.75 ± 0.20	-3.06a	-2.36	-2.80ab	-3.47
p-value	*1.0000*	*0.0011*	*0.3993*	*0.0040*	*0.9984*

^{ns} No significant differences

^{z} Values are the mean ± S.D. of measurements.

^{y} Means with a different letter within a column differ significantly at the 5% level (LSD).

Table 17

Fruit rind water potential of ‘Nadorcott’ mandarin harvested from Riebeeck Kasteel in 2015, as recorded at various intervals during the postharvest chain, after being exposed to different postharvest treatments. The treatments entailed control fruit waxed five days postharvest (A), whereas other fruit were first dehydrated for two days and then exposed to a rehydration protocol for one day, followed either by a wax (B) or no wax treatment (D), five days postharvest; or fruit was kept at room temperature waxed on day 3 and again left at room temperature (C). The rind water potential was measured by means of a Psypro water potential system.

Treatment	Rind water potential (Ψ_p)				
	After harvest (-MPa)	After dehydration (-MPa)	After rehydration (-MPa)	After 30 days cold storage at 4 ˚C (-MPa)	After 7 days shelf life at ambient temperatures (-MPa)
A	-3.03 ± 0.34^{z}	-1.99^{ns}	-2.35^{ns}	-3.19^{ns}	-2.18^{ns}
B	-3.03 ± 0.34	-2.91	-2.00	-3.40	-3.86
C	-3.03 ± 0.34	-2.74	-1.49	-2.29	-2.05
D	-3.03 ± 0.34	-2.72	-1.81	-3.02	-2.19
p-value	*1.0000*	*0.6906*	*0.8036*	*0.2830*	*0.3667*

[ns] No significant differences.

[z] Values are the mean ± S.D. of measurements.

Appendix 1

Minimum regquirements of mandarins for export (Department of Agriculture and forestry [DAFF] 2015).

Variety	Juice content (%)	°Brix	Titracible acid (TA) (%)	°Brix:Acid
‘Nules Clementine'	48%	9.0+	0.8 - 1.5	8.0:1
‘Nadorcotts'	48%	9.0+	0.8 - 1.5	8.0:1

Appendix 2: Photographic evidence of pitting lesions observed in ‘Nadorcott ‘mandarin fruit at harvest as produced in Riebeeck Kasteel in the 2014 season.

GENERAL DISCUSSION AND CONCLUSION

The external appearance of fresh citrus fruit determines its market value and therefore it should be free of any physical blemishes. Postharvest physiological disorders such as rind breakdown or rind pitting affect the external quality of all citrus cultivar groups, at non-chilling postharvest storage temperatures. The negative impact of postharvest rind disorders in citriculture experienced widely in South Africa is responsible for severe financial losses throughout the entire citrus supply chain.

The primary reasons for an increased focus on postharvest rind disorders lie with a greater awareness and demand from consumers for high quality fruit, along with the growth in mandarin production and export of these cultivars which are normally more prone to rind disorders than orange types. In just three years, southern African citrus exporters have increased their volumes from 100 million 15 kg cartons to 120 million (Chadwick, 2015). This initiative was on the back of an aggressive drive to retain and optimise existing markets as well as open new markets in Asia. However, the need to apply cold sterilisation protocols for export to previously untapped markets, greatly increased the incidences of physiological disorders. Citrus fruit can normally be stored for relatively long periods. However two major problems limit the long-term storage capability of citrus fruit, namely pathological (decay) and physiological breakdown resulting in the appearance of various rind disorders. Decay development problems can, to a large extent, be solved by applying fungicides. There is, however, no reliable commercial method/application to prevent the development of most types of rind disorders. Rind disorders can generally be divided into two main groups: chilling injuries (CI) that develop following storage at sub-optimal temperatures and non-chilling related physiological rind disorders.

The correct identification of a physiological disorder is the first step to rectify either a detrimental production practice or a postharvest handling procedure. Many of these rind disorders display similar symptoms and make accurate identification/classification very difficult. However, associations with certain descriptions of the lesions as well as developmental conditions are useful. The incidence of these disorders are, however, erratic and unpredictable, with high variability between seasons as well as among orchards and even fruit from a given tree. Postharvest handling and storage practices have been recorded to be the predominant cause of non-chilling related rind disorders. It is believed that these disorders are induced or aggravated by transferring fruit from a low to a high relative humidity (RH) environment, leading to a decreased vapour pressure deficit (VPD). In previous studies, the more severe the dehydration of fruit before transferring to high RH, the more prone citrus fruit became to rind staining, pitting or rind breakdown. Observations therefore suggest that variations in rind water status are determinants of rind disorder induction and development in sensitive citrus fruit. Industry feedback indicates that fruit exposed to moderate dehydration in the orchard are more prone to develop rind damage after storage at high RH, similar to fruit dehydrated under postharvest storage. Disorders are, however, an integration of pre- and postharvest conditions to which the fruit is subjected and resulted in the reason to investigate the effect of factors such as pre-harvest water stress and nitrogen application on rind quality and rind disorder incidence. Also, morphological changes in the citrus rind and the potential relation to alterations in water status induced by moderate dehydration of mandarin fruit during postharvest handling are to a large extent unknown. As part of the ongoing research focus in the South African citrus industry to reduce the development of postharvest rind disorders in citrus fruit, the objective of this project was to examine the influence of rind water content and alteration thereof by means of pre and postharvest stress on the internal and external quality of mandarin fruit.

This research was conducted on 'Nules Clementine' and 'Nadorcott' mandarins due to the complete lack of information on the impact of rind water balance on disorder incidences in these two cultivars. 'Nules Clementine' was the first major mandarin to be cultivated in South Africa and is harvested mid-May to mid-June. The 'Nadorcott' mandarin, referred to in Morocco as 'Affourer', is a late maturing mandarin harvested mid-July to mid-August. Plantings and production of this extremely valuable cultivar has increased exponentially, being driven by previously unheard-of high prices for citrus fruit. However, the cultivar is susceptible to the development of postharvest rind disorders (developing from harvest till sale in the market), a disorder that couldt threaten its market sustainability. The experiments thus focused on these two widely planted mandarin cultivars as they have two distinct commercial harvest dates and are known to develop postharvest rind disorders.

In addition to the impact of rind water status on rind disorders, the study assessed the influence of late nitrogen soil applications on Mandarin (*Citrus reticulata* blanco) fruit quality. Nitrogen application during stage II of mandarin fruit growth is reported to impact negatively on rind quality (Iglesias et al., 2001) and by applying additional nitrogen viz. 20 and 40kg·ha^{-1} early (Jan.) and late (March) in the growing season, the influence on pitting and rind breakdown (RBD) was determined. The nitrogen trial was conducted in an attempt to clarify whether late N fertilization has a negative impact on rind quality. If no negative impact exists, it would enable producers to increase N-levels in trees which could potentially improve flower quality, which is difficult to do via the standard N foliar application on 'Nadorcott' which is harvested late in the season. Aspects measured were rind colour and incidence of disorders. In general across the areas, seasons and cultivars studied, no significant difference in fruit colour or size were recorded. Furthermore, no consistent differences were seen after the fruit had been stored at either -0.6 °C or 4 °C among the N treatments. Mineral nutrient analysis recorded that N applications did not increase the N levels in the leaves, indicating that these N amounts used (and which were chosen on advice from nutrition consultants), could be increased in future projects to determine a threshold level. From this study, the expected decreased rind colour due to increased nitrogen application was therefore not confirmed. Although the incidence of pitting was recorded, it was generally at too low levels for all the treatments to warrant being included in the data analysis and therefore could not be correlated with N treatments. However, the lack of disorders did indicate that these two treatments (N and storage temperature) did not lead to higher pitting susceptibly. It was also not possible to link pre-harvest rind mineral content with the eventual incidence of rind pitting during subsequent storage with additional N applications. The interaction between N application rate and time should be investigated in subsequent studies as the lack of higher N levels in leaves and rind of treated trees could indicate that even higher N could be applied between Jan-March. In addition the impact on flowering and fruit set should be measured.

Pre-harvest water stress seemed to have little or no effect on fruit susceptibility to postharvest physiological disorders in this study. It was, however, observed that an early wax application after harvest may significantly decrease moisture loss, coinciding with lower incidences of rind disorders. The pre-harvest water stress treatment needs to be re-evaluated and an attempt should be made to apply more severe stress environments to the trees. When repeating this trial, plastic soil coverage can be laid earlier in order to cut off water supply to the trees for longer periods. Also to be included is the comparison between fruit harvested from non-water stressed trees which receive the same postharvest treatments, to serve as a control. This should give a better indication of the effect of pre-harvest water stress on fruit’s susceptibility to postharvest rind disorders.

Although the experimental systems and the storage conditions used during the evaluation of the influence of postharvest handling on mandarin fruit quality, do not correspond with the standard postharvest procedures for mandarin fruit, they were effective in inducing fruit rind pitting. The experimental procedure of dehydration followed by rehydration mirrors the commercial conditions and the higher incidence of disorders shown under these conditions were detrimental to rind quality and should be improved upon in future studies on factors responsible for these disorders. It is recommended that moisture loss rather be expressed in terms of rind and pulp (than whole fruit). Values and norms were

RDI meet practical significance, and have a commercial impact on the industry, must also be deteremined.

All of these trials were an attempt to change production and postharvest practices to ensure better quality fruit can be delivered to the market. Being part of a holistic value chain, all facets from production up to sales will eventually determine the end quality of the fruit. Preventative measures should therefore begin at orchard level. Postharvest fluctuations in RH and temperature lead to changes in vapour pressure deficit (VPD) that will drive water loss from the fruit rind. It is common to experience fluctuations in RH during the citrus harvest season in the orchards in South Africa, because it is normally very dry with temperatures between 20-30 °C. Moreover, harvested fruit may experience up to 24 hours of low RH exposure during transportation to the pack house and subsequent handling.

Citrus fruit and tree physiology are known to be influenced by the environment. Microclimatic conditions during the growing season and within the canopy may influence the physico-chemical and biochemical properties of the fruit rind. The incidence of rind disorders could therefore be impacted on by light penetration within the canopy. In a preliminary study the impact of shade netting was evaluated. Caution should, however, be taken when viewing these results, as it is based on only one season's data sampled from a commercial covered orchard. This project was however initiated to establish the first link between 'Nadorcotts' produced under shade nets and postharvest rind quality, particularly as pertaining to the incidence of disorders. Although a slightly higher % moisture loss was observed in fruit harvested from orchards produced under nets in Patensie, the incidence of rind disorders were negligibly low. No negative influence on rind quality could be seen in fruit compared between production under net or open orchard from either the Patensie or Bonnievale orchards. A more positive impact of nets will undoubtedly be seen in areas with an arid climate (as found in areas such as Upington) or high light intensity (light reflecting off the white sand in the Sandveld area). With the increasing popularity of the use of shade nets, it is suggested that further research evaluating the effect thereof on vegetative growth, export quality and disorder incidence (whether chilling injury or physiological rind disorders) be conducted. Trees planted at a wider spacing mature earlier than fruit from trees planted at higher densities. This difference is attributed to higher air temperatures (which could lead to lower micro environmental RH) and greater light penetration at the wider spacing. When establishing a new orchard, tree spacing should therefore be kept in mind due to the possible impact on the occurrence of rind disorder incidence, especially since orchard practices are much more difficult when trees are already covered with nets.

For future studies, trials could to be done to establish the influence of harvest date on rind sensitivity so as to determine green vs over mature fruit. It has been noted that the first and last picking is usually responsible for more rind disorders. Trials should also include more orchards (different areas) and different cultivars. Comparing data in these types of experiments will contribute towards understanding rind maturity and possibly help in identifying a method to ensure quality rind maturity, a phenomenon which is still eludes the citrus industry.

The results on the nitrogen treatment call for a revision of the industry' prescribed recommendations on nitrogen application, specifically for the new group of cultivars harvested in the last part of the season i.e. "late mandarins". Citrus rootstocks have well-known effects on tree size, crop load, fruit size and various fruit quality factors and the need exists to test the impact of various rootstocks combined with different cultivars on rind disorder incidence. New rootstock cultivars may have the potential for decreasing the occurrence of rind disorders. The application and different concentrations of postharvest fungicides (e.g. Thiabendazole) in citrus packhouses should also be evaluated to ultimately optimize its effectiveness in reducing the incidence of rind disorders. The expansion of new cultivars is made difficult through the availability of plants and the payment of royalties resulting in a very high financial impact on the producer. In order to make informed decisions, farmers must therefore have as much information as possible regarding cultivars. Although thorough evaluation of the trees might be difficult when evaluating new cultivars, the susceptibility of their fruit to chilling injury and rind disorders should at least be screened and included in the information upon release of the cultivars. Certain cultivars can then be recommended for specific markets.

To conclude from these studies: Considering the interesting results from the nitrogen trial it is suggested that the industry's prescribed recommendations be revisited after further validation in large scale experiments. Pre-harvest water stress seemed to have little or no effect on fruit susceptibility to postharvest physiological disorders. This theory must, however, be further investigated in summer rainfall areas and under more severe stress conditions. Through postharvest dehydration, fruit susceptibility for pitting development can be increased. Dehydration prior to wax application increased rind disorder incidence whereas wax application 2-3 days after harvest reduced the incidence of pitting/staining. It should be remembered that due to the large volume of citrus fruit shipped, a small % decrease in rind disorder incidence can have a large positive financial impact.

Rind disorder development was found to differ between cultivars, areas and seasons and all parties in the production and processing chain should be aware of these variations. It is of utmost importance that the industry keeps on developing practices and continues to ask relevant questions and address problems associated with these disorders. The ideal situation will be that a protocol be specifically developed for each cultivar per area to reduce rind disorders. Some preliminary recommendations have been made from these studies which can form part of this protocol and hopefully with future research, more information can be added and in the long run add value to the industry. It is concluded that commercial strategies to reduce rind disorders should involve a change in pre-harvest management practices i.e. fertilizer application, rootstock choice, irrigation, as well as postharvest handling practises such as temperature and RH management of the fruit from the orchard to the packhouse. Maintaining constant RH conditions during postharvest handling and storage are of key importance in preventative rind disorder protocols. Harvesting susceptible cultivars at high RH and minimizing exposure to low RH after harvest could reduce the commercial impact of postharvest rind disorders, although implementing this on day-to-day farming operations is not always practical.

Studies on postharvest disorders are made extremely difficult due to their erratic (and low) incidence between seasons. Within this study certain correlations could be made between factors influencing rind quality (climatic influence, moisture loss and colour) and rind disorder incidence. However, no single factor could be identified as the main underlying cause of rind disorders. Even though some results lack explanation, the “induction” of rind disorders in some inctances by stressing the fruit pre-harvest or the lack of expected decreased rind colour due to increased nitrogen application, can be regarded as a positive discovery. These data can, therefore, help serve in unravelling the factors predisposing the citrus fruit rind to progressive postharvest disorders.

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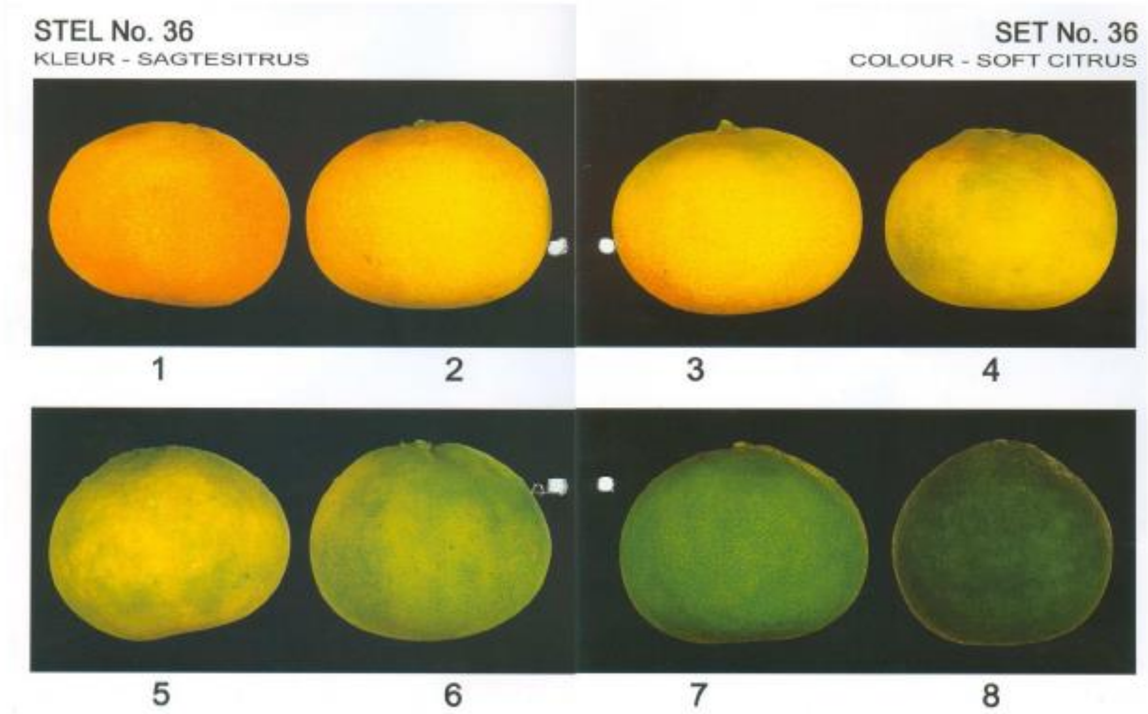

Appendix A: Colour chart for soft citrus (1: Orange, 8: Green) (CRI, 2004).